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THE EFFECT OF THE MAIN METEOROLOGICAL ELEMENTS ON THE 24 HOUR VARIABILITY OF MOISTURE OF SOIL UNDER RYE AND POTATO

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K e y w o r d s: soil moisture, meteorological factors, rye, potato.

A b s t r a c t

The aim of the study was to assess the variability of light soil moisture in relation to the course of main meteorological elements. This relationship was described by the analysis of linear regression, using the Statistica 6.1 program. The material in the study was based on the 24 hr results of moisture measurements of soil under the cultivation of rye and potato in the periods of their vegetation and on the values of meteorological elements in the years 2001-2003 gathered at the Lipki Agrometeorological Station near Stargard Szczecinski. It was observed that the variability of soil moisture to a depth of 10 cm under rye and potato cultivation is mainly formed by precipitation totals. Whereas moisture in deeper soil layers under rye depends, first of all, on the air humidity, and under potato cultivation, on thermal conditions of air and soil.

DOBOWA ZMIENNOŚĆ WILGOTNOŚCI GLEBY POD UPRAWAMI ŻYTA I ZIEMNIAKA W ZALEŻNOŚCI OD PRZEBIEGU GŁÓWNYCH ELEMENTÓW METEOROLOGICZNYCH

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S ł o w a k l u c z o w e: wilgotność gleby, elementy meteorologiczne, żyto, ziemniak.

A b s t r a k t

Celem pracy była ocena zmienności wilgotności gleby lekkiej w zależności od przebiegu głównych elementów meteorologicznych. Zależność tę opisano za pomocą analizy regresji liniowej, z wykorzystaniem programu Statistica 6.1. W pracy uwzględniono dobowe wyniki wilgotności gleby pod

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uprawami żyta i ziemniaka w okresach ich wegetacji oraz wartości elementów meteorologicznych z lat 2001-2003, pochodzące ze Stacji Agrometeorologicznej w Lipkach k. Stargardu Szczecińskiego. Stwierdzono, że zmienność wilgotności gleby do głębokości 10 cm, pod uprawami żyta i ziemniaka, kształtują głównie sumy opadów. Natomiast wilgotność w głębszych warstwach gleby pod uprawą żyta zależy, przede wszystkim, od warunków wilgotnościowych powietrza, a pod uprawą ziemniaka – od warunków termicznych powietrza i gleby.

Introduction

Soil moisture depends first of all on the course of the weather, whereas in the case of the soil with plants growing on it, its moisture also depends on the plant species and the plant growth phase. As the plants grow they modify to a large extent the course of soil moisture, particularly during the periods of the strongest water requirement.

The knowledge of soil moisture allows for the determination of the water reserves of soil and thus for the assessment of fulfillment of the plants' water requirements. Generally, to achieve this aim, indirect methods are used consisting in working out models based on different statistic-mathematical methods, from simple ones of linear regression to artificial neuron nets and sophisticated mechanistic simulative models (DANALATOS et al. 1994, KOŹMIŃSKI, MICHALSKA 1999, WIGNERON et al. 1999, ŻYROMSKI 2001a,b, LAMORSKI, WALCZAK 2002, SAU et al. 2002, QIU et al. 2003, KOŹMIŃSKI et al. 2003, EITZINGER et al. 2004).

The attempts of indirect estimation of soil moisture are made not only on the basis of selected meteorological elements (KOŹMIŃSKI, MICHALSKA 1999, KOŹMIŃSKI et al. 2003, ŻYROMSKI 2001a), but also on the basis of the level of ground waters (ŻYROMSKI 2001b), satellite photos (TANSEY et al. 1999), thermovision readings (LUBECKI, ŁACHACZ 1994), or on the basis of a leaf surface index (LAI) and the growth rate of dry matter (IGRAS, KUBSIK 1999). There are also models for calculating retention of soils using only those parameters which define their physical properties (DANALATOS et al. 1994, WITKOWSKA-WALCZAK, WALCZAK 2002). Depending on the applied method the obtained soil moisture forecasts are from satisfactory to very good ones.

The most accurate results are obtained by direct measurements, which in a continuous way have been taken in our country only at a few university research stations using a traditional laborious dryer method, most often every ten days. New technological solutions make it possible to record automatically and continuously soil moisture changes what, along with more and more common automatic meteorological measurements, may be the basis for a detailed analysis of the effect of weather conditions on this element of the soil climate.

The aim of this study was to assess the relationship between the moisture of soil under rye and potato in the periods of their vegetation and the course of the main meteorological elements in the 24 hour system on the basis of three year automatic measurements of light soil moisture carried out at the Lipki Station near Stargard Szczeciński.

Materials and Methods

In the study the data from the Lipki Agrometeorological Station near Stargard Szczeciński were used. The station is situated in the central part of Nizina Szczecińska (Szczecin Lowlands) ($\varphi = 53^{o}21'$; $\lambda = 14^{o}58'$), about 1,8 km NE of Miedwie Lake, 30 m above sea level. In the area of the station there is light, sour brown soil formed from boulder sand with clay inserts at a depth of 70-80 cm without a rise in ground water (NIEDŹWIECKI, KOŹMIŃSKI 1994). The field water volume for the layer to a depth of 100 cm equals 169 cm and average 10 day reserves of useful water in fallow soil vary from 88 to 122 mm during vegetation (KOŹMIŃSKI, MICHALSKA 1995).

The basic material for the study were the 24 hour period results of automatic measurements of volumetric soil moisture ($m^3 \cdot m^{-3}$), presented as a mean of measurements taken at 2 a.m., 8 a.m., 2 p.m. and 8 p.m. The results of soil moisture measurements were obtained in three measurement series for the soil under rye: 26 April – 29 July 2001; 11 May – 28 July 2002 and 4 May – 29 July 2003 and two series for the soil under potato: 11 May – 30 August 2002 and 4 May – 2 Sept. 2003. The soil moisture measurements were made by means of the Theta Probe sensors manufactured by the Delta-T firm, which were installed at the depths of 5, 10, 20, 30, 50 cm and additionally at the depth of 70 cm in the soil under rye. For the analysis, the 24 hour period values of the following meteorological elements were taken into consideration: the temperature of the soil under rye at the depths of 5, 10, 20 and 50 cm, and under potato only at the depths of 10 and 50 cm, the precipitation, the relative humidity and the deficiency of air humidity.

Statistical relationships between the soil moisture and the meteorological elements were determined by the analysis of a simple linear regression at the level of significance of at least $\alpha = 0.1$. Since soil moisture is not an immediate effect of actual weather conditions, the meteorological elements taken into account in the analysis concerned not only the same days as the dependent variable but also one to nine days in advance.

24 hr variability of soil moisture was determined by means of a random coefficient of variation (%) being a quotient of a mean value and a standard deviation.

Results and Discussion

Thermo-precipitation classification of individual months in the period from 1961 to 2003 at the Lipki Station near Stargard Szczeciński carried out by MICHALSKA and KALBARCZYK (2005), shows that the years of the studies, i.e. 2001-2003 can be regarded as a group of warm years. Most of the analysed months (except for July 2001, which was a cold month) were even very warm. August in 2002 and also August in 2003 and July in 2003 turned out to be extremely warm. Whereas, due to the precipitation totals most of the months were qualified as dry or very dry. Only June in 2001 and July in 2003 were marked by the precipitation totals exceeding the value of the many year period by 30 and 14 mm, respectively.

During the analysed period the average moisture under rye in the soil profile to a depth of 70 cm varied from 8.5 to 18.0%, while under potato, but to the depth of 50 cm, it ranged from 4.8 to 13.6%.

The moisture of top layers of soil is the best illustration of actual weather conditions, thus it undergoes the strongest changes from day to day, as is shown in Figure 1. A stronger reaction of moisture to precipitation is characteristic of soil under potato. For example, on 20th June 2003 after 24 hr precipitation of about 25 mm, the soil moisture at a depth of 5 cm under potato increased from about 3 to 30%. A large increase in moisture at this depth can be also observed after smaller precipitation, but occurring in the period of a few successive days (e.g. from 1st to 6th July). Whereas a long non-precipitation period and at the same time high temperatures (the average summer temperature was by 3.3^{o}C higher than the standard) caused a decrease in moisture at a depth of 5 cm to about 2% and at a depth of 10 cm – to 0%.

The analysis of soil moisture both under rye and potato in the studied years shows that the 24 hour totals of precipitation of the value below 2.5 mm do not, generally, affect the growth of soil moisture and this is convergent with the results reported by KOŹMIŃSKI and MICHALSKA (1999) and KOŹMIŃSKI et al. (2003). Only 24 hr precipitation above 5 mm (on individual days or in a series of successive days) changed the soil moisture in the top layer, and if the precipitation was above 10 mm it changed the moisture at larger depths. ŻYROMSKI (1990) also reports that the precipitation totals not exceeding 10 mm in a ten day period do not contribute to an effective supply of soil profile with water.

In the years of studies the coefficient of 24 hr variability of soil under rye equalled about 48% at a depth of 5 cm and it showed a drop to about 18% at a depth of 70 cm. The moisture changes in the soil under potato to a depth of 5 cm and also 10 cm were still larger, as the coefficients of random variation amounted respectively to 83 and 82% and they showed a decrease to about 38% in the deeper layers.

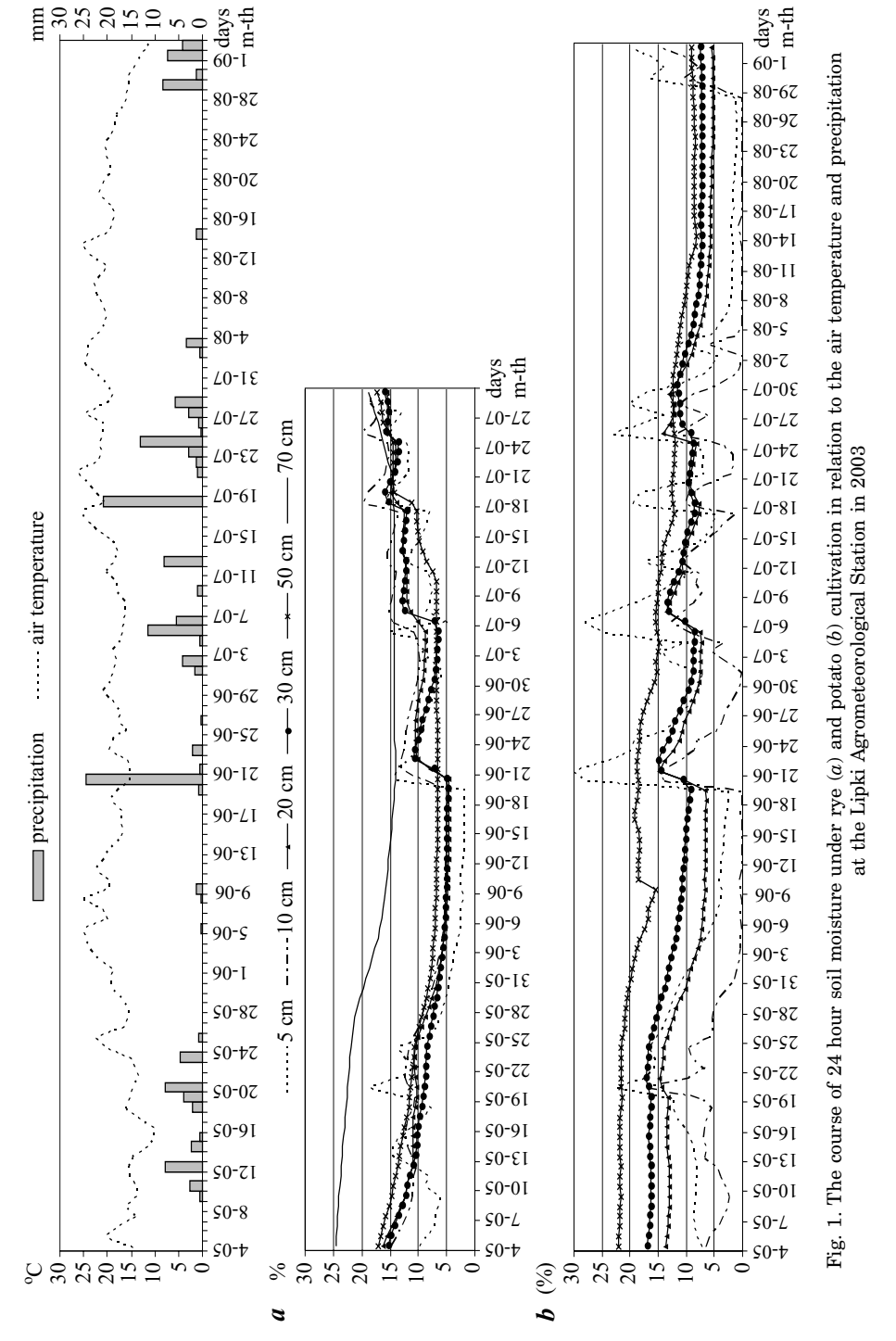
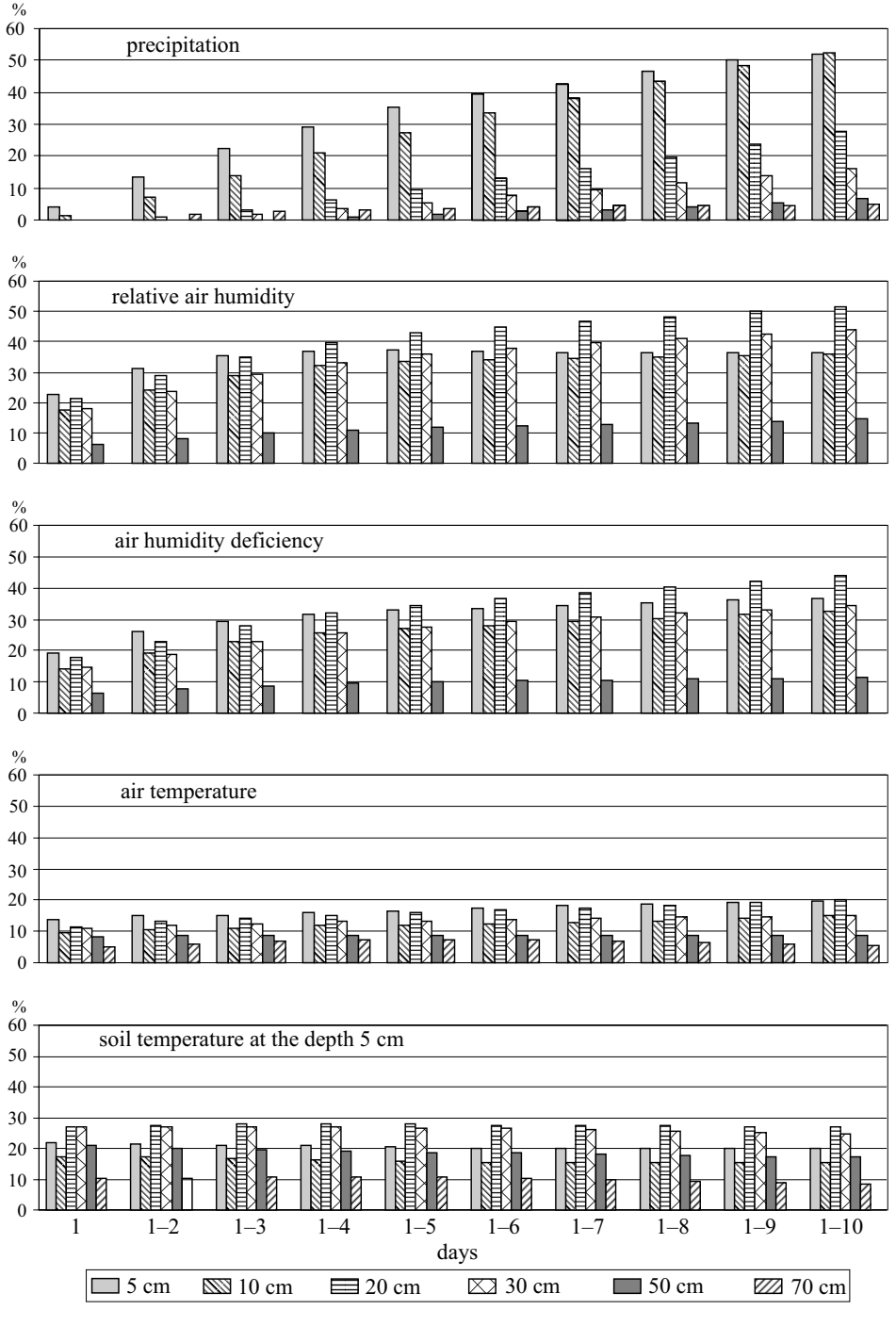


Fig. 1. The course of 24 hour soil moisture under rye (*a*) and potato (*b*) cultivation in relation to the air temperature and precipitation at the Lipki Agrometeorological Station in 2003


%
precipitation
relative air humidity
air humidity deficiency
air temperature
soil temperature at the depth 5 cm
0
10
20
30
40
50
60
1
1–2
1–3
1–4
1–5
1–6
1–7
1–8
1–9
1–10
days
5 cm
10 cm
20 cm
30 cm
50 cm
70 cm

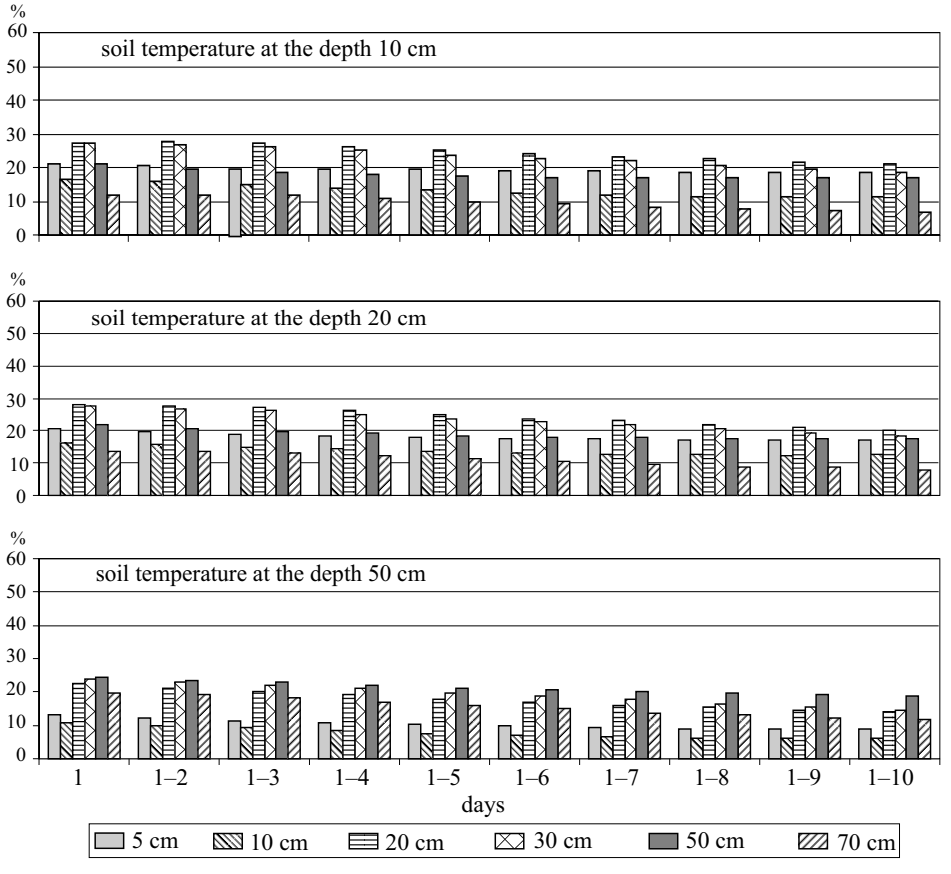
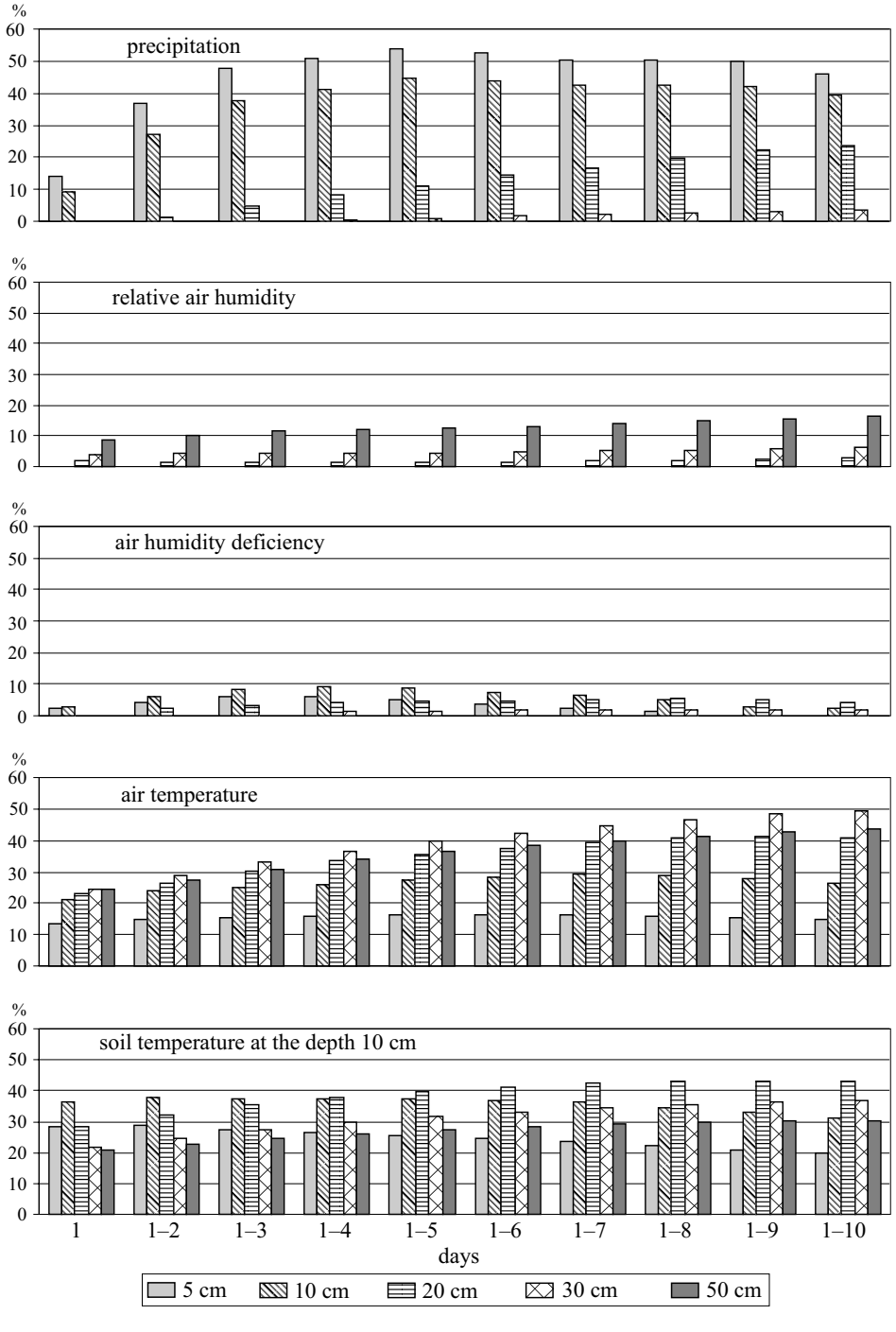


Fig. 2. Coefficients of determination (R^2 in %) for the 24 hour relationship between the soil moisture under rye cultivation and the meteorological elements of the day on which the measurements were taken (1) and also of the successive days, one to ten (1-2, ... 1-10), preceding the evaluation of the soil moisture (years 2001-2003)

In the 24 hr course of the values of soil moisture under rye and potato cultivation, a consequent decrease in these values, despite periodical fluctuations, can be observed at all the analysed depths. Independently of current weather conditions, the periods of the average smallest moisture correspond to the occurrence of critical periods of water administration in rye and potato. For example, on 30th May 2003 soil moisture at a depth of 50 cm under rye, which was flowering, amounted to 8.2%, while under potato, in which full seedlings were observed, it was more than two times larger.

The coefficients of determination for the relationship between the moisture of soil under rye and potato and selected meteorological elements are pres-


%
precipitation
relative air humidity
air humidity deficiency
air temperature
soil temperature at the depth 10 cm
0
10
20
30
40
50
60
1
1–2
1–3
1–4
1–5
1–6
1–7
1–8
1–9
1–10
days
5 cm
10 cm
20 cm
30 cm
50 cm

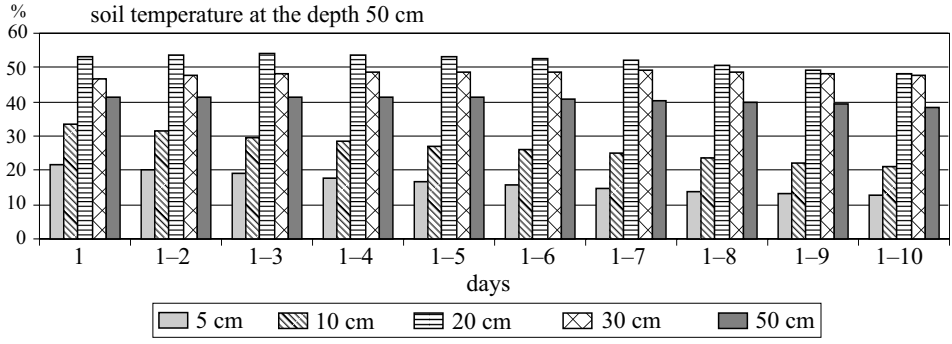


Fig. 3. Coefficients of determination (R^2 in %) for the 24 hour relationship between the soil moisture under potato cultivation and the meteorological elements of the day on which the measurements were taken (1) and also of the successive days, one to ten (1-2, ... 1-10), preceding the evaluation of the soil moisture (years 2002-2003)

ented in Figures 2 and 3. They include only the results statistically significant at least of $\alpha = 0.1$. The 24 hr variability of soil moisture under rye was formed first of all by precipitation conditions and air humidity, whereas thermal conditions of air and soil had a less significant role. However, a dominant role of precipitation was observed only in the 10 cm layer, as earlier was reported in a 24 hour outline of results by KOŹMIŃSKI and MICHALSKA (1999), and also KOŹMIŃSKI et al. (2003). The effect of precipitation on soil moisture at a depth of 20 cm was two times smaller than at the depths of 5 or 10 cm, and at deeper parts of the profile, i.e. 50 or 70 cm, it was insignificant. It is worthwhile to notice that independently of the range of precipitation effects on soil moisture, there is a clear successive improvement in the role of precipitation as the period of the 24 h precipitation totals lengthens. The best effect, concerning all the depths, was obtained on the basis of precipitation total of the ten day period (including the day of the measurements and nine preceding days). In the layer to a depth of 10 cm the coefficient of determination increased to about 50%, taking into account precipitation totals of 10 days, whereas when the precipitation totals covered only five days before the measurements, it amounted to about 27%.

A better description of the variability of soil moisture was obtained on the basis of relative air humidity, while a weaker description was achieved if moisture deficiency was considered (Figure 2). However, both elements, contrary to precipitation, had also a large influence on the soil moisture in deeper soil layers, mainly to 30 cm. As in the case of precipitation, the best description was obtained using the characteristics of the ten day periods.

As regards thermal conditions, a stronger relation with the moisture of soil under rye shows a soil temperature and a weaker one, a temperature of air.

While, with the lengthening of the period of averaged data concerning the air temperature, a slight improvement of the soil moisture description is observed, in the case of soil temperature (except for the shallowest layer), it is quite the opposite. For the largest coefficients of determination are obtained when the temperature of the same day as soil moisture is taken into account or of the period of one to three days before the measurements. It is particularly marked in the case of the soil temperature at a depth of 10 cm and then at a depth of 20 cm. Moreover, the results presented in Figure 2 show on the whole that it is less possible to describe the moisture by means of all the analysed meteorological elements in deeper soil layers, particularly at the depths of 50 and 70 cm.

As far as the soil under potato is concerned and similarly as that under rye cultivation, the atmospheric precipitation affected mainly a top layer of soil to a depth of 10 cm (Figure 3). However, while in the soil under rye the precipitation total of ten successive days was needed to obtain a coefficient of determination amounting to about 50%, in the case of soil under potato a description of similar accuracy was already assured by the precipitation total of 4 or 5 successive days. Whereas the largest coefficients of determination for the depth of 20 cm were obtained when the precipitation totals of ten day periods were taken into consideration, but they were half the value of those in the 5 and 50 cm layers.

In 2003-2005 the air humidity had an insignificant influence on the moisture of soil under potato, contrary to the case of the soil under rye. Thermal conditions of air and soil had a stronger effect (Figure 3). However, it is interesting that the air temperature and the temperature of soil described variability of soil moisture better at larger depths and worse – in the shallower layers. The air temperature determined the soil moisture at the depth of 30 cm and its best illustration for the accuracy of statistical description of soil moisture, also at the depths of 20 and 50 cm were the averages of ten days (Figure 3). The strongest relationship between soil moisture and soil temperature was observed at a depth of 20 cm, and the largest coefficients of determination, exceeding 50% concerned the soil temperature measured at a depth of 50 cm. Contrary to the air temperature, the differences between coefficients of determination obtained for various lengths of the periods of averaging the soil temperature were on the whole insignificant.

Among the considered meteorological elements, higher precipitation totals and larger relative air humidity resulted in an increase in the moisture of soil under both rye and potato cultivation, whereas a soil moisture decrease was caused by an increase in the air and soil temperature and also the deficiency of air humidity.

Conclusions

1. The largest 24 hr variability of soil moisture under both rye and potato cultivation was characteristic of the top soil layer to a depth of 10 cm. The 24 hour fluctuation in moisture was markedly higher in the soil under potato cultivation.

2. The influence of the weather on soil moisture depends to a large extent on the type of cultivation. The 24 hour variability of the soil moisture under rye cultivation was above all affected by precipitation and air humidity, whereas that under potato cultivation depended on precipitation and thermal conditions of air and soil.

3. As regards the type of cultivation and the depth, a better description of the soil moisture was assured by the meteorological elements of the same days as the results concerning soil moisture or of the periods even nine days before the measurements of moisture were taken.

4. A distinct increase in soil moisture caused by atmospheric precipitation, both in the soil under rye and potato cultivation was observed in the top soil layer, to 10 cm. However, under rye cultivation a better description of soil moisture was achieved as the period of precipitation totals was lengthened to ten days, whereas in the soil under potato cultivation a similar correlation of accuracy was assured by the precipitation totals of both a three day and ten day period.

5. Thermal conditions of air and soil had considerably larger influence on the soil moisture under potato than under rye cultivation and their effect was mainly marked in deeper layers of soil.

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THE EFFECTS OF SOIL CONTAMINATION WITH FUNGICIDES ON MICROORGANISM COUNTS

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K e y w o r d s: soil contamination, fungicides, microorganism counts.

A b s t r a c t

A pot experiment was performed to determine the effects of soil contamination by fungicides, Unix 75 WG and Swing Top 183 SC, on the counts of soil microorganisms. The experiment was established on brown soil developed from heavy loamy sand with pH_{KCl} = 6.7. The variable experimental factors were the type of fungicide and the dose of fungicide (0, 1 – recommended dose, 10-fold dose and 100-fold dose), the method of soil management (soil cropped to spring barley and uncropped soil) and the time of analysis (14, 28, 42 and 56 days),

It was found that the counts of microorganisms in soil contaminated by fungicides, Unix 75 WG and Swing Top 183 SC, were affected by the type and dose of fungicides, the method of soil use and the time of analysis. The fungicides, applied at recommended doses, stimulated the proliferation of copiotrophic bacteria and actinomycetes, but inhibited (irrespective of the dose) the growth of *Azotobacter* spp. and *Pseudomonas* spp. Higher counts of oligotrophic and copiotrophic bacteria, actinomycetes and *Azotobacter* spp. were recorded in soil cropped to spring barley.

WPŁYW ZANIECZYSZCZENIA GLEBY FUNGICYDAMI NA LICZEBNOŚĆ MIKROORGANIZMÓW

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S ł o w a k l u c z o w e: zanieczyszczenie gleby, fungicydy, liczebność drobnoustrojów.

A b s t r a k t

W doświadczeniu wazonowym badano wpływ zanieczyszczenia gleby (brunatnej właściwej wytworzonej z piasku gliniastego mocnego o pH_{KCl} = 6,7) fungicydami Unix 75 WG oraz Swing Top 183 SC na liczebność drobnoustrojów glebowych. Czynnikami zmiennymi były: rodzaj fungicydu

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i jego dawka wyrażona jako krotność dawki zalecanej przez producentów (0, 1, 10 i 100), sposób użytkowania gleby (gleba obsiana jęczmieniem jarym i nieobsiana) oraz termin analizy (14, 28, 42 i 56 dni).

Stwierdzono, że liczebność drobnoustrojów w glebie zanieczyszczonej fungicydami Unix 75 WG oraz Swing Top 183 SC zależała od rodzaju i dawki zastosowanego środka grzybobójczego, sposobu użytkowania gleby oraz terminu analizy. Fungicydy, stosowane w ilości zalecanej przez producentów, stymulowały namnażanie się bakterii kopiotroficznych i promieniowców, ale hamowały – i to niezależnie od dawki – namnażanie *Azotobacter* spp. i *Pseudomonas* spp. Większą liczebność bakterii oligotroficznych, kopiotroficznych, promieniowców i *Azotobacter* spp. odnotowano w glebie pod uprawą jęczmienia jarego.

Introduction

The quantities of xenobiotics that penetrate into the natural environment are related to the intensity of agricultural production in a given area, crop species and the form in which a given chemical is applied (VEGA et al. 2005). Too low environmental awareness may result in soil contamination by fungicides, starting from their production, through distribution, to agricultural use (OLSZAK et al. 2003). In Poland the amount of fungicides used for agricultural purposes increased from 3350 Mg in 1995 to 7038 Mg in 2004 (DMOCHOWSKA 2005). The active substances in pesticides that penetrate into the environment may be further translocated and accumulated, thus causing gradual degradation of ecosystems (WYSZKOWSKA, KUCHARSKI 2004). Ill-considered application of crop protection chemicals may have negative results, including their accumulation in crops (DROŻDŻYŃSKI 2004) and a negative impact on non-pathogenic microorganisms present on plant surface and in the soil (JOHNSEN et al. 2001, MONKIEDJE et al. 2002, NIEWIADOMSKA, SAWICKA 2002, WACHOWSKA 2005).

Microorganisms are integral components of the soil. They contribute to matter circulation, organic matter mineralization, liberation of elements indispensable for the growth of other living organisms, as well as to the degradation of anthropogenic organic compounds penetrating into the soil (WINDING et al. 2005), including crop protection chemicals. The effect of fungicides on microorganisms depends on the chemical constitution and dose of the agent, as well as on the properties of these microorganisms. Fungicides applied on a non-rational basis may disturb the processes occurring in the soil, thus reducing its fertility (CHEN, EDWARDS 2001). In some cases fungicides may reduce soil respiration and microorganism biomass (CHEN et al. 2001).

It is very difficult to develop biodegradable preparations that would affect pathogens only, and would not accumulate in the soil or penetrate into ground waters (JOHNSEN et al. 2001). Due to the fact that microorganisms are sensitive to all environmental changes, they are used as indicators of the biological activity of the soil (WINDING et al. 2005). Therefore, the aim of the present

study was to determine the effects on fungicides on the counts of soil microorganisms.

Materials and Methods

A pot experiment was performed in a greenhouse of the University of Warmia and Mazury in Olsztyn, in four replications, on brown soil developed from heavy loamy sand (pH_{KCl} = 6.7, hydrolytic acidity Hh – 9.0 mmol · kg^{-1} soil, organic carbon content C_{org} – 8.50 g · kg^{-1}). The soil was contaminated by two fungicides known under trade names Unix 75 WG and Swing Top 183 SC. The biologically active substances in these preparations were: cyprodinil (750 g · dm^{-3}) in the fungicide Unix, and dimoxystrobin (133 g · dm^{-3}) and epoxiconazole (50 g · dm^{-3}) in the fungicide Swing.

The variable experimental factors were as follows:

1. the type of fungicide: Unix 75 WG and Swing Top 183 SC;

2. the dose of fungicide (0, 1 – recommended dose, 10-fold dose and 100-fold dose); the dose recommended by the producer was 1 kg · ha^{-1} for Unix 75 WG and 1.5 dm^3 · ha^{-1} for Swing Top 183 SC;

3. the time of analysis: 14, 28, 42 and 56 days (from the moment the experiment was established and the fungicides were introduced into the soil);

4. the method of soil management: soil cropped to spring barley cv. Start and uncropped soil.

Soil samples weighing 3.2 kg were thoroughly mixed with the fungicides as well as with macroelements and microelements, and then placed in polyethylene pots. Identical mineral fertilizers were applied in all treatments. The fertilization rate expressed as the weight of pure elements per unit area was as follows: macroelements (g · kg^{-1} soil): N – 0.12 ($CO(NH_2)_2$), P – 0.096 (KH_2PO_4), K – 0.12 (KH_2PO_4+KCl), Mg – 0.02 ($MgSO_4 \cdot 7H_2O$), microelements (mg · kg^{-1} soil): Zn – 5.0 ($ZnCl_2$), Cu – 5.0 ($CuSO_4 \cdot 5H_2O$), Mn – 5.0 ($MnCl_2 \cdot 5H_2O$), Mo – 5.0 ($Na_2MoO_4 \cdot 2H_2O$), B – 0.33 (H_3BO_3). The soil in half of the pots was cropped to spring barley (*Hordeum vulgare L.)* cv. Start (15 plants per pot), and the soil in the other half of the pots remained uncropped. Over the entire experimental period soil moisture content was 60% of the capillary water capacity of the soil. Treatments without fungicide application served as control.

The experiment lasted for 56 days, i.e. until spring barley was harvested at the flowering stage. Soil samples were collected four times, at 14-day intervals, starting from seed sowing. The samples were taken from the cross section of the soil profile, using the Egner Riehm stick. Each soil sample was a mixture of soil coming from 4 different pots. Bacterial counts: oligotrophic bacteria

(Olig), copiotrophic bacteria (Cop), actinomycetes (Act), *Pseudomonas* spp. (Ps), *Azotobacter* spp. (Az) and fungi (Fun) were determined in the soil by the plate method (in three replications) as described by KUCHARSKI and JASTRZĘBSKA (2005).

The results were verified statistically by a four-factor analysis of variance, using Statistica software (StatSoft, Inc... 2003) and the Duncan's multiple range test. Regression equations were developed and determination coefficients were calculated for the sizes of particular microorganism groups and the time of analysis.

Results and Discussion

The counts of microorganisms in fungicide-treated soil were affected by all experimental factors, i.e. the type and dose of fungicides, the method of soil use and the time of analysis – which determined the duration of the effect of active substances in fungicides on soil microorganisms. With the passage of time, their soil concentrations could decrease, both due to degradation and sorption by organic matter (JOHNSEN et al. 2001).

Both Unix 75 WG and Swing Top 183 SC, applied at recommended doses, stimulated the proliferation of copiotrophic bacteria and actinomycetes, irrespective of the method of land use (Figure 1). Actinomycetes were more effectively stimulated by Swing, whereas copiotrophic bacteria – by Unix. Their counts increased by 48% in cropped soil and by 20% in uncropped soil in the case of Unix, and by 20% and 17% respectively in the case of Swing, as compared with the control treatments. Swing Top 183 SC had a similar effect on oligotrophic bacteria. The responses of the other microorganisms depended on the kind of fungicide and on the method of soil management.

The impact of doses higher than recommended (10-fold and 100-fold) on soil microorganisms was less positive. The tenfold dose of both preparations stimulated the proliferation of copiotrophic bacteria and actinomycetes in soil cropped to spring barley, but the hundredfold dose had an inhibitory effect.

Bacteria of the genera *Azotobacter* and *Pseudomonas* responded negatively to soil contamination by fungicides, but the response of *Pseudomonas* spp. was much stronger. All doses of the fungicide Swing (1, 10-fold, and 100-fold) reduced the counts of these bacteria by 35, 51 and 70% respectively in soil cropped to spring barley, and by 29, 52 and 59% in uncropped soil. Following the application of Unix, the counts of *Pseudomonas* spp. decreased by 6, 39 and 67%, and by 48, 66 and 64% in cropped and uncropped soil respectively, as compared with the control treatments.

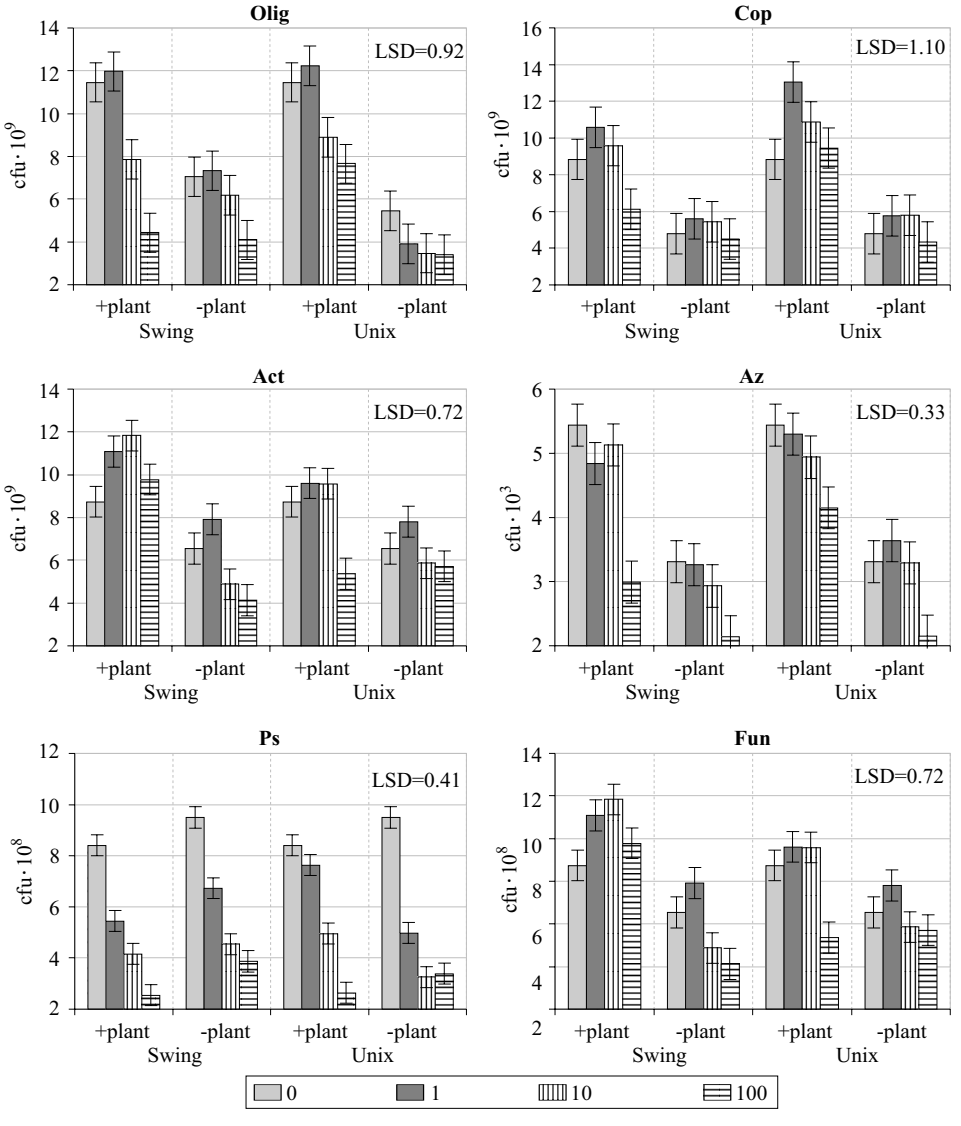

Fig. 1. Counts of microorganisms in fungicide-contaminated soil; + plant – soil cropped to spring barley, – plant – uncropped soil, LSD – statistically significant for $P < 0.01$

Fungi responded in a different way to soil contamination by fungicides. In cropped soil both fungicides, regardless of the dose, stimulated their growth for the first 28 days of the experiment, and then inhibited it.

Various responses of particular microorganism groups to the fungicides resulted most probably from their different chemical structures and different

properties of the active substances contained in both preparations, as well as from the properties of these microorganisms. Some microorganisms use pesticides as the only source of energy and nutrients, while the same pesticides may negatively affect other microorganisms (JOHNSEN et al. 2001).

The active substances contained in both fungicides differed in chemical constitution and in the mechanism of their effect on fungi. The fungicide Swing top 183 SC contains dimoxystrobin and epoxiconazole. Epoxiconazole is a triazole derivatives. It has a broad spectrum of activity, involving disturbances in the biochemical processes of sterol biosynthesis. The most common of them is demethylation of C-14 during lanosterol conversion into ergosterol or its functional replacer – sterol (ELMHALT 1992). Dimoxystrobin belongs to strobilurins, known as Q_o inhibitors, because they inhibit respiration in fungi on the mitochondrial level, blocking electron transport between cytochromes *b* and *c* (GRASSO et al. 2006, KARADIMOS et al. 2005). Cyprodinil, the active substance in the fungicide Unix 75 WG, is a anilinopyrimidine in terms of chemical constitution, which contains a phenolic ring. The mechanism of its action consists in inhibiting methionine biosynthesis (OTERO et al. 2002).

Literature on the subject provides scant information on the effects of the above active substances on microorganisms. BERTELSEN et al. (2001), who studied the effects of azoxystrobin and epoxiconazole on the saprophytic fungi *Alternaria alternata* and *Cladosporium macrocarpum,* found that azoxystrobin inhibited both mycelium growth and spore production in both fungal species. Epoxiconazole had a weaker effect on spore formation, and a stronger effect on mycelium growth, in comparison with azoxystrobin. HART and BROOKES (1996) reported that epoxiconazole reduced the C-biomass of soil microorganisms.

The impacts of other fungicides on microorganisms have been investigated in more detail. According to MONKIEDJE et al. (2002), the application of fungicides may increase the total bacterial count in the soil, but at the same time inhibit the growth of sensitive microorganisms, such as e.g. nitrifying bacteria, bacteria of the genera *Bradyrhizobium* (HASHEM et al. 1997) or *Rhizobium* (NIEWIADOMSKA et al. 2005). Fungicide application may not only limit the proliferation of microorganisms, but also affect their biochemical properties, which manifests itself by the ability to fix nitrogen (NIEWIADOMSKA, SAWICKA 2001). STRZELEC and DEC-PLEWKA (1992) observed a slight stimulation of the growth of *Azotobacter* bacteria at low doses of fungicides (1 and 10 $mg \cdot kg^{-1}$), whereas fungicides applied at high doses (50, 100 and 500 $mg \cdot kg^{-1}$) had a negative effect on microorganisms. WACHOWSKA (2001) recorded a decrease in the counts of bacteria of the genus *Azotobacter* following the application of the fungicides Amistar 250 SC and Brawo 500 S.C., and an increase in the counts of bacteria of the genus *Actinomycetales*. Bacteria of the genera *Pseudomonas* and *Bacillus*, reducing plant infection by pathogenic

fungi, were found to be tolerant of the fungicides tested (applied at recommended terms and doses).

In this experiment the method of land use determined the counts of all groups of soil microorganisms (Figure 1). Greater counts of oligotrophic and copiotrophic bacteria, as well as actinomycetes and *Azotobacter* spp., were recorded in soil cropped to spring barley, as compared with uncropped soil. The counts of fungi and bacteria of the genus *Pseudomonas* were higher in uncropped soil treated with Swing.

The stimulation of the growth of the majority of microorganism groups by spring barley growing was most probably caused by substances being the source of carbon (especially low-molecular weight compounds, like amino saccharides, saccharides and organic acids), secreted by the roots of spring barley plants. These compounds, released by the roots of most plants, may stimulate bacterial growth (GLICK 2003). Their kind and quantity depend not only on plant species, but also on developmental stage (BLAGODATSKAYA at al. 2004). BAIS et al. (2004) demonstrated that low-molecular metabolites secreted by plants may show antibacterial and fungicidal activity, as well as affect the formation of soil aggregates. In addition, plants can influence the microorganism populations developing around their root systems through modification of other factors, such as soil pH (BLAGODATSKAYA at al. 2004) and moisture content, which impacts not only microorganisms, but also the rate of organic matter mineralization (PAUL, CLARK 2000). According to PAUL and CLARK (2000), nutrient intake from the soil by plants makes microorganisms mineralize persistent substances.

The counts of soil microorganisms depended also on the time of analysis (Figures 2 and 3). A positive effect of fungicides on copiotrophic bacteria and fungi observed at the beginning of the experiment was followed by a decrease in their counts, which was directly proportional to the dose of fungicides.

The response of oligotrophic bacteria and actinomycetes was different. At the first stage of the study (14 days) the counts of oligotrophic bacteria were lower in soil treated with Swing Top 183 SC, irrespective of the dose, than in the control soil. Later on this fungicide applied at the recommended dose stimulated the growth of these bacteria. In the case of actinomycetes such a responses was observed not only for the standard dose, but also for a tenfold higher one, in both fungicides. Regardless of the time of analysis, the counts of bacteria of the genus *Pseudomonas* in soils with various levels of fungicide contamination were lower than in the control treatment.

The counts of soil microorganisms, changing in time, are affected by a variety of factors, including temperature, moisture content, crop growing (PAUL, CLARK 2000) and fungicide persistence in the soil. According to BLAGODATSKAYA et al. (2004), the biomass of rhizosphere-colonizing microorgan-

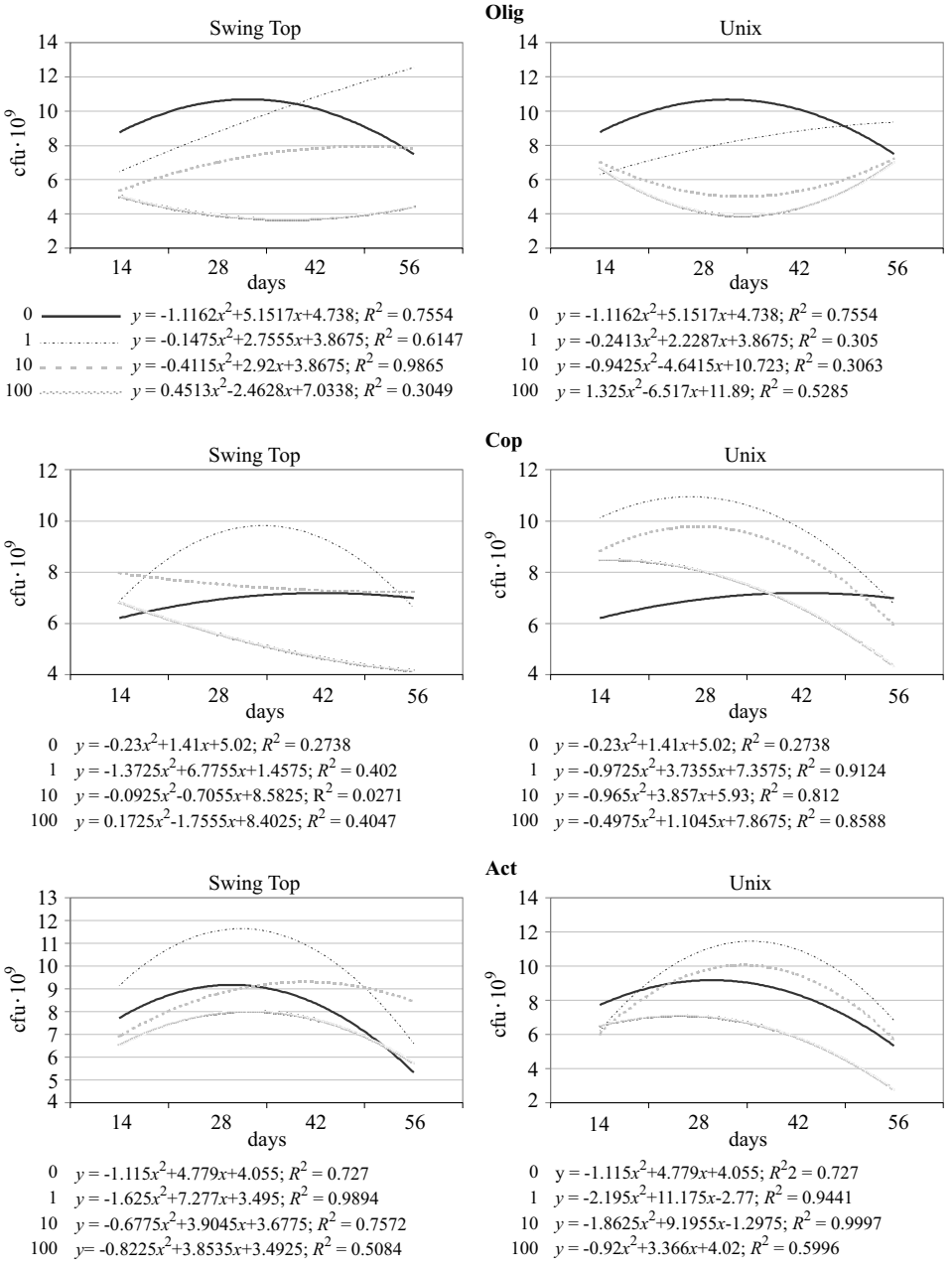

Fig. 2. Counts of oligotrophic bacteria (Olig), copiotrophic bacteria (Cop) and actinomycetes (Act) in fungicide-contaminated soil

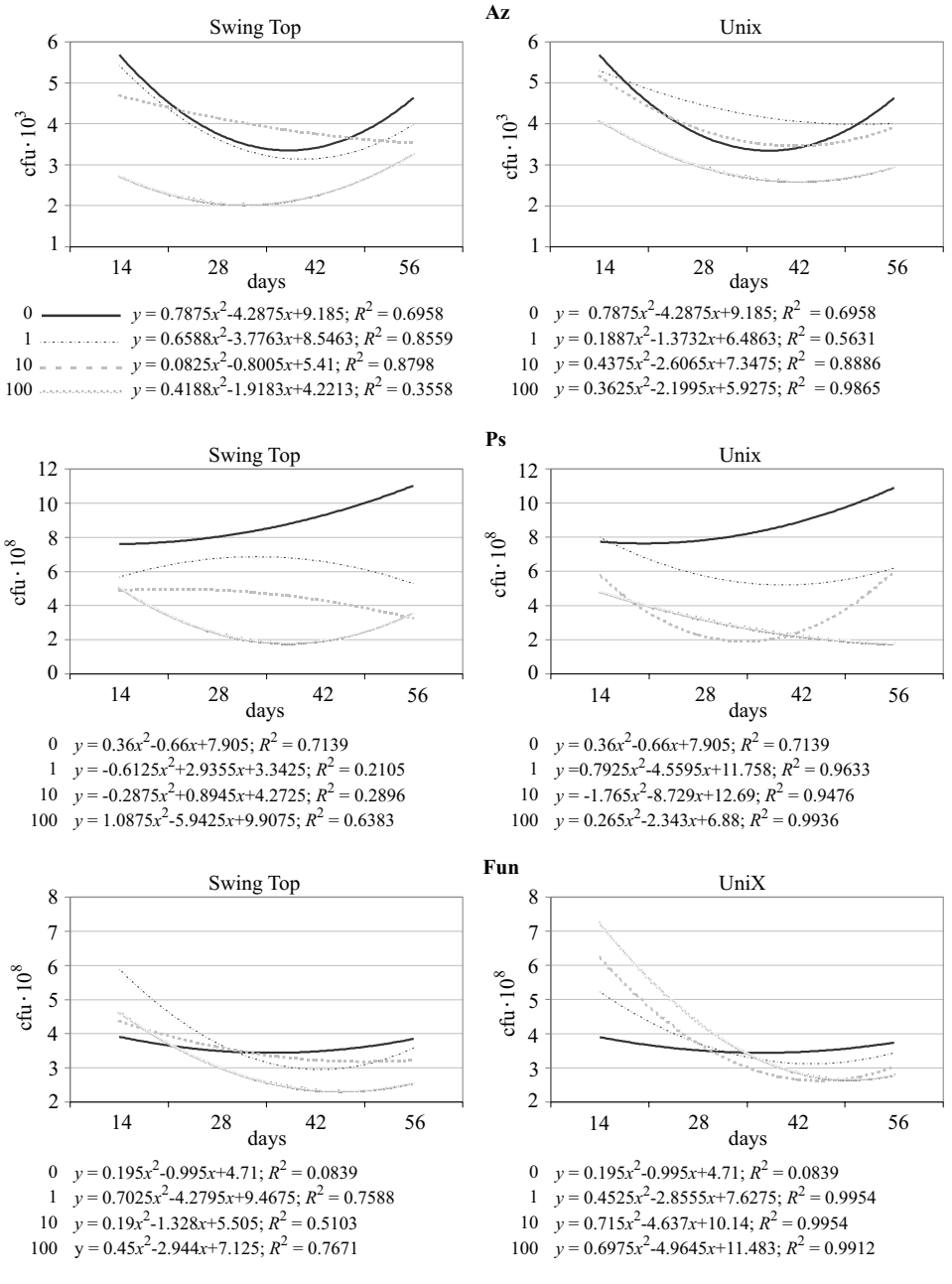

Fig. 3. Counts of *Azotobacter* spp. (Az), *Pseudomonas* spp. (Ps) and fungi (Fun) in fungicide--contaminated soil

isms is also related to the developmental stage of plants. Pesticide persistence in the soil depends primarily on the chemical constitution of the active substance, which determines the rate of its chemical and biological degradation. The process of its decomposition in the soil is also related to other factors, such as the type and physicochemical properties of soil or organic matter content, as well as to climate conditions, including humidity and temperature (NOWAK 1996). In the present study the active substances contained in both fungicides could gradually undergo degradation, which in turn could modify their effect on microorganisms.

Biodegradation of many substances in the soil, including pesticides, is dependent on the joint action of numerous microorganism groups. The numbers and species composition of these populations have an effect not only on the rate of biodegradation, but also on the type of secondary metabolites formed. These metabolites may be more toxic than the original product (NIEWIADOMSKA, SAWICKA 2002, TIXIER et al. 2002). This was confirmed by TIXIER et al. (2002), since in their study 4-isoproturon formed as a result of isoproturon (herbicide) biodegradation was found to be more toxic than the initial compound. On the other hand, the substances secreted by the roots of plants may react with crop protection chemicals introduced into the soil, thus stimulating the growth of microorganisms (WACHOWSKA 2001). The plant itself may also influence soil microorganisms. Thus, the count of soil microorganisms recorded in the present experiment was probably affected by all of the above factors.

Conclusions

1. The counts of microorganisms in soil contaminated by fungicides, Unix 75 WG and Swing Top 183 SC, were affected by the type and dose of fungicides, the method of soil management and the time of analysis.

2. The fungicides, applied at the recommended dose, stimulated the proliferation of copiotrophic bacteria and actinomycetes. Higher doses (especially the 100-fold dose) had a negative effect on the oligotrophic bacteria, *Azotobacter* spp. and *Pseudomonas* spp.

3. Both Unix 75 WG and Swing Top 183 SC reduced the counts of *Azotobacter* spp. and *Pseudomonas* spp.

4. Higher counts of oligotrophic and copiotrophic bacteria, actinomycetes and *Azotobacter* spp. were recorded in soil cropped to spring barley, as compared with uncropped soil.

Translated by ALEKSANDRA POPRAWSKA

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BIOLOGICAL DIVERSITY OF CEREAL FIELDS

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K e y w o r d s: biological diversity, cereal mix, weeds, yield.

A b s t r a c t

On the basis of field experiment maintained during the years 1990-2000 the diversity of phytocenosis of standing mix of barley and oats to fields of single species and the relation of that diversity to cereal yield was determined. The density and biomass of weeds, their biomass, abundance and composition of species, structure of domination in phytocenoses were determined, the continuity of appearance of species in consecutive years, species diversity and evenness as well as similarity of communities ratios were calculated.

In total 33 species of weeds (4 to 18 in individual standing cereals and depending on time of determination) appeared in the cereals. The species present all the time were: *Chenopodium album, Thlaspi arvense, Stellaria media, Fallopia convolvulus, Capsella bursa-pastoris*, *Matricaria maritima* ssp. *inodora*, *Polygonum aviculare* and *Lamium amplexicaule*. In the spring the following species were classified as dominants and subdominants: *Chenopodium album, Thlaspi arvense, Stellaria media* and *Fallopia convolvulus,* before harvest *Chenopodium album* was the dominant. In spring the numbers of weeds in communities were similar; before harvest the number of weeds in barley was significantly higher than in oats and in the mix. In the mix the weeds produced a smaller biomass. The diversity and evenness of species in the mix field were significantly higher than in oats and barley. The communities of weeds in cereals did not differ in those aspects. In the spring, the community of weeds in the mix showed a larger similarity to those developing in barley and oats and the similarities between communities developing in oats and barley alone. In the summer the least similarity was recorded usually between weeds in barley and in the mix. The mix offered the highest yield. The yield showed negative correlation to the biomass of weeds but it did not depend on the numbers and diversity of weeds.

RÓŻNORODNOŚĆ BIOLOGICZNA ŁANÓW ZBÓŻ

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Abstrakt

Porównano różnorodność fitocenozy łanu mieszanki jęczmienia i owsa z zasiewami jednogatunkowymi oraz określono jej związek z wydajnością zbóż, opierając się na eksperymencie polowym prowadzonym w latach 1990-2000. Ustalono liczebność i biomasę chwastów, ich bogactwo i skład gatunkowy, strukturę dominacji w fitocenozach, wyliczono stałość pojawiania się gatunków w latach, wskaźniki różnorodności i równomierności gatunkowej oraz podobieństwo zbiorowisk.

W zbożach pojawiły się łącznie 33 gatunki chwastów (4-18 w poszczególnych latach i terminach oznaczeń). Stale występowały: *Chenopodium album, Thlaspi arvense, Stellaria media, Fallopia convolvulus, Capsella bursa-pastoris*, *Matricaria maritima* ssp. *inodora*, *Polygonum aviculare* i *Lamium amplexicaule*. Wiosną do dominantów lub subdominantów zakwalifikowano: *Chenopodium album, Thlaspi arvense, Stellaria media* i *Fallopia convolvulus,* przed zbiorem – *Chenopodium album*. Wiosną liczebność zbiorowisk chwastów była podobna, przed zbiorem – w jęczmieniu istotnie większa niż w owsie i mieszance. W mieszance chwasty wytworzyły najmniejszą biomasę. Różnorodność i równomierność gatunkowa łanu mieszanki była istotnie wyższa niż owsa i jęczmienia. Zbiorowiska chwastów w zbożach nie różniły się pod tym względem. Wiosną zbiorowiska chwastów w mieszance wykazywały większe podobieństwo do tworzących się w jęczmieniu i owsie w porównaniu ze zbiorowiskami w zasiewach jednogatunkowych. Latem najmniejsze podobieństwo notowano zwykle między chwastami w jęczmieniu i w mieszance. Z mieszanki uzyskano najwyższe plony. Wydajność zbóż ujemnie korelowała z biomasą chwastów, a nie zależała od ich liczebności i różnorodności.

Introduction

The biodiversity has been the focus of interest among biologists for over 50 years (RICOTTA 2003). The process of extinction of numerous species observed during that period was the main reason for that interest (WILSON 1990). The duty of identifying biodiversity, monitoring it and educating the society in that aspect has been imposed by the convention on protection of biological diversity signed in 1992. According to that convention, biodiversity means genetic differentiation of organisms within a species and the differentiation of species in ecological units and ecosystems. The issue of rational Earth surface use was another cause of interest in the subject (TRZCIŃSKA-TACIK 2003).

Biodiversity is an important characteristic that determines functioning of ecosystems. That applies in particular to plant communities that form the energy base for ecological systems (HOOPER et al. 2005). Studies prove that reduction of the richness of vegetable species influences a decrease in their productivity, stability of biomass, resistance and flexibility to stress conditions as well as intake of nutrients (SCHLAPFER, SCHMID 1999).

Biodiversity should also be an important element in agricultural studies as limitation of those studies reaching as fat as genetic homogeneity of crops has lead to numerous negative, generally known consequences (FINCKH et al. 2000). It should be pointed out that during the recent years scientific interest in mixed sowing as a form of biological enrichment of cultivated fields increased significantly (WANIC, NOWICKI 2000). Cultivation of two or more crop species, even if they are not too distant genetically (e.g. various species of

cereals), in one field determines development of a different system of interactions between components of agrocenosis and their relations with organisms of neighboring biocenoses (HOOPER et al. 2005, WANIC 1997).

The attitude to weeds has also changed. Besides their harmful effects, also their other role in the ecosystems started to be recognized (GEROWITT et al. 2003, MIKLASZEWSKA, ADAMCZEWSKI 2004). On one hand the weeds are components of biodiversity in the standing crop while on the other their importance in maintaining or increasing biodiversity through expansion of trophic relations with other organisms is stressed (MARSHALL et al. 2003).

In Polish studies the diversity of agricultural phytocenoses has so far been defined by the number of weed species appearing in the standing crop (TRZCIŃSKA-TACIK 2003). According to ecological criteria the biological diversity at the level of species is the function of their number and relative abundance (RICOTTA 2003). Shannon-Wiener's index, which increasingly frequently appears in Polish agricultural publications is one of the most often used measures of biodiversity described in that way (JĘDRUSZCZAK, ANTOSZEK 2004, STUPNICKA-RODZYNKIEWICZ et al. 2004, WANIC et al. 2005).

This paper aimsaring the diversity in phytocenoses of the mixed crop of barley and oats measured by biological indicators with single crop fields as well as determination of its relation to the cereals yield levels.

Material and Methods

The input data for this paper originate from a closed statistical field experiment implemented during the years 1990-2000 at the Production – Experimental Enterprise in Bałcyny (belonging to the University of Warmia and Mazury in Olsztyn). The experiment was set on typical grey-brown podzolic soil formed of light clay dust classified as medium soil with humus content in cultivated layer at from 1.49 to 1.61% and medium abundance with available forms of macro and microelements. That soil represents fertility class IIIa and very good rye soil-agricultural complex.

The subject of the covered phytocenoses of barley, oats and their mix. Those cereals were cultivated yearly in rotation systems with the following selection and order of crops:

A: potato – Persian clover – spring barley – spring barley;

B: potato – Persian clover – oats – oats;

C: potato – Persian clover – mix – mix.

Standing cereals cultivated on plots after clover were subject to detailed analysis.

Every year barley was sown at 350 germinating seeds per 1 m^2, oats – 550, and the mix – 175 and 275 germinating seeds respectively. Cereals were

fertilized with differentiated doses of mineral elements (NPK) depending on position in the rotation system. The total input of those elements ($kg \cdot ha^{-1}$) was: 240 – after clover, 280 – in case of the second year of cultivation of the same cereal. In the experiment (during its entire duration) no weeds removal treatment was applied to present the defensive properties of individual cereal species more clearly.

The density of cereals and the presence of weeds were determined every year in the spring (during full germination of cereals) and before harvest in two repetitions on every field defining the number of plants and composition of weed species on the marked, constant surfaces (1 m^2). In the analysis conducted before harvest in case of cereals the number of productive blades and in case of weeds the number of shots were counted. The results obtained were used to determine the richness of species, calculation of constancy of appearance of species in consecutive years (BRAUN-BLANQUET 1964), structure of domination in the communities (TROJAN 1980), Shannon-Wiener indexes of diversity and evenness of species (SHANNON 1948, WIENER 1948) and communities; similarity index according to SORENSEN (1948). The diversity and evenness indexes were calculated for whole phytocenoses (crop plant and weeds) and separately for communities of weeds.

The method of phytosociological constancy identifying classes according to Braun-Blanquet scale: V – species appearing constantly or very frequently (present in 80.1-100% of years covered) IV – frequent species (in 60.1-80%), III – medium frequent (40.1-60%), II – infrequent (20.1-40%) and I – sporadically or rarely (0.1-20% of the years) (BRAUN-BLANQUET 1964) was used for determination of constancy of appearance of weed species in consecutive years.

Within the phytocenosis of the cereal field encompassing the crop and the weeds, and according to the concept by Tarwid forming a competitive group, the following groups of plants were identified according to TROJAN (1980):

1. Dominants – covering the species representing over 5% of the individuals in the community.

2. Subdominants – species representing 2-5% of the individuals.

3. Influents – represented by species forming 1-2% of the individuals each.

4. Accessory and foreign species – representing by random individuals (below 1%).

The Shannon-Wiener indexes were calculated using the formulas:

– diversity of species (H'): $H' = -\Sigma\ (p_i \cdot \ln p_i)$,

– evenness of species (J'): $J' = H' \cdot (\ln S)^{-1}$,

where:

p_i – proportion of i-species individuals in the community to the number of all individuals in the community;

S – community abundance of species.

The weed communities similarity indices were calculated by adjusting the formula by Sorensen: $P = 2c \cdot 100 \cdot (a + b)^{-1}$, where:

P – similarity index expressed in percent,

c – sum of common numbers of species for a given pair of communities,

a – sum of the number of weeds in the first community,

b – sum of the number of weeds in the second community.

The characteristics of plant communities were subjected to variance analysis and the averages were compared using the Duncan test at $p = 0.05$.

The relations between studied characteristics of plant communities (fields and weed communities) and between characteristics of communities and the volumes of precipitations and average temperature during the period covered were determined using the linear correlation ratios. The relation between the characteristics of weed communities and yields of cereals were also analyzed.

For the studied parameters the trends across the years were determined according to the formula: $y = a \cdot x + b$, where:

x – value of independent variable (here: consecutive years of studies);

y – value of the dependent variable corresponding to the x value (here the number of weeds, abundance of species, diversity and evenness ratios respectively);

a – regression constant (free expression) – defines the point of intersection between the determined regression straight and the axis of the dependent variable *y;*

b – tangens of the angle of regression axis to the independent variable x axis; it shows by how much the dependent variable y will change if the independent variable x changes by a single unit.

The Latin names of weed species were assumed after MIREK et al. (1995).

The weather conditions under which the vegetation of spring cereals and accompanying weeds progressed during the analyzed period of 11 years were quite differentiated. On the basis of the sum of precipitations during the period from April until August, according to the criteria developed by KACZOROWSKA (1962) and PRZEDPEŁSKA (1973), years 1997 and 1999 were classified as very wet, 1998 as wet, 1990, 1993, 1996 and 2000 as average seasons, 1991, 1994 and 1995 were classified as dry and 1992 as very dry. The seasons of 1992, 1994, 1995 were warm as for northeastern Poland, 1990, 1993, 1999 and 2000 were moderate and 1991, 1996, 1997 an 1998 were cool.

Results

Biodiversity of fields of single (oats or barley) and two-crop species (mix of oats with barley) fields had been definitely determined by presence of weeds.

Table 1

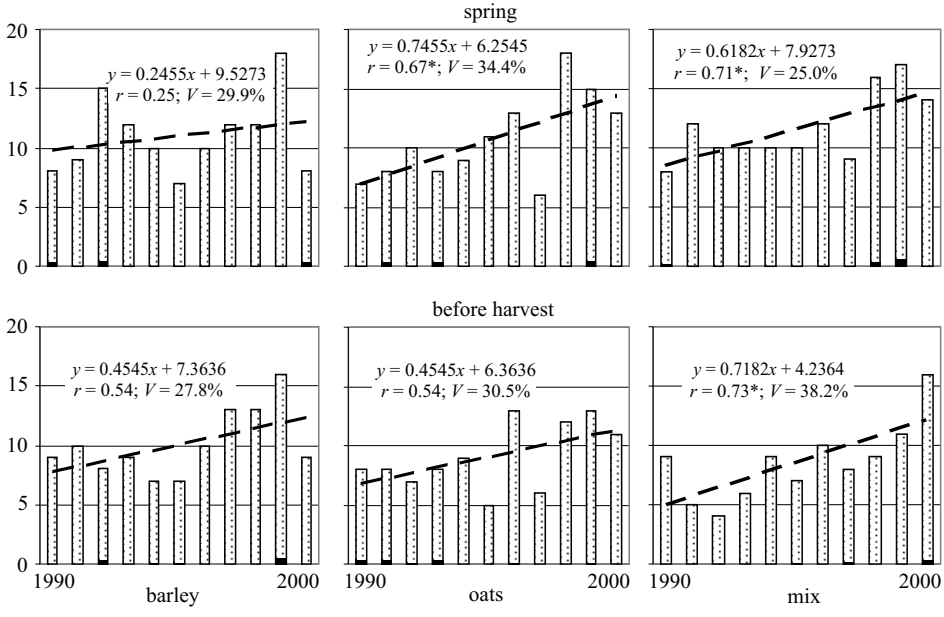
Density (plants · m^{-2}) and constancy of appearance of weed species in cereal fields (average from 11 years of experiment)

Weed species	Plant								
	barley			oats			mix		
	density		*S*	density		*S*	density		*S*
	w	*z*		*w*	*z*		*w*	*z*	
Chenopodium album L.	58.2^{xx}	38.0^{x}	V	50.9^{xx}	29.5^{xx}	V	44.4^{xx}	26.0^{x}	V
Thlaspi arvense L.	36.2^{xx}	3.2	V	67.8^{xx}	5.5	V	48.4^{xx}	3.6	V
Stellaria media (L.) Vill.	25.7^{xx}	10.9	V	23.8^{x}	3.0	V	19.8^{x}	4.5	V
Fallopia convolvulus (L.) Á. Löve	11.2^{x}	12.2	V	12.4^{x}	8.5	V	11.5^{x}	6.5	V
Matricaria maritima L. ssp. *inodora* (L.) Dostàl	5.5	7.3	V	5.9	1.6	V	4.5	2.3	IV
Sonchus arvensis L.	1.5	7.8	III	5.5	2.6	III	4.0	3.2	IV
Capsella bursa-pastoris (L.) Medik.	1.7	6.4	V	0.6	5.8	V	2.4	3.5	V
Galinsoga parviflora Cav.		6.3	II	0.1	2.6	II	0.5	4.9	II
Veronica arvensis L.	3.2	2.1	III	3.0	0.7	III	2.6	0.4	III
Polygonum aviculare L.	1.2	4.2	V	1.9	2.4	IV	1.8	0.4	III
Lamium amplexicaule L.	3.1	0.4	V	5.3	0.1	IV	2.8	0.1	IV
Spergula arvensis L.	5.4	1.0	IV	1.1		II	2.0	0.3	II
Polygonum lapathifolium L.	0.7	1.3	II	4.4	1.8	III	1.4		II
Equisetum arvense L.	0.4	2.8	III	1.0	1.4	III	0.9	1.9	IV
Echinochloa crus-galli (L.) P. Beauv.		2.1	II	2.5	2.9	II	0.5	0.4	I
Viola arvensis Murray	1.5	1.5	IV	0.4	0.4	II	0.9	0.5	II
Galium aparine L.	0.2	0.1	II	1.2	0.3	II	1.5	1.5	IV
Myosotis arvensis (L.) Hill	1.1	0.9	III	0.9	0.4	III	0.6	0.7	IV
Cirsium arvense (L.) Scop.	0.3	1.3	I	0.8	1.0	III	1.0	0.1	II
Plantago lanceolata L.	0.1	1.4	II		0.4	I	0.3	0.1	I
Galeopsis tetrahit L.	0.1	0.1	II	0.1	0.4	II	1.2	0.3	II
Anchusa arvensis (L.) M. Bieb.	0.3	0.2	II	0.5	0.5	III	0.5	0.1	II
Fumaria officinalis L.	0.4	0.1	II	0.6	0.2	III	0.7		II
Agropyron repens (L.) P. Beauv.					0.4	I		1.2	I
Sinapis arvensis L.	0.8		III					0.4	I
Veronica persica Poir.							0.6		II
Erodium cicutarium (L.) L'Hér.				0.1		I	0.4		I
Apera spica-venti (L.) P. Beauv.	0.5		II						
Conyza canadensis (L.) Cronquist		0.1	I					0.3	II
Raphanus raphanistrum L.		0.3	I						
Poa annua L.				0.1		I		0.1	I
Mentha arvensis L.		0.2	I						
Vicia hirsuta (L.) S. F. Gray	0.1		I						
Total number of species	29			26			29		

w – spring (tillering stage), *z* – before harvest (end of vegetation), *S* – constancy;
^{xx} – dominants, ^{x} – subdominants

In total, during 11 years of studies, 33 species of weeds were recorded of which 26 were recorded in oats and 29 in barley and the mix (Table 1). Among them, 22 species appeared at least once in all the three studied cereal fields. The real composition of phytocenoses in individual years and times of studies

was less abundant: cereals were accompanied by 4-18 synanthropic species (Figure 1). *Chenopodium album, Thlaspi arvense, Stellaria media, Fallopia convolvulus* and *Capsella bursa-pastoris* were constantly present in the fields while *Matricaria maritima* ssp. *inodora* was constantly present in the single crop fields while – *Polygonum aviculare* and *Lamium amplexicaule* were present in barley alone fields (Table 1). In case of the mix the group of infrequent and accidentally present weeds was the most numerous (16 taxa), which means larger temporary differentiation of those phytocenoses. In the spring 4 following species were outstanding in the numbers of individuals: *Chenopodium album, Thlaspi arvense, Stellaria media* and *Fallopia convolvulus*. On the basis of the average numbers of individuals those taxa were classified as dominants or subdominants in all three phytocenoses. During the summer it was possible to include *Chenopodium album* only in those categories.



r – linear correlation ratio defining the significance of the year-to-year linear trend,
* – *r* significant at $p < 0.05$; *V* – year-to-year variance ratio, %

Fig. 1. Number of weed species in cereal field

The number of species in the plant community offers the simplest measurement of species diversity, however, it is a poor measure as it gives the same rank to the taxa represented in large numbers and those appearing as random individuals and, as a consequence, not representative for real quantitative relations. The Shannon-Wiener diversity index based on system entropy

increases with the number of species forming the community and the uniformity of distribution of individuals among species. Its value calculated for plant communities (cereal + weeds) is decisively determined by the number of crop species individuals. Its domination as concerns the numbers is determined already at the moment of sowing and as a consequence, certain differences in the values of indices in individual years result from possible poorer densities (poorer germination, poorer productive spreading) and depend on characteristics of weed communities.

In average, for 11 years of studies, the numbers of weeds in weeds communities developing in the spring in the studied cereals did not differ, which was confirmed by variance analysis (Table 2). During the individual years, however, that characteristic assumed values within a very wide range (59-433 individuals $\cdot$ m^{-2}) and in slightly different ways in individual fields (Figure 2). The communities of weeds in barley were outstanding in their highest stability. That is confirmed by the variance index (V = 37.8%) and the lowest value of trend straight slope angle relative to the x-axis. The significant increasing year-to-year trend was indicated by the numbers of weed individuals in weed communities in oats. On the other hand, in case of the mix the highest variance index was recorded (V = 63.6%). The increasing trend resulted from particularly abundant presence of weeds in the mix in 1999. The number of weeds in the spring was strongly correlated with the abundance

Table 2

Characteristics of field phytocenoses and weed communities (11 years averages)

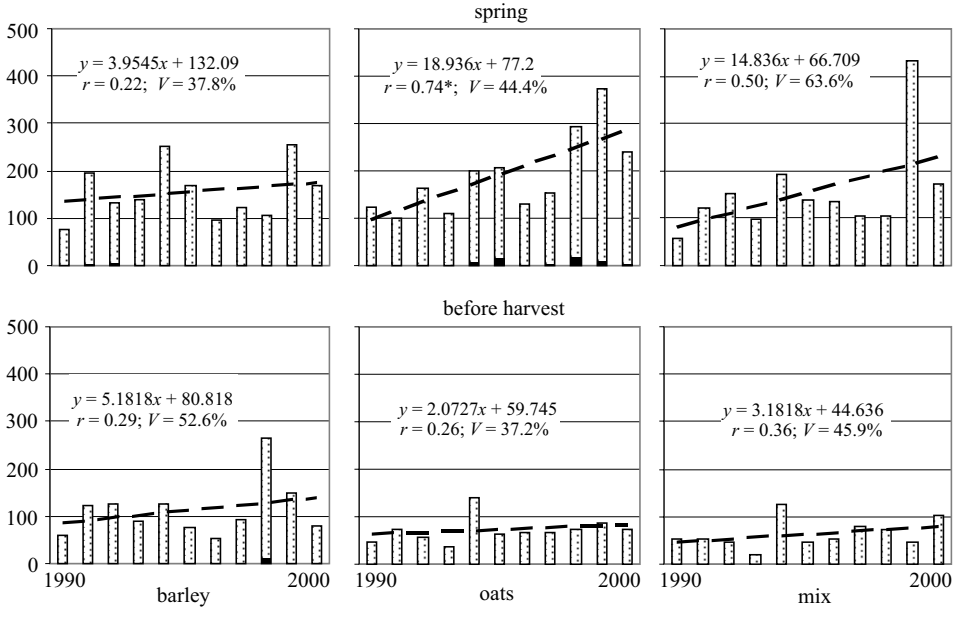
Characteristic – indicator	Plant	Time of analysis			
		spring		before harvest	
		field phytocenosis	weed community	field phytocenosis	weed community
Density plants $\cdot$ m^{-2}	barley	417.9*	155.8^a	875.0^b	111.9^b
	oats	549.7	190.8^a	573.5^a	72.2^a
	mix	509.5	155.7^a	830.8^b	63.7^a
Number of species	barley	12.0^a	11.0^a	11.1^a	10.1^a
	oats	11.7^a	10.7^a	10.1^a	9.1^a
	mix	13.6^a	11.6^a	10.5^a	8.5^a
H'	barley	1.20^a	1.56^a	0.62^a	1.74^a
	oats	1.18^a	1.63^a	0.58^a	1.61^a
	mix	1.58^b	1.78^a	0.99^b	1.58^a
J'	barley	0.49^a	0.66^a	0.26^a	0.73^a
	oats	0.49^a	0.70^a	0.25^a	0.75^a
	mix	0.61^b	0.73^a	0.43^b	0.76^a

H' – Shannon-Wiener's diversity index

J' – Shannon-Wiener's evenness index

a, b – significance of differences between averages: within a given factor and characteristic numbers marked with the same letter do not differ significantly at $p < 0.05$

* – differences result from sowing technique and the averages were not compared



r, V – as in Figure 1

Fig. 2. Density of weeds in cereal fields, plants · m^{-2}

of precipitations in April (Table 3). Year 1999 was moderately warm and very wet, which supported development of weeds. In all fields the numbers of weeds during that season was the highest in the entire period of 11 years.

The communities of weeds developing before harvest usually consisted of smaller numbers of individuals than those developing during the spring (Figure 2). The average number of weeds in barley for the period of study was 111.9 weed individuals per unit of area (Table 2). That number was significantly lower than the corresponding value for oats (72.2 individuals · m^{-2}) and the mix (63.7 individuals · m^{-2}). The highest year-to-year variance was reached by the number of weeds in barley (V = 52.6%), the lowest – in oats (V = 37.2%). The variance index for that characteristic in case of the mix was almost exactly in the middle between the values for single crop fields (Figure 2). The year-to-year trends proved to be insignificant. The number of weeds in the summer did not show correlation with temperatures and precipitations during vegetation (Table 3). It is interesting that the numbers of individuals in segetal spring and summer communities did not have any documented correlation with the density of crop plants (only trends – positive in the spring and negative during the summer – Table 4). It is generally believed that weeds take every free space in the field. In the analyzed experiment a quite good density of cereals caused that their invasiveness did not appear. It is worth pointing out in this place

that during the spring analyses the number of weed species in oats and barley increased and the diversity index increased significantly with increase in density of crop plant. Considering that domination of a species is the opposite of diversity, that means that less dense field supported increase of domination of a species, i.e. created better conditions for germination for some, ecologically stronger, species only. In the mix it was more moderately marked. Before harvest of the cereals no correlation between density of cereal and the number of weed species present, their diversity, evenness and their generated biomass was noticed.

Table 3
Linear correlation ratios between ecological indicators of weed communities and precipitations (mm) and temperatures (°C) during the period of study

Item		Density of weed plants	Richness of weed species	Indexes		Biomass of weeds
				H'	*J'*	
Spring (tillering stage)						
April	– precipitations	0.77*	0.56*	0.43*	0.09	
	– air temperature	0.17	0.15	-0.01	-0.20	
Before harvest (end of vegetation)						
June	– precipitations	0.17	0.46*	0.57*	0.52*	0.29
	– air temperature	-0.00	-0.18	-0.36*	-0.48*	-0.18
July	– precipitations	-0.24	0.05	0.21	0.32	0.27
	– air temperature	0.16	-0.42*	-0.64*	-0.67*	-0.10
August	– precipitations	-0.14	0.16	0.19	0.20	-0.15
	– air temperature	-0.21	-0.54*	-0.66*	-0.61*	-0.24
April-August	– precipitations	0.05	0.51*	0.64*	0.61*	0.34
	– air temperature	-0.06	-0.45*	-0.67*	-0.71*	-0.44*

H', *J'* – explanations as in Table 2
* – correlation significant at $p < 0.05$

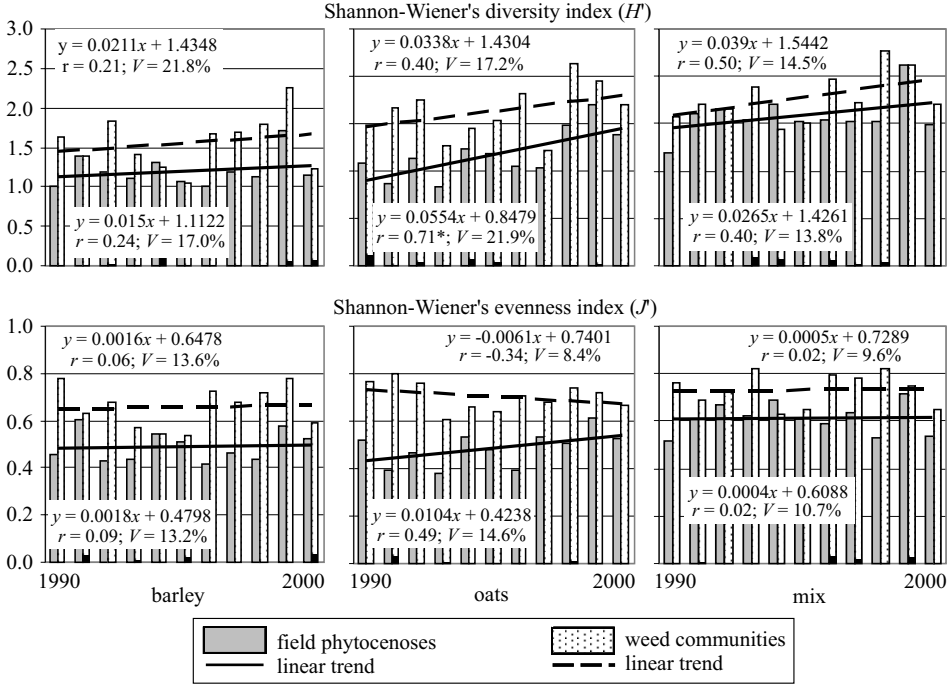
Tabela 4
Relation between cereal density and characteristics of weed communities expressed by simple correlation ratios

Weed community characteristic – indicator	Time of analysis					
	spring			before harvest		
	plant					
	barley	oats	mix	barley	oats	mix
Density, plants · m^{-2}	0.24	0.34	0.25	-0.28	-0.40	-0.10
Number of species	0.64*	0.70*	0.58	-0.12	-0.40	-0.04
H'	0.40	0.66*	0.38	-0.28	-0.06	0.22
J'	0.03	0.11	-0.00	-0.41	0.29	0.37
Biomass				-0.01	0.27	-0.07

H', *J'* – explanations as in Table 2
* – correlation significant at $p < 0.05$

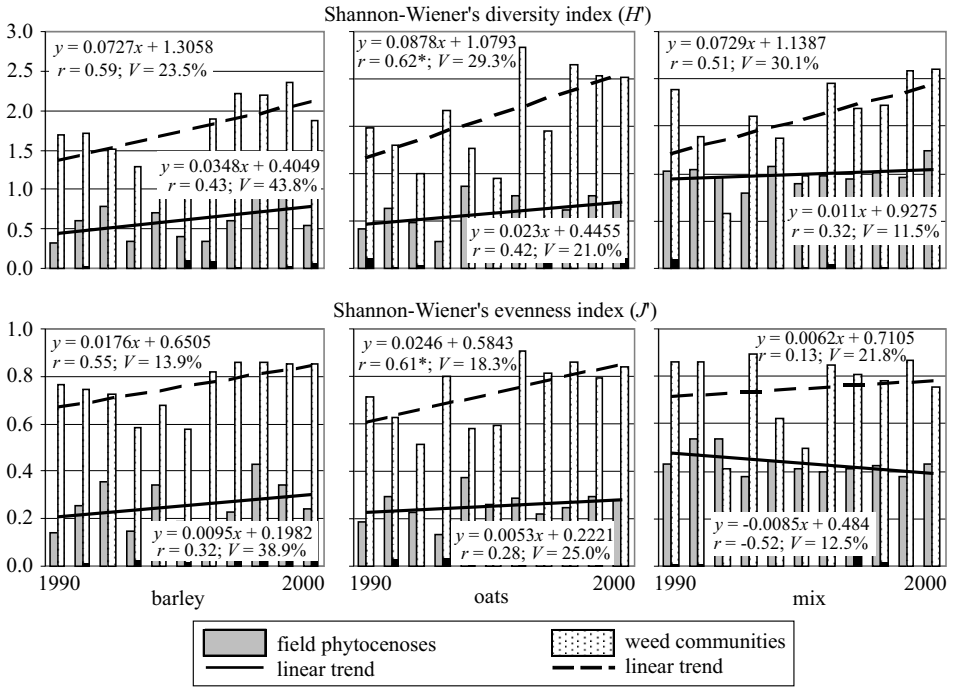
The number of weed species appearing in the spring in cereals was from 6 to 18 (Figure 1) and the averages for the period of study in the analyzed fields were similar (insignificant differences – Table 2). The lines of year-to-year trends in oats and mix were increasing (significant trends – Figure 1). In the fields of oats that characteristic showed the largest year-to-year variance. The abundance of species in the spring increased significantly in case of higher soil humidity (Table 3). Segetal communities developing before harvest consisted of slightly fewer species a compared to the spring. During individual years from 4 to 16 taxa appeared and that number in all cereals showed an increasing trend – in case of the mix that trend was confirmed statistically (Figure 1). Increased abundance of species in summer communities was supported by abundant precipitations in June and the period from June until harvest (Table 3); higher temperature in July and August had opposite consequences as it accelerated ripening and elimination of some species from communities.

The average numbers of species in the entire studied phytocenoses, i.e. fields of barley, oats and mix, did not differ significantly; this applies to both times of testing (Table 2). On the other hand, sowing of two crops instead of



r, V – as in Figure 1

Fig. 3. Shannon-Wiener's diversity and evenness indices for phytocenoses of cereal fields and weed communities at spring determination time



r, V – as in Figure 1

Fig. 4. Shannon-Wiener's diversity and evenness indices for phytocenoses of cereal fields and weed communities before harvest of cereals

one changed the value of Shannon-Wiener diversity index calculated for the entire plants community both in the spring and before harvest of cereals significantly. In the spring the diversity of mix field measured with that index averaged 1.58, and in the summer – 0.99 and in both cases it was significantly higher than the corresponding results for oats and barley in single crop fields. The phytocenosis of the mix was also characterized by a significantly higher evenness of species. Evidently lower values of diversity indices of phytocenoses in the spring than those obtained before harvest result from methodological assumptions (before harvest the density of productive lades resulting from spreading was determined). The values of diversity indices for weeds communities were usually higher than for the entire communities as the situation where the segetal species achieved the numbers matching those of the cultivated crop at relatively low numbers of weed species in the community were extremely rare. As average for the period of study the weed communities in the fields covered did not differ in values of their diversity and evenness indices. No clear differences between the diversity of weed communities in the spring and before harvest were found. The variance of the discussed indices and the direction of

that variance year-to-year are presented in Figures 3 and 4. It should be stressed that the increasing trend of phytocenosis diversity index was confirmed in the fields of oats in the spring only. Also in oats the diversity and evenness indices for weeds measured before harvest increased with the passage of years.

The diversity of three assessed fields depended highly on the numbers of weeds as its increase weakened the domination of the cultivated crop; the number of species was of a little lesser importance (Table 5). The diversity of weeds increased with the abundance of species and evenness of distribution of individuals among species (Table 6). The diversity and evenness of weed communities measured by Shannon-Wiener indices indicated a high correlation with weather conditions (Table 3). Spring and pre-harvest communities were more diversified in case of more abundant precipitations during their formation. Higher temperatures during that time limited the diversity of summer communities. The weather influenced the evenness of species in summer communities in the same way.

Tabela 5

Dependence of field diversity (*H'*) on characteristics of weed communities expressed using simple correlation ratios

Weed community characteristic – indicator	Time of analysis					
	spring			before harvest		
	plant					
	barley	oats	mix	barley	oats	mix
Density, plants · m^{-2}	0,82*	0,97*	0,91*	0,91*	0,82*	0,74*
Number of species	0,65*	0,77*	0,59	0,57	0,67*	0,69*
H'	0,48	0,68*	0,35	0,55	0,39	0,34
J'	0,17	0,06	-0,11	0,47	0,09	-0,23

H', *J'* – explanations as in Table 2
* – correlation significant at $p < 0.05$

Tabela 6

Dependence of weed community diversity (*H'*) on their other characteristics expressed using simple correlation ratios

Weed community characteristic – indicator	Time of analysis					
	spring			before harvest		
	plant					
	barley	oats	mix	barley	oats	mix
Density, plants · m^{-2}	-0.04	0.36	0.23	0.41	-0.03	0.07
Number of species	0.86*	0.90*	0.79*	0.91*	0.90*	0.78*
J'	0.85*	0.50	0.72*	0.83*	0.91*	0.86*

H', *J'* – explanations as in Table 2
* – correlation significant at $p < 0.05$

Table 7

Similarity of weed communities in corn field (%)

Plants Classification*	1990 *P/U*	1991 *S/Z*	1992 *BS/C*	1993 *P/U*	1994 *S/C*	1995 *S/C*	1996 *P/Z*	1997 *BM/Z*	1998 *M/Z*	1999 *BM/U*	2000 *P/U*	Average
Spring												
*J-M***	82.4	59.0	76.0	64.1	78.3	74.7	76.2	78.3	68.9	50.6	71.0	70.86
O-M	60.4	73.0	79.2	69.5	86.7	76.3	70.9	64.6	49.7	81.3	73.9	71.41
J-O	69.0	61.0	50.8	73.1	80.0	74.6	64.6	71.9	51.0	47.1	63.6	64.25
Before harvest												
J-M	43.6	43.7	49.7	27.5	78.1	63.4	37.7	76.0	41.7	44.7	49.5	50.51
O-M	62.0	53.1	80.0	45.6	70.7	69.0	62.2	65.3	47.9	42.6	62.9	60.12
J-O	54.7	67.3	58.2	46.8	82.4	80.0	57.9	63.8	39.6	47.3	64.9	60.26

* – *BM* – very wet, *M* – wet, *P* – average, *S* – dry, *BS* – very dry; *C* – warm, *U* – moderate, *Z* – cool
** – *J* – barley, *O* – oats, *M* – mix

Communities of weeds developing during spring in fields of barley, oats and mix showed a relatively high similarity: from 47.1 to 86.8% (Table 7). A slightly larger spread of similarity ratios was obtained comparing communities of weeds before harvest (27.5-80.0%). In average during the study period, the communities of weeds in the mix showed larger similarity than those developing in barley and oats, i.e. in single crop fields. During the summer the lowest similarity was usually recorded between weeds in barley and the mix. During individual years however, Sorensen ratios developed in a different way for both times of determination. During 11 years in the spring on 6 occasions the communities in oats and the mix were the most similar, four times the largest similarities were recorded between the communities in barley and the mix and only once the largest similarities were recorded between those communities in single crop fields. Before harvest only once the highest similarity ratio applied to segetal communities of barley and the mix, 4 times it applied to communities in oats and the mix and 6 times the compared single crop fields. No major correlations between similarity of communities and precipitation and temperature conditions during the covered seasons were observed.

Taking the average values for the years of studies, in the mix, that is the phytocenosis with the highest biological diversity, the weeds generated the smallest biomass (Figure 5). Although it did not differ statistically from that found in oats, it was lower from it by 32.6%. Weeds in the fields of barley generated around three times larger biomass. The biomass of weeds in that crop increased significantly with the diversity of species in the summer community (Table 8). In oats and the mix no correlation between biomass and studied characteristics of weed communities was confirmed. No correlation between biomass of weeds and density of cereals (Table 4) not the intensity of

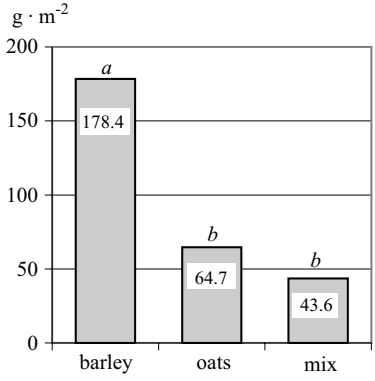


Fig. 5. Biomass (air dried) of weeds in fields of studied cereals (average for years): *a, b* – significance of differences between averages: numbers marked with the same letter do not differ significantly at $p < 0.05$

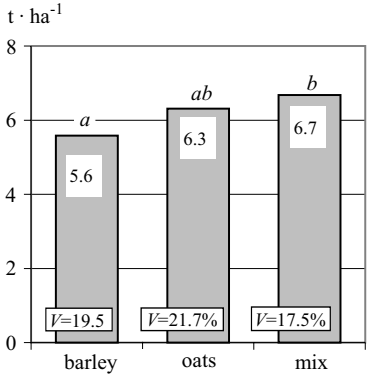


Fig. 6. Yield of studied cereals (average for years): *V* – year-to-year variance ratio (%), *a, b* – significance of differences between averages: numbers marked with the same letter do not differ significantly at $p < 0.05$

Tabela 8

Dependence of weed biomass on studied characteristics of weed communities expressed using simple correlation ratios

Weed community characteristic – indicator	Time of analysis					
	spring			before harvest		
	plant					
	barley	oats	mix	barley	oats	mix
Density, plants · m^{-2}	0.01	0.33	0.25	0.63	0.53	0.40
Number of species	0.29	0.30	0.38	0.69*	0.12	0.17
H'	0.32	0.14	0.04	0.67*	0.29	0.16
J'	0.20	-0.14	-0.41	0.51	0.44	-0.24

H', *J'* – explanations as in Table 2
* – correlation significant at $p < 0.05$

Tabela 9

Dependence of yield on characteristics of weed communities expressed using simple correlation ratios

Weed community characteristic – indicator	Time of analysis					
	spring			before harvest		
	plant					
	barley	oats	mix	barley	oats	mix
Density, plants · m^{-2}	-0.40	-0.39	-0.41	-0.53	-0.26	-0.25
Number of species	-0.30	-0.17	-0.37	-0.51	0.11	-0.11
H'	-0.20	0.03	-0.14	-0.47	0.08	0.05
J'	0.01	0.42	0.17	-0.36	-0.00	0.19
Biomass				-0.66*	-0.62	-0.66*

H', *J'* – explanations as in Table 2
* – correlation significant at $p < 0.05$

precipitation during its generation (Table 3) was found. It was influenced negatively by higher temperature at the end of vegetation.

The mix offered the highest average yields – 6.68 $t \cdot ha^{-1}$ of grain, higher than those of oats and barley by 0.37 and 1.10 t respectively (Figure 6) as well as the lowest year-to-year variance (V = 17.5% – as compared to 19.5% for barley and 21.7% – for oats). The correlation between the yield and the numbers or diversity of weeds in cereal fields was confirmed. The yield, on the other hand, correlated negatively with the biomass of weeds (Table 9).

Discussion

Biological diversity, next to mass thermodynamic and bio-geo-chemical phenomena, is currently one of the two major fields in ecological studies in the world (WEINER 2000). Poland has its share in it: in 2003 the report on "Biological diversity of Poland" (*Różnorodność...* 2003) was published. That report documented the status of diversity at the three levels: within species, species and higher than species (plant communities and landscapes). Crops and wild growing species have different sections devoted to them. The diversity of crop plants cultivated according to the range of localized crop species is determined while the breeding sector works on the qualitative aspect of diversity within species. Also the segetal flora has been described by specialists for years including highlighting the qualitative and quantitative changes occurring in it (LATOWSKI 2002). There are fewer studies concerning diversity within plant communities in agricultural systems treated from the structural point of view. The number of articles using biological indicators considering the relative shares of individual taxa for assessment of weed communities increases (JĘDRUSZCZAK, ANTOSZEK 2004, STUPNICKA-RODZYNKIEWICZ et al. 2004). However, they do not encompass complete phytocenoses including the crop plants, which was proposed by the authors of this paper.

In publications concerning weeds generally the major species of weeds are indicated frequently calling them the "dominating species". Their numbers are mainly determined arbitrarily determined by the author on the basis of count (PAWŁOWSKI, WOŹNIAK 2000, RADECKI et al. 2003, ZAWIŚLAK 1997) or surface coverage (ŁABZA et al. 2003). For example STUPNICKA-RODZYNKIEWICZ et al. (2004) in their work name as many as 17 of them. In the presented paper treating phytocenoses of cereal fields as competitive communities, at maximum 4 taxa only, beside the cultivated crop, were classified as dominants and subdominants. The species of weeds considered here as presented constantly or co-dominating together with the cereal are those very common or common all over Poland (LATOWSKI 2002).

In own studies the abundance of species forming the phytocenosis of the mix field did not differ clearly from those in fields of oats and barley sown as single crops. On the other hand SNARSKA (2004) monitoring weeds in cereals in a selected municipality of Podlaskie province (the largest share of mixes in the structure of sown crops in Poland) stated that the largest number of weed species was present in the mix of oats and barley.

Mixed crops are nowadays considered a form of biological enrichment of fields (FINCKH et al. 2000, WANIC, NOWICKI 2000). That is confirmed by the diversity indices based on Shannon-Wiener formula, which takes into account the relative abundance of species in phytocenosis. It seems obvious that the distribution of domination in the fields between to cereal species results in an increase of diversity defined by the Shannon-Wiener index, however, it should be pointed out that the same effect can be offered by the compensating segetal taxon matching in numbers the cultivated species. In the studies by ROLA and ROLA (2002) the number of *Apera spica-venti* panicles in winter wheat reached even more than 600 pieces $\cdot$ m^{-2}. Confrontation of own results with other results in that aspect is difficult because of lack of studies employing a compatible approach to the research material. Comparing, on the other hand the diversity of weed communities determined in our studies to that measured by that index in existing studies by other authors (JĘDRUSZCZAK, ANTOSZEK 2004, STUPNICKA-RODZYNKIEWICZ et al. 2004) would have no scientific dimension because those communities developed in other plants and under influence of other factors. The procedural reasons are also of importance.

In literature on cereal mixes their influence on limiting the general development of weeds is highlighted (WANIC 1997, WANIC, HRUSZKA 2000). The regulating role of mixes with participation of oats (RUDNICKI et al. 1996) as well as appropriate density of that species (IDZIAK, MICHALSKI 2003) are highlighted in particular. In studies by IDZIAK and MICHALSKI (2003) the numbers of weeds in mixes of barley and oats at different proportions were lower than in barley sown alone and closer to oats while their fresh mass was lower than in single crop fields of each of the components. That partly matches our observations that showed that both the number and biomass of weeds were at the lowest in the mix.

These studies are consistent with other studies also as concerns higher yields and higher yield stability of mixes as compared to single crop fields (IDZIAK, MICHALSKI 2003), particularly under more difficult habitat and agricultural technique conditions (RUDNICKI et al. 1996, WANIC 1997).

In the presented experiment a large year-to-year variance of studied characteristics and their dependence on weather were observed. Similar effects were obtained in the experiment with oats cultivated after potato and after oats (WANIC et al. 2005). JĘDRUSZCZAK and ANTOSZEK (2004) stress that

development of phytocenoses does not depend on the experimental factor only but that it is modified by meteorological conditions and other, less known factors as well as their combined influence.

Conclusions

1. During 11 years of studies 33 species of weeds appeared in cereals. The communities during individual years and at different times of determination (spring, harvest), however, were much poorer consisting of 4-18 taxa.

2. *Chenopodium album, Thlaspi arvense, Stellaria media, Fallopia convolvulus* and *Capsella bursa-pastoris* were constantly occurring elements of communities. In single crop fields *Matricaria maritima* ssp. *inodora* was present constantly and in barley *Polygonum aviculare* and *Lamium amplexicaule* were additional constantly present weed species.

3. In the studied cereals the numbers of weeds in weed communities developing in the spring did not differ. Before harvest a significantly higher numbers of weeds in their communities were recorded in barley than in oats and the mix. The communities of weeds before harvest were usually less numerous than during the spring.

4. The weeds generated the smallest biomass in the cereal mix.

5. As concerns the numbers in the spring *Chenopodium album, Thlaspi arvense, Stellaria media* and *Fallopia convolvulus* were classified as the dominants and subdominants while before harvest only *Chenopodium album* was classified as such.

6. The mixed crop field achieved higher diversity and evenness of species than oats and barley as opposed to the communities of weeds, which did not differ in those aspects.

7. In the spring the communities of weeds in the mix showed a larger similarity to communities developing in barley and oats than the similarity level between communities developing in single crop fields. In the summer the lowest similarities were usually recorded between weeds in barley and in the mix.

8. The mix offered the highest yields with the lowest year-to-year variance. The yield of cereals was negatively correlated with the biomass of weeds and did not depend on their numbers or diversity.

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A POTENTIAL DECREASE IN THE YIELD OF POTATO IN POLAND CAUSED BY DEFICIENCY IN SUNSHINE

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K e y w o r d s: potato, a decrease in the yield, deficiency in sunshine, Poland.

A b s t r a c t

In the study an attempt was undertaken to determine the increase in the potato yields caused by deficiency of actual sunshine. This relationship was described by the equation of square regression. The strongest relationship between the potato yields in production conditions and the actual sunshine total was observed in the period from 21st June to 20th August both in relation to the whole country and to a province. A decrease in the national potato yields below the average of the many year period caused by deficiency of actual sunshine in the period from 21st June to 20th August occurred when the sunshine conditions were below 365 h. Actual sunshine deficiency in the period from 21st June to 20th August can cause a fall in the potato yields on average by 7-11%. The largest decrease may occur in the eastern and north-western part of the country and in Kotlina Płocka. In the period from 21st June to 20th August, the actual sunshine deficiency below 365 h occur most often in the north and the west of the country and the rarest in the east.

POTENCJALNE ZMNIEJSZENIE PLONU ZIEMNIAKA W POLSCE POWODOWANE NIEDOBORAMI USŁONECZNIENIA

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S ł o w a k l u c z o w e: ziemniak, zmniejszenie plonu, niedobory usłonecznienia, Polska.

A b s t r a k t

W pracy podjęto próbę określenia zmniejszenia plonu ziemniaka spowodowanego niedoborami usłonecznienia rzeczywistego. Zależność tę opisano za pomocą analizy regresji kwadratowej. Najsilniejszy związek między plonem ziemniaka w warunkach produkcyjnych a sumą usłonecznienia

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rzeczywistego stwierdzono w okresie od 21 VI do 20 VIII, i to zarówno w skali kraju, jak i województwa. Zmniejszenie krajowych plonów ziemniaka poniżej średniej wieloletniej, powodowane niedoborami usłonecznienia w okresie od 21 VI do 20 VIII, wystąpiło w warunkach usłonecznienia poniżej 365 h. Niedobory usłonecznienia rzeczywistego w okresie od 21 VI do 20 VIII mogą powodować zmniejszenie plonów ziemniaka przeciętnie o 7-11%, przy czym największe we wschodniej i północno-zachodniej części Polski oraz w Kotlinie Płockiej. W okresie od 21 VI do 20 VIII niedobory usłonecznienia rzeczywistego poniżej 365 h występują najczęściej na północy i zachodzie Polski, najrzadziej zaś na wschodzie.

Introduction

In the period of the last ten years the area of potato cultivation has decreased in Poland from 1.3 million ha in 1996 to about 0.6 million ha in 2005. Despite that, the cultivation area of potato in 2005 was smaller only than that of cereals, except for maize and oats. The contribution of potato to the structure of crops was 6.0%. The limitation of cultivation area resulted not only from a decrease in the use of potato for fodder, but also from the lack of possibility to increase sales, and a part of area that has been used for potato cultivation so far, is now used for cultivation of maize. In Poland potato yields do not usually exceed 20 $t \cdot ha^{-1}$ and in this respect they are smaller by about 50% than the average yields in EU countries in which potato cultivation has a very important role (Roczniki Statystyczne GUS).

The growth and yields of potato are determined to a large extent by the distribution of meteorological conditions during vegetation of plants (GRABOWSKA et al. 1997, BOMBIK 1998, SZWEJKOWSKI et al. 2005), including, among others, light conditions and particularly the time during which solar radiation reaches a given point on the Earth. In Poland actual sunshine is characterized by large time and spatial variability and by a significant time trend, positive in May and August and negative in September (GLUZA 2000, KOŹMIŃSKI, MICHALSKA 2004). The relation between potato yield and actual sunshine was reported, among others, by DZIEŻYC et al. (1997), GRABOWSKI (1995) and KALBARCZYK (2003b, 2004). According to DZIEŻYC et al. (1997), sunshine has a significant influence on the yields of potato tubers in most regions of the country on rye and wheat soil complexes during the period from sprouting to the drying of haulms. In the period from sprouting to flowering the effect is advantageous, whereas from flowering to haulms drying, it is disadvantageous. According to SAWICKA and MIKOS-BIELAK (1995), small totals of actual sunshine in the period from July to August cause low accumulation of dry matter in tubers and an increase in sugar totals.

A relation of potato yields and light conditions, particularly those described by actual sunshine, has rarely been presented so far, taking into consideration both the whole country and individual provinces. Hence an attempt has been

made to determine the effect of actual sunshine on potato yields and to assess the risk of cultivating this plant from deficiency of sunshine.

Material and Methods

The basis for the study were the results concerning the level of potato yields in production conditions in the years 1971-1998 and ten day period totals of actual sunshine in 1971-2002 gathered at 49 IMGW stations (Biuletyn Agrometeorologiczny), evenly distributed throughout Poland. Potato production was characterized by the data of Central Statistical Office from 44 former provinces according to the administrative division from 1975 (Roczniki Statystyczne GUS). Due to a small number of meteorological stations and large microregional variability of weather conditions, the mountain areas situated within the administrative borders of 5 former provinces of Jelenia Góra, Wałbrzych, Bielsko-Biała, Nowy Sącz and Krosno were not included in the study.

Due to the change of the administrative division of Poland in 1999 and in consequence lack of access to the Central Statistical Office data, the effect of actual sunshine on potato yields was examined on the basis of a shorter period (1971-1998) than the analysis of actual sunshine characteristics was done (1971-2002).

For the determination of statistical relations of the average national yield of potato and the total of actual sunshine in the period from May to September the analysis of regression was used, the parameters of which were determined by means of the smallest squares method. The significance of regression parameters was examined using the t-Student test, whereas the significance of the whole equation by means of the F-Snedecor test, assuming the level $p = 0.05$. For choosing the function, a decisive factor was the postulate of the minimum total of deviations. For the analysis of regression only those provinces were chosen which consisted of at least 65% of the general area of arable land, typical of potato cultivation (soil-agricultural complex 4, 5 and 6). Thus the number of basic materials (the number of provinces x the number of years) amounted to 336 elements for the whole country and 28 – for a province.

To define the threat to national cultivation of potato from disadvantageous conditions of actual sunshine, a threshold value of deficiency in the number of hours with the sun was determined on the basis of the dependence described by the function of square regression, the value that may be the cause of the decrease in the potato yields below the average level of the many year period. Then the average value of actual sunshine for each of 49 IMGW stations was calculated, but only for those years in which the threshold value determined

earlier, was exceeded. By substituting the average value of actual sunshine into the equation of square regression, the yields conditioned by an average occurrence of deficiency in the number of hours with the actual sunshine in the discussed period were calculated. The differences between actual national yields and the yields calculated according to the procedures described above made it possible to determine a potential decrease in the yield caused by the deficiency in actual sunshine.

All the statistical analysis were carried out using the Statistica 6 program.

Results and Discussion

The closest relationship between the national yield of potato in the production conditions and the actual sunshine was proved in the period from 21st June to 20th August in which flowering occurs and that is related to the strongest sensitivity of this plant to the changeable weather conditions (KALBARCZYK 2003a, KALBARCZYK, KALBARCZYK 2004). The largest coefficient of determination (R^2 = 29.7%) and at the same time the smallest standard error of estimation of the equation (2.6 $t \cdot ha^{-1}$) was obtained by means of the function of square regression which has a form of a convex parabola reaching the maximum at the number of hours with actual sunshine 440 during the period from 21st June to 20th August. The above relationship can be presented in the following form:

$$y = -0.000195U_{21\text{June-}20\text{Aug}}{}^{2***} + 0{,}1719U_{21\text{June-}20\text{Aug}}{}^{***} - 18.9392^{***} \quad (1)$$

where:
y – potato yields in production conditions ($t \cdot ha^{-1}$),

$U_{21\text{June-}20\text{Aug}}$ – totals of actual sunshine during the period from 21st June to 20th August (h),
*** significant at p = 0.01.

According to GRABOWSKI (1995), the most advantageous effect of actual sunshine in Pojezierze Olsztyńskie during the whole period of vegetation on the potato yields occurs in the interval 1100-1200 h. SAWICKA'S (1993) study shows that the decrease in actual sunshine in the period of vegetation by affecting photosynthesis and plant breathing is not favourable to the process of tuberization and as a result new tubers do not grow and at the same time there is an increase in the mass of the tubers formed earlier. Table 1 shows the

characteristics of variables used in the above equation of regression (1) in relation to the whole country. The yields of potato vary from 7.1 to 25.1 t · ha^{-1}, and on average they equal 17.9 t · ha^{-1}, while the totals of actual sunshine in the period from 21st June to 20th August range from 262 to 759 h, and on average they amount to about 519 h. Variability of the yields and sunshine is equal to about 20% each.

Table 1

Statistical characteristics of variables used in the regression equation (1)

Variable	Characteristics					
	mean	min.	max.	standard deviation	coefficient of variation (%)	linear trendline
y (t · ha^{-1})	17.9	7.1	25.1	3.5	19.8	ns
U (h)	519.4	262.0	759.0	99.0	19.1	+***

y – potato yields in production conditions (t · ha^{-1}), U – totals of real sunshine during the period from 21st June to 20th August (h), ns – not significant at α = 0.1, +/ – positive effect, *** – significant at α = 0.01.

The strongest relation of the potato yield and sunshine in the period from 21st June to 20th August was confirmed by the results of the regression for all the discussed provinces in Poland. As it is illustrated in Figure 1, the best

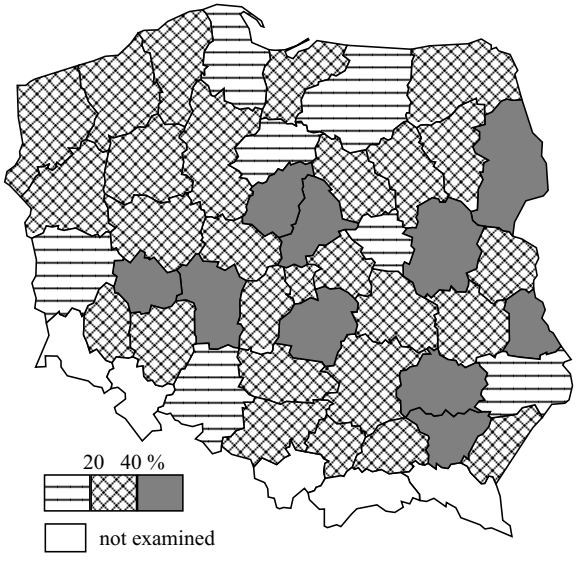

Fig. 1. Coefficients of determination (%) for the dependence of potato yields on actual sunshine in the period from 21st June to 20th August in the years 1971-1998

description of the potato yields was obtained in ten provinces situated in the central and mid-eastern part of Poland in which the coefficients of determination exceeded 40%, whereas the worst one, in seven provinces: the provinces of Gdańsk, Olsztyn, Toruń, Warszawa, Zamość, Zielona Góra and Opole in which R^2 did not exceed 20%. In all the analysed provinces the dependence of potato yields on actual sunshine was described by the second degree polynomial regression with a negative square coefficient of regression.

Figure 2 shows that the largest totals of actual sunshine, in the period in which their significant effect on potato yields was observed, occurred in the east of the country (above 460 h) and particularly in Polesie Lubelskie (above 480 h), while the smallest ones – in Wyżyna Śląska (Silesian Highlands) and

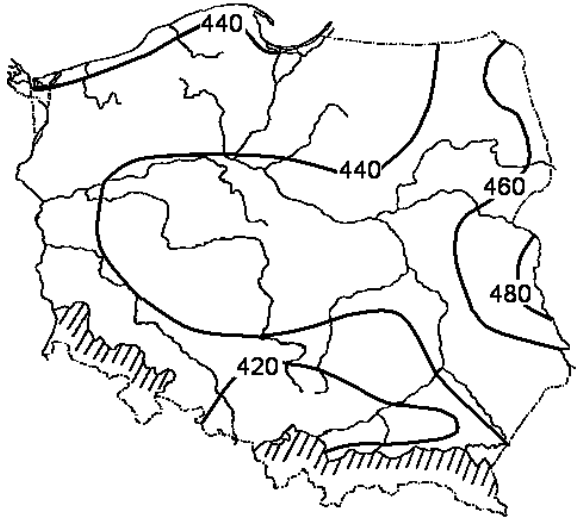

Fig. 2. Actual sunshine totals (h) in the period from 21st June to 20th August over 1971-2002

Wyżyna Krakowsko-Częstochowska (Kraków-Częstochowa Highlands) and in the region of Tarnów (below 420 h). According to KOŹMIŃSKI and MICHALSKA (2001) an approximate distribution of annual totals occurred in the years 1966-1995. The coefficient of variation calculated for actual sunshine in the period from 21st June to 20th August was at the level of about 16-22% and it increased in the north-western direction. The largest differentiation of actual sunshine in individual years was characteristic of Pojezierze Pomorskie, except for the coastal zone and the region of Elbląg, whereas the smallest differences were in Wyżyna Lubelska (the Lublin Highlands) and Pogórze Karpackie (Carpathian Uplands) – Figure 3. A significant increase in the totals of actual

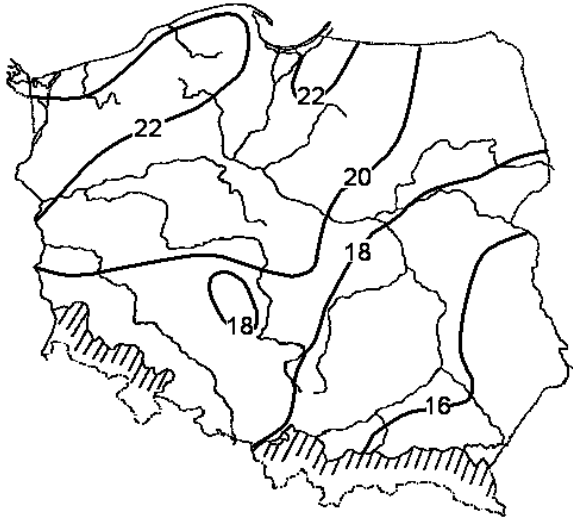

Fig. 3. The coefficient of variation (%) of the actual sunshine total in the period from 21st June to 20th August over 1971-2002

sunshine in the years 1971-2002 was observed only at six stations situated in the south-western part of the country. The average rise in the period from 21st June to 20th August amounted to 14 h per 10 years.

In order to determine the threat to the potato cultivation from disadvantageous light conditions in the period from 21st June to 20th August, a threshold value for the deficiency of actual sunshine was determined on the basis of the regression equation (1) included in the presented study. Potato yields, lower than the many year average i.e. 17.9 $t \cdot ha^{-1}$, occurred when the totals of sunshine were smaller than 365 h. In order to determine accurately a potential decrease in potato yields due to unfavourable distribution of light conditions described by totals of actual sunshine, a graph for their deficiency was plotted. The basis for the plotting of the graph shown in Figure 4 was a percentage estimation of the difference between national yields and the yields calculated from the equation (1), but only for those values of the discussed factor at which a decrease in potato yields caused by deficiency of sunshine occurs. At the sunshine values equal to 315 h, the national potato yield will fall by 11% in relation to an average from the years 1971-1998, and at 295 h – by 17% (Figure 4). The risk of occurrence of sunshine deficiency in the period from 21st June to 20th August decreased with a fall in the number of hours with the sun from the point of the determined threshold value (< 365 h). At the determined threshold value unfavourable light conditions for potato cultivation throughout Poland occurred with frequency of nearly 20% (Figure 5).

Figure 4 allows the assessment of the decrease in potato yields caused by deficiency of sunshine only in relation to the whole country. Actual sunshine determined below the threshold value which causes a decrease in potato yields below the level of the many year period may be the cause of differentiated losses in the yields depending on a region of the country. The spatial distribu-

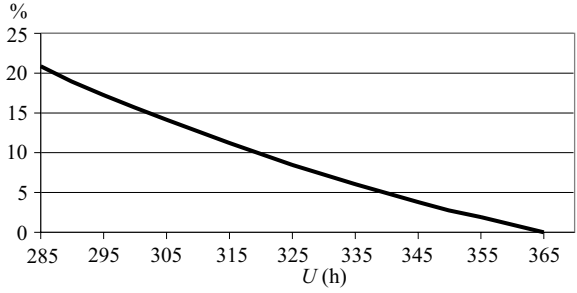

Fig. 4. The potential decrease (%) in the national potato yields caused by deficiency of actual sunshine (U) in the period from 21st June to 20th August according to the regression equation (1)

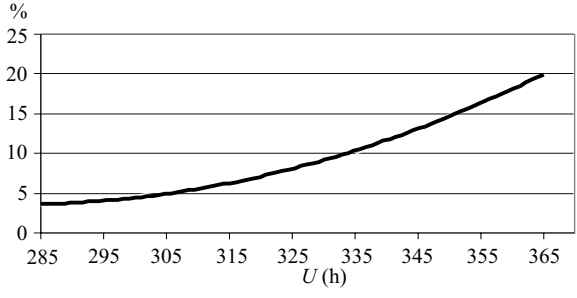

Fig. 5. Frequency (%) of actual sunshine deficiency (< 365 h) in Poland in the period from 21st June to 20th August

tion of a potential drop in the potato yields caused by deficiency of sunshine (< 365 h) in the period from 21st June to 20th August is irregular and the losses of the yields may range from < 7 to > 11% (Figure 6). The largest fall in the yields may occur both in the east of Poland – in Wyżyna Lubelska, Polesie Lubelskie, Nizina Podlaska and in the north – in Pojezierze Kaszubskie, Pojezierze Bytowskie and also in the west – in Pojezierze Myśliborskie, Pojezierze Lubuskie and in central Poland – in Równina Kutnowska, Kotlina Płocka and Kotlina Sandomierska. The lowest losses of the yields can be expected in the south-west and in Pojezierze Olsztyńskie and in the southern part of Pojezierze Pomorskie. An average frequency of sunshine totals below 365 h in the period from 21st June to 20th August throughout the country varies

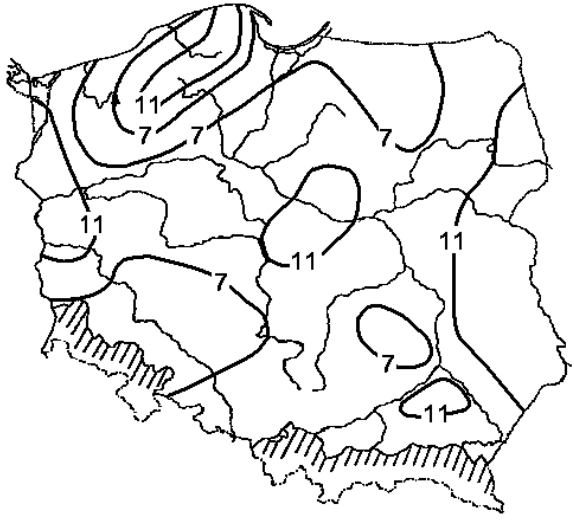

Fig. 6. The potential decrease (%) in the national potato yields caused by deficiency of actual sunshine (< 365 h) in the period from 21st June to 20th August

Fig. 7. Frequency (%) of the years with actual sunshine deficiency (< 365 h) in the period from 21st June to 20th August

from < 10 to > 30%, growing mainly towards the west and the north (Figure 7). Sunshine values below 365 h occur most frequently in the western part of the country and in Pojezierze Olsztyńskie, Pojezierze Drawskie and Pojezierze Bytowskie and also in Wyżyna Śląska and they occur least frequently in the

eastern part of the country. The strongest threat to potato yields from the potential decrease in the yields of potato and the frequency of sunshine totals < 365 h in the period from 21st June to 20th August can occur in western parts of Poland and in morainal hills of Pojezierze Pomorskie (Figures 6-7). In this area the losses amounting to 11% can occur on average every 3 years.

Conclusions

1. The strongest relationship between the potato yields in production conditions and the actual sunshine total was observed in the period from 21st June to 20th August both in relation to the whole country and to a province.

2. A decrease in the national potato yields below the average of the many year period caused by deficiency of actual sunshine in the period from 21st June to 20th August occurred when the sunshine conditions were below 365 h.

3. Actual sunshine deficiency in the period from 21st June to 20th August can cause a fall in the potato yields on average by 7-11%. The largest decrease may occur in the eastern and north-western part of the country and in Kotlina Płocka.

4. In the period from 21st June to 20th August, the actual sunshine deficiency below 365 h occur most often in the north and the west of the country and the rarest in the east.

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EFFECT OF FOLIAR APPLICATION OF ECOSOL U ON THE YIELD OF RED BEET (*Beta vulgaris* L. ssp. *esculenta*) AND THE CONCENTRATIONS OF MINERALS IN STORAGE ROOTS

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K e y w o r d s: red beet, foliar application of fertilizer, yield, minerals.

A b s t r a c t

A three-year experiment was conducted to determine the effect of foliar application of 0.1% and 0.2% aqueous solutions of Ecosol U on total yield, marketable yield and marketable yield expressed as a percentage of total yield, as well as on the concentrations of nitrogen, phosphorus, potassium, magnesium, calcium, sodium, copper and iron in the storage roots of local and foreign red beet (*Beta vulgaris* L. ssp. *esculenta)* cultivars. It was found that Ecosol U significantly affected the total yield of storage roots. Differences in marketable yield related to red beet cultivar and the cultivar x fertilizer interaction were statistically significant. Foliar application of 0.2% Ecosol U increased marketable yield expressed as a percentage of total yield of storage roots. The concentrations of total nitrogen, potassium, sodium, copper and iron in red beet storage roots depended on the variant of foliar application of fertilizer. Particular red beet cultivars differed in the levels of potassium, calcium and copper.

WPŁYW DOKARMIANIA DOLISTNEGO NA PLONOWANIE I ZAWARTOŚĆ SKŁADNIKÓW MINERALNYCH W KORZENIACH SPICHRZOWYCH BURAKA ĆWIKŁOWEGO (*Beta vulgaris* L. ssp. *esculenta*)

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S ł o w a k l u c z o w e: burak ćwikłowy, dokarmianie dolistne, plon, składniki mineralne.

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Abstrakt

W trzyletnim doświadczeniu badano wpływ dokarmiania dolistnego w polowej uprawie buraka ćwikłowego (*Beta vulgaris* L. ssp. *esculenta*) na plon ogółem, handlowy i udział plonu handlowego w plonie ogółem oraz zawartość azotu, fosforu, potasu, magnezu, wapnia, sodu, miedzi i żelaza w korzeniach spichrzowych. Badaniom poddano odmiany pochodzenia krajowego i zagranicznego. *Beta vulgaris* L. ssp. *esculenta*, które dokarmiano preparatem Ekosol U w stężeniu 0,1% i 0,2% wodnego roztworu. Stwierdzono istotny wpływ badanych czynników na plon ogółem korzeni spichrzowych. Różnice w wielkości plonu handlowego w zależności od uprawianej odmiany buraka ćwikłowego oraz jej współdziałania z zastosowanym roztworem Ekosolu U potwierdzono statystycznie. Zastosowanie w uprawie buraka ćwikłowego 0,2% roztworu Ekosolu U spowodowało zwiększenie udziału plonu handlowego korzeni w plonie ogółem w porównaniu z innymi obiektami badawczymi. Zawartość azotu ogólnego, potasu, sodu oraz miedzi i żelaza w korzeniach spichrzowych zależała od dokarmiania dolistnego. Odmiana była czynnikiem różnicującym zawartość K, Ca oraz Cu.

Introduction

Mineral fertilizers, applied at high rates in large-scale intensive vegetable growing, enable to increase the yield but also affect the biological value of edible parts of vegetables (SADY, ROŻEK 2001, SADY, SMOLEŃ 2004). Mineral fertilization has a considerable effect on nutrient concentrations in red beet roots, thus deciding about their nutritive value (KANNER et al. 2001, MICHAŁOJĆ, SZEWCZUK 2003). The biological quality of red beet storage roots depends not only on the levels of organic elements, but also on the content of mineral salts, which may vary greatly both in local and foreign red beet cultivars.

Foliar and soil application of fertilizers complement each other. The efficiency of the former depends on the type and rate of fertilizer, development stage of plants, air temperature and humidity. According to reference data, supplementary foliar application of fertilizers to field-grown vegetables allows to reduce the rate of total nitrogen introduced into the soil by 30% to 40% (KOŁOTA, BIESIADA 2000, SZEWCZUK, MICHAŁOJĆ 2003, KRĘŻEL 2005).

The aim of the present study was to determine the effect of foliar application of Ecosol U on red beet yield and nutrient levels in storage roots as dependent on fertilizer concentration and cultivar.

Materials and Methods

The experiment was conducted during the years 2001 to 2003, at the Experimental Garden of the University of Warmia and Mazury in Olsztyn, on brown soil of good rye complex, developed from loamy sand (pH 6.7) containing

2.8% of humus. The concentrations of minerals were as follows: N-NO_3 – 38, K – 127, P – 90, Ca – 1840, Mg – 194 mg · dm^{-3}. The experiment was performed in a randomized split-plot design in three replications. Experimental factor I was fertilizer concentration, i.e. a 0.1% or 0.2% aqueous solution of Ecosol U (N – 15%, P_2O_5 – 5%, K_2O – 3.2%). Ecosol U was not applied in the control treatment. Experimental factor II was red beet cultivar, i.e. Lithuanian cultivars Ainiai, Kamuoliai 2, Nevėzis and Vytėnų Bordo, Polish cultivars Chrobry and Rywal and Dutch cultivar Wodan F_1.

Plot size was 8.1 m^2 (2.7 x 3.0 m). Based on the results of a chemical analysis of the soil surface layer, nitrogen (80 kg N · ha^{-1}) in the form of ammonium salpeter was applied in all treatments 10 days prior to sowing. Cucumbers served as forecrop. Seeds dressed with Seed Dressing T (5 g · kg^{-1} of seeds) were sown in rows, 30 cm apart, in the middle of May. Standard cultivation measures applied during the growing season included mechanical weed control and soil loosening. 0.1% and 0.2% solutions of Ecosol U were applied at a rate of 1 dm^{-3} per 10 m^2 to the leaves of red beet plants six times, at two-week intervals, from the beginning of June to mid-August.

One-phase beet harvesting was carried out in the middle of October. The roots were divided into fractions. Total yield, marketable yield (t · ha^{-1}) and marketable yield expressed as a percentage of total yield were determined. Marketable yield consisted of healthy, well-developed roots more than 8 cm in diameter. 20 such roots were selected of each treatment for chemical analysis. The roots were crushed, dried to constant mass at 65°C in a dryer, model KBC G 65/250, and milled in an electric mill. The plant material was mineralized at the laboratory of the Chemical and Agricultural Station in Olsztyn, and the concentrations of the following macro- and micronutrients were determined: total nitrogen by the potentiometric method, phosphorus by the vanadium-molybdenum method, potassium, calcium and sodium by flame photometry, magnesium, copper and iron by atomic absorption spectrometry (AAS). The experiment was conducted in accordance with Accreditation Certificate no. AB 277, granted by the Polish Center for Accreditation in Warsaw.

Results were verified statistically by an analysis of variance. Differences between means of three years (2001 to 2003) were estimated by the Tukey's test at a significance level of $\alpha = 0.05$.

Results

The yields of all red beet cultivars used in the study are given in Table 1. The highest mean total yield (91.61 t · ha^{-1}) was attained in the control treatment. Foliar application of 0.2% Ecosol U resulted in a statistically significant decrease in red beet yield (on average 78.69 t · ha^{-1}). Differences in

marketable yield related to the concentration of foliar fertilizer were found to be statistically non-significant. However, a tendency towards higher yields was observed in the treatment where Ecosol U was applied at a concentration of 0.1%. A higher concentration of this fertilizer (0.2%) caused a decrease in marketable yield by 11.57 $t \cdot ha^{-1}$. The Dutch cultivar Wodan F_1 provided the highest total and marketable yield, whereas the Lithuanian cultivar Nevėzis – the lowest.

Table 1

Yield of red beet storage roots as dependent on cultivar and foliar application of fertilizer (means of 2001-2003)

Concentrations Ecosol U	Cultivar	Yield ($t \cdot ha^{-1}$)		Decrease in the total and marketable yield (%)
		total	marketable	
Control	Ainiai	102.50	100.00	97.56
	Chrobry	82.10	81.00	98.66
	Kamuoliai 2	84.00	84.00	100.00
	Nevėzis	78.40	75.80	96.69
	Rywal	114.20	111.30	97.46
	Vytėnų Bordo	81.90	80.30	98.05
	Wodan F_1	98.20	96.30	98.07
Average		91.61	89.82	98.07
Ecosol U 0.1%	Ainiai	99.30	97.70	98.39
	Chrobry	88.80	87.74	98.81
	Kamuoliai 2	81.40	81.40	100.00
	Nevėzis	83.70	81.10	96.90
	Rywal	92.10	92.00	100.00
	Vytėnų Bordo	86.10	86.10	100.00
	Wodan F_1	105.40	105.40	100.00
Average		90.97	90.21	99.16
Ecosol U 0.2%	Ainiai	70.50	70.00	99.29
	Chrobry	69.50	69.96	99.95
	Kamuoliai 2	80.90	80.90	100.00
	Nevėzis	77.90	77.90	100.00
	Rywal	82.10	82.10	100.00
	Vytėnų Bordo	81.30	81.00	99.63
	Wodan F_1	88.60	88.60	100.00
Average		78.69	78.64	99.84
Average	Ainiai	90.77	89.24	98.42
	Chrobry	80.13	79.57	99.14
	Kamuoliai 2	82.10	82.10	100.00
	Nevėzis	80.00	78.27	97.87
	Rywal	96.13	95.14	99.16
	Vytėnų Bordo	83.10	82.47	99.23
	Wodan F_1	97.10	96.77	99.36
LSD $\alpha_{=0.05}$				
Concentrations Ecosol U		13.57	ns.	–
Cultivar		16.13	10.60	
Interaction		20.42	16.42	

The interaction of the experimental factors also had a statistically significant effect on red beet yield, noticeable particularly in the local cultivar Rywal grown in control plots. A significant yield decrease was recorded in the local cultivar Chrobry fertilized with 0.2% Ecosol U.

Despite a decrease in the total and marketable yield of *Beta vulgaris* L. ssp. *esculenta,* foliar application of Ecosol U at a concentration of 0.2% contributed to an increase in marketable yield expressed as a percentage of total yield. The difference between this treatment and the control treatment was 1.77%. All storage roots of the Lithuanian cultivar Kamuoliai 2 were classified as marketable yield.

Apart from yield height, another important factor is the concentration of macro-and micronutrients in red beet roots. The experimental factors had a significant effect on the levels of particular minerals contained in storage roots (Table 2). Foliar application of Ecosol U considerably affected the concentrations of nitrogen, potassium, sodium, copper and iron. 0.1% Ecosol U caused a decrease in the levels of macro- and micronutrients in red beet storage roots, as compared with the other treatments. The only exception was sodium, since the highest amounts of this element accumulated in the edible parts of red beets in the control treatment. The application of Ecosol U at a concentration of 0.2% had the reversed effect, i.e. contributed to an increase in the content of nitrogen, phosphorus, potassium, magnesium and copper in storage roots.

Particular red beet cultivars differed in the levels of potassium, calcium and copper. The storage roots of the local cultivar Chrobry contained the largest amounts of potassium (0.53% of fresh weight) and copper (1.32 $mg \cdot kg^{-1}$ of fresh weight).

The interaction of both experimental factors also affected nutrient concentrations in red beet storage roots. Significant differences were observed for total nitrogen, potassium, sodium, copper and iron. The total nitrogen content of storage roots ranged from 0.17 to 0.39% of fresh weight. The lowest concentration of this nutrient was recorded in cv. Ainiai in the control treatment, and the highest – in cv. Chrobry fertilized with 0.2% Ecosol U. An identical tendency was observed in the potassium content of edible parts of red beet plants. Sodium concentrations varied between 0.02% and 0.11% of fresh weight. 0.1% Ecosol U had a positive impact on the accumulation of this element in storage roots when applied to cv. Rywal, but a negative when applied to cv. Vytėnų Bordo. The levels of copper and iron in red beet storage roots ranged from 0.56 $mg \cdot kg^{-1}$ of fresh weight to 1.85 $mg \cdot kg^{-1}$ of fresh weight, and from 5.87 $mg \cdot kg^{-1}$ of fresh weight to 29.37 $mg \cdot kg^{-1}$ of fresh weight respectively. The highest concentrations of copper and iron were recorded in cv. Chrobry fertilized with 0.2% Ecosol U and in cv. Vytėnų Bordo in the control treatment respectively.

Table 2
Concentrations of macro- and micronutrients in red beet storage roots (means of 2001-2003)

Concentrations Ecosol U	Cultivar	N	P	K	Mg	Ca	Na	Cu	Fe
		% f.m.						mg · kg^{-1} f.m.	
Control	Ainiai	0.17	0.03	0.25	0.03	0.02	0.04	0.84	25.56
	Chrobry	0.23	0.05	0.51	0.03	0.03	0.03	1.07	10.80
	Kamuoliai 2	0.25	0.05	0.45	0.03	0.03	0.12	1.13	14.47
	Nevėzis	0.31	0.06	0.46	0.04	0.03	0.04	1.84	17.02
	Rywal	0.22	0.04	0.42	0.03	0.03	0.11	0.85	14.15
	Vytėnų Bordo	0.25	0.05	0.47	0.02	0.04	0.06	0.97	16.62
	Wodan F_1	0.25	0.05	0.49	0.03	0.02	0.09	0.85	29.37
Average		0.24	0.05	0.44	0.03	0.03	0.07	1.08	18.28
Ecosol U 0.1%	Ainiai	0.22	0.04	0.40	0.03	0.02	0.05	0.91	5.87
	Chrobry	0.21	0.05	0.43	0.03	0.03	0.04	1.05	9.75
	Kamuoliai 2	0.23	0.05	0.42	0.03	0.03	0.04	0.96	8.62
	Nevėzis	0.23	0.02	0.47	0.03	0.02	0.06	0.77	6.96
	Rywal	0.22	0.04	0.43	0.03	0.04	0.06	1.12	9.10
	Vytėnų Bordo	0,24	0.05	0.38	0.03	0.03	0.06	0.82	16.78
	Wodan F_1	0.23	0.03	0.36	0.02	0.02	0.02	0.56	6.65
Average		0.23	0.04	0.41	0.03	0.03	0.05	0.88	9.11
Ecosol U 0.2%	Ainiai	0.31	0.06	0.57	0.04	0.04	0.10	1.52	18.30
	Chrobry	0.39	0.06	0.63	0.04	0.04	0.05	1.85	11.54
	Kamuoliai 2	0.29	0.06	0.50	0.04	0.03	0.05	1.55	13.96
	Nevėzis	0.24	0.06	0.45	0.03	0.03	0.05	1.29	11.14
	Rywal	0.31	0.06	0.57	0.04	0.07	0.06	1.25	11.99
	Vytėnų Bordo	0.30	0.06	0.54	0.03	0.04	0.07	1.85	17.65
	Wodan F_1	0.20	0.05	0.44	0.02	0.02	0.05	0.74	7.72
Average		0.29	0.06	0.53	0.04	0.04	0.06	1.44	13.19
Average	Ainiai	0.23	0.04	0.41	0.03	0.03	0.06	1.09	16.57
	Chrobry	0.28	0.05	0.53	0.03	0.03	0.04	1.32	10.70
	Kamuoliai 2	0.26	0.05	0.46	0.03	0.03	0.07	1.21	12.35
	Nevėzis	0.26	0.05	0.46	0.03	0.03	0.05	1.30	11.71
	Rywal	0.25	0.05	0.47	0.03	0.05	0.08	1.07	11.75
	Vytėnų Bordo	0.26	0.05	0.46	0.03	0.04	0.06	1.21	17.02
	Wodan F_1	0.23	0.04	0.43	0.02	0.02	0.06	0.72	14.58
LSD $\alpha_{=0.05}$									
Concentrations Ecosol U		0.03	ns.	0.04	ns.	ns.	0.02	0.19	2.93
Cultivar		ns.	ns.	0.07	ns.	0.02	ns.	0.33	ns.
Interaction		0.06	ns.	0.05	ns.	ns.	0.03	0.14	1.79

The nutritive value of vegetables is also dependent on ratios between particular nutrients. The concentration of Ecosol U had no statistically significant effect on the ratios between the following elements: K:Mg, K:(Mg+Ca), Ca:P and Ca:Mg (Table 3). However, these ratios were found to be related to red beet cultivar and the cultivar x fertilizer interaction. The K:Mg and K:(Mg+Ca) ratios were significantly widened in all cultivars (Figure 1). The Ca:P and Ca:Mg ratios (Figure 2) displayed a different tendency.

The effect of cultivar on the Ca:P ratio was statistically non-significant. The highest and the lowest Ca:Mg ratio, confirmed by a statistical analysis, was noted in the edible parts of cv. Rywal (1.51) and cv. Ainiai (0.81). The interaction between red beet cultivar and fertilizer concentration had a significant effect on the K:(Mg+Ca), Ca:P and Ca:Mg ratios (Figures 3, 4). The widest K:(Mg+Ca) ratio was achieved in cv. Wodan F_1 fertilized with 0.2% Ecosol U. The fertilizer applied at this concentration positively affected also the Ca:P and Ca:Mg ratios in cv. Rywal. The lowest K: (Mg+Ca) ratio was observed in cv. Ainiai grown in the control treatment. When fertilized with 0.1% Ecosol U, this cultivar was also characterized by the lowest Ca:P and Ca:Mg ratios.

Table 3

Effect of Ecosol U concentration on nutrient ratios in red beet storage roots

Concentrations Ecosol U	K:Mg	K:(Mg+Ca)	Ca:P	Ca:Mg
Control	15.72	7.62	0.64	1.06
Ecosol U 0.1%	15.33	7.58	0.64	1.02
Ecosol U 0.2%	15.77	7.29	0.69	1.16
LSD $\alpha_{=0.05}$ concentrations Ecosol U	ns.	ns.	ns.	ns.

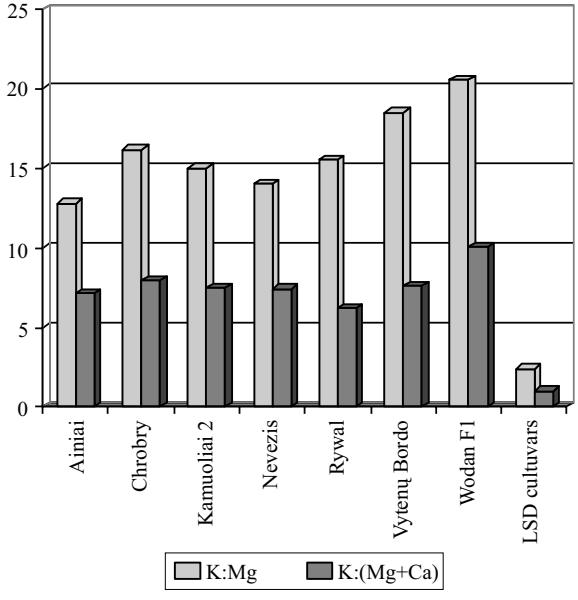

Fig. 1. K:Mg and K:(Mg+Ca) ratios in red beet storage roots as dependent on cultivar (means of 2001-2003)

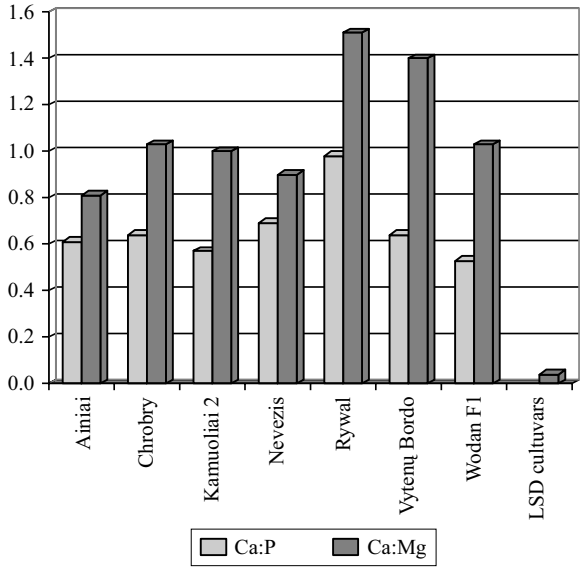

Fig. 2. Ca:P and Ca:Mg ratios in red beet storage roots as dependent on cultivar (means of 2001-2003)

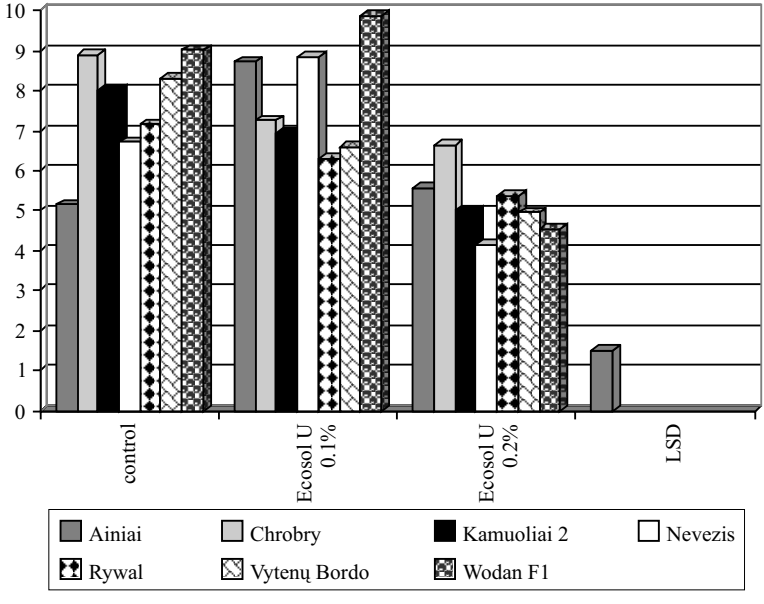

Fig. 3. K:(Mg+Ca) ratio in red beet storage roots as dependent on the concentration of Ecosol U and cultivar (means of 2001-2003)

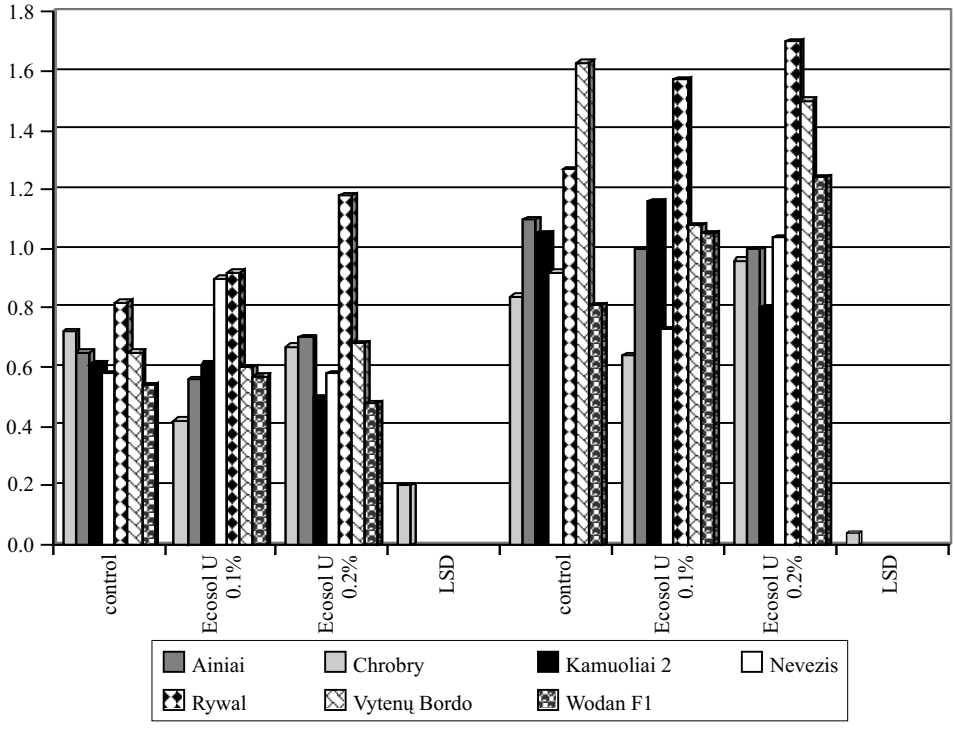

Fig. 4. Ca:P and Ca:Mg ratios in red beet storage roots as dependent on the concentration of Ecosol U and cultivar (means of 2001-2003)

Discussion

Foliar application of fertilizers is an important component of cultivation technology in intensive horticultural production. This form of fertilization permits the supply of essential minerals, both in case of their deficiencies in the soil and problems with nutrient uptake by plants. This concerns primarily such macronutrients as nitrogen and magnesium, and such micronutrients as boron, copper, zinc, manganese, molybdenum and iron (MICHAŁOJĆ, SZEWCZUK 2003). Fertilization that fully meets the requirements of field crops contributes to a higher yield and a better quality of raw material (SUCHORSKA-ORŁOWSKA, JURGIEL-MAŁECKA 2001, WIERZBICKA, MAJKOWSKA 2003).

Red beet yield attained in this study was comparable to that achieved by FRANCZUK (2006). The yield of storage roots as dependent on fertilization was different than reported previously. CHWIL, SZEWCZUK (2003) demonstrated that foliar application of Rolvit B at increasing rates resulted in the increment of sugar beet yield. In the experiment conducted by ROŻEK et al. (2000) variable

rates of soil fertilizers combined with foliar application of Microvit 2 had a similar affect on the total yield of carrots as in our study.

The concentrations of macronutrients (nitrogen, phosphorus, potassium, magnesium, calcium and sodium) and micronutrients (copper, iron) in red beet storage roots were comparable to those obtained by KUNACHOWICZ et al. (2001). However, the levels of these elements were significantly affected by the experimental factors. A similar relationship was also observed by KRĘŻEL, and KOŁOTA (2000), and JADCZAK, and GRZESZCZUK (2004). According to these authors, cultivars of vegetables showed a strong response to the form and rate of fertilizers.

KOTOWSKA and WYBIERALSKI (1999) demonstrated that the quality of the edible parts of crops depends not only on the concentrations of macro- and micronutrients, but also on the ratios between them. The K:Mg, Ca:Mg and K:(Mg+Ca) ratios are of primary importance. It was found in the present study that red beet storage roots were characterized by wide K:Mg and K:(Mg+Ca) ratios, and narrow Ca:P and Ca:Mg ratios. According to RADKOWSKI et al. (2005), these ratios should be as follows: K:Mg – 6:1, K:(Mg+Ca) – 1.6-2.2, Ca:P – 2:1 and Ca:Mg – 3:1.

Conclusions

1. Foliar application of a 0.2% solution of Ecosol U caused a significant decrease in red beet yield, but increased marketable yield expressed as a percentage of total yield, as compared with the other treatments.

2. The Dutch cultivar Wodan F_1 provided the highest yield of all *Beta vulgaris* L. ssp. *esculenta* cultivars tested in the study.

3. Foliar application of Ecosol U, especially at a concentration of 0.2%, enabled to increase the levels of nitrogen, potassium, sodium, copper and iron in red beet storage roots.

4. The red beet cultivars tested in the study differed significantly in the content of potassium, calcium and copper in edible parts.

5. Red beet storage roots were characterized by wide K:Mg and K:(Mg+Ca) ratios, and narrow Ca:P and Ca:Mg ratios.

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CORRELATION BETWEEN MALTING QUALITY OF BARLEY CULTIVARS AND DEVELOPMENT OF FLOUR MITE (*ACARUS SIRO* L.)

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Key words: *Acarus siro*, malting barley, malting quality.

Abstract

Among the factors which disqualify barley grain from being used for malting purposes is damage caused by grain and flour insect pests, as well as presence of live or dead grain and flour pests, including various species of mites. The aim of the study has been to find out whether grain of malting barley cultivars is a suitable habitat for the development of flour mite. The authors also tried to establish the correlation between the development of flour mite and the malting quality of several barley cultivars. The experiment has demonstrated that crushed grain of malting barley cultivars is a suitable habitat for the development of flour mite. By knowing the correlation coefficient for the malting quality of a given barley cultivar, it is possible to specify the suitability of its grain for the development of flour mite, although the specification thus obtained must be viewed in the aspect of grain fragmentation. The content of protein in finely ground grain (F1) did not affect the development of flour mite. In a combination with the grain ground to fraction F2, higher contribution of protein resulted in depressed abundance of the flour mite's offspring population. It seems possible that not all protein substances of barley grain are available to flour mite.

KORELACJA MIĘDZY WARTOŚCIĄ BROWARNĄ ODMIAN JĘCZMIENIA A ROZWOJEM ROZKRUSZKA MĄCZNEGO (*ACARUS SIRO* L.)

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Słowa kluczowe: *Acarus siro*, jęczmień browarny, wartość browarna.

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Abstrakt

Jedną z głównych cech dyskwalifikujących ziarno jęczmienia na cele browarne są uszkodzenia przez szkodniki zbożowe i mączne oraz obecność żywych lub martwych szkodników zbożowo-mącznych, w tym również różnych gatunków roztoczy. Celem badań było ustalenie, czy ziarno browarnych odmian jęczmienia jest siedliskiem, w którym może rozwijać się rozkruszek mączny. Podjęto również próbę ustalenia korelacji między rozwojem badanego gatunku roztocza a wartością browarną odmian jęczmienia. Stwierdzono, że rozdrobnione ziarno browarnych odmian jęczmienia jest dobrym siedliskiem do rozwoju *A. siro*. Dzięki znajomości współczynnika wartości browarnej można opisać jakość siedliska dla rozwoju rozkruszka mącznego, ale tylko w kontekście czynnika, jakim jest stopień rozdrobnienia ziarna. Zawartość białka w ziarnie silnie rozdrobnionym (F1) nie wpłynęła na rozwój rozkruszka mącznego. W ziarnie rozdrobnionym do frakcji F2 stwierdzono, że wyższa zawartość tego składnika powodowała spadek liczebności populacji potomnej badanego gatunku roztocza. Prawdopodobnie nie wszystkie substancje białkowe ziarna są przyswajalne przez *A. siro*.

Introduction

Barley is one of the first cereal crops used for human consumption. At present barley is the fourth cereal, after wheat, maize and rice, in terms of the total cultivation area and yields. In Poland, barley makes up 12% of the cereals sown, which means that average annual barley yields reach 3.3 million tons. Barley grain is used for production of groats, bran and extract flour. But most of the barley grain produced (70-80% of the yield) is used for farm animals' consumption. However, barley is still irreplaceable as raw produce to make malt, which is used in brewing, sugar, fermentation, pharmaceutical and other industries (PECIO 2002). Barley grain used for brewing must meet certain technological requirements, which in terms of their values and range are specified by a synthetic index known as malting quality (MQI). This index is expressed on a 9-point scale. When assessing grain quality for brewing purposes malters consider for example grain uniformity (minimum value of 85%), impurities (up to 6%, including up to 1% of kernel-foreign grain), moisture content (up to 16%), protein content (maximum 85%), germination energy (minimum 92%) and cultivar uniformity (minimum 85%). One of the parameters which disqualify barley grain from further brewing is damage caused by grain and four pests or presence of live or dead grain and flour pests, including various species of mites. Flour mite is one of the most noxious mites foraging on stored cereal grains and grain products (NIETUPSKI, CIEPIELEWSKA 2005). There are may difficulties with reducing its number in storehouses (IGNATOWICZ 1996). Its presence is favoured by high moisture level of grain, infestation by other storage pests and mechanical damage which can occur during grain harvest, transport and storage.

The aim of this study has been to find whether the grain of malting barley cultivars is a suitable habitat for development of flour mite. Another objective

has been to establish if there is some correlation between the development of flour mite and the malting quality of several barley cultivars.

Material and Methods

Material

The experiment focused on the development of flour mite on grain of several malting barely cultivars. The material for the tests comprised seeds of eleven barley cultivars: Granal, Sezam, Binal, Barke, Blask, Scarlett, Annabell, Gwarek, Brenda, Stratus and Refren. The grain was harvested in 2003 and 2004 at the Experimental Station in Wrocikowo near Olsztyn. The above barely cultivars were selected for the tests because they could be used in brewing industry, which was demonstrated by their synthetic malting quality index (MQI) described by Research Centre For Cultivar Testing in Slupia Wielka (Table 1).

Table 1
Some factors defining the malting suitability of the barley cultivars and the parameters of flour mite's populations

Cultivar	Malting quality*	Protein content (% d.m.)*	Grain resistance index (GRI)		Number of flour mite's offsprings		
			F1**	F2***	F1	F2	mean
Granal	8.30	11.6	14.38	12.42	74.70	31.80	53.25^{a****}
Sezam	8.10	11.9	18.08	14.80	227.00	84.90	155.95^{d}
Binal	8.00	11.8	15.81	12.14	114.90	38.15	67.15^{ab}
Barke	8.00	11.7	17.01	13.58	164.40	58.80	111.6^{c}
Brenda	7.90	11.5	15.49	13.64	104.40	59.80	82.1^{abc}
Blask	7.70	11.3	15.93	15.35	118.90	100.10	109.5^{c}
Scarlett	7.45	11.3	17.48	9.63	189.50	18.00	103.75^{bc}
Annabell	7.40	10.9	16.22	13.49	129.70	57.30	93.5^{bc}
Gwarek	7.15	11.7	18.69	13.75	272.40	61.90	167.15^{d}
Refren	7.15	11.7	16.33	13.06	134.10	47.30	90.7^{abc}
Stratus	6.55	11.1	18.47	13.15	255.00	51.60	153.3^{d}
Control	–	15.0	17.10	10.72	168.60	24.90	96.75^{bc}
Mean					162.8^{b}	51.32^{a}	

* according to COBORU 2004, ** grain fraction (F < 1 mm), *** grain fraction (2,2 mm < F < 3,15 mm), **** means marked with the same letter do not differ statistically (Student's test)

The development of flour mite was observed on barley grain ground to two fractions. The fractions (F) used for the experiment were sieved through mesh of:

F* < 1.0 mm (F1)
2.2. mm < F <3.15 mm (F2)

F* – size of the barley grain fraction analysed

The control combination consisted of cv Mewa wheat grain. The grain for the tests was ground at the Chair of Food Plant Processing and Chemistry of the University of Warmia and Mazury in Olsztyn.

Methods

Acarological observations

Whole and ground barley grains were placed in glass breeding chambers size 3 x 2 cm and 6 mm in thickness, with a drilled conical hole. The entry hole diameter was 8 mm, and the exit hole was 3 mm in diameter. The cone was filled with a sample of barley grain mass examined, on which 16 adult individuals of flour mite were placed. The mites originated from maternal populations bred at the Chair of Phytopathology and Entomology, the UWM Olsztyn. The experiment was established in 10 replications and was conducted under controlled conditions, in a Protherm HS – 2/M climatic chamber, at constant temperature of 20°C and relative air humidity of 75%.

In order to assess whether the barley cultivars tested were suitable for the foraging by flour mite, the grain resistance index (GRI):

$$GRI = (\log_n F1 \cdot 100\%) / D,$$

F1 – number of offspring, D – time of the offspring's development (days)

The results were subjected to analysis of variance. The estimation of the differences between the means was performed on real data, using *t*-Student's test. The correlation coefficient was calculated between the abundance of the offspring generation of flour mite, and the malting value index and protein content in barley grain.

Results and Discussion

The observations revealed that the grain of the eleven malting barley cultivars was a suitable habitat for the development of flour mite. This was

evidenced by the high value of grain resistance index (GRI) derived for the barley cultivars, depending on a grain fraction (Table 1). Analysis of variance showed that the experimental factors analysed as well as their mutual interactions had highly significant influence ($p = 0.05$) on the development of flour mite populations.

Development of flour mite on the fractions of barley grain

Barley kernels can suffer mechanical damage during grain harvest, transport and storage. Uniform mass of grain can contain kernels with split hulls or broken kernels. Such grain is likely to be settled by secondary storage pest insects. In the present experiment we examined the development of flour mite on barley grain with damaged hull or broken kernels (fraction F2; 2.2 mm < 2.2 f < 3.15 mm) and on dust obtained as overtails from a sieve with a mesh size less than 1 mm (F1). The most favourable conditions for the development of flour mite occurred on flour and finely ground cereal grain (BOCZEK, DAVIES 1998, THIND, CLARKE 2001). Similar results were obtained in our investigations. Flour mite developed more numerous populations on finely ground barley grain (F1) – Table 1. The analysis of the population abundance on finely ground barley kernels suggests that this type of habitat is as attractive for flour mite as wheat grain of a comparable size fraction. The grain of four barley cultivars (Scarllett, Sezam, Stratus and Gwarek) proved to be even more attractive for flour mite than the control combination. A change in the conditions under which flour mite developed, caused by increasing the granulation of grain (F2), resulted in highly significant changes in the numbers of the flour mite's offspring (Table 1). Flour mite developing on F2 grain produced less abundant offspring generations than on F1 grain. Although the abundance of offspring populations decreased significantly, the flour mite continued to develop, which means that any small damage appearing during the grain transport or storage could be a chance for flour mite to grow. This risk is even higher in the case barley grain. Out of the eleven barley cultivars tested, ten proved to create more suitable conditions for the development of flour mite than the control wheat grain.

Malting quality of the barley cultivars versus the development of flour mite

Brewing industry makes use of barley grain which maintains certain parameters, specified by the malting quality index (MQI), which is expressed

on a 9-point scale (COBORU 2004). Among the 11 barley cultivars examined, 7 qualify as yielding grain suitable for brewing purposes, that is their values of MQI were higher than 7.4 (Table 1). The present experiment involved a comparison of the development of flour mite on barley grain characterised by different malting quality. It was found out that the malting quality index and its value could be helpful in assessing the suitability of grain as a habitat for the development of flour mite, but only in conjunction with the degree of grain fragmentation. The analysis of correlation and regression between the experimental factors revealed the relationship described by the correlation coefficient at r = -0.45. Finely ground grain (F1) created the best conditions for the development of flour mite on the barley grain representing the lowest malting quality value (cv. Gwarek, Stratus) – Figure 1a. The grain of such cultivars as Granal, Binal and Barke, which are willingly used for production of malt, proved to be the least favourable habitat for the development of flour mite. A reverse correlation, however, was observed when analysing the relationships between the abundance of populations produced by flour mite and the malting quality index of barley grain in the combinations with flour mite foraging on coarser grain fraction (F2). The correlation coefficient there attained a positive value (r = 0.35), which implied a tendency for higher malting quality grain to create better conditions for flour mite (Figure 1b).

One of the essential parameters affecting the malting quality of barley grain is the protein content (GARCIA DEL MORAL et al. 1998, PECIO 2002, BERNE 2005). Ground and broken kernels are more readily settled by flour mite, as it is easier for the pest to access nutrients. The results of our tests show that the correlation between the protein content in grain and the abundance of the flour mite's populations depended on the degree of grain fragmentation. Finely ground barley grain (F1) created very good conditions for flour mite. Easy access to nutrients that the foraging mites had found eliminated any larger differences in the number of offspring individuals from each combination, even though the grain differed in the protein content (Figure 2a). Higher granulation of grain (F2) as a habitat for the development of flour mite revealed the effect of protein content on the development of mites. Unexpectedly, the analysis of correlation and regression showed that for the combination with higher protein content in barley grain the abundance of the flour mite's offspring population was lower (Figure 2b). This perhaps could be explained through analysis of chemical composition of barley grain proteins. It is possible that not all protein substances in cereal grain are absorbable by phytophages (WARCHALEWSKI et al. 2002).

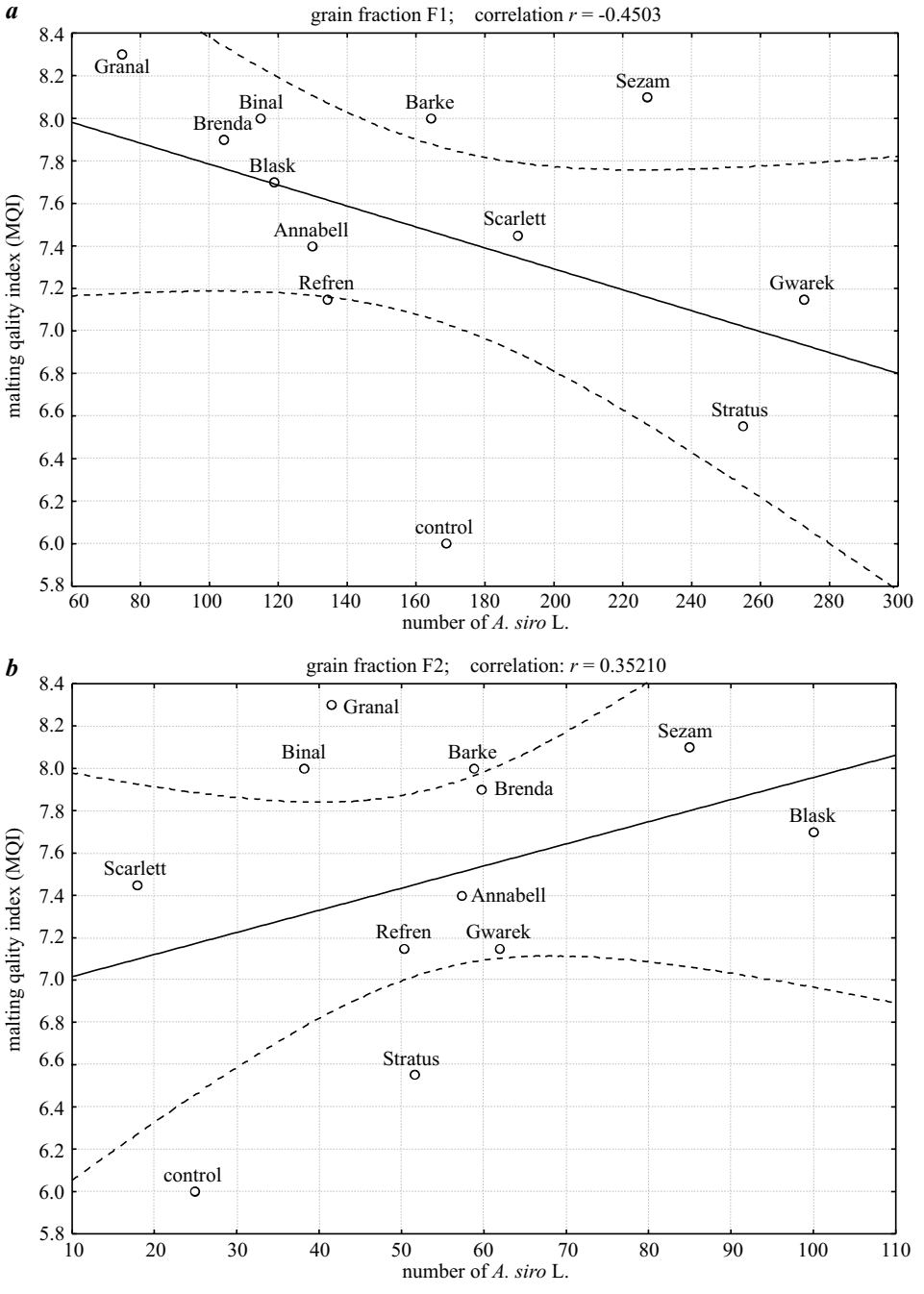

Fig. 1. Correlation between the malting quality index (MQI) for the grain of the eleven barley cultivars examined and the abundance of flour mite offsprings

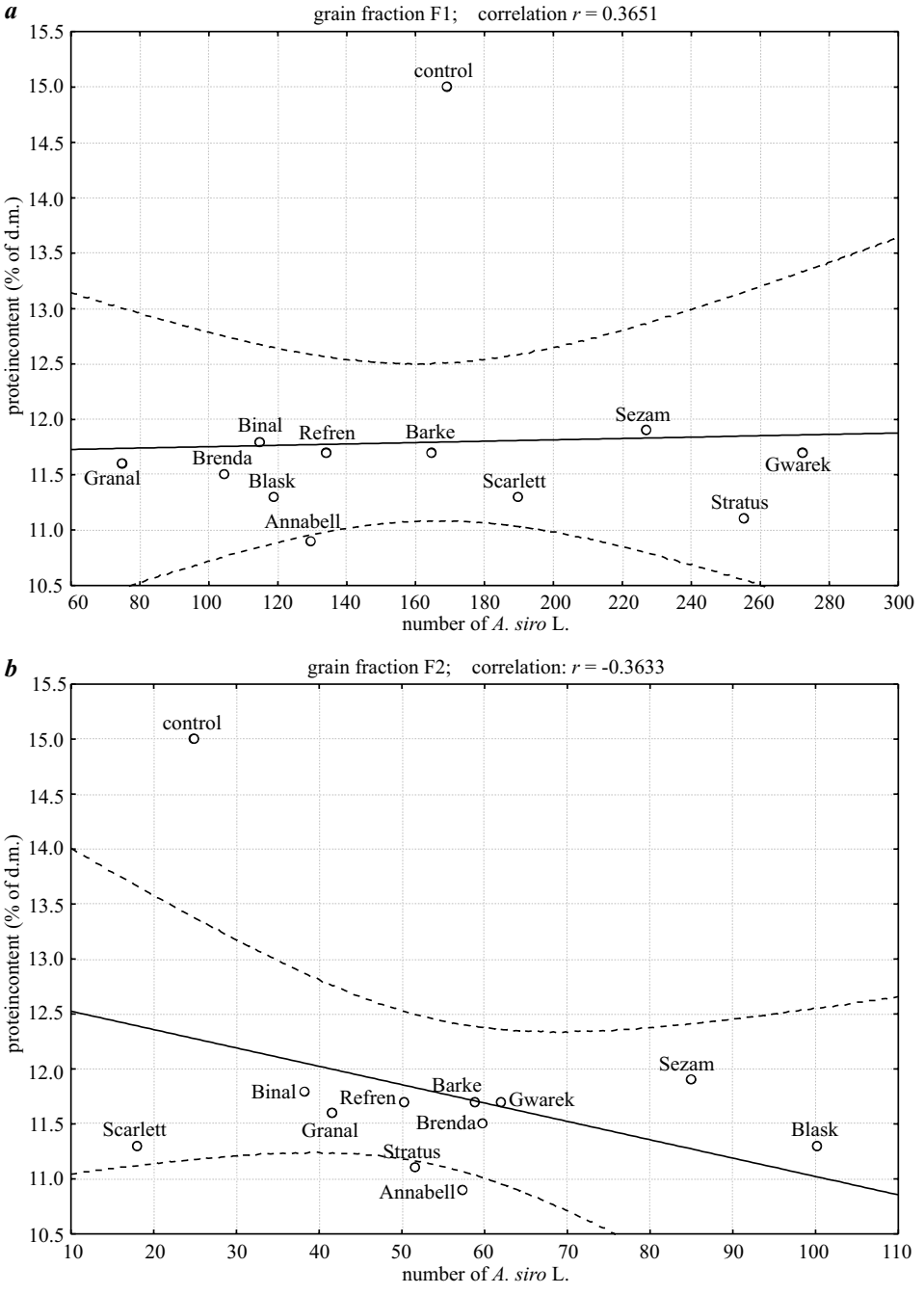

Fig. 2. Correlation between the protein content (%) in the grain of the barley cultivars and abundance of flour mite offsprings

Conclusions

1. Finely ground (F1) grain of malting barley cultivars is a suitable substract for development of flour mite as wheat grain.

2. The value of malting quality index could be useful in describing barley grain as a potential habitat for flour mite, provided that a degree of grain fragmentation is included in the analysis.

3. The development of flour mite is not affecting by higher protein content in finely ground grain (F1). In a combination with the grain ground to fraction F2, it was noticed that higher protein content resulted in lower abundance of the flour mite's offspring population. It is possible that not all protein substances contained in grain are available to flour mite.

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EFFECT OF WATER STRESS ON PHYSIOLOGICAL PROCESSES, LEAF GREENNESS (SPAD INDEX) AND DRY MATTER YIELD OF *LOLIUM PERENNE* AND *DACTYLIS GLOMERATA*

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K e y w o r d s: dry matter yield, leaf greenness (SPAD index), orchard grass, perennial ryegrass, photosynthesis, transpiration, water stress, WUE.

A b s t r a c t

A greenhouse pot experiment was performed at two soil moisture levels: 80% field water capacity (optimum moisture content) and 40% field water capacity (water stress) to study some varieties of perennial ryegrass (Maja, Argona) and orchard grass (Dala, Areda). Rates of photosynthesis and transpiration were measured during vegetation with a Li-Cor 6400 gas analyzer, and chlorophyll concentration was determined with a SPAD-502 chlorophyll meter. Photosynthetic water use efficiency (WUE) was also calculated.

It was found that a decrease in soil moisture from 80% to 40% field water capacity reduced the rate of photosynthesis by on average 42%. The strongest response was recorded in var. Maja, where the rate of photosynthesis decreased by 46%. Var. Dala evaporated the most water, and var. Areda – the least. Higher values of WUE and SPAD were recorded in the tested grass varieties under water deficiency conditions. Perennial ryegrass varieties were characterized by a higher chlorophyll content of leaves. All varieties compared in the study responded by yield decrease to a lower soil moisture. The strongest response was noted in var. Argona, which was also characterized by the lowest water use efficiency.

WPŁYW STRESU WODNEGO NA PRZEBIEG PROCESÓW FIZJOLOGICZNYCH, INDEKS ZIELONOŚCI LIŚCI (SPAD) ORAZ PLON SUCHEJ MASY *LOLIUM PERENNE* I *DACTYLIS GLOMERATA*

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S ł o w a k l u c z o w e: fotosynteza, transpiracja, indeks zieloności liści (SPAD), *Lolium perenne, Dactylis glomerata*, plon suchej masy, stres wodny, WUE.

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Abstrakt

W szklarniowym doświadczeniu wazonowym, z uwzględnieniem dwóch poziomów wilgotności gleby: 80% ppw (wilgotność optymalna) i 40% ppw (stres wodny), badano *Lolium perenne* (odmiany Maja i Argona) oraz *Dactylis glomerata* (odmiany Dala i Areda). W okresie wegetacji mierzono intensywność fotosyntezy i transpiracji za pomocą urządzenia Li-Cor 6400 do pomiaru parametrów wymiany gazowej oraz zieloność liści za pomocą chlorofilometru SPAD-502. Wyliczono również fotosyntetyczny współczynnik wykorzystania wody (WUE).

Wykazano, że obniżenie wilgotności gleby z 80 do 40% ppw spowodowało zmniejszenie intensywności fotosyntezy średnio o 42%, przy czym największą reakcję wykazywała życica trwała odmiany Maja, u której nastąpiło zmniejszenie fotosyntezy o 46%. Najwięcej wody wyparowywała kupkówka pospolita odmiany Dala, najmniej Areda. U badanych gatunków traw wykazano większe wartości WUE i SPAD w warunkach niedoboru wody. Większy poziom chlorofilu w liściach stwierdzono u życicy trwałej. Analizowane trawy reagowały ograniczeniem plonu suchej masy na niższą wilgotność gleby, najbardziej życica trwała odmiany Argona, u której jednocześnie wykazano najmniejszy współczynnik wykorzystania wody.

Introduction

Water availability to plants depends, to a great extent, on the type of soil in which they are grown. Peat-muck soils are characterized by considerable fluctuations in groundwater levels. Rainfall deficiency causes peat decomposition and overdrying, followed by reduced capillary elevation and moisture capacity of the soil. Intensive evaporation, stimulated by strong soil heating caused by solar radiation absorption, contributes to ground over-desiccation (GAWLIK et al. 1997). Under such conditions plants suffer from water deficit. A rapid decrease in water supply makes plants close their stomata. This enables to rapidly reduce water losses during transpiration, but this process is accompanied by increased diffusion resistance for CO_2, leading to photosynthesis inhibition. This allows the plant to survive drought, but leads to yield decrease.

The aim of the present study was to determine the effects of water stress on the rates of photosynthesis and transpiration, leaf greenness (SPAD index), water relations and dry matter yield in some *Lolium perenne* and *Dactylis glomerata* varieties grown in organic (peat-muck) soil.

Materials and Methods

A pot experiment was performed in a greenhouse of the University of Warmia and Mazury in Olsztyn, at two soil moisture levels: 80% field water capacity (optimum moisture content) and 40% field water capacity (water stress) to study the responses of two *Lolium perenne* varieties: tetraploid Maja and diploid Argona, and two *Dactylis glomerata* varieties: tetraploid Dala and

diploid Areda to water stress. Soil moisture content was differentiated after emergence. In order to maintain the appropriate soil moisture, water losses were made up on a daily basis, to achieve a specified weight of the pot with soil. Kick-Braukmann pots were filled with 8 kg peat-muck soil containing 29.7% organic matter. The available nutrient content of the soil was as follows: 320 mg P, 540 mg K and 300 mg Mg soil, and 5 mg Cu, 28.6 mg Zn, 88.6 mg Mn and 112.6 mg $Fe \cdot kg^{-1}$. Soil reaction in 1 mol $KCl \cdot dm^{-3}$ was 4.9. The experiment was performed in four replications. Two to three seeds were sown at 10 points of each pot. After emergence poorly developed seedlings were removed, leaving eight plants per pot. Nitrogen fertilization, in the amount of 0.5 g per pot, was applied at three rates, before sowing, after the first and second cutting. Nitrogen was applied in the form of a $CO(NH_2)_2$ solution. Phosphorus, potassium and magnesium were applied presowing, in the forms of solutions: KH_2PO_4, K_2SO_4 and $MgSO4 \cdot 7\ H_2O$, respectively, at the following rates: 0.15 g P, 0.6 g K and 0.15 g Mg per pot. In addition, a nutrient solution, in the amount of 20 ml per pot, containing $C_{10}H_{12}FeN_2NaC_8$, $MnCl_2 \cdot 4\ H_2O$, $ZnCl_2$, $CuCl_2 \cdot 2\ H_2O$, H_3BO_3, $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$, was applied presowing. Over the vegetation season, the rates of photosynthesis and transpiration were measured with a LI-COR 6400 portable gas analyzer, and leaf greenness (SPAD index) – with a SPAD 502 optical chlorophyll meter (Minolta). This device measures the difference between light absorption by a leaf at a wavelength of 650 and 940 nm, and the quotient of these values represents indexed leaf greenness correlated with chlorophyll content (BLACKMER and SCHEPERS 1994, GREGORCZYK and RACZYŃSKA 1997, SAMBORSKI and ROZBICKI 2002). The rates of photosynthesis and transpiration, and chlorophyll concentration, were measured on the youngest, fully developed leaf of shoots selected randomly of each pot. The measurements were taken for the first time five weeks after sowing, at the tillering stage. Four measurements were performed for each regrowth, and readings were taken every week. Photosynthetic water use efficiency (WUE) was calculated based on instantaneous values of photosynthesis and transpiration. The plants were defoliated three times over the growing season. Successive defoliations were carried out at six-week intervals. The results presented in the paper are means of particular regrowths. They were analyzed statistically using Statistica software (StatSoft 2005). The significance of difference was verified by the Tukey test at a significance level $p = 0.99$.

Results and Discussion

Measurements of photosynthetic activity revealed significant differences between species and varieties. Photosynthetic rate was higher in orchard grass varieties than in perennial ryegrass varieties. This process was the most intensive in the leaves of var. Dala (Table 1). Water stress considerably reduced photosynthetic activity (on average by 42%). The strongest response was recorded in var. Maja, where photosynthetic rate decreased by 46%, as compared with the control treatments. Reduced photosynthetic activity in grasses resulting from water stress was also reported by McFARLANE et al. (2001), PSZCZÓŁKOWSKA et al. (2003) and WYSZYŃSKI et al. (2002). An analysis of the course of photosynthesis in particular regrowths showed that photosynthetic activity was the highest in the second regrowth. Over this period the rate of photosynthesis ranged between 9.38 and 11.12 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, depending on variety, whereas in the first and third regrowth it varied from 5.42 to 8.36 and from 5.31 to 5.79 respectively.

Table 1

Intensity of photosynthesis (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)

Species and cultivar	Soil moisture (%)	1st regrowth	2nd regrowth	3rd regrowth	Mean
Lolium perenne Maja	40	4.25^{*ab}	7.10^{a}	4.24^{c}	5.20^{a}
	80	8.98^{d}	13.10^{f}	7.09^{d}	9.72^{e}
Lolium perenne Argona	40	3.95^{a}	7.06^{a}	3.97^{b}	4.99^{a}
	80	6.90^{c}	11.70^{d}	7.62^{ef}	8.74^{d}
Dactylis glomerata Dala	40	7.50^{c}	8.36^{b}	3.18^{a}	6.35^{c}
	80	9.22^{d}	13.21^{f}	7.44^{e}	9.96^{e}
Dactylis glomerata Areda	40	4.92^{b}	9.70^{c}	3.01^{a}	5.87^{b}
	80	9.73^{d}	12.53^{e}	7.68^{f}	9.98^{e}
Mean for cultivar					
Maja		6.61^{b}	10.10^{b}	5.67^{b}	7.46^{b}
Argona		5.42^{a}	9.38^{a}	5.79^{b}	6.87^{a}
Dala		8.36^{d}	10.79^{c}	5.31^{a}	8.15^{d}
Areda		7.32^{c}	11.12^{d}	5.34^{a}	7.93^{c}
Mean for soil moisture					
	40	5.15^{a}	8.05^{a}	3.60^{a}	5.60^{a}
	80	8.71^{b}	12.64^{b}	7.46^{b}	9.60^{b}

* homogeneous groups

The decrease in photosynthetic rate, caused by water deficit in the soil, was accompanied by reduced transpiration in the experimental varieties, since both processes are interrelated, as confirmed by high correlation coefficients. On

average, drought stress reduced transpiration rate by 66% (Table 2). According to JONES et al. (1980), a decrease in the transpiration rate of plants exposed to moisture stress is related to changes in leaf structure. Under water deficit conditions leaves are shorter, their surfaces are smaller, their tissue is more compact, and stomata are shorter, but densely arranged. Such leaf morphology limits transpiration, which makes plants more resistant to drought. Among the tested varieties, Maja showed the lowest transpiration rate under moisture deficiency conditions. The heaviest water losses were recorded in the second regrowth. Var. Dala evaporated the most water, and var. Areda – the least. In all tested varieties the values of water use efficiency (WUE) were found to be statistically higher under water stress conditions, and ranged from 10.47 to 14.99 μmol $CO_2 \cdot mmol^{-1}$ H_2O, while under optimum soil moisture conditions these values varied between 6.83 and 7.69 μmol $CO_2 \cdot mmol^{-1}$ H_2O (Table 3). These results correspond to those obtained by PIETKIEWICZ et al. (2005), who demonstrated that in sugar beets photosynthetic WUE depends on water availability to plants. It is higher in the case of water deficiency and lower when water availability increases. In this experiment var. Maja was characterized by high water use efficiency (11.16), while the lowest WUE was observed in var. Argona (8.65). Irrespective of soil moisture content, water use efficiency increased in successive regrowths.

The experimental factors investigated in the study had a significant effect on the chlorophyll content of leaves. Perennial ryegrass varieties were gen-

Table 2

Intensity of transpiration (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)

Species and cultivar	Soil moisture (%)	1st regrowth	2nd regrowth	3rd regrowth	Mean
Lolium perenne Maja	40	0.59^{b}	0.63^{a}	0.16^{a}	0.46^{a}
	80	2.13^{g}	1.79^{d}	0.68^{ef}	1.53^{e}
Lolium perenne Argona	40	0.45^{a}	0.89^{c}	0.27^{c}	0.54^{b}
	80	2.01^{f}	2.36^{f}	0.63^{d}	1.67^{g}
Dactylis glomerata Dala	40	1.00^{c}	0.72^{b}	0.19^{b}	0.64^{c}
	80	1.91^{e}	2.32^{f}	0.66^{e}	1.63^{f}
Dactylis glomerata Areda	40	0.57^{b}	0.65^{a}	0.18^{ab}	0.47^{a}
	80	1.78^{d}	1.93^{e}	0.69^{f}	1.47^{d}
Mean for cultivar					
Maja		1.36^{c}	1.21^{a}	0.42^{a}	1.00^{b}
Argona		1.23^{b}	1.62^{d}	0.45^{b}	1.10^{c}
Dala		1.45^{d}	1.52^{c}	0.43^{a}	1.13^{d}
Areda		1.18^{a}	1.29^{b}	0.43^{a}	0.97^{a}
Mean for soil moisture					
	40	0.65^{a}	0.72^{a}	0.20^{a}	0.53^{a}
	80	1.96^{b}	2.10^{b}	0.67^{b}	1.57^{b}

Table 3

Water use efficiency (μmol CO_2 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)

Species and cultivar	Soil moisture (%)	1st regrowth	2nd regrowth	3rd regrowth	Mean
Lolium perenne Maja	40	7.20^{d}	11.27^{e}	26.50^{e}	14.99^{f}
	80	4.22^{a}	7.32^{d}	10.43^{a}	7.32^{b}
Lolium perenne Argona	40	8.78^{e}	7.93^{d}	14.70^{c}	10.47^{c}
	80	3.43^{a}	4.96^{a}	12.10^{b}	6.83^{a}
Dactylis glomerata Dala	40	7.50^{d}	11.61^{e}	16.73^{d}	11.95^{d}
	80	4.83^{b}	5.69^{b}	11.27^{a}	7.26^{b}
Dactylis glomerata Areda	40	8.63^{e}	14.92^{f}	16.72^{d}	13.42^{e}
	80	5.46^{c}	6.49^{c}	11.13^{a}	7.69^{b}
Mean for cultivar					
Maja		5.71^{a}	9.30^{c}	18.47^{b}	11.16^{d}
Argona		6.11^{a}	6.45^{a}	13.40^{a}	8.65^{a}
Dala		6.17^{a}	8.65^{b}	14.00^{a}	9.61^{b}
Areda		7.04^{b}	10.71^{d}	13.93^{a}	10.56^{c}
Mean for soil moisture					
	40	8.03^{b}	11.43^{b}	18.66^{b}	12.71^{b}
	80	4.49^{a}	6.12^{a}	11.23^{a}	7.28^{a}

Table 4

Leaf greenness index (SPAD)

Species and cultivar	Soil moisture (%)	1st regrowth	2nd regrowth	3rd regrowth	Mean
Lolium perenne Maja	40	39.88^{d}	46.56^{e}	45.18^{e}	43.87^{f}
	80	41.53^{d}	42.57^{c}	41.75^{b}	41.95^{e}
Lolium perenne Argona	40	37.66^{c}	40.02^{b}	43.93^{d}	40.54^{cd}
	80	37.81^{c}	37.28^{a}	38.50^{a}	37.87^{b}
Dactylis glomerata Dala	40	35.58^{b}	43.73^{d}	42.83^{c}	40.72^{d}
	80	34.35^{ab}	36.86^{a}	37.89^{a}	36.37^{a}
Dactylis glomerata Areda	40	32.79^{a}	43.16^{cd}	43.56^{cd}	39.84^{c}
	80	34.41^{ab}	37.66^{a}	37.74^{a}	36.60^{a}
Mean for cultivar					
Maja		40.70^{d}	44.57^{c}	43.46^{c}	42.91^{c}
Argona		37.75^{c}	38.65^{a}	41.22^{b}	39.20^{b}
Dala		34.97^{b}	40.30^{b}	40.36^{a}	38.54^{a}
Areda		33.60^{a}	40.41^{b}	40.65^{a}	38.22^{a}
Mean for soil moisture					
	40	36.48^{a}	43.34^{b}	43.87^{b}	41.24^{b}
	80	37.03^{a}	38.59^{a}	38.97^{a}	38.20^{a}

erally characterized by higher values of leaf greenness than orchard grass varieties, and the highest values of the SPAD index were recorded in var. Maja (Table 4). All tested varieties showed higher values of the SPAD index under conditions of soil moisture deficiency. Chlorophyll concentration in leaves

increased on average by 7%, compared with the control treatments. The highest increase was recorded in var. Dala (over 10%). However, the increase in SPAD index values was not accompanied by higher photosynthesis rates, which was reflected by negative values of correlation coefficients (Table 6). In var. Maja and Argona the coefficients of correlation between these traits were -0.9377** and -0.9494** respectively, while in var. Dala and Areda -0.9218** and -0.9705** respectively. Photosynthetic pigments are usually present in excess in plant tissues, and only part of them participate in photosynthesis. Thus, the chlorophyll content of leaves does not affect photosynthetic rate (STREBEYKO 1972). In the present study the highest SPAD index values were found in the third regrowth. Reference data indicate that chlorophyll concentration is a genetic, varietal character. Some varieties accumulate more chlorophyll in spring, others in autumn (GÁBORČIK 2003, MACHUL 2003, SAMBORSKI and ROZBICKI 2002).

Yield is usually strongly correlated with the activity in physiological processes. However, the physiology of yield formation is very complex and related to numerous factors, such as the rates of photosynthesis and transpiration, and activity of enzymes (RAKOWSKI 2003, ZBIEĆ et al. 1989). The dry matter yield of all tested varieties examined was at a comparable level, except for var. Areda, whose dry matter yield was statistically significantly lower. Water stress considerably affected dry matter yield. The mean decrease in dry matter yield was 35%, in comparison with control treatments (Table 5). The

Table 5

Dry matter yield ($g \cdot pot^{-1}$)

Species and cultivar	Soil moisture (%)	1st regrowth	2nd regrowth	3rd regrowth	Mean
Lolium perenne Maja	40	14.28^{a}	9.73^{a}	7.23^{ab}	31.23^{ab}
	80	20.18^{b}	13.80^{b}	13.28^{f}	47.25^{bc}
Lolium perenne Argona	40	14.18^{a}	10.35^{a}	6.20^{a}	30.73^{ab}
	80	20.75^{b}	15.55^{d}	13.70^{f}	50.00^{c}
Dactylis glomerata Dala	40	13.00^{a}	11.25^{a}	8.33^{cd}	32.58^{ab}
	80	22.90^{b}	15.38^{cd}	10.48^{e}	48.75^{c}
Dactylis glomerata Areda	40	12.08^{a}	10.23^{a}	7.38^{bc}	29.68^{a}
	80	21.55^{b}	13.90^{bc}	9.33^{d}	44.77^{b}
Mean for cultivar					
Maja		17.23^{a}	11.76^{a}	10.25^{c}	39.24^{b}
Argona		17.46^{a}	12.95^{bc}	9.95^{bc}	40.36^{b}
Dala		17.95^{a}	13.31^{c}	9.40^{b}	40.66^{b}
Areda		16.81^{a}	12.06^{ab}	8.35^{a}	37.23^{a}
Mean for soil moisture					
	40	13.38^{a}	10.39^{a}	7.28^{a}	31.05^{a}
	80	21.34^{b}	14.66^{b}	11.69^{b}	47.69^{b}

strongest response was observed in var. Argona, where yield decrease was approx. 38%. At the same time, this variety showed the lowest water use efficiency. A significant yield decrease, resulting from water deficit, was also observed in grasses by SZOSZKIEWICZ et al. (1991), and MADZIAR and LATANOWICZ (1996). Dry matter yield height was also related to the course of photosynthesis and transpiration. The correlation coefficients calculated in the study indicate a close interdependence between the rates of these two processes and dry matter yield (Table 6). However, a positive correlation between photosynthetic activity per unit area or weight and yield occurs in some cases only. Very often varieties showing a high photosynthetic rate have a lower yield than those characterized by limited photosynthetic activity. This is due to the fact that biomass production is dependent not only upon photosynthetic rate, but also upon assimilation area, especially the period of time during which assimilation organs are capable of efficient photosynthesis WOJCIESKA-WYSKUPAJTYS (1996).

Table 6

Relationship between parameters examined in the study

Correlation between	*Lolium perenne*		*Dactylis glomerata*	
	Maja	Argona	Dala	Areda
Intensity of photosynthesis and intensity of transpiration	0.9590**	0.9574**	0.9773**	0.9487**
Intensity of photosynthesis and index SPAD	-0.9377**	-0.9494**	-0.9218**	-0.9705**
Intensity of photosynthesis and dry matter yield	0.8947**	0.7887**	0.8458**	0.7775**
Intensity of transpiration and dry matter yield	0.9592**	0.9198**	0.9627**	0.9011**
Water use efficiency and dry matter yield	-0.9933**	-0.9863**	-0.9805**	- 0.992**

** correlation significant at the level $\alpha = 0.01$

Conclusions

1. A decrease in moisture from 80% to 40% FC reduced the rate of photosynthesis, on average by 42%. The strongest response was recorded in var. Maja, where photosynthetic activity decreased by 46%.

2. Water stress considerably limited transpiration in all varieties examined in the study (on average by 66%). Var. Dala evaporated the most water, and var. Areda the least.

3. All tested varieties used water more economically under water stress

conditions, and WUE was significantly higher than in the case of optimum soil moisture content.

4. Both grass species showed higher values of leaf greenness under moisture deficiency conditions. Perennial rye grass varieties were characterized by higher values of the SPAD index than orchard grass varieties.

5. All varieties responded to water stress by a decrease in dry matter yield. The strongest response was observed in var. Argona, which was also characterized by the lowest water use efficiency.

6. Photosynthesis and transpiration had a highly significant effect on dry matter yield in the varieties analyzed. A close correlation was also observed between the processes of photosynthesis and transpiration.

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IDENTIFICATION OF *FUSARIUM* SPECIES FROM WINTER WHEAT GRAIN USED FOR FLOUR PRODUCTION BY THE CULTURE-PLATING METHOD AND BIO-PCR TECHNIQUE

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A b s t r a c t

A mycological analysis of wheat grain, harvested in 2003 and 2004 and stored in elevators in northern Poland, revealed the presence of toxin-producing fungi of the genus *Fusarium*. Higher infection rates were observed in 2003. *Fusarium* species were identified by the culture – plating method and by the molecular, PCR-based method. *Fusarium poae* and *F. avenaceum* were commonly found in wheat grain, whereas *F. graminearum* occurred much less frequently.

K e y w o r d s: *Fusarium* spp., winter wheat, artificial culture method, BIO-PCR technique.

IDENTYFIKACJA NIEKTÓRYCH GATUNKÓW GRZYBÓW Z RODZAJU *FUSARIUM* Z ZIARNA PSZENICY OZIMEJ PRZEZNACZONEJ DO PRZETWÓRSTWA MŁYNARSKIEGO METODĄ SZTUCZNYCH KULTUR I BIO-PCR

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S ł o w a k l u c z o w e: *Fusarium* spp., pszenica ozima, metoda sztucznych kultur, metoda BIO-PCR.

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Abstrakt

Analiza mikologiczna ziarna, pochodzącego z magazynów z terenu Polski północnej ze zbioru w latach 2003 i 2004, wykazała obecność toksynotwórczych grzybów z rodzaju *Fusarium*. Większe nasilenie porażenia ziarna przez te grzyby stwierdzono w 2003 roku. Obecność gatunków z rodzaju *Fusarium* stwierdzono metodą sztucznych kultur i molekularną, opartą na reakcji PCR. *Fusarium poae* i *Fusarium avenaceum* występowały powszechnie na ziarnie pszenicy, natomiast *Fusarium graminearum* znacznie rzadziej.

Introduction

Today the vast majority of flour consumption is wheat flour. This results from the exceptional baking properties and chemical composition of wheat grain (CACEK-PIETRZAK et al. 1999). The high value of wheat is related to its technological properties (good-quality raw material for milling and baking purposes) as well as to a combination of the high nutritive value of processed products (rich chemical composition of grain) and desirable flavor characteristics.

According to CHEŁKOWSKI (1985), the presence of fusariotoxins in cereal grain and other products of natural origin may be a serious problems in Poland. Fungi of the genus *Fusarium* colonize crops causing toxin contamination, which has adverse effects on human and animal health. Many *Fusarium* species are toxic and produce trichothecene mycotoxins, zearelone and fumonisins (YU 2000, EDWARDS 2004). Major metabolites produced by fungi of the genus *Fusarium* are trichothecene compounds whose presence in cereal grain and animal feed is the reason for poisoning in humans and domestic animals. The most toxic metabolites are T-2 toxin, HT-2 toxin, neosolaniol and diacetoxyscirpenol. Attention should be also paid to other compounds of this group, like nivalenol and deoxynivalenol (so called vomitoxin), which may cause vomiting and lack of appetite in animals. The risk of poisoning is the greatest in the epidemiological seasons characterized by high intensity of head blight and corn cob fusariosis (KWAŚNA et al. 1991).

Fungi of the genus *Fusarium* represent a numerous group of species posing a serious threat to crops worldwide. The identification of fusariosis by the artificial culture method (consisting in observations of morphological characters) at species level may be difficult, especially in the case of closely related species, such as *Fusarium graminearum* and *Fusarium culmorum*. Another problem is wide variation in morphological characters, dependent on culture conditions and differences between classification systems (SCHILLING et al. 1996, YODER and CHRISTIANSON 1998, LESLIE et al. 2001). Species identification with the use of the artificial culture method is both time-consuming and laborious (STEVENS et al. 1997b) since fungal cultures are obtained by placing

seeds of agar media or on moist filter paper, and then identified under an optical microscope through observations of the mycelium and spores (SCHAAD et al. 1997). That is why methods based on polymerase chain reaction (PCR) are more and more often applied to diagnose fusariosis. Molecular methods are considered reliable, cost-effective, highly sensitive and rapid (SCHILLING et al. 1996, PAVELEY et al. 1997). Another advantage of these methods is that in contrast to conventional morphological markers they are not affected by environmental factors (CZEMBOR 1995). During the identification of pathogens obtained from seeds certain problems may be caused by PCR inhibitors and the presence of dead cells in the matrix (PAVELEY et al. 1997, STEVENS et al. 1997a). A viable alternative is here BIO-PCR technique, with amplification of DNA from living cells of the pathogen only (SCHAAD et al. 1997).

The aim of the resent study was to determine the severity of infection caused by fungi of the genus *Fusarium* in winter wheat grain used for flour production by the artificial culture method and BIO-PCR technique.

Materials and Methods

Samples of winter wheat grain used for flour production were taken in 12 grain elevators located in northern Poland (Pomeranian Province) in 2003 (5 elevators) and 2004 (7 elevators).

Sample collection procedure

Samples of winter wheat grain (1 kg) used for flour production were taken in accordance with the Polish Standard PN-ISO 13690 (2000). A representative laboratory sample was separated from a total sample taken from the grain lot stored in a given elevator. Each average laboratory sample weighing approx. 1 kg was put into a paper bag.

Identification of some fungal species of the genus *Fusarium* by the artificial culture method

In order to determine the health status of wheat grain by the artificial culture method, 100 kernels were selected randomly of each laboratory sample. The kernels were rinsed under running water for 15 to 20 minutes and surface disinfected with 70% ethyl alcohol and 1% sodium hypochlorite to remove impurities, and next rinsed three times in sterile distilled water. Then the

kernels were put in Petri dishes with a PDA solid medium in a laminar flow chamber. The dishes were stored in a thermostat at 20 to 23°C for 7 to 10 days, and next mycelium hyphae were transferred to PDA slants. Finally the fungal cultures were identified to genus and species based on their morphological characters observed under an optical microscope, as described in the monographs by ELLIS (1971), GILMANS (1957), and KWAŚNA et al. (1991).

Identification of some fungal species of the genus *Fusarium* by BIO-PCR technique

Mycelium hyphae (i.e. live pathogen inoculum with potential infectious properties) obtained from kernels cultured on a PDA medium (artificial culture method), characteristic of *Fusarium* spp., were immediately isolated in separate Petri dishes for BIO-PCR analysis. After 2 to 3 days mycelium pieces were taken with a scalpel, placed in porcelain mortars and ground in liquid nitrogen. DNA was isolated by the CTAB method (NICHOLSON et al. 1996).

Isolation of genomic DNA by the CTAB method

The material (0.2 g) ground in liquid nitrogen (hyphae of infectious mycelium) was transferred to 1.5 ml Eppendorf tubes. Following the addition of 0.6 ml CTAB homogenization buffer and β-mercaptoethanol, the tubes were boiled at 100°C for 2 minutes and incubated at 65°C for 30 minutes. Then 0.6 ml of a chloroform-octanol mixture (24:1) was added, the samples were stirred and centrifuged at 14 000 rpm for 10 minutes. Next the liquid phase was transferred to a sterile tube with ethyl alcohol and centrifuged at 14 000 rpm. The supernatant was poured off and the DNA pellet was rinsed twice with 70% ethanol, dried and suspended in 100 µl of TE. DNA was stored at 4°C for further analysis (NICHOLSON et al. 1996, modified).

Polymerase chain reaction (PCR)

Polymerase chain reaction was performed using 25 µl of a reaction mixture consisting of Tfl 20x reaction buffer, magnesium ions (2 mM $MgCl_2$), free nucleotides (0.2 mM of each), 1 µM of each primer, PCR Enhancer, 0.2 U Tfl DNA polymerase (Epicentre), deionized water and 5 µl of matrix DNA. The samples prepared for analysis were subjected to cyclical temperature changes in a thermocycler (GeneAmp PCR System 2400). Species-specific SCAR

Table 1

Primers used for detection and identification o some fungal pathogens by BIO-PCR technique.

No.	Fungal species	Symbols and sequences of primers	Expected product size	PCR conditions	Reference
1	*Fusarium* spp.	P58SL 5' AGT ATT CTG GCG GGC ATG CCT GT 3' P28SL 5' ACA AAT TAC AAC TCG GGC CCG AGA 3'	339 bp	94°C 5 min; [94°C 1 min, 68°C 1 min, 72°C 1 min]x40; 72°C 5 min.	HUE et al. (1999)
2	*Fusarium poae*	Fp82F, 5' CAA GCA AAC AGG CTC TTC ACC TGT 3' Fp82R 5' TGT TCC ACC TCA GTG ACA GGT T 3'	220 bp	94°C 2 min; [94°C 1 min, 55°C 1 min, 72°C 2 min]x40; 72°C 5 min	PARRY, NICOLSON (1996)
3	*Fusarium avenaceum*	FaF 5' CAA GCA TTG TCG CCA CTC TC 3' FaR 5' GTT TGG CTC TAC CGG GAC TG 3'	920 bp	94°C 2 min; [94°C 30s, 58°C 30s, 72°C 2 min]x40; 72°C 5 min	DOOHAN et al. (1998)
4	*Fusarium culmorum*	OPT18F470 5' GAT GCC AGA CCA AGA CGA AG 3' OPT18R470 5' GAT GCC AGA CGC ACT AAG AT 3'	472 bp	94°C 2 min; [94°C 1 min, 55°C 1 min, 72°C 2 min. x 40; 72°C 5 min	SCHILLING et al. (1996)
5	*Fusarium graminearum*	Fg16F 5' CTC CGG ATA TGT TGC GTC AA 3' Fg16R 5' GGT AGG TAT CCG ACA TGG CAA 3'	400-500 bp	94°C 5 min; [94°C 20 sec., 66°C 1 min, 72°C 45 sec.] x 5; [94°C 20 sec., 64°C 1 min., 72°C 45 sec.] x 5, [94°C 20 sec. 62°C 1 min., 72°C 45 sec.] x 25; 72°C 5 min	NICHOLSON et al. (1998)

Table 2

Identification of fungi of the genus *Fusarium* by the artificial culture method and by BIO-PCR technique

Grain elevator no.	Fungal species									
	Fusarium spp.		*Fusarium avenaceum*		*Fusarium poae*		*Fusarium culmorum*		*Fusarium graminearum*	
	method									
	T	M	T	M	T	M	T	M	T	M
I	+ (19)	+ (19)	+ (8)	+ (7)	+ (2)	+ (1)	–	–	+ (5)	+ (2)
II	+ (16)	+ (16)	+ (4)	+ (5)	+ (11)	+ (11)	–	–	–	–
III	+ (9)	+ (9)	–	+ (2)	+ (6)	+ (6)	–	–	–	–
IV	+ (1)	+ (1)	–	–	+ (1)	+ (1)	–	–	–	–
V	+ (2)	+ (2)	–	–	+ (2)	+ (2)	–	–	–	–
VI	+ (4)	+ (1)	–	–	+ (1)	+ (1)	–	–	–	–
VII	+ (4)	+ (4)	–	–	+ (2)	+ (2)	+ (1)	–	+ (1)	–
VIII	+ (8)	+ (6)	+ (3)	+ (2)	+ (3)	+ (3)	–	–	–	–
IX	+ (12)	+ (10)	–	–	+(9)	+(7)	–	–	+ (1)	–
X	+ (10)	+ (4)	+ (2)	–	+ (2)	+ (2)	–	–	+(1)	–
XI	+ (8)	+ (7)	+ (1)	+ (1)	+ (2)	+ (1)	–	–	+ (4)	+ (2)
XII	+ (3)	+ (3)	–	–	+ (1)	+ (1)	–	–	–	–

Explanations:
T – artificial culture method
M – molecular method BIO-PCR
(+) – positive result
(–) – negative result
grain elevators I-V – wheat grain harvested in 2003, grain elevators VI-XII – wheat grain harvested in 2004
number of fungal cultures of a given species is given in brackets

primers known from literature (Table 1) were used to identify some fungal species of the genus *Fusarium*.

Electrophoresis and visualization of the PCR product

Electrophoresis was performed in 1.5% agarose gel with ethidium bromide, in an electric field at a voltage of 50 V, for 1.5 hours. The size of the PCR product was estimated by referring to the molecular mass standard M 100-1000.

Results and Discussion

The results of studies on the infection of winter wheat grain used for flour production by fungi of the genus *Fusarium*, obtained applying the artificial culture method and the molecular method, were found to be consistent (Table 2). The artificial culture method, based on morphological characters, permitted the identification of major pathogens of the genus *Fusarium* and other fungal species (pathogenic and saprotrophic) in grain samples taken during the years 2003 to 2004 in 12 elevators located in northern Poland (Table 3).

Table 3

Fungi isolated from winter wheat grain stored in 12 elevators in northern Poland during the years 2003-2004

Fungal species	Number of isolates												Total fungal isolates	Infestation rate (%)
	2003					2004								
	grain elevator no.													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fusarium avenaceum (Corda ex Fr.) Sacc.	8	4						3		2	1		18	1.8
Fusarium culmorum (W.C. Smith) Sacc.							1						1	0.1
Fusarium chlamidosporum (Wollenw. et Reinking.)						1			1	1		1	4	0.4
Fusarium graminearum Schwabe	5						1		1	1	4		12	1.2
Fusarium poae (Peck) Wollenw.	2	11	6	1	2	1	2	3	9	2	2	1	42	4.1
Fusarium solani (Mart.) Appel et Wollenw. emend. Snyd et Hans.										2			2	0.2

cont. table 3

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fusarium tricinctum (Corda) Sacc.	4	1	3					2	1		1	1	13	1.3
Fusarium spp.						2				2			4	0.4
Microdochium nivale (Fr.) Sammuel et Hallett						3	2	2	1				8	0.8
Alternaria alternata (Fr.) Keissler	66	68	75	92	79	50	50	38	53	21	48	47	687	67.6
Alternaria spp.						3		19			5	1	28	2.8
Aspergillus niger van. Tieghem							1						1	0.1
Botrytis cinerea Pers								1					1	0.1
Bipolaris biseptata Shoemaker			1										1	0.1
Bipolaris sorokiniana (Sacc. in Sorok.) Shoem.			1										1	0.1
Epicoccum nigru, m Link Schol-Schwarz	16	15	18	12	3	7	4	6	25	5	10	13	134	13.2
Mucor spp.										1			1	0.1
Nigrospora oryzae (Berk. et Br.) Petch.		2								3		3	8	0.8
Papularia sphaerospherma (Pers.) Höhnel.	1	1	4		1					4		3	14	1.4
Penicillium spp.						3	2	10					15	1.5
Rhizoctonia solani Kühn							1						1	0.1
Stemphylium botryosum Wallr.		2		4						1			7	0.7
Torula spp.		1											1	0.1
Non sporulating colonies							3	1		1		4	9	0.9
Total *Fusarium* spp.	19	16	9	1	2	4	4	8	12	10	8	3	96	9.5
Total other species	83	89	99	108	83	66	63	77	79	36	63	71	920	90.5
Total	102	105	108	109	85	70	67	85	91	46	71	74	1016	100

The mycological analysis showed that winter wheat grain used for flour production harvested in 2003 was infected by fungi to a higher degree than grain harvested in 2004 (Table 3). This could result from heavy precipitation (113 mm, Table 4) and high mean daily air temperatures in July 2003 (Table 5), as well as from storage conditions. CHAMPEIL et al. (2004) also reported that crops may be contaminated by toxins not only during the growing season, but also during harvest, transportation and storage. GOLIŃSKI and co-authors (1999) demonstrated that the susceptibility of 10 tested winter wheat varieties to fusariosis depended on environmental conditions (location

of the plantation and years of study). These authors found that the weather conditions prevailing in 1996 were more conducive to the development of *Fusarium* spp. in cereals, as compared with 1995 (GOLIŃSKI et al. 1999).

Table 4

Meat precipitation total (Bałcyny)

Month	Precipitation (mm)			
	2002	2003	2004	means of the years 1961-2000
January	–	14.1	28.9	27.4
February	–	6.0	60.7	21.6
March	–	11.8	28.2	28.5
April	–	23.6	51.5	35.4
May	–	77.1	87.1	57.6
June	–	60.7	90.6	69.5
July	–	113.2	78.8	81.6
August	–	34.9	89.3	75.2
September	4.3	19.1	–	59.0
October	6.9	66.1	–	53.5
November	4.0	39.4	–	48.9
December	0.9	48.6	–	41.8

Table 5

Mean daily air temperatures (Bałcyny)

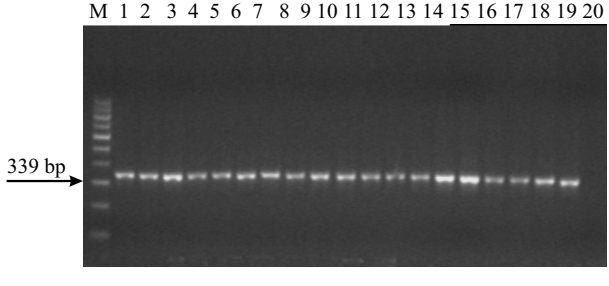
Month	Mean daily air temperatures (°C)			
	2002	2003	2004	means of the years 1961-2000
January	–	–3.8	–6.9	–3.2
February	–	–5.2	–1.1	–2.3
March	–	1.4	2.4	1.4
April	–	6.1	7.7	7.0
May	–	14.2	11.0	12.5
June	–	16.5	14.5	15.8
July	–	18.9	16.2	17.2
August	–	17.3	18.2	16.8
September	12.5	13.7	–	12.6
October	6.4	4.8	–	8.1
November	3.1	4.9	–	2.7
December	–6.6	1.3	–	–1.3

In this study the highest *Fusarium* infection rates were recorded in 2003 in elevators I, II and III (18.63%, 15.24% and 8.33% respectively), while in 2004 in elevators IX, XI and VIII (13.19%, 11.27% and 9.41% respectively) – Table 3.

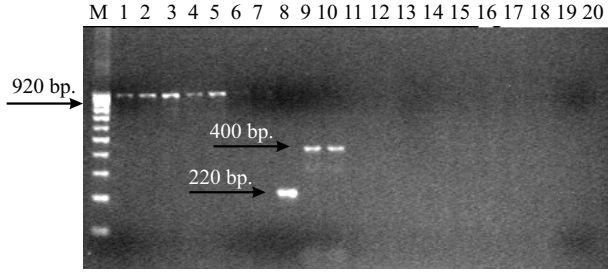
PERKOWSKI and KIECANA (1998) found that some kernels from heads infected by *F. crookwellense*, *F. culmorum* and *F. graminearum* were poorly developed, shriveled, pink-gray in color, soft and lighter than non-inoculated kernels, although their size was identical. Thousand seed weight was also lower in inoculated heads, in comparison with control ones. In addition, barley grain of the tested varieties contained fusariotoxins. The cited authors observed a tendency to a significant correlation between yield decrease caused by grain inoculation by strongly pathogenic *Fusarium* spp. strains and the amount of fusariotoxins accumulated in kernels (PERKOWSKI and KIECANA 1998).

Molecular diagnostics based on DNA polymorphism, applied in the present study, enabled to detect fungi of the genus *Fusarium* spp. in grain samples as well as to identify the species *Fusarium poae*, *Fusarium graminearum* and *Fusarium avenaceum* (Table 2, Photographs 1-24). SCAR (Sequence Characterized Amplified Regions) markers proved to be particularly useful for the detection and identification of *Fusarium* fungi. The primers employed in this method are species-specific and during PCR amplify only one, specified fragment of the genome of a pathogen. Such primers have already been developed for the majority of *Fusarium* species considered important from the economic perspective (PARRY and NICHOLSON 1996, SCHILLING et al. 1996, DOOHAN et al. 1998, NICHOLSON et al. 1998, TURNER et al. 1998, YODER and CHRISTIANSON 1998, HUE et al. 1999).

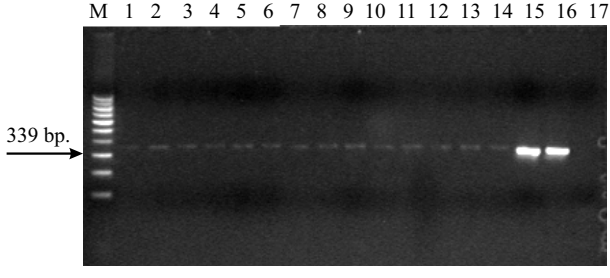
The results of analysis performed by the artificial culture method and the molecular method with the use of P58SL and P28SL primers, concerning the presence of *Fusarium* spp. in winter wheat grain stored in 12 elevators, were found to be identical in the case of 7 elevators. These results differed slightly



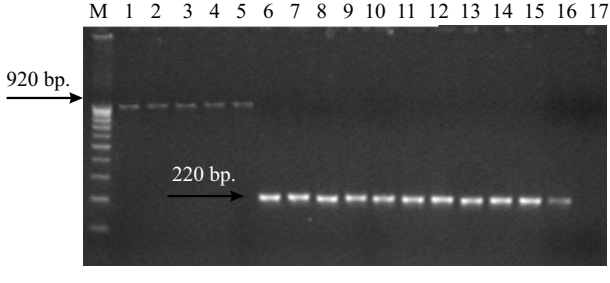
Phot. 1. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no I (molecular mass standard M 100-1000, 1-19 *Fusarium* spp., 20 – negative control)



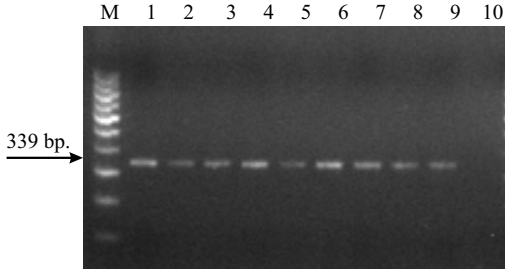
Phot. 2. BIO-PCR products obtained from winter wheat grain with *Fusarium avenaceum*-specific primers FaF, FaR, *Fusarium poae*-specific primers Fp82F, Fp82R and *Fusarium graminearum*-specific primers Fg16F, Fg16R. Elevator no I (molecular mass standard M 100-1000, 1-7 – *Fusarium avenaceum*, 8 – *Fusarium poae*, 9-10 – *Fusarium graminearum*, 11-19 – negative result, 20 – negative control)



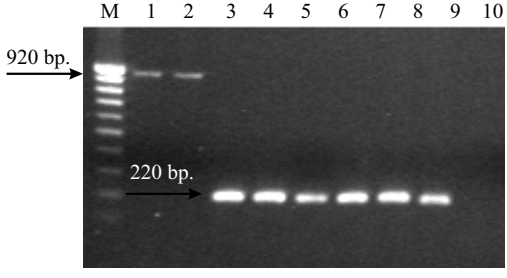
Phot. 3. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no II (molecular mass standard M 100-1000, 1-16 – *Fusarium* spp., 17 – negative control)



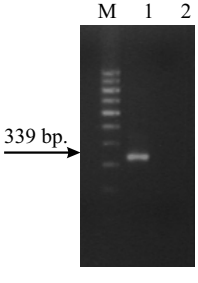
Phot. 4. BIO-PCR products obtained from winter wheat grain with *Fusarium avenaceum*-specific primers FaF, FaR and *Fusarium poae*-specific primers Fp82F, Fp82R. Elevator no II (molecular mass standard M 100-1000, 1-5 *Fusarium avenaceum*, 6-16 – *Fusarium poae*, 17 – negative control)



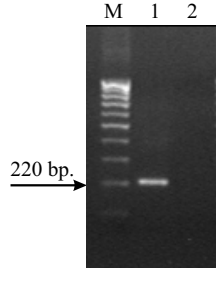
Phot. 5. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no III (molecular mass standard M 100-1000, 1-9 – *Fusarium* spp., 10 – negative control)



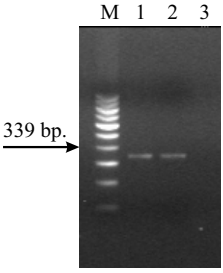
Phot. 6. BIO-PCR products obtained from winter wheat grain with *Fusarium avenaceum*-specific primers FaF, FaR and *Fusarium poae*-specific primers Fp82F, Fp82R. Elevator no III (M 100-1000, 1-2 – *Fusarium avenaceum*, 3-9 – *Fusarium poae*, 10 – negative control)



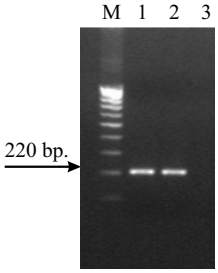
Phot. 7. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no IV (molecular mass standard M 100-1000, 1 – *Fusarium* spp., 2 – negative control)



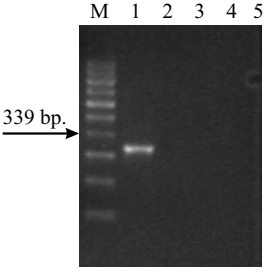
Phot. 8. BIO-PCR products obtained from winter wheat grain with *Fusarium poae*-specific primers Fp82F, Fp82R. Elevator no IV (molecular mass standard M 100--1000, 1 – *Fusarium poae*, 2 – negative control)



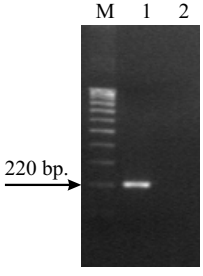
Phot. 9. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no V (molecular mass standard M 100-1000, 1-2 – *Fusarium* spp., 3 – negative control)



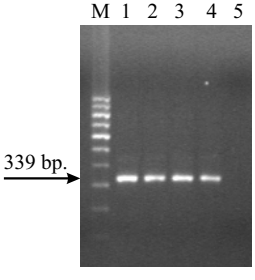
Phot. 10. BIO-PCR products obtained from winter wheat grain with *Fusarium poae*-specific primers Fp82F, Fp82R. Elevator no V (molecular mass standard M 100--1000, 1-2 – *Fusarium poae*, 3 – negative control)



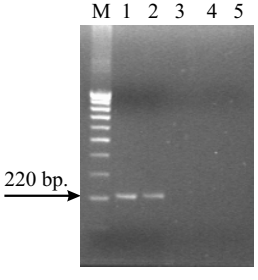
Phot. 11. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no VI (molecular mass standard M 100-1000, 1 – *Fusarium* spp., 2-4 – negative result, 5 – negative control)



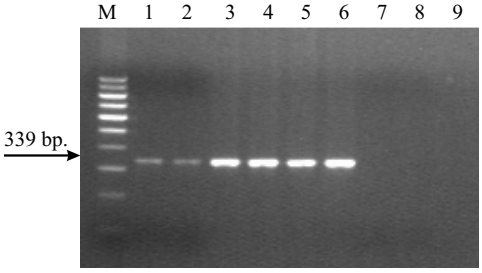
Phot. 12. BIO-PCR products obtained from winter wheat grain with *Fusarium poae*-specific primers Fp82F, Fp82R. Elevator no VI (molecular mass standard M 100--1000, 1 – *Fusarium poae*, 2 – negative control)



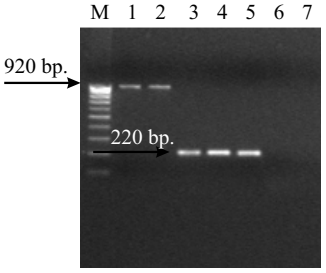
Phot. 13. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no VII (molecular mass standard M 100-1000, 1-4 – *Fusarium* spp, 5 – negative control)



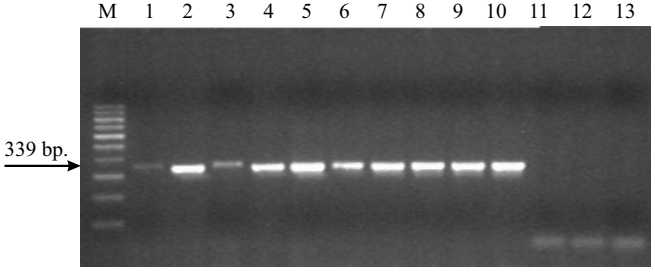
Phot. 14. BIO-PCR products obtained from winter wheat grain with *Fusarium poae*-specific primers Fp82F, Fp82R. Elevator no VII (molecular mass standard M 100--1000, 1-2 – *Fusarium poae*, 3-4 – negative result, 5 – negative control)



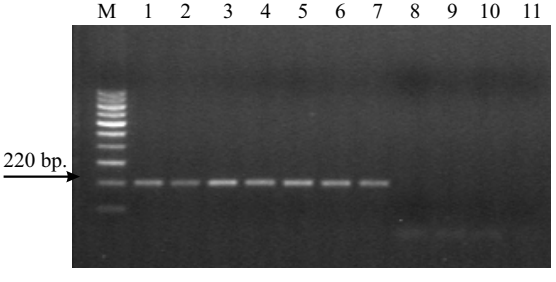
Phot. 15. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no VIII (molecular mass standard M 100-1000, 1-6 – *Fusarium* spp, 7-8 – negative result, 9 – negative control)



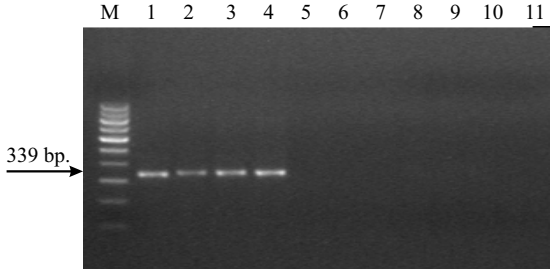
Phot. 16. BIO-PCR products obtained from winter wheat grain with *Fusarium avenaceum*-specific primers FaF, FaR and *Fusarium poae*-specific primers Fp82F, Fp82R. Elevator no VIII (molecular mass standard M 100-1000, 1-2 – *Fusarium avenaceum*, 3-5 – *Fusarium poae*, 6 – negative result, 7 – negative control)



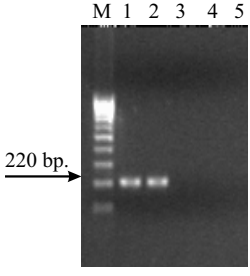
Phot. 17. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no IX (molecular mass standard M 100-1000, 1-10 – *Fusarium* spp, 11-12 – negative result, 13- negative control)



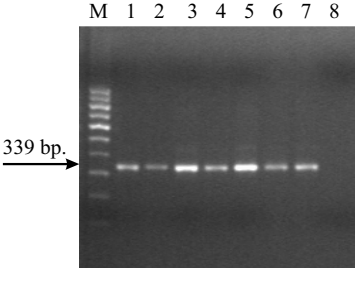
Phot. 18. BIO-PCR products obtained from winter wheat grain with *Fusarium poae*-specific primers Fp82F, Fp82R. Elevator no IX (molecular mass standard M 100-1000, 1-7 – *Fusarium poae*, 8-10 – negative result, 11 – negative control)



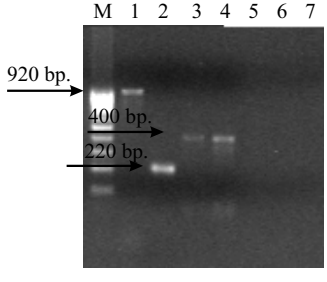
Phot. 19. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no X (molecular mass standard M 100-1000, 1-4 – *Fusarium* spp., 5-10 – negative result, 11 – negative control)



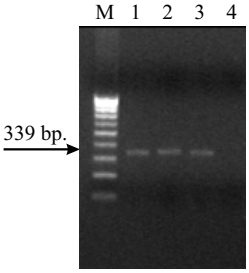
Phot. 20. BIO-PCR products obtained from winter wheat grain with *Fusarium poae*-specific primers Fp82F, Fp82R. Elevator no X (molecular mass standard M 100-1000, 1-2 – *Fusarium poae*, 3-4 – negative result, 5 – negative control)



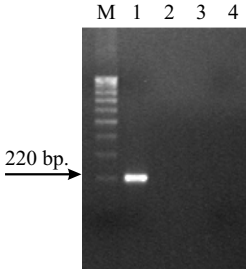
Phot. 21. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no XI (molecular mass standard M 100-1000, 1-7 – *Fusarium* spp., 8 – negative control)



Phot. 22. BIO-PCR products obtained from winter wheat grain with *Fusarium avenaceum*-specific primers FaF, FaR, *Fusarium poae*-specific primers Fp82F, Fp82R and *Fusarium graminearum*-specific primers Fg16F, Fg16R. Elevator no XI (molecular mass standard M 100-1000, 1 – *Fusarium avenaceum*, 2 – *Fusarium poae*, 3-4 – *Fusarium graminearum*, 5-7 – negative result, 8 – negative control)



Phot. 23. BIO-PCR products obtained from winter wheat grain with universal primers P58SL, P28SL. Elevator no XII (molecular mass standard M 100-1000, 1-3 – *Fusarium* spp., 4 – negative control)



Phot. 24. BIO-PCR products obtained from winter wheat grain with *Fusarium poae*-specific primers Fp82F, Fp82R. Elevator no XII (molecular mass standard M 100--1000, 1 – *Fusarium poae*, 2-3 – negative result, 4 – negative control)

for samples taken in three elevators (VIII, IX and XI), whereas more considerable differences were noted for samples collected in elevators VI and X. The product obtained following PCR carried out for all samples was 339 bp in length, which is consistent with the results reported by HUE et al. (1999) – Table 2, Photographs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23.

In the case of *Fusarium avenaceum* certain differences in isolate identification were recorded between samples taken in elevators I, II, VIII (Table 2, Photographs 2, 4, 16). Moreover, *F. avenaceum* was not detected by the artificial culture method in wheat grain stored in elevator III, while BIO-PCR technique with FaF and FaR primers allowed to identify two isolates of this species (Table 2, Photograph 6). The PCR product was 920 bp in length, which agrees with the results obtained by DOOHAN et al. (1998). In wheat grain stored in elevator XI the presence of *F. avenaceum* was confirmed using both methods (Table 2, Photograph 22), while *Fusarium avenaceum* was not detected by the PCR-based method in wheat grain stored in elevator X (Table 2).

The presence of *Fusarium poae* was confirmed by both the artificial culture method and BIO-PCR technique with Fp 82F and Fp 82R primers developed by PARRY and NICHOLSON (1996); the amplified product was 220 bp in length in wheat grain stored in elevators II, III, IV, V, VI, VII, VIII, X and XII (Table 2, Photographs 4, 6, 8, 10, 12, 14, 16, 20 and 24). Seven *F. poae* isolated were detected in wheat grain stored in elevator IX (Table 2, Photograph 18), while single isolates of this species were identified by BIO-PCR technique in grain samples taken in elevators I and XI (Table 2, Photographs 2 and 22).

Fusarium culmorum (1 isolate) was identified only by the artificial culture method in wheat grain stored in elevator VII (Tables 2 and 3). This could be caused by a too low quantity of the material used for DNA isolation.

The presence of *Fusarium graminearum* was detected by the artificial culture method in grain samples collected in elevators I, VII, IX, X and XI (Tables 2 and 3), and by the molecular method using species-specific primers in the samples taken in elevators I and XI (Table 2, Photographs 2 and 22). It was found, based on a molecular analysis, that the PCR product was approximately 400 bp in length. Such a size of the amplified DNA fragment (400 bp), obtained using *Fusarium graminearum*-specific primers Fg16F/R, is characteristic of the European type 6 isolate, as demonstrated by CARTER et al. (2002) who examined variation in pathogenicity associated with the genetic diversity of *Fusarium graminearum* isolates from different geographical regions.

Fungal species identification by the artificial culture method and by the molecular method showed a high contribution of *Fusarium poae* to wheat grain infection. KULIK et al. (2005) also observed high levels of infection by this species in seeds of some crops. *Fusarium poae* is more and more frequently isolated from cereal grain. This species is able to synthesize trichothecene mycotoxins in affected grain, including nivalenol (NIV), diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS), scirpenol (STO), fusarenon X (FUS-X) T-2 toxin, HT-2 toxin and neosolaniol (NEO) (PERKOWSKI et al. 1997, KIECANA et al. 2005). KIECANA and co-authors (2005) demonstrated that *Fusarium poae* not only caused oat yield decrease, but also contributed to trichothecene synthesis within the tested genotypes of this cereal species.

Its seems that the differences in pathogen detection by the artificial culture method and BIO-PCR technique, observed in our experiment, could result from the fact that fungi of the genus *Fusarium* are often accompanied by other fungi, mainly members of *Alternaria* spp. and *Epicoccum nigrum*. DNA was isolated from a relatively low number of hyphae, which could be insufficient for effective amplification. Moreover, DNA isolation may be difficult due to the characteristic structure of cell walls in the tested fungal species.

Among molecular methods particular attention should be paid to BIO-PCR, widely applied to detect seed pathogens. The advantage of BIO-PCR technique over other methods based on PCR following DNA isolation is that it permits the elimination of positive results related to the presence of dead cells (amplification from live pathogen inoculum only) as well as the elimination of wrong results caused by PCR inhibitors. In addition, it is 100-fold more sensitive (SCHAAD et al. 1997). In the case of toxin-producing *Fusarium* species, the rapid and reliable PCR technique based on species-specific SCAR primers, enables to detect even very small amounts of a specific DNA fragment (LEE et al. 2002). SCHILLING et al. (1996) reported that as low quantity as 5 pg from the genomic DNA of *Fusarium graminearum* was sufficient to provide reliable results in agarose gel.

In our study all tested wheat grain samples were infected by fungal species of the genus *Fusarium*, which poses a threat of food contamination by secondary metabolites of these fungi, dangerous to human health.

Conclusions

1. Winter wheat grain for flour production stored in 12 elevators tested in the study was infected by fungi of the genus *Fusarium*.

2. Fusarium poae and *F. avenaceum* were commonly found in wheat grain, whereas *F. graminearum* occurred much less frequently.

3. Both the artificial culture method and the PCR-based method were found to be suitable for the detection and identification of fungal species.

4. The diagnostic procedures applied in the study complemented each other.

Translated by Aleksandra Poprawska

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EFFECT OF MINERAL FERTILIZATION AND GROWTH REGULATORS ON NITROGEN BALANCE AND LEVEL OF CARBOHYDRATES IN SEED PEA

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Key words: pea cultivars, fertilization, growth regulators, nitrogen, content, accumulation, monosaccharides, disaccharides.

Abstract

The aim of the study has been to determine the influence of mineral fertilization and growth regulators on nitrogen balance and accumulation of reducing sugars in pea seeds. The research was based on a two-factor pot experiment. Two pea cultivars were grown: Poker (a sugar cultivar with the traditional type of leaves) and Wenus (a general use, narrow-leaved cultivar). The effects produced by traditional mineral fertilization with single NPK fertilizers were compared to those generated by complex fertilizers – Polifoska 6 and Amofoska 3 (the latter was applied alone or with growth regulators). The sugar pea cv. Poker contained more nitrogen in seeds than the universal cv. Wenus. Mineral fertilization and growth regulators decreased the concentration of nitrogen in seeds, while raising it mainly in leaves. Cultivar Wenus took up 16% more nitrogen than cv. Poker. However, the latter accumulated more nitrogen in its seeds. Mineral fertilization increased the uptake and accumulation of nitrogen in seeds of both cultivars. The effect produced by the growth regulators depended on a cultivar. In cv. Poker the growth regulators depressed the uptake and accumulation of nitrogen in seeds, whereas in cv. Wenus the effect was opposite. Mineral fertilization and growth regulators decreased the contribution of seeds in nitrogen accumulation. Seeds of the sugar pea cultivar Poker contained more reducing sugars than those of the general-use cv. Wenus. The dominant fraction of reducing sugars consisted of disaccharides. The content of reducing sugars was negatively correlated with the uptake of nitrogen by plants.

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WPŁYW NAWOŻENIA MINERALNEGO I REGULATORÓW WZROSTU NA GOSPODARKĘ AZOTEM I ZAWARTOŚĆ CUKRÓW W GROCHU SIEWNYM

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S ł o w a k l u c z o w e: odmiany grochu, nawożenie, regulatory wzrostu, azot, zawartość, akumulacja, cukry proste, dwucukry.

A b s t r a k t

Określono wpływ nawożenia mineralnego i regulatorów wzrostu na gospodarkę azotem oraz gromadzenie cukrów redukujących w nasionach grochu. Podstawę badań stanowiło 2-czynnikowe doświadczenie wazonowe. Uprawiano 2 odmiany grochu: Poker (odmianę cukrową o tradycyjnym typie ulistnienia) oraz Wenus (odmianę ogólnoużytkową, wąskolistną). Porównywano działanie tradycyjnego nawożenia nawozami pojedynczymi NPK oraz nawozami wieloskładnikowymi – Polifoską 6 oraz Amofoską 3 (bez dodatków i z dodatkiem regulatorów wzrostu). Groch cukrowy odmiany Poker zawierał więcej azotu w nasionach niż ogólnoużytkowa odmiana Wenus. Nawożenie mineralne i regulatory wzrostu zmniejszały koncentrację azotu w nasionach, a zwiększały przede wszystkim w liściach. Groch odmiany Wenus pobrał o 16% więcej azotu niż odmiana Poker, jednakże to odmiana Poker zgromadziła więcej tego składnika w nasionach. Nawożenie mineralne zwiększyło pobranie i akumulację azotu w nasionach obu odmian grochu. Działanie regulatorów wzrostu było uzależnione od odmiany – u odmiany Poker zmniejszyło pobranie i akumulację N w nasionach, u odmiany Wenus zaś zwiększyło. Nawożenie mineralne i regulatory wzrostu zmniejszyły udział nasion w akumulowaniu azotu. Nasiona cukrowej odmiany Poker zawierały więcej cukrów redukujących niż ogólnoużytkowa odmiana Wenus. Dominującą formą cukrów redukujących były dwucukry. Zawartość cukrów redukujących była ujemnie skorelowana z pobraniem azotu przez rośliny grochu.

Introduction

Under the soil and climatic conditions of Poland, pea is the most widespread leguminous crop. Pea cultivars are divided into edible, field and general use pea varieties. Seed pea is a rich source of many nutrients essential for human and animal nutrition (LAMPART-SZCZAPA 1997, MARISCAL-LADIN et al. 2002, SALGADO et al. 2002). The nutritional value of pea seeds is high owing to a large concentration of protein they contain, which includes 1.5% of lysine – an amino acid deficient in other foodstuffs. Variations in the chemical content of pea seeds depend on species-specific genetic traits, cultivation technologies and fertilization regimes.

The aim of this study has been to determine the effect of fertilization and growth regulators on nitrogen balance and accumulation of reducing sugars in pea seeds.

Materials and Methods

A two-factor pot experiment was established in a greenhouse at the University of Warmia and Mazury in Olsztyn. The experiment was set up in a completely random block design, with three replications. Pea was grown in modified Kick-Braukmann's pots filled with 10 kg of light soil of the particle distribution representing heavy loamy sand. The soil was characterised by slightly acidic reaction (pH = 5.52 in 1 mol KCl · dm^{-3}) and very high content of plant-available elements (170 mg · kg^{-1} P, 207 mg · kg^{-1} K and 100 mg · kg^{-1} Mg).

The first experimental factor was a pea species: the traditional sugar variety Poker and the general use, narrow-leaved cv. Wenus. The second factor consisted of the type of fertilization: no fertilization (C), traditional NPK fertilization (NPK), Polifoska 6 fertilizer (P6), Amofoska 3 fertilizer (A3) and multi-component fertilizers with growth regulators: Amofoska 3 + IBA (β-indolebutyric acid) (A 3 + IBA), Amofoska 3 + NAA (α-naphthylacetic) (A 3 + NAA), Amofoska 3 + tria (triacontanol) (A 3 + tria), Amofoska 3 + try (L-tryptophan) (A 3 + try), Amofoska 3 + ade (adenine) (A 3 + ade), Amofoska 3 + BA (benzyladenine) (A 3 + BA).

The NPK fertilization was applied at a rate of 0.3 g N (ammonium nitrate), 1.0 g P (triple superphosphate) and 2.8 g K (potassium salt) per pot. Polifoska was applied at 6 g per pot and Amofoska – at 10 g per pot. Acryl amide gel, used to coat Amofoska 3 directly prior to application, served as a carrier of growth regulators. The fertilizers were point-applied before seed sowing. The regulators were used at the following rates: 10 mg IBA and NAA, 5.2 g tria, 90 mg try, 40 mg ade and 30 mg BA per pot.

Twelve pea seeds were sown per pot. The soil moisture was maintained at 60% of field water capacity. Plants were harvested at the full maturity stage and dissected into the following organs: pea pods, leaves and stems.

The following determinations were performed in the plant material: nitrogen with the subchlorine method in colorimetry and the content of carbohydrates with the reduction titration method using a weakly alkaline copper reagent (Luff-Schoorl method).

Discussion and the Results

The content of nitrogen in particular plant parts is a species-specific character. Seeds of the consumption cultivar Poker contained on average 5 g · kg^{-1} d.m. more nitrogen than those of the universal cultivar Wenus (Table 1). In addition, compared to cv. Wenus, cv. Poker had much more N in stems and the difference in nitrogen concentration was even more evident between

N concentrations in leaves (4.12 g · kg^{-1} d.m. more N in cv. Poker stems). Only of the pods shell revealed higher N concentrations in cv. Wenus than in the other variety. Mineral fertilization and the growth regulators applied together with the fertilizers decreased the amounts of nitrogen in seeds while increasing its levels in vegetative organs, and in leaves particularly.

Table 1

Mean N content (± confidence interval) in aboveground parts of pea (g · kg^{-1} d.m.)

Treatment	Parts of plant			
	seeds	pods shell	stems	leaves
Poker				
Control	37.32 ± 0.42	4.34 ± 0.71	8.52 ± 0.09	7.52 ± 0.12
NPK	33.28 ± 0.71	3.22 ± 0.53	8.54 ± 0.17	10.60 ± 0.36
Polifoska 6	29.92 ± 0.24	3.60 ± 0.06	10.34 ± 0.62	9.48 ± 0.33
Amofoska 3	31.16 ± 0.06	3.14 ± 0.42	8.88 ± 0.39	12.20 ± 0.36
Amofoska 3+IBA	29.60 ± 0.48	5.04 ± 0.24	10.22 ± 0.59	12.40 ± 0.45
Amofoska 3+NAA	31.52 ± 0.24	5.14 ± 0.36	9.72 ± 0.15	11.84 ± 0.42
Amososka 3+tria	30.04 ± 0.42	4.90 ± 0.18	9.64 ± 0.74	12.04 ± 0.17
Amofoska 3+try	29.68 ± 0.24	4.58 ± 0.24	10.82 ± 0.77	12.24 ± 0.42
Amofoska 3+ade	31.56 ± 0.30	4.52 ± 0.75	9.14 ± 0.34	10.60 ± 0.27
Amofoska 3+BA	29.72 ± 0.30	3.98 ± 0.59	11.66 ± 0.30	14.24 ± 0.18
Mean for cultivar	31.38 ± 0.77	4.25 ± 0.60	9.75 ± 0.35	11.32 ± 0.24
Wenus				
Control	29.56 ± 0.30	4.32 ± 0.86	6.52 ± 0.45	4.64 ± 0.09
NPK	24.44 ± 0.42	5.54 ± 0.94	7.78 ± 0.36	6.96 ± 0.36
Polifoska 6	25.52 ± 0.24	5.36 ± 0.06	8.76 ± 0.33	7.72 ± 0.15
Amofoska 3	26.52 ± 0.18	5.58 ± 0.59	8.86 ± 0.69	8.80 ± 0.12
Amofoska 3+IBA	26.32 ± 0.24	7.36 ± 0.12	8.90 ± 0.65	8.00 ± 0.74
Amofoska 3+NAA	24.40 ± 0.24	7.58 ± 0.48	8.16 ± 0.21	9.20 ± 0.18
Amososka 3+tria	26.64 ± 0.12	6.38 ± 0.24	8.80 ± 0.33	8.08 ± 0.11
Amofoska 3+try	27.72 ± 0.53	6.94 ± 0.77	7.46 ± 0.18	6.04 ± 0.10
Amofoska 3+ade	27.08 ± 0.65	6.86 ± 0.89	8.02 ± 0.06	5.88 ± 0.53
Amofoska 3+BA	28.48 ± 0.59	6.58 ± 0.24	8.30 ± 0.24	6.64 ± 0.36
Mean for cultivar	26.67 ± 0.53	6.25 ± 0.48	8.16 ± 0.26	7.20 ± 0.33

Some authors claim that the chemical composition of plants, including the total protein content in seeds, is not related to the type of leaves on pea plants (ANDRZEJEWSKA et al. 2002, KOTECKI 1994, SAWICKI et al. 2000). In contrast, KOTECKI and GRZĄDKOWSKA (1997) as well as STĘPNIAK-SOŁYGA and WOJTASIK (2003) demonstrated cultivar-specific variation in the content of nitrogen in grains, stubble and roots, although no such differences related to a crop cultivar were observed in the case of straw. Cultivation conditions, such as plant density per 1 m^2, had no effect on this factor. The same authors suggested that pea seeds, as compared with straw, contained more N and P, but less K, Ca and Mg. According to MICHAŁOJĆ (1997), nitrogen-potassium

fertilization rates had no effect on the content of nitrogen in pea seeds and leaves, neither did the chemical form of the fertilizers.

Numerous reports on the effect of growth regulators on the chemical composition of plants are ambiguous. Some authors claim that IBA causes a decline in the amount of nitrogen in horse bean and yellow lupine seeds, which is in compliance with the results presented in this report. At the same time, the same researchers report that nitrogen concentrations in vegetative parts of plants increase, which may suggest that there are certain disorders in nitrogen metabolism and transport of nitrogen compounds (KLASA et al. 1996, PRUSIŃSKI and BOROWSKA 2002). However, NOWAK et al. (1997) showed a stimulating effect produced by phytohormones, especially NAA and BA, on the content of nitrogen in seeds as well as in vegetative parts of horse bean. Other reports have shown that GA3, NAA, IBA and BAP contributed to an increase in the concentration of nitrogen in triticale grain while depressing N levels in the culm as well as in seeds, stems and leaves of soybean (IBA, NAA and IBA + NAA) (CZAPLA et al. 2000, 2003). KUCHARSKI and WYSZKOWSKA (2000) reported that soil application of benzyladenine and its precursors, isopentyl alcohol and adenine, led to an increase in the content of nitrogen in aerial parts of buckwheat; in buckwheat root such a decline was also observed under the influence of BA. In some other studies, cytokinin precursors, adenine and isopnetyl alcohol as well as benzyladenine itself depressed the concentration of nitrogen in the aerial mass of horse bean (KUCHARSKI et al. 1999). Trials involving flax (KUKRESH, KHODYANKOWA 2001) showed that complex forms of mineral fertilizers with an addition of bioactive substances (Fenomelan) significantly increased both the yield of seeds and straw and the content of fat in seeds as well as the quality of fibre.

The data presented in Figures 1 and 2 show that pea plants of the universal cultivar Wenus took up 16% more nitrogen than cv. Poker. Regarding cv, Wenus (Figure 2), the mineral fertilization and the growth regulators applied increased the uptake of this element by 17-43% relative to the pea plants grown without any fertilization. Cultivar Poker, however, responded differently (Figure 1). In was only under the influence of single NPK fertilizers and Polifoska 6 that the uptake of nitrogen rose by 12-17%, whereas the application of Amofoska 3 decreased the N uptake by ca 20%. The growth regulators introduced together with Amofoska 3 alleviated the adverse effect of this fertilizer, while triacontanol, a synthetic auxin (NAA), as well as cytokinin (BA) resulted in the uptake of nitrogen on a level comparable to that attained by control plants. Despite the lower total nitrogen uptake, cv. Poker (Figure 1) accumulated about 20% more nitrogen than cv. Wenus (Figure 2), which was attributed to a much higher nitrogen content in plants of the former cultivar. Both cultivars responded to fertilization with single NPK fertilizers and with

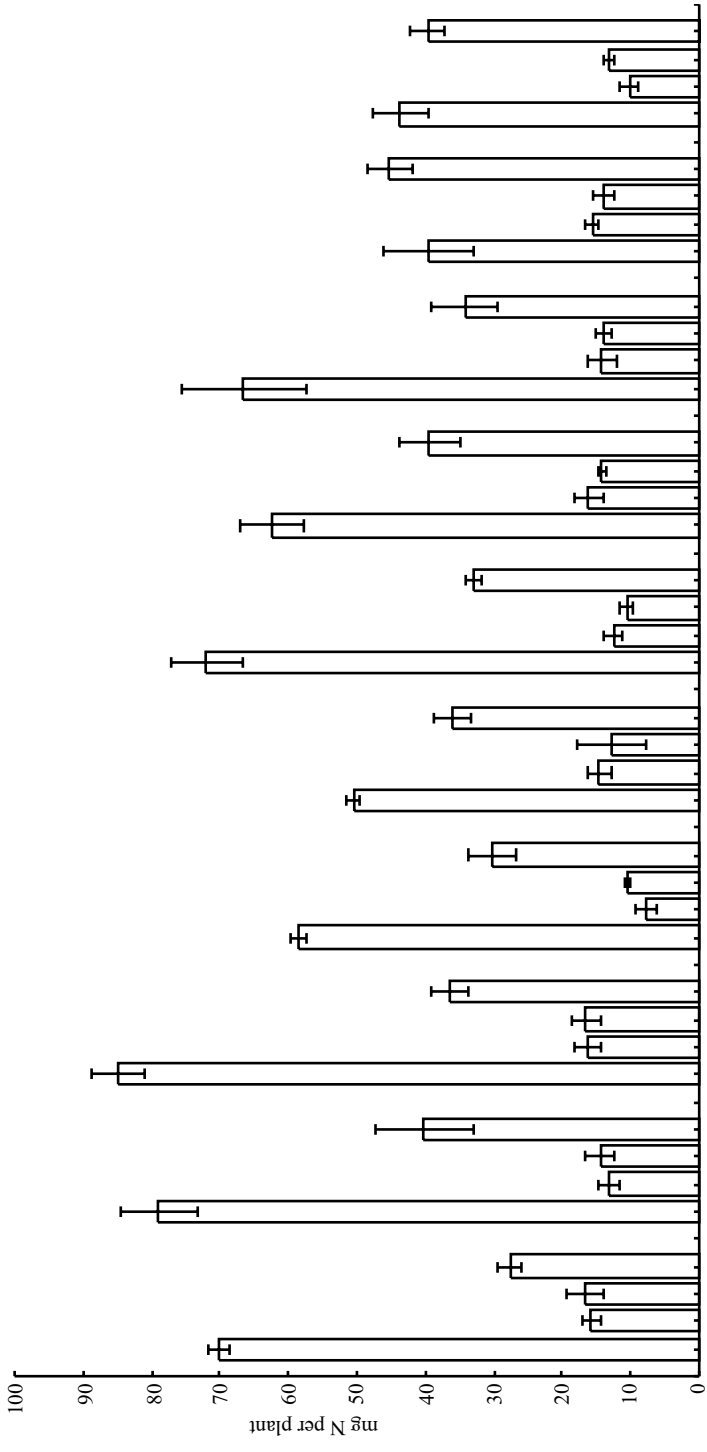

Fig. 1. Nitrogen accumulation (± confidence interval, $p = 0.99$) in aboveground parts of *cv.* Poker: *s* – seeds, *p* – pods shell, *st* – stems, *l* – leaves

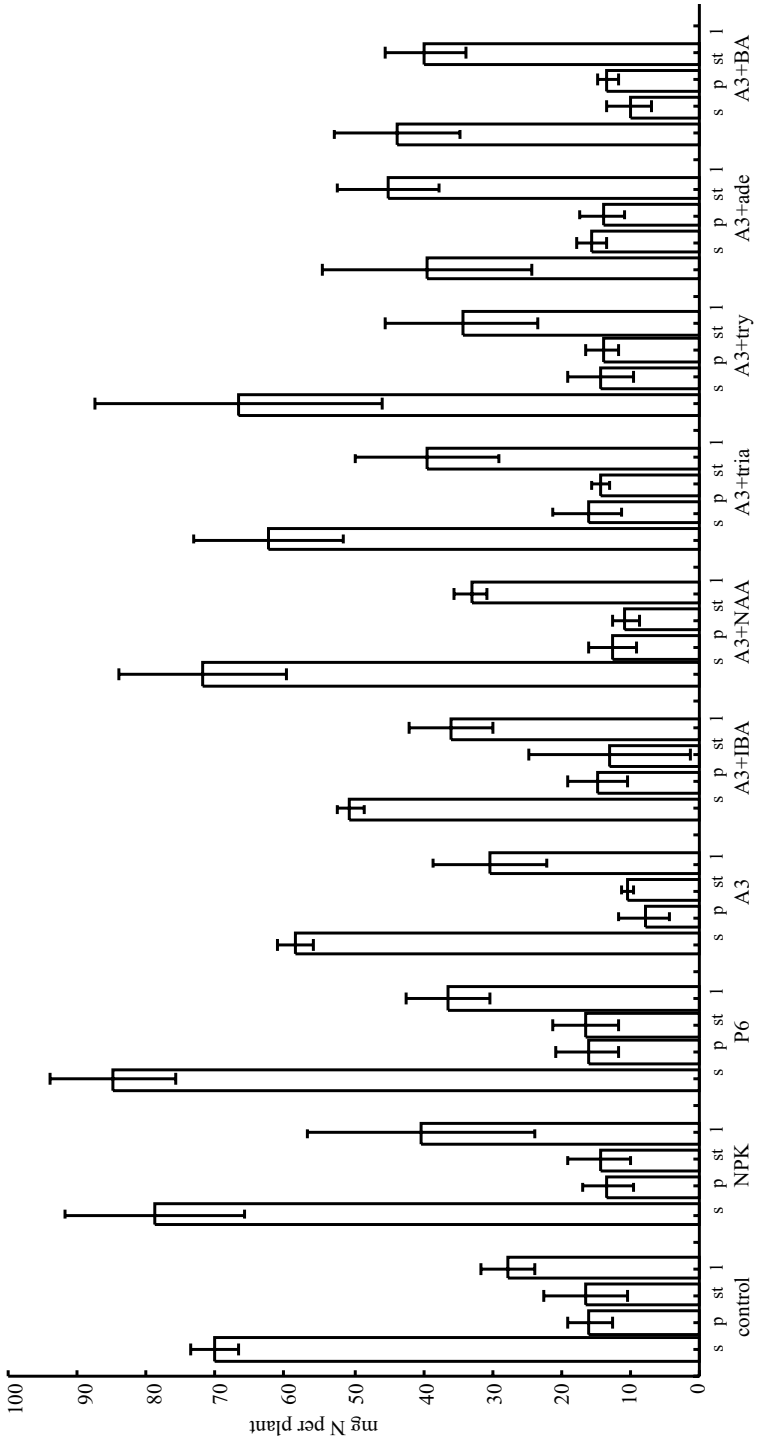

Fig. 2. Nitrogen accumulation (± confidence interval, $p = 0.99$) in aboveground parts *cv.* Wenus

Explanations see Fig. 1

Polifoska 6 by accumulating more nitrogen in seeds (5% more nitrogen was accumulated in cv. Wenus and 12% in cv. Poker seeds after NPK fertilization; 20% in cv. Poker and 31% in cv. Wenus following Polifoska 6 treatments). The response of the cultivars to the fertilization with Amofoska 3 and addition of the growth regulators was just as varied as that revealed in the case of nitrogen uptake. Cultivar Poker (Figure 1) depressed the N accumulation in seeds under the effect of Amofoska 3, and the growth regulators IBA, BA and adenine added to Amofoska 3 caused a further reduction in nitrogen accumulation in seeds. The remaining growth regulators levelled the negative effect of the above fertilizer, with NAA (auxin) increasing the amount of nitrogen in seeds above the level determined in the control plants. Mineral fertilization and the growth regulators increased the accumulation of nitrogen in leaves, especially in cv. Wenus (over 2-fold more nitrogen gathered in leaves under the effect of Amofoska 3 applied in conjunction with IBA or with triacontanol) (Figure 2).

The contribution of particular organs of pea plants to the total accumulation of nitrogen is presented in Figure 3. Cultivar Poker (a sugar pea variety with traditional type of leaves) grown without any fertilization accumulated nearly 54% of the nitrogen taken up from soil in seeds, 12-13% of the total nitrogen was found in pods and stems while *ca* 21% was gathered in leaves. The universal, narrow-leaved cv. Wenus contained 43, 11, 20 and 25% of

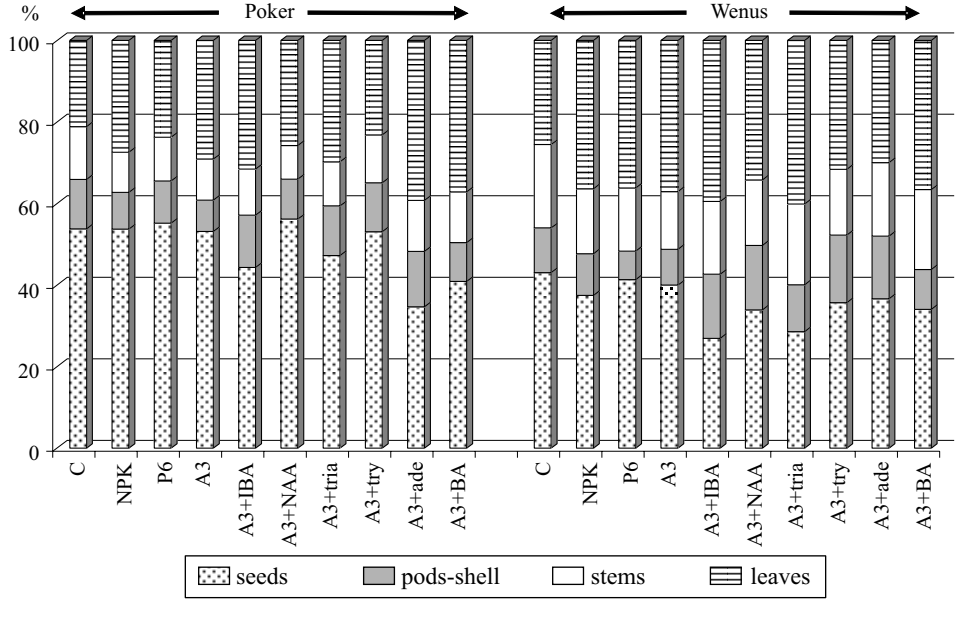

Fig. 3. Nitrogen distribution in aboveground parts of pea

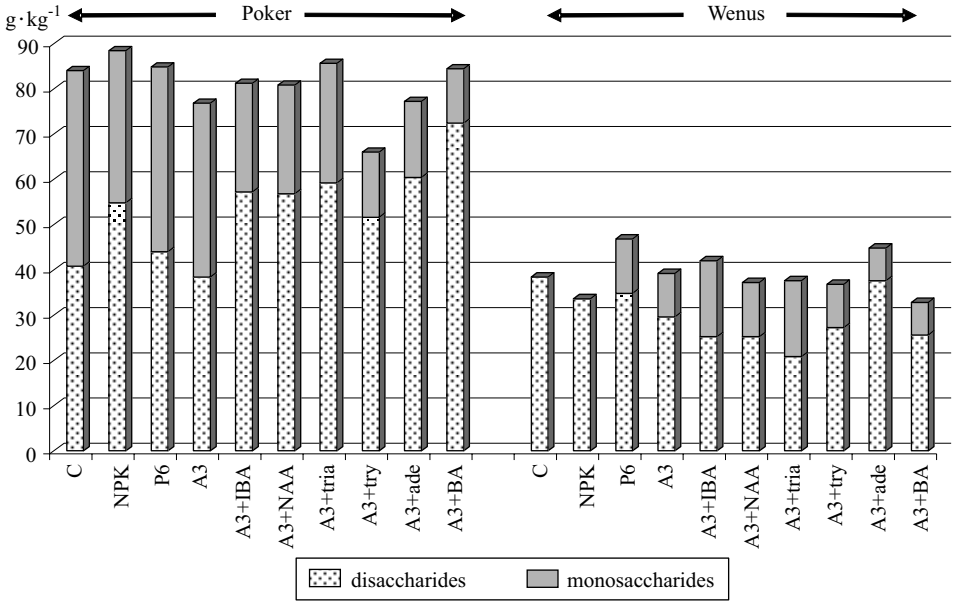

Fig. 4. Sugars reducing in seeds of pea

nitrogen in the respective plant organs. In both pea cultivars, mineral fertilization only slightly changed the above percentages of nitrogen accumulated in particular organs. The growth regulators produced somewhat stronger, and in general negative effect on the accumulation of nitrogen. Only one growth regulator, auxin (NAA), increased very slightly the percentage of nitrogen accumulated in seeds produced by cv. Poker, while the per cent amounts of nitrogen determined in pods and stems slightly declined. In the other variants of the trials involving the growth regulators, those substances typically favoured the accumulation of nitrogen in leaves.

The results reported by KOTECKI and GRZĄDKOWSKA (1997) show that under field conditions, pea seeds accumulate *ca* 67% of nitrogen taken up by plants (the aboveground part + the roots).

Cultivar Poker, a sugar form of pea with traditional leaves, contains about 2-fold more reducing sugars in seeds than the narrow-leaved cultivar Wenus (Figure 4). A much larger difference between the two cultivars was observed in the content of monosaccharides: cv. Poker contained three times as many monosaccharides as cv. Wenus. The dominant fraction among reducing sugars consisted of disaccharides. Mineral fertilization and the growth regulators modified the content of reducing sugars but their effect depended on a cultivar. In the seeds of the sugar pea Poker, versus the control, the application of mineral fertilization and the growth regulators increased by about 1/3

the content of disaccharides, while the concentration of simple sugars declined. The content of monosaccharides was negatively correlated with the accumulation of nitrogen in the aerial mass of pea plants (Figure 5).

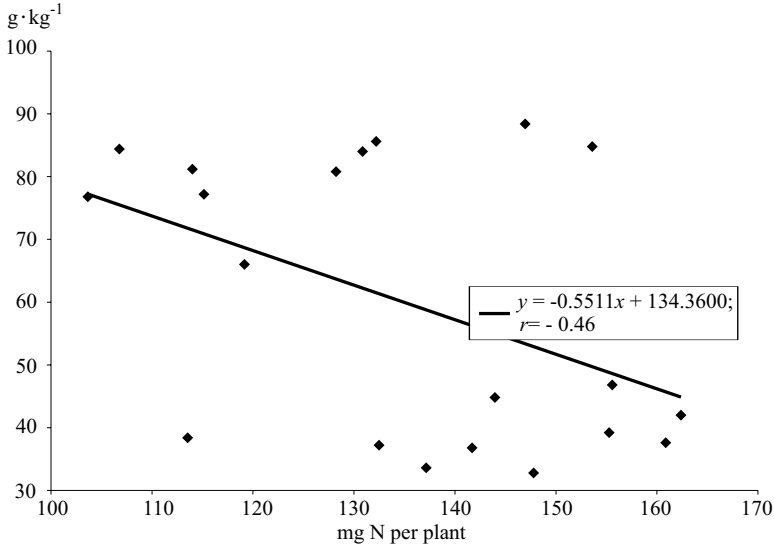

Fig. 5. Sugars reducing in seeds of pea independence on accumulation in aboveground parts of plant

According to TARASEWICZ (1998), oligosaccharides isolated from seeds of narrow-leaved lupine, which was used as a fodder for quail, improved the birds' egg-laying potential and depressed cholesterol in egg yolk. SZYNAL et al. (2001) suggested that growth inhibitors stimulated the intensity of photosynthesis, as a results of which they contributed to higher accumulation of reducing sugars in wheat and triticale seedlings.

Conclusions

1. The sugar pea Poker contained more nitrogen in seeds than the general use cultivar Wenus. Mineral fertilization and the growth regulators decreased the concentration of nitrogen in seeds while increasing it mainly in leaves.

2. Pea plants of the cultivar Wenus took up about 16% more nitrogen than the sugar cultivar Poker. Nonetheless, cv. Poker accumulated more nitrogen in seeds.

3. Mineral fertilization improved the uptake and accumulation of nitrogen in seeds of both pea cultivars. The effect of the growth regulators depended on a pea cultivar: in cv. Poker they decreased the uptake and accumulation of N in seeds, while in cv. Wenus they acted the opposite.

4. Mineral fertilization and the growth regulators depressed the contribution of seeds to total nitrogen accumulation in pea plants.

5. Seeds of the sugar pea cultivar Poker contained more reducing sugars that the general use cultivar Wenus. The dominant fraction among reducing sugars consisted of disaccharides. The content of reducing sugars was negatively correlated with the uptake of nitrogen by pea plants.

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EFFECT OF NICKEL ON THE PROLIFERATION OF NITROGEN-FIXING BACTERIA*

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K e y w o r d s: *Arthrobacter* spp., *Azotobacter* spp., *Rhizobium, Bradyrhizobium*, nickel.

A b s t r a c t

A laboratory experiment was conducted to test the effect of nickel on the proliferation of the following soil bacteria suspended in liquid media: *Azotobacter* spp., *Arthrobacter* spp., *Rhizobium leguminosarum* bv. *viciae* and *Bradyrhizobium* spp. (lupini). The experiment was performed on 10 strains of each species, in three replications. Two types of microbiological media were used, i.e. standard and with an additional carbon source. Nickel was applied in the form of two compounds, $NiCl_2 \cdot 6H_2O$ and $NiSO_4 \cdot 7H_2O$, in the following doses (mg $Ni \cdot dm^{-3}$ of the medium): 2, 4, 6, 8, 10, 100, 200, 300, 400.

Laboratory studies revealed that the tested microorganisms showed various degrees of sensitivity to nickel introduced into the growth media. Among the bacteria analyzed, *Azotobacter* spp. was found to be most sensitive to nickel compounds, followed by *Arthrobacter* spp., *Bradyrhizobium* spp. (lupini) and *Rhizobium leguminosarum* bv. *viciae*, which was most nickel-resistant. $NiCl_2 \cdot 6H_2O$ was more toxic to *Arthrobacter* spp. and *Rhizobium leguminosarum* bv. *viciae*, while $NiSO_4 \cdot 7H_2O$ to *Azotobacter* spp. and *Bradyrhizobium* spp. (lupini).

WPŁYW NIKLU NA NAMNAŻANIE BAKTERII WIĄŻĄCYCH AZOT ATMOSFERYCZNY

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S ł o w a k l u c z o w e: *Arthrobacter* spp., *Azotobacter* spp., *Rhizobium, Bradyrhizobium*, nikiel.

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Abstrakt

W doświadczeniu laboratoryjnym testowano wpływ niklu na namnażanie się w hodowlach płynnych następujących bakterii glebowych: *Azotobacter* spp., *Arthrobacter* spp., *Rhizobium leguminosarum* bv. *viciae*, *Bradyrhizobium* spp. (lupini). Badania każdego gatunku wykonano na 10 szczepach w 3 powtórzeniach. W doświadczeniu wykorzystano 2 rodzaje podłóż mikrobiologicznych: standardowe oraz wzbogacone w dodatkowe źródło węgla. Nikiel zastosowano w postaci dwóch związków: $NiCl_2 \cdot 6H_2O$ i $NiSO_4 \cdot 7H_2O$ w następujących dawkach (mg $Ni \cdot dm^{-3}$ pożywki): 2, 4, 6, 8, 10, 100, 200, 300, 400.

Wykazano zróżnicowaną wrażliwość testowanych mikroorganizmów na wprowadzany do podłoża nikiel. Wśród badanych bakterii najbardziej wrażliwy na jego związki okazał się *Azotobacter* spp., następnie *Arthrobacter* spp., *Bradyrhizobium* spp. (lupini), a najmniej *Rhizobium leguminosarum* bv. *viciae*. Bardziej toksyczny dla *Arthrobacter* spp. i *Rhizobium leguminosarum* bv. *viciae* był nikiel stosowany w postaci $NiCl_2 \cdot 6H_2O$, a dla *Azotobacter* spp. i *Bradyrhizobium* spp. (lupini) w postaci $NiSO_4 \cdot 7H_2O$.

Introduction

Nickel belongs to heavy metals that are widespread environmental contaminants (DOSANJH, MICHEL 2006). The most common oxidation states of nickel are +2 and +3, though +4 Ni complexes are also observed. In soil this metal occurs mostly in the form of the Ni^{2+} cation (TERELAK, PIOTROWSKA 1997). The distribution and behavior of nickel in the natural environment are determined by its geochemical association with iron and cobalt. As a result of weathering, the highly mobile ionic form of nickel is released, capable of long-distance migrations in solutions. However, most often nickel is bound to iron hydroxides or manganese hydroxides (TERELAK et al. 2000, WĘGLARZY 2001).

Microbes are characterized by different sensitivity to heavy metals, including nickel. The toxic effect of heavy metals on microorganisms may be related to their tendency to form covalent bonds with a variety of biomolecules (BARABASZ et al. 1997). According to GILLER et al. (1998), the occurrence of elevated concentrations of metal ions or metabolites in the natural environment leads to their bioaccumulation in microbial cells. The quantity of a metal penetrating into the microbial cell is dependent primarily on the permeability of the cytoplasmic membrane and on the presence of the resistance factor in plasmids. Upon penetration into the cell, the metal may bind to protein thus blocking the active groups of enzymes (NOWAK et al. 2004, LIHOR et al. 2006). Heavy metal uptake and accumulation by cells, especially microbial cells, cause their death unless they possess special metabolites able to bind and inactivate a given metal (GUIBAUD et al. 2005).

Many elements that belong to heavy metals are indispensable for the proper functioning of cells (BARABASZ et al. 1997, DOSANJH, MICHEL 2006,

GILLER et al. 1998). However, they may also disturb metabolic processes when present in excess. This group includes nickel, which is a component of urease – the enzyme responsible for urea hydrolysis.

The aim of the present study was to determine the effect of various nickel concentrations in the medium on the proliferation of free-living and symbiotic nitrogen-fixing bacteria.

Materials and Methods

A laboratory experiment was conducted to test the effect of nickel on the proliferation of pure bacterial cultures in liquid media. The following soil bacteria, from the collection of the Department of Microbiology, University of Warmia and Mazury in Olsztyn, were used: *Azotobacter* spp., *Arthrobacter* spp., *Rhizobium leguminosarum* bv. *viciae* and *Bradyrhizobium* spp. (lupini). Nickel was applied in the form of two compounds, $NiCl_2 \cdot 6H_2O$ and $NiSO_4 \cdot 7H_2O$, in the following doses (mg $Ni \cdot dm^{-3}$ of the medium): 2, 4, 6, 8, 10, 100, 200, 300, 400. The experiment was performed on 10 strains of each species, in three replications. Two types of media were used, i.e. standard and with an increased (by 50%) carbon content. The composition of the growth media was as follows: *Azotobacter* spp. – K_2HPO_4 – 1.5 g, $MgSO_4 \cdot 7H_2O$ – 0.3 g, NaCl – 0.3 g, $FeSO_4 \cdot 7H_2O$ – 2 mg, $MnSO_4 \cdot 7H_2O$ – 2 mg, $CaCO_3$ – 3.0 g, saccharose – 15.0 g, H_2O – 1 dm^3, agar – 7 g, pH – 7-8 (FENGLEROWA 1965); *Arthobacter* ssp.: $Ca(H_2PO_4)_2$ – 0.25 g, K_2HPO_4 – 1.0 g, $MgSO_4 \cdot 7H_2O$ – 0.25 g, $(NH_4)_2SO_4$ – 0.25 g, casein – 1.0 g, yeast extract – 0.7 g, glucose – 1.0 g, agar – 15 g, pH – 7.0 (MULDER, ANTHEUMISSE 1963); *Bradyrhizobium* spp. (lupini) and *Rhizobium leguminosarum* bv. *viciae*: mannitol – 10 g, KH_2PO_4 – 0.5 g, $MgSO_4 \cdot 7H_2O$ – – 0.2 g, NaCl – 0.1 g, $CaCO_3$ – 3.0 g, yeast extract – 0.4 g, H_2O – 1 dm^3, agar – 15 g, pH – 6.8 (YEMB medium – VINCENT 1970). The media whose carbon content was increased by 50% were supplemented as follows: *Azotobacter* spp. – 7.5 g of saccharose, *Arthobacter* ssp. – 0.5 g of glucose and 0.5 g of casein, *Bradyrhizobium* spp. (lupini) and *Rhizobium leguminosarum* bv.*viciae* – 5.0 g of mannitol. Liquid culture media contained no agar.

In order to observe the multiplication of soil bacteria, 2 cm^3 of suspensions of bacteria grown on agar slants for 96 hours were transferred to 50 cm^3 flasks containing 28 cm^3 of respective liquid media. The flasks with the media and bacterial suspensions were incubated at 28°C for 48 hours. Then cell suspension density was measured with a spectrophotometer and 1 cm^3 of the suspension was transferred to test tubes containing 14 cm^3 of a liquid medium and nickel at a specified concentration. Suspension density was measured with a spectrophotometer following 48-hour incubation at 28°C. Results were

converted into initial extinction units, according to the formula (WYSZKOWSKA, KUCHARSKI 2004):

$$E_w = \frac{E_{t1}}{E_{t0}},$$

where:
E_w – initial extinction of the bacterial culture per unit of initial extinction,
E_{t0} – initial extinction at time t_0 (beginning of culture),
E_{t1} – final extinction after time t_1 (completion of culture – 48 h).

The conversion of experimental results into initial extinction units enabled to compare bacterial strains and species, irrespective of the range of extinction of suspended cells used for culturing.

Results were verified statistically by Duncan's multiple range test and three-factorial analysis of variance. Computations were performed using Statistica software (StatSoft, Inc. 2003).

Results and Discussion

It was found that under laboratory conditions *Azotobacter* spp., *Arthrobacter* spp., *Rhizobium leguminosarum* bv. *viciae* and *Bradyrhizobium* spp. (lupini) responded negatively to increasing doses of nickel introduced into the media in the form of chloride and sulfate. This phenomenon was observed both in standard media and in media with an additional carbon source (Table 1). The negative impact of nickel was noted as soon as after the application of the lowest dose (2 mg Ni · dm^{-3}), and increased along with the increasing contamination with this metal. The 100 to 400 mg Ni · dm^{-3} doses almost completely inhibited bacterial growth. WYSZKOWSKA (2002), WYSZKOWSKA and KUCHARSKI (2004), STRZELEC and OGOŃ (1987) also reported that heavy metals introduced into growth media had a negative effect on the tested bacterial strains (*Azotobacter* spp., *Arthrobacter* spp., *Rhizobium leguminosarum* bv. *viciae, Bradyrhizobium* spp. lupini). In addition, xenobiotics (including nickel) are known for their acidifying activity: they reduce the pH of a medium thus inhibiting bacterial growth, as demonstrated by KUCHARSKI and KUCZYŃSKA-KROGULEC (1996) in experiments on *Rhizobium leguminosarum* and *Bradyrhizobium* sp. (lupini), which require a certain pH level to survive and grow. In this study a significant negative correlation was observed between nickel concentrations in the medium and the rate of cell proliferation in all tested microbes. In the medium contaminated with $NiCl_2 \cdot 6H_2O$ the correlation coefficient ranged from -0.51 for *Azotobacter* spp. to -0.75 for *Bradyrhizobium*

Table 1

Effect of nickel on bacterial proliferation in liquid media as dependent on the type of nickel compound, its dose and the type of medium, converted into initial extinction units (*Ew*)

Ni dose ($mg \cdot dm^{-3}$)	*Bradyrhizobium* sp. (lupini)		*Rhizobium leguminosarum* bv. *viciae*		*Azotobacter* spp.		*Arthrobacter* spp.	
	type of medium							
	S	S + C	S	S + C	S	S + C	S	S + C
$NiCl_2 \cdot 6H_2O$								
0	7.59	3.05	9.31	7.20	2.17	2.30	2.30	4.32
2	5.49	2.77	6.62	4.98	0.59	0.32	1.53	3.01
4	4.75	2.65	3.60	3.39	0.45	0.32	1.14	2.47
6	3.66	2.46	2.57	2.41	0.45	0.32	0.95	1.43
8	2.93	2.43	1.96	1.33	0.39	0.32	0.61	1.23
10	2.57	2.23	0.76	1.00	0.42	0.32	0.00	0.77
100	0.03	0.00	0.02	0.04	0.02	0.00	0.00	0.00
200	0.02	0.00	0.02	0.04	0.02	0.00	0.00	0.00
300	0.02	0.00	0.02	0.04	0.01	0.00	0.00	0.00
400	0.02	0.00	0.02	0.04	0.01	0.00	0.00	0.00
r	-0.75	-0.85	-0.59	-0.62	-0.51	-0.43	-0.61	-0.66
$NiSO_4 \cdot 7H_2O$								
0	3.39	3.84	13.37	8.32	1.51	2.47	2.45	4.05
2	3.15	3.36	8.95	6.50	0.00	0.37	2.21	3.82
4	2.61	3.17	3.74	2.20	0.00	0.31	1.95	3.69
6	1.62	3.05	2.20	1.55	0.00	0.31	1.50	3.56
8	1.24	2.84	1.39	1.06	0.00	0.31	1.24	3.35
10	0.00	2.63	1.42	0.77	0.00	0.31	1.10	0.71
100	0.00	0.05	0.03	0.02	0.00	0.01	0.05	0.00
200	0.00	0.04	0.03	0.02	0.00	0.00	0.04	0.00
300	0.00	0.04	0.03	0.02	0.00	0.00	0.04	0.00
400	0.00	0.03	0.03	0.01	0.00	0.00	0.03	0.00
r	-0.64	-0.85	-0.52	-0.52	-0.25	-0.42	-0.79	-0.76
$LSD_{0.01}$*	*a* – 0.09; *b* – 0.04; *c* – 0.04; *a* · *b* – 0.12; *a* · *c* – 0.12; *b* · *c* – 0.05; *a* · *b* · c – 0.17		*a* – 0.81; *b* – 0.36; c – 0.36; *a* · *b* – 1.15; *a* · *c* – 1.15; *b* · *c* – 0.51; *a* · *b* · c – 1.62		*a* – 0.05; *b* – 0.02; c – 0.02; *a* · *b* – 0.07; *a* · *c* – 0.07; *b* · *c* – 0.03; *a* · *b* · c – 0.10		*a* – 0.12; *b* – 0.05; c – 0.05; *a* · *b* – 0.17; *a* · *c* – 0.17; *b* · *c* – 0.07; *a* · *b* · c – 0.24	

S – standard medium, S + C – medium enriched with an additional carbon source;
LSD for: *a* – Ni dose, *b* – nickel compound; *c* – type of medium;
r – coefficient of correlation significant at $p < 0.01$; $n = 270$.

spp. (lupini), and in the medium contaminated with $NiSO_4 \cdot 7H_2O$ this coefficient varied from -0.25 for *Azotobacter* spp. to -0.79 for *Arthrobacter* spp. Among the bacteria analyzed, *Azotobacter* spp. was found to be most sensitive to nickel compounds, since even the lowest dose of this metal (2 mg Ni · dm^{-3}) introduced into the standard medium in the form of $NiSO_4 \cdot 7H_2O$ completely inhibited the growth of these bacteria. The negative impact of nickel was not related to medium composition modifications (Figure 1). The inhibitory activity of this metal was observed in both standard media and carbon-enriched

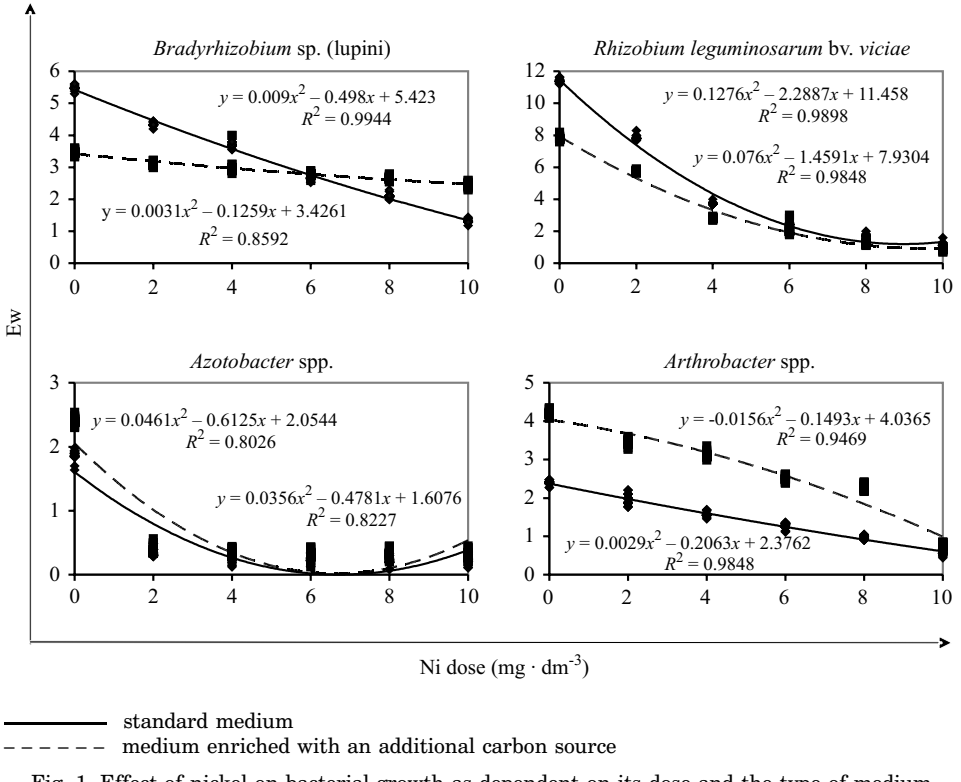

Fig. 1. Effect of nickel on bacterial growth as dependent on its dose and the type of medium

ones. The negative effect of nickel on the multiplication of soil bacteria was reflected by significant coefficients of determination between nickel concentrations in the medium and the rate of bacterial proliferation, which ranged from $R^2 = 0.7949$ for *Azotobacter* spp. in the carbon-enriched medium to $R^2 = 0.9958$ for *Arthrobacter* spp. in the medium with the optimal composition. The reason for such a strong negative impact of nickel on bacterial growth may be the fact that it blocks the functional groups of enzymes, displaces metals indispensable

for normal cell metabolism and induces conformational changes in polymers (SKŁODOWSKA 2000, SŁABA, DŁUGOŃSKI 2002). Medium enrichment with an additional carbon source did not bring the expected results, i.e. did not alleviate the negative effect of nickel on the proliferation of bacteria co-existing with legumes, i.e. *Rhizobium leguminosarum* bv. *viciae, Bradyrhizobium* spp. (lupini) and *Azotobacter* spp. A positive influence of the increased carbon content of a medium was noted only in the case of *Arthrobacter* spp.

The negative effect of nickel on the growth of bacteria in liquid media was also dependent on the type of chemical compounds (Figure 2) applied in the study. Nickel chloride more considerably inhibited the proliferation of *Rhizobium leguminosarum* bv. *viciae* and *Arthrobacter* spp., while nickel sulfate inactivated the growth of *Bradyrhizobium* spp. (lupini) and *Azotobacter* spp. to a higher degree. *Azotobacter* spp. was found to be most sensitive to nickel present in excess, followed by *Arthrobacter* spp. *Bradyrhizobium* spp. (lupini) and *Rhizobium leguminosarum* bv. *viciae* were most nickel-resistant. These results are consistent with reference data, according to which nickel causes

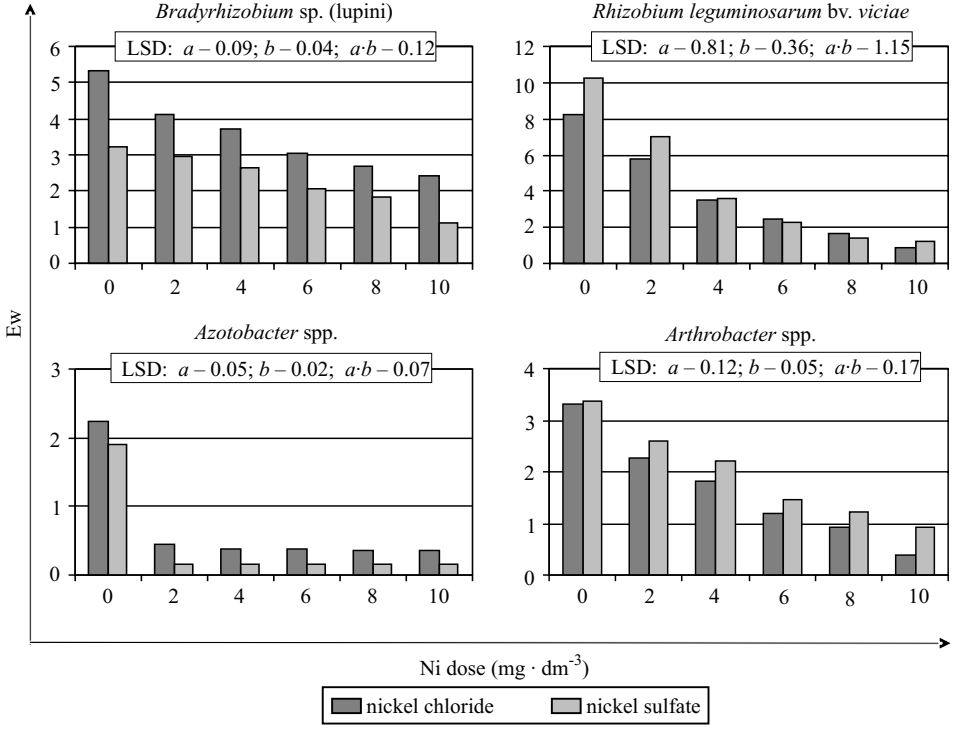

Fig. 2. Effect of nickel on bacterial growth as dependent on its dose and the type of nickel compound
LSD for: *a* – Ni dose, *b* – nickel compound

substantial changes in bacterial populations (DOSANJH, MICHEL 2006, GILLER et al. 1998, GUIBAUD et al. 2005, LIHOR et al. 2006, LOPES et al. 2005, SCHMIDT et al. 2005).

Conclusions

1. Nickel strongly inhibited the multiplication of all tested bacteria already when applied at a dose of 2 to 4 mg Ni · dm^{-3} of the medium. Doses above 10 mg Ni · dm^{-3} completely inhibited bacterial growth.

2. Taking into account their negative response to increasing levels of nickel contamination, the tested bacteria can be ordered as follows: *Azotobacter* spp. > *Arthrobacter* spp. > *Bradyrhizobium* sp. (lupini) > *Rhizobium leguminosarum* bv. *viciae*.

3. Nickel chloride more considerably inhibited the proliferation of *Rhizobium leguminosarum* bv. *viciae* and *Arthrobacter* spp., while nickel sulfate restricted the growth of *Bradyrhizobium* spp. (lupini) and *Azotobacter* spp. to a higher degree.

4. Medium enrichment with an additional carbon source had no significant effect on bacterial proliferation. A positive influence of the increased carbon content of a medium was noted only in the case of *Arthrobacter* spp.

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ECONOMIC AND ENERGY EFFECTIVENESS OF RED CLOVER SEED PRODUCTION

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K e y w o r d s: economic and energy effectiveness, red clover, seeds.

A b s t r a c t

The aim of the study was to determine the economic and energy effectiveness of seed production in red clover sown into spring barley, as dependent upon variety (diploid or tetraploid) and sowing rate. In the production technology analyzed the main cost group included machinery- and tractor-operating costs, mineral fertilizer costs and labor costs. Seeds had a relatively high share in the production value of undersown red clover. The total production value was higher in the case of diploid varieties, as compared with tetraploid forms, and ranged between 2 024.6 and 2 088.9 EUR · ha^{-1}. The energy effectiveness ratio was 2.72-3.03. Sowing rate had no significant influence on the economic effectiveness of red clover production. Red clover production in a two-year cycle was characterized by high energy effectiveness irrespective of sowing rate. Higher values of the energy effectiveness ratio were recorded in the case of tetraploid varieties.

EKONOMICZNO-ENERGETYCZNA EFEKTYWNOŚĆ UPRAWY NASIENNEJ KONICZYNY CZERWONEJ

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S ł o w a k l u c z o w e: efektywność ekonomiczna i energetyczna, koniczyna czerwona, nasiona.

A b s t r a k t

Określono opłacalność oraz wskaźniki efektywności energetycznej produkcji nasion koniczyny czerwonej w siewie z jęczmieniem jarym w zależności od diploidalnej i tetraploidalnej formy i gęstości siewu. W analizowanej technologii produkcji główne koszty były związane przede wszystkim z ek-

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sploatacją ciągników i maszyn, nawozami mineralnymi oraz nakładami pracy. Nasiona stanowiły relatywnie wysoki udział w wartości produkcji koniczyny czerwonej uprawianej jako wsiewka w jęczmień jary. Ogólna wartość produkcji była wyższa przy uprawie odmian diploidalnych niż tetraploidalnych. Kształtowała się na poziomie 2 024.6-2 088.9 Euro · ha^{-1}, a wskaźnik efektywności energetycznej wynosił 2.72-3.03. Ilość wysiewu nie miała znaczącego wpływu na efektywność ekonomiczną uprawy koniczyny czerwonej. Produkcja koniczyny czerwonej w cyklu dwuletnim sianej w jęczmień jary charakteryzowała się wysoką efektywnością energetyczną, niezależną od ilości wysiewu koniczyny. Wyższy poziom wskaźnika efektywności energetycznej stwierdzono przy uprawie odmian tetraploidalnych.

Introduction

Yield effectiveness is related primarily to biological progress (variety) and technological progress (cultivation technology). An important role is also played by market factors, such as the prices of agricultural products and means of production (CHOTKOWSKI 1995, NASALSKI 2001, SKARŻYŃSKA et al. 2005). One of the major research and analytical tasks to be undertaken in order to improve plant cultivation technologies is to optimize the process of production technology in terms of economic end energy effectiveness. One of the most synthetic measures of economic effectiveness is profitability ratio. In the case of agricultural production, this ratio may be optimized through the optimization of expenditure of human labor and machine-operating costs as well as material inputs, accompanied by technological loss reduction and rational organization of cropland expanse (KRASOWICZ 1996). Changes in the levels of outlays and crop yields affect the value of the energy effectiveness ratio.

Financial decisions should be made based on a systematic analysis of economic production conditions. Professional literature on the subject provides scant information on the cost-effectiveness analysis in red clover production. Expenditures for red clover production depend on a cultivation system. The most common method is sowing a fodder crop (red clover) into a cover crop (cereals, e.g. spring barley) (WILCZEK et al. 1999, ŻUK-GOŁASZEWSKA et al. 2006). Spring barley, grown as a cover crop, enables to increase cultivation efficiency in the first year, providing grain yield (KANKANEN et al. 2001). Potential benefits of such a cultivation regime result also from a lower disease incidence and reduced pest and weed infestation (ANIL et al. 1998). In addition, red clover sowing into a cover crop is more economical due to, among others, lower fertilizer (especially nitrogen) rates (KELLY et al. 1996).

The aim of the study was to determine the economic and energy effectiveness of seed production in red clover sown into spring barley, as dependent upon form (diploid or tetraploid) and sowing rate.

Materials and Methods

Empirical studies were conducted at the Experimental Station in Bałcyny (53°40' N, 19°50' E), in three series (2001/2002, 2002/2003 and 2003/2004). Data on crop productivity, expenditure of labor, machine-operating costs, and material inputs were collected during an exact experiment. Red clover was sown into spring barley (1st year). Spring barley seed yields were obtained in 2001, 2002 and 2003 (without straw yield), and stubble-clover yield only in 2002 (ŻUK-GOŁASZEWSKA et al. 2006). In the second year, green matter of clover was harvested from the first-cut and seeds were harvested from the second-cut. Red clover seeds were obtained from second-cut plants, while first cut of clover Mean yields of three experimental series were adopted for calculations.

Separate sowing was applied – red clover was sown after spring barley. The sowing rate of spring barley was 100 kg · ha^{-1}. The experimental factor were Polish red clover varieties – diploid (Krynia, Parada) and tetraploid (Bona, Jubilatka). Sowing rates were identical for all varieties, i.e. 4, 8, 12, 16 kg · ha^{-1}. The following fertilizer rates were applied:

– year of sowing spring barley and red clover: 60 kg · ha^{-1} N, 70 kg · ha^{-1} P_2O_5, 100 K_2O pre-sowing;

– year of red clover utilization: 30 kg N (ammonium nitrate), 70 kg P_2O_5 (boronated superphosphate), 100 kg · ha^{-1} K_2O (potassium salt).

Machine-operating costs and costs of technical equipment were determined by the cost account method (MUZALEWSKI 1998). Machinery- and tractor--operating costs constituted a sum of maintenance costs (K_{utrz}) and costs of operational use ($K_{uż}$). The unit operating cost was calculated based on the following data: current price of a tractor/machine, normative operational use during service life, service life, costs of repair, and machine performance. Maintenance costs included amortization costs, costs of storage and insurance. The unit amortization cost (EUR · h^{-1}) was calculated by dividing the price of a machine by the number of years of its operational use and the estimated number of working hours per year. The costs of storage and repair amounted to 2% per year, in relation to the purchase price of machinery. Taking into account the above items, the unit cost of machine maintenance per working hour was calculated from the following formula:

$$K_{utrz} = \frac{C_m / T + k_k \cdot C_m + U}{W_r},$$

where:

K_{utrz} – unit cost of machine maintenance (EUR · h^{-1}),

C_m – price of a machine (EUR),
T – expected service life (years),
k_k – index of storage and repair costs (% · year^{-1}),
U – insurance costs (EUR · year^{-1}),
W_r – expected number of working hours per year (h · year^{-1}).

Another group of machinery- and tractor-operating costs are costs of operational use, including costs of repair, fuels, lubricants and indirect materials. The costs of repair were calculated based on the formula:

$$K_n = \frac{k_n \cdot C_m}{T \cdot W_r},$$

where:
K_n – unit repair cost (EUR · h^{-1}),
k_n – index of repair costs (%),
C_m – price of a machine (EUR),
T – expected service life (years),
W_r – estimated number of working hours per year (h · year^{-1}).

The costs of fuels and lubricants are the dominant position in the total machinery- and tractor-operating costs. They were calculated using the formula:

$$K_p = Z_p \cdot 1.2 \cdot C_p$$

where:
K_p – unit cost of fuels and lubricants (EUR · h^{-1}),
Z_p – fuel consumption (l · h^{-1}),
C_p – fuel price (EUR · l^{-1}).

The conversion factor 1.2 takes into account the value of used lubricants in relation to the value of used fuels at a level of 20%. The unit cost of operational use ($K_{uż}$) per working hour was calculated as a sum of partial costs:

$$K_{uż} = K_n + K_p + K_{mp} \text{ (EUR} \cdot \text{h}^{-1}),$$

where:
K_n – unit repair cost (EUR · h^{-1}),
K_p – unit cost of fuels and lubricants (EUR · h^{-1}),
K_{mp} – unit cost of indirect materials (EUR · h^{-1}).

Material costs were calculated as a product of material consumption (fertilizers, seeds, etc.) and price per unit (KISIEL, KALISZEWICZ 1996). Current prices (average PLN/EUR exchanged rate of 3.8, fixed by the National Bank of Poland for the first quarter of 2006) were considered. The labor cost account included a parity rate per working hour calculated based on an average wage, assuming that a full-time worker employed in the agricultural sector works for 2200 h · year^{-1} (SKARŻYŃSKA, SADOWSKA 1998). The pricing of means of production and non-market products (regular price quotations) was based upon a comparison with market products. The loading for indirect costs (15%) was added to direct production costs. Cost-effectiveness analysis was carried out for a surface area of 1 ha. The basic measure of economic effectiveness was profitability ratio (the relationship between the production value and the total costs incurred to obtain it, expressed as a percentage).

Energy inputs were calculated as a product of means and materials used in the production process or work time, expressed as mass units and time units respectively, and corresponding to them unit cumulated energy consumption. The following energy consumption ratios were used in the study (WÓJCICKI 1981): labor – 40 MJ · l^{-1} man-hours, tractors and agricultural machinery – 112 MJ · kg^{-1}, fuels – 48 MJ · kg^{-1}, seeds – 7.5 – 10.0 MJ · kg^{-1}, green forage – 0.7 MJ · kg^{-1}, nitrogen fertilizers (N) – 77 MJ · kg^{-1}, phosphate fertilizers (P_2O_5) – 14 MJ · kg^{-1}, potassium fertilizers (K_2O) – 10 MJ · kg^{-1}. The economic effectiveness ratio was the ratio between the energy value of yield per ha and energy inputs made to produce this yield. The yield value energy was calculated as yields and some energy ratio.

Results and Discussion

Red clover seed production was based on an integrated cultivation technology. The cost analysis was performed for a two-year cycle. Various sowing rates (4 to 16 kg · ha^{-1}) caused slight fluctuations in the levels of expenditures and costs. The total cost of red clover seed production was 930.2 to 989.3 EUR · ha^{-1} (Table 1). In the years of sowing particular emphasis was placed on spring barley production. The costs incurred for this purpose in the first year ranged between 55.5 and 58.2 % of the total costs, depending on sowing rate. The highest costs were recorded at the highest sowing rate of red clover seeds (989.3 EUR · ha^{-1}).

In the year of full utilization of red clover the production results of the experiment included green matter harvest and seed harvest. In the second year the costs accounted for 41.8 to 44.5% of the total costs. The highest costs were incurred for tractor- and machine-operating, mineral fertilizers and labor.

Table 1

Costs of red clover production in a cycle (EUR · ha^{-1})

Specification	Sowing rate			
	4	8	12	16
Labor costs	158.1	158.1	158.1	158.1
Tractor- and machine-operating costs	408.9	408.9	408.9	408.9
Fertilizer costs	207.2	207.2	207.2	207.2
Other direct costs	34.7	51.8	68.9	86.1
Indirect costs	121.3	123.9	126.5	129.0
Total costs	930.2	949.9	969.6	989.3
Structure of total costs in a cycle (%):				
– year of sowing	55.5	56.5	57.4	58.2
– year of red clover utilization	44.5	43.5	42.6	41.8

Source: own calculation

Table 2

Profitability of red clover production

Specification	Sowing rate (kg · ha^{-1})			
	4	8	12	16
Diploid varieties				
Production value in a two-year cycle (EUR · ha^{-1})	2 088.9	2 072.2	2 024.6	2 088.4
Structure of production value (%):				
– red clover seeds	52.2	51.9	53.5	53.7
– red clover green matter	28.6	29.5	28.2	28.6
– spring barley seeds	19.2	18.6	18.3	17.7
Total costs (EUR · ha^{-1})	930.2	949.9	969.6	989.3
Profit (EUR · ha^{-1})	1 158.7	1 122.3	1 055.0	1 099.1
Profitability ratio (%)	2.25	2.18	2.09	2.11
Tetraploid varieties				
Production value in a two-year cycle (EUR · ha^{-1})	1 833.1	1 860.7	1 890.6	1 866.9
Structure of production value (%):				
– red clover seeds	42.4	41.5	43.6	43.5
– red clover green matter	35.8	36.9	36.8	36.7
– spring barley seeds	21.8	21.6	19.6	19.8
Total costs (EUR · ha^{-1})	930.2	949.9	969.6	989.3
Profit (EUR · ha^{-1})	902.9	910.8	921.0	877.6
Profitability ratio (%)	1.97	1.96	1.95	1.89

Source: own calculation

Similar relationships were reported by DOBEK (2004) in a study on the economic and energy effectiveness of wheat and rapeseed production.

The profitability ratio of red clover production varied from 1.89 to 2.25 (Table 2). The total production value was higher in the case of diploid varieties, as compared with tetraploid forms, and ranged between 2 024.6 (at a sowing rate of 12 kg · ha^{-1}) and 2 088.9 EUR · ha^{-1} (at a sowing rate of 4 kg · ha^{-1}). Red

clover seeds had the greatest share in this value. In diploid varieties seeds constituted (at all sowing rates) over 50.0% of the production value achieved during a two-year series. Tetraploid forms were characterized by a considerably lower (by nearly 10.0%) proportion of red clover seeds in the yield structure.

Spring barley generated a relatively low income. Based on the yield attained, the value of spring barley production accounted for 17.7 – 19.2% and for 19.6 – 21.8% of the total production value in diploid and tetraploid varieties respectively. In pure sowing the cost of spring barley production was 350 EUR · ha^{-1} (NASALSKI 2001).

A higher income per ha was generated by diploid varieties (1 055.0 – 1 158.7 EUR · ha^{-1}). Red clover seeds accounted for 51.9 – 53.7% of the total yield value. Worse economic results were achieved in tetraploid varieties (877.6 – – 921.0 EUR · ha^{-1}). STOYKOVA (2002) demonstrated that seed production of forage crops is economically and ecologically efficient. It was characterized by a net income of 358.83 to 895.87 EUR · ha^{-1}.

Production profitability ratios were comparable with the income. Over the entire experimental period, the highest mean profitability ratio was recorded in diploid forms, at a sowing rate of 4 kg · ha^{-1}. The lowest economic effectiveness was observed in tetraploid varieties, at a sowing rate of 16 kg · ha^{-1}.

Energy inputs were at a comparable level during the experimental period (23 428.4 – 23 545.4 MJ · ha^{-1}) (Table 3). Fertilizers and fuels had the highest share in their structure. In a two-year cycle higher energy inputs, related to the technique of spring barley production, were recorded in the first year.

Table 3

Energy inputs in red clover production in a cycle (MJ · ha^{-1})

Specification	Sowing rate (kg · ha^{-1})			
	4	8	12	16
Labor	2 640.0	2 640.0	2 640.0	2 640.0
Tractors and machines	1 587.4	1 587.4	1 587.4	1 587.4
Fuels	6 432.0	6 432.0	6 432.0	6 432.0
Fertilizers	11 870.0	11 870.0	11 870.0	11 870.0
Other expenditures	899.0	938.0	977.0	1 016.0
Total expenditures	23 428.4	23 467.4	23 506.4	23 545.4
Structure of energy inputs in a cycle (%):				
– year of sowing	58.5	58.6	58.7	58.8
– year of red clover utilization	41.5	41.4	41.3	41.2

Source: own calculation

The energy value of total spring barley and red clover yields was higher in the case of tetraploid varieties (Table 4). Red clover green matter had the highest, and red clover seeds the lowest proportion in the energy value

of yields. In diploid forms the percentage of red clover seeds was slightly higher. The highest energy effectiveness was observed when red clover and ryegrass were sown into spring barley in the spring (MACIEJEWSKI et al. 1998). This was reflected by the costs of energy acquisition. The lowest costs occurred at the highest energy effectiveness.

Table 4

Energy effectiveness of red clover production

Specification	Sowing rate (kg · ha^{-1})			
	4	8	12	16
Diploid varieties				
Energy value of production in a two-year cycle (MJ · ha^{-1})	67 618.5	67 103.9	63 854.0	65 412.5
Structure of production energy value (%):				
– red clover seeds	6.9	6.8	7.3	7.4
– red clover green matter	48.3	49.6	48.5	49.6
– spring barley seeds	44.8	43.6	44.2	43.0
Energy inputs (MJ · ha^{-1})	23 428.4	23 467.4	23 506.4	23 545.4
Energy surplus (MJ · ha^{-1})	44 190.1	43 636.5	40 347.6	41 867.1
Energy effectiveness ratio	2.89	2.86	2.72	2.78
Tetraploid varieties				
Energy value of production in a two-year cycle (MJ · ha^{-1})	69 337.2	71 078.0	69 437.4	68 718.0
Structure of production energy value (%):				
– red clover seeds	4.8	4.7	5.1	5,1
– red clover green matter	51.5	52.4	54.4	54.0
– spring barley seeds	43.7	42.9	40.5	40.9
Energy inputs (MJ · ha^{-1})	23 428.4	23 467.4	23 506.4	23 545.4
Energy surplus (MJ · ha^{-1})	45 908.8	47 610.6	45 931.0	45 172.6
Energy effectiveness ratio	2.96	3.03	2.95	2.92

Source: own calculation

The energy effectiveness ratios obtained in the experiment were generally high (2.72 – 3.03). Their higher levels were found in tetraploid varieties (2.92 – 3.03), as compared with diploid forms (2.72 – 2.89). Sowing rates had no influence on the energy effectiveness of production.

Conclusions

1. In Poland, the total costs of seed production in red clover sown into spring barley ranged between 930.2 to 989.3 EUR · ha^{-1}. Seeds had a relatively high share in the production value of undersown red clover. In the production

technology analyzed the main cost group included machinery- and tractor-operating costs, and mineral fertilizer costs.

2. The total production value was higher in the case of diploid varieties, as compared with tetraploid forms, and ranged between 2 024.6 and 2 088.9 EUR · ha^{-1}. The economic effectiveness of production was 23 428.4 – – 23 545.4 MJ · ha^{-1}. Sowing rate had no significant influence on the economic effectiveness of red clover production.

3. Red clover production in a two-year cycles was characterized by high energy effectiveness irrespective of sowing rate. Higher values of the energy effectiveness ratio were recorded in the case of tetraploid varieties.

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SEED YIELDS OF DIPLOID AND TETRAPLOID VARIETIES OF RED CLOVER AS DEPENDENT UPON SOWING RATE

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K e y w o r d s: red clover, seed production, bee species.

A b s t r a c t

The purpose of this study was to compare diploid and tetraploid varieties of red clover in terms of seed yield-forming factors, taking into account sowing rate. No significant differences were found in the above parameters after overwintering. Tetraploid varieties were characterized by a higher green matter yield and higher 1000 seed weight. The yield of generative parts was on average by 28% higher in diploid varieties, as compared with tetraploid ones. An increase in sowing rate, from 4 to 16 kg · ha^{-1} seeds had no significant effect on seed yield.

PLONY NASION DI- I TETRAPLOIDALNYCH ODMIAN KONICZYNY CZERWONEJ W ZALEŻNOŚCI OD ILOŚCI WYSIEWU

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S ł o w a k l u c z o w e: koniczyna czerwona, uprawa na nasiona, owady zapylające.

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Abstrakt

Celem pracy było porównanie di- i tetraploidalnych odmian koniczyny czerwonej w zależności od gęstości siewu oraz analiza wybranych cech kształtujących plon nasion. Nie wykazano istotnych różnic w zakresie badanych cech po przezimowaniu roślin. W przypadku dmian tetraploidalnych stwierdzono wyższy plon zielonki oraz wyższą masę 1000 nasion. Plon generatywny odmian diploidalnych był średnio o 28% wyższy od plonu odmian diploidalnych. Zwiększenie ilości wysiewu z 4 do 16 kg · ha^{-1} nasion nie wpływało istotnie na wysokość ich plonu.

Introduction

In Poland red clover is considered a valuable fodder crop, so it is rarely grown for seed. Due to numerous problems encountered by producers, the production of red clover seeds is gradually declining (ARSENIUK and OLEKSIAK 2002, OLEKSIAK 2003). The decline in seed production observed in Poland is related to an imperfect seed reproduction system and seed unavailability on the market. The soil area under red clover grown for seed decreased over thirteen-fold during the years 1988-1999 (BRONIARZ 2000, NOWAK et al. 1999). In addition, the seed yields of this species are generally low, unstable and highly dependent on weather conditions and the numbers of pollinators, which is another reason for the lack of interest in red clover seed production (SĄDEJ et al. 2005).

The yielding potential of red clover is much greater than suggested by the seed yields attained. Both yielding stability and yield height are affected by a variety of factors, such as regionalization, cultivation system, plantation management strategies, habitat, agricultural practices, fertilization, crop cultivation, and biological progress (OLIVA et al. 1994a,b, STEINER et al. 1995, 1997, STEINER and ADERMAN 2003, WILCZEK et al. 1999).

According to many researchers, the key determinants of high and stable yields of red clover seeds are weather conditions, especially during flowering (WILCZEK et al. 2000, WILCZEK and ĆWINTAL 2003), followed by the numbers and activity of pollinators and the method of flower pollination (BORISOV et al. 2005, JABŁOŃSKI 2001, KLEPACZ-BANIAK and CZEKOŃSKA 2005, LOKUTOVA et al. 2005, SĄDEJ et al. 2005, STEINER et al. 1997). These factors, in most cases objective, are accompanied by the quality of available varieties and agronomic factors, including sowing rate (RYBAK et al. 1994, WILCZEK et al. 2000).

Seed yields are also modified by meteorological and habitat conditions. During dry years higher yields are usually attained on fertile soils, whereas during wet years – on sandy-loamy soils (WILCZEK et al. 2000). The process of seed setting is considerably influenced by weather conditions during flowering, since temperature and access to light over this period affect flower abundance, duration of flowering, pollination rate, and seed quality. WILCZEK (1999) and

JABŁOŃSKI (2001) reported that high temperatures and a good solar exposure of a plantation attract more pollinators, and that short corolla tubes are accessible to a broader spectrum of pollinators, including bees.

Trifolium pratense is a major food component for bumblebees (*Bombus* Latr.), which therefore are very effective pollinators of this species (JABŁOŃSKI 2001, SĄDEJ et al. 2003). Bumblebees have high body weight and are strongly built. Long-tongue species are the most effective pollinators of red clover. The honeybee *Apis mellifera* L. and wild bees are of lesser importance (JABŁOŃSKI 2001).

The aims of this study were as follows: (1) to determine whether under climate conditions of NE Poland the red clover forms with various ploidity levels and the agronomic factor – sowing rate significantly affect seed yields; (2) to determine the relationships between green matter yields and seed yield; (3) to analyze the variety x sowing rate interaction as dependent upon weather conditions over the three successive vegetation periods, in order to find the optimum variant of this interaction, enabling to achieve the maximum red clover seed yield.

Materials and Methods

The results of long-term experiments performed on red clover by the Department of Plant Production during the years 2001-2004 at the Experimental Station in Bałcyny, provided the basis for this study.

The experiment was established on pseudopodsolic soil of quality class IIIa, developed from light loam of very good rye complex 4. In the years when the experiment was laid out, the cover crop for red clover was spring barley grown after winter wheat.

The experiment involved combinations of two experimental factors, i.e. diploid (Krynia, Parada) and tetraploid (Bona, Jubilatka) varieties, and sowing rate (4, 8, 12 and 16 kg · ha^{-1} seeds), and was performed in a split-plot design. Red clover seeds were sown in the spring, with a drill, at the spacing of 10 cm; plot area was 14.4 m^2. Pre-plant soil treatment was typical of spring barley grown as a cover crop for red clover. In the 1st year plants were applied at the following rates: 30 kg · N ha^{-1} (ammonium nitrate), 70 kg · ha^{-1} P_2O_5 (20% boronated superphosphate) and 60 kg · K_2O (potassium salt). The first cut of clover was taken for green matter yield. Second cut plants were grown for seeds. The plants were harvested when 85% of red clover heads on the plantation reached maturity.

Root crown thickness, dry matter content of the rosette and roots, and leaf greenness index were determined after winter 2002. During all years, 20 plants

of the second cut were collected randomly of each plot before harvest, to determine their morphological characters and yield components, including plant height, number of shoots per plant, root crown thickness before harvest, number of heads per plant, 1000 seed weight and seed yield. The numbers of representatives of the family *Apidae* were determined during red clover flowering.

The results were verified statistically by an analysis of variance for split-plot designs with years of study as the additional source of variation. The significance of differences between means was estimated by the Tukey's T test. Diploid and tetraploid varieties of red clover and their interactions with sowing rate were compared using orthogonal contrasts.

Weather conditions and densities of members of the family *Apidae* on the plantation

In the first year of red clover growing (2002) mean monthly temperatures were higher than mean multiannual temperatures, and precipitation total during the growing season was lower than or comparable to mean multiannual precipitation. The red clover seed yield recorded in 2002 was the highest, and ranged 518.87 kg · ha^{-1}. The growing season was warm and dry, which contributed to intensive flowering and encouraged members of the family *Apidae* to visit the plantation (Table 1, Figure 1). The total mean density of all pollinators was 4333 insects ha^{-1}, including 3167 bumblebees (*Bombus*) · ha^{-1}, 833 honeybees (*Apis mellifera*) · ha^{-1}, and 333 solitary bees · ha^{-1}.

Table 1

Mean daily air temperatures and precipitation over the experimental period as compared with mean multiannual temperatures and precipitation (Meteorological Station in Bałcyny)

Month	Mean daily air temperature (°C)				Precipitation (mm)			
	2002	2003	2004	multiannual average 1961-2000	2002	2003	2004	multiannual average 1961-2000
January	-1.1	-3.8	-6.3	-3.5	41.8	14.1	28.9	27.4
February	2.9	-5.2	-0.3	-2.6	54.5	6.0	60.7	21.6
March	3.5	1.4	3.0	1.2	37.0	11.8	28.2	28.5
April	7.3	6.1	8.9	6.6	10.0	23.6	51.5	35.4
May	16.1	14.2	11.8	12.4	90.1	78.6	87.1	57.6
June	15.9	16.5	15.3	15.7	72.5	60.7	90.6	69.5
July	19.3	18.9	17.0	16.9	43.2	118.2	78.8	81.6
August	19.8	17.3	19.2	16.5	87.3	34.9	89.3	75.2
September	12.5	13.7	14.1	12.6	60.5	19.1	41.9	59.0
October	6.4	4.8	–	8.1	143.5	66.1	–	53.5
November	3.1	5.0	–	2.8	28.2	39.4	–	48.9
December	-6.6	1.3	–	-1.3	6.8	48.6	–	41.8

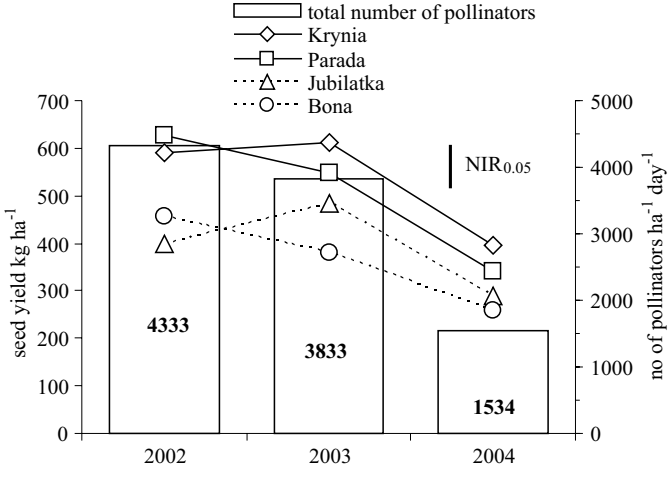

Fig. 1. Seed yields of particular red clover varieties versus the numbers of pollinators of the family *Apidae* (bumblebees, honeybees and solitary bees)

In the study conducted by JABŁOŃSKI (2001) *Bombus* Latr., *Apis mellifera* L. and solitary bees accounted for 82.2%, 17.7% and 0.1%, respectively, of all insects pollinating red clover. In other studies (KLEPACZ-BANIAK, CZEKOŃSKA 2005) one of pollen plants visited most frequently by *Apis mellifera* L. in the summer was red clover (*Trifolium* – 6%).

The temperatures recorded in 2003 were higher than mean multiannual temperatures, and their distribution was similar to that noted in the previous year. Greater disproportions were observed between the total precipitation recorded in 2003 and mean multiannual precipitation; July was wet, but in August and September precipitation was light. It was dry and sunny during the summer months, the plants were flowering and the plantation was eagerly visited by pollinators. The total number of members of the family *Apidae* was 3833. Bumblebees were the dominant group – 3233 · ha^{-1}, with only 367 honeybees and 233 solitary bees · ha^{-1} (Figure 1).

The year 2004 was extremely wet and cold. A wet winter was followed by heavy precipitation in April and in subsequent months of the growing season. In May and June precipitation amounted to 87.1 and 90.6 mm respectively, and was much higher than mean multiannual precipitation (56.7 and 68.3 mm). Heavy rainfall in July and August resulted in a longer flowering period and low yields. The mean temperature registered in 2004 was similar to the mean temperature of forty years, but a relatively cold and wet summer was not conducive to pollinator flights and seed setting. In this year the total number of pollinators was 1534, including 1167 bumblebees ha^{-1}, 267 honeybees · ha^{-1} and

only 100 solitary bees ha^{-1}. Flowering patterns were non-uniform, and the flowering period lasted until pod setting. The seed yield was approximately 321.94 $kg \cdot ha^{-1}$.

The above data show that in 2002 and 2003 the densities of pollinators exceeded the numbers that guarantee the economic profitability of growing red clover for seeds, whereas in 2004 their density was much too low (SĄDEJ et al. 2005).

Results and Discussion

The plants entered successive developmental stages almost simultaneously, regardless of variety and sowing rate. In second-cut plants the duration of the growing season depended on meteorological conditions. In 2002 the growing season lasted for 78 days, and in 2004 – for 99 days (Table 2).

Table 2

Major developmental stages of red clover in particular years of the study

Developmental stages	2002	Day number	2003	Day number	2004	Day number
Beginning of the growing season – rosette development	29 March – – 22 April	25	25 March – – 24 April	32	18 March – – 25 April	29
Stem development, leaf development, budding	23 April – – 26 May	34	25 April – – 1 June	38	26 April – – 2 June	38
1st cut of red clover	27 May (beginning of flowering)	1	2 June (beginning of flowering)	1	3 June (middle of bud--formation)	1
Rosette formation	28 May – – 05 June	9	3-14 June	12	4-17 June	14
Bud formation	6 June – – 15 July	30	15 June – – 10 July	26	18 June – – 17 July	30
Flowering	16-29 July	14	11 July – – 1 August	22	18 July – – 16 August	30
Seeds setting, maturity	30 July – – 22 August	24	2 August – – 4 September	34	17 August – – 9 September	24
Harvest	23 August	1	5 September	1	10 September	1
Vegetative period (day)		138		166		167

Lower precipitation total and higher temperatures in 2002 and 2003 resulted in a shorter growing period of red clover grown for seed. Whereas heavy rainfall in 2003 (July) and 2004, lower temperatures in 2004 extended

the growing season in these years. In 2002 first-cut plants were harvested as early as on May 27. In subsequent years they were cut at the beginning of June. The flowering period lasted from 14 days in 2002, 22 days in 2003 to 30 days in 2004.

Table 3

Values of red clover parameters in the spring, after winter 2002

Form	Variety	Sowing rate ($kg \cdot ha^{-1}$)	Root crown thickness (mm)	Dry matter content of the rosette (g)	Dry matter content of roots (g)	Leaf greenness index SPAD
Diploid	Krynia	4	13.75	0.66	1.19	39.72
		8	11.50	0.93	0.50	36.62
		12	9.62	0.62	0.68	38.05
		16	12.00	0.87	0.67	40.15
		mean	11.71	0.99	0.76	38.63
	Parada	4	10.50	0.87	0.73	26.47
		8	11.62	1.18	1.25	37.76
		12	13.62	0.78	0.67	30.17
		16	11.00	0.05	0.96	35.75
		mean	11.68	0.97	0.90	32.51
Tetraploid	Bona	4	11.50	1.42	1.05	35.32
		8	13.87	1.02	0.90	33.27
		12	12.87	0.63	0.61	36.27
		16	10.12	0.88	0.81	28.10
		mean	12.09	0.99	0.84	33.24
	Jubilatka	4	13.37	1.88	1.50	32.47
		8	12.62	0.92	0.77	35.82
		12	14.00	0.10	0.77	33.42
		16	12.00	0.73	0.78	29.27
		mean	13.00	1.16	0.96	32.75
Means for sowing rate		4	12.28	1.26	1.12	33.50
		8	12.40	0.94	0.86	35.85
		12	12.53	0.86	0.68	34.48
		16	11.28	0.82	0.81	33.31

The characters of diploid and tetraploid red clover plants, grown at various spacing, determined after overwintering, such as root crown thickness, dry matter content of the rosette and roots and leaf greenness index, did not differ significantly. Even considerable differences between them remained within experimental error (Table 3). The mean root crown thickness was 11.7 mm in the diploid varieties Krynia and Parada, and 12.1 mm and 13.0 mm in the tetraploid varieties Bona and Jubilatka respectively. This parameter was generally not affected by plant density per unit area, but a tendency towards a decrease in root crown thickness was observed at the highest sowing rate

(16 kg · ha^{-1}). The dry matter content of the rosette and roots ranged in the varieties analyzed from 0.97 to 1.16 g, and from 0.70 to 0.90 respectively. The mean values of these parameters obtained for various plant density exhibited the opposite tendency to that observed in the case of root crown thickness – the highest dry matter content of the rosette and roots was recorded at the lowest sowing rate (4 kg · ha^{-1}). Chlorophyll content (leaf greenness index, SPAD) varied widely from 32.47 to 38.63, but both the lower and higher values of this parameter indicated a relatively good nutritional status of plants.

Table 4

Green matter yields (t · ha^{-1}) of red clover varieties as dependent on sowing rates during the years 2002-2004 (1st cut of red clover)

Form	Variety	Sowing rate (kg · ha^{-1})	2002	2003	2004	Mean
			Year x variety x sowing rate (nsd)			variety x sowing rate (nsd)
Diploid	Krynia	4	25.9	38.5	52.3	38.9
		8	36.5	42.7	51.4	43.5
		12	26.5	40.1	49.8	38.8
		16	28.1	43.5	51.0	40.8
	Parada	4	33.1	43.6	53.7	43.5
		8	33.3	44.1	45.6	41.0
		12	32.0	44.6	44.8	40.5
		16	34.0	44.3	47.1	41.8
Tetraploid	Bona	4	31.6	48.7	61.2	47.2
		8	36.0	45.9	63.2	48.4
		12	37.6	49.2	61.0	49.3
		16	36.2	46.7	63.9	49.0
	Jubilatka	4	33.8	42.3	55.4	43.8
		8	36.5	43.4	62.9	47.6
		12	41.3	32.6	61.8	45.2
		16	36.4	42.6	64.6	47.9
			Year x variety, LSD_{LxO} = 8.59			variety LSD_O = 3.80
	Krynia		41.2	51.1	43.4	40.5
	Parada		44.1	47.8	44.9	41.7
	Bona		47.6	62.3	43.3	48.4
	Jubilatka		40.2	61.2	45.1	46.1
			Year x sowing rate, LSD_{LxG} = 9.44			sowing rate (nsd)
		4	31.1	43.2	55.6	35.4
		8	35.6	44.0	55.8	37.0
		12	34.4	41.6	54.4	29.2
		16	33.7	44.3	56.7	33.1
			Year (LSD_L = 6.85)			
			33.7	43.3	55.6	

Table 5

Morphological characters of plants, seed yield components and red clover seed yield

Research year, form, variety	Sowing rate ($kg \cdot ha^{-1}$)	Plant height (cm)	Number of shoots per plant	Root crown thickness before harvest (mm)	Number of heads per plant	1000 seeds weight (g)	Seed yield ($kg \cdot ha^{-1}$)
2002		99.08	4.86	13.58	11.08	2.24	518.87
2003		97.53	3.49	8.73	12.97	1.93	506.29
2004		127.29	7.23	11.64	14.98	2.39	321.94
$LSD_{0.05}$ for years		7.94	2.93	2.66	2.66	0.12	87.55
di-, Krynia	4	101.83	6.61	15.77	21.52	1.83	530.65
	8	105.08	5.52	12.45	12.97	1.76	534.12
	12	103.16	4.81	14.25	12.94	1.81	509.99
	16	107.84	4.63	12.58	9.19	1.65	559.01
	mean	104.48	5.39	13.76	14.15	1.77	533.44
di-, Parada	4	111.35	5.69	13.04	19.00	1.88	505.77
	8	109.59	4.97	11.12	10.94	1.89	488.41
	12	10.50	3.98	13.41	10.95	1.89	521.97
	16	101.10	4.33	11.45	8.75	1.87	505.77
	mean	106.64	4.74	12.26	12.41	1.89	505.48
tetra-, Bona	4	109.09	4.41	15.62	14.22	2.77	358.20
	8	111.75	5.25	13.50	13.91	2.66	345.47
	12	115.59	4.43	14.27	11.70	2.70	379.61
	16	111.86	10.78	13.79	13.72	2.65	380.77
	mean	112.07	6.21	14.29	13.39	2.70	366.01
tetra-, Jubilatka	4	108.10	5.77	14.83	13.44	2.35	383.09
	8	107.67	4.55	14.62	13.00	2.44	388.87
	12	109.55	3.38	13.58	10.63	2.44	402.76
	16	109.38	3.97	12.95	11.25	2.34	402.76
	mean	108.67	4.42	14.00	12.08	2.39	391.18
$LSD_{0.05}$ for varieties		5.64	ns	1.51	ns	0.11	39.78
Contrasts (significant at $p \leq 0.05$)							
Forms, varieties							
di- vs. tetra-		-9.63	ns	ns	ns	-1.44	281.7
Krynia vs. Parada		ns	ns	ns	ns	0.12	ns
Bona vs. Jubilatka		ns	ns	ns	ns	-0.31	ns
Sowing rate							
linear effect		ns	ns	-5.41	-20.1	ns	ns
Form x sowing rate							
di- vs. tetra- x linear effect		ns	ns	ns	55.1	ns	ns
Krynia vs. Parada x linear effect		-51.92	ns	ns	ns	ns	ns

ns – non significant differences and contrasts

Red clover plants were cut at the onset of flowering (2002, 2003) or in the middle of bud formation (2004), due to various responses of varieties to weather conditions in particular years of the study. The year x variety interaction was significant and impacted the green matter yield of red clover (Table 4). The highest green matter yield was achieved in 2004. It was by almost 65% higher than that obtained in 2002. Tetraploid varieties produced higher yields than diploid forms (Bona – 48.4, Jubilatka – 46.1 t · ha^{-1}). Var. Krynia provided the lowest green matter yield (40.5 t · ha^{-1}). Both experimental factors, i.e. variety and sowing rate, were modified by weather conditions.

Diploid (Krynia and Parada) and tetraploid (Bona and Jubilatka) varieties of red clover grown for seed differ significantly in plant height, 1000 seed weight and yield (Table 5). Plants of the tetraploid variety Bona were the tallest (112.07 cm), irrespective of sowing rate, while plants of the diploid variety Krynia were the shortest. The mean height difference between diploid and tetraploid red clover plants was 9.63 cm. It should be noted that the interaction: variety x linear effect of sowing rate was significant in diploid forms, since plants of var. Krynia were taller, and plants of var. Parada – shorter at a higher sowing rate (Figure 2a).

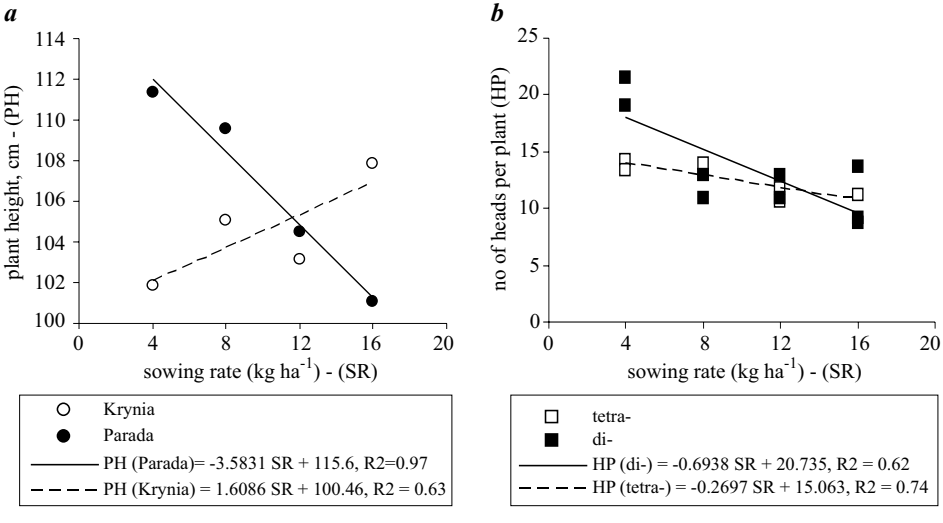

Fig. 2. Graphical interpretation of the significant interaction variety x sowing rate in the estimation of plant height (*a*) and the number of heads per plant (*b*) linear regression between sowing rate per ha and the number of heads per plant in diploid and tetraploid forms of red clover

Sowing rate had no effect on the number of shoots per plant, which confirmed the ability of red clover to "control" stand density. At high stand density plants are weaker and produce fewer shoots, whereas at low stand

density plants are stronger and produce more fertile shoots (ŚCIBOR and BAWOLSKI 1994, WILCZEK 1986).

Var. Bona had the highest 1000 seed weight (2.70 g), and var. Krynia – the lowest (1.77 g). Particular attention should be paid to the great contrast in this parameter (-1.44) to the advantage of tetraploid varieties, and to the opposite relationship in seed yield, at an average level of +281.7 kg · ha^{-1}, to the advantage of diploid varieties. Among the varieties tested, Krynia provided the highest yield (533.44 kg · ha^{-1}), despite the finest seeds. This variety produced on average 5.39 shoots and set the greatest number of inflorescences (14-15). The tetraploid variety Bona produced the lowest seed yield – on average 366.01 kg · ha^{-1}. These results correspond to the findings of TOMASZEWSKI-JUNIOR (1989). In his experiment seed yields were by 30% to 60% higher in diploid varieties than in tetraploid varieties. In the study performed by WILCZEK and WILCZEK (2002) red clover seed yields ranged from 347 to 533 kg · ha^{-1}, and were affected primarily by weather conditions during flowering and ripening. The lower productivity of tetraploid varieties results from less intensive flowering and a lower number of inflorescences per plant (GÓRAL and SPISS 1992). In our study the number of heads per plant was similar in all red clover varieties (12-14). Taking into account the average effect of both forms, among the traits analyzed only root crown thickness and the number of heads per plant exhibited an inverse linear correlation with increasing sowing rates. In addition, a significant effect of the interaction: variety x linear effect of sowing rate was noted for the number of heads per plant (Figure 2). The number of heads per plant decreased along with increasing sowing rates; an increase in sowing rate from 4 to 16 kg of seeds ha^{-1} was followed by a decrease in the mean number of heads per plant, by 8.4 and 3.2 in diploid and tetraploid varieties respectively.

Sowing rate had no significant effect on seed yields. The highest red clover seed yield was attained in the year 2002, characterized by sunny weather during flowering, and the lowest – in the wet year 2004. These years differed significantly in precipitation total over the period from the harvest of first-cut plants to flowering, which affected the numbers of pollinators and seed yields. The results of this experiment are consistent with those obtained by WILCZEK et al. (2000). Sowing rate had no considerable influence on seed yields, which were significantly affected by 1000 seed weight and the number of seeds per head. RYBAK et al. (1994) reported that red clover seed yields were highly dependent on rainfall total during the growing of second-cut plants for seed. The optimum sowing rate was 4 kg · ha^{-1}. According to NOWAK et al. (1999), when red clover is grown for seed sowing rate should be reduced to 6 kg/ha (at the row spacing of 12 cm), and according to RYBAK et al. (1994) – even to 4 kg · ha^{-1}.

RYBAK et al. (1993) observed a correlation between the green matter yield and the seed yield of red clover – a higher green matter yield was accompanied by a lower seed yield. MAUNTEAN and SAVATTI (2003) demonstrated a negative correlation between a seed yield and green matter yield. In our study the coefficients of correlation and simple regression for red clover varieties varied depending on sowing rate.

Table 6

Coefficients of correlation (r_{xy}) and simple regression ($b_{y/x}$) between the yields of vegetative parts (green matter) and generative parts (seed weight) for diploid and tetraploid forms of red clover at various sowing rates

Sowing rate (kg · ha^{-1})	Diploid forms		Tetraploid forms	
	r_{xy}	$b_{y/x}$	r_{xy}	$b_{y/x}$
4	0.099	0.96	-0.291	-3.15
8	0.101	1.15	0.267	4.09
12	-0.713	-8.08	-0.154	-1.45
16	-0.089	-0.81	-0.831	-15.94

Strong negative relationships between green matter yield and seed yield were recorded only in the variant with a sowing rate of 12 kg · ha^{-1} of seeds in diploid forms, and in that with a sowing rate of 16 kg · ha^{-1} of seeds in tetraploid varieties. The values of regression coefficients show that an increase in green matter yield by one ton per ha was followed by a decrease in seed yield, by 8.08 and 15.94 kg · ha^{-1} of seeds in diploid and tetraploid varieties respectively (Table 6).

Conclusions

1. There is a close relation between weather conditions during flowering and the numbers of members of the family *Apidae*, which are the main determinants of red clover yield variability in the climatic conditions of a particular year. The best weather conditions were observed in 2002 and 2003. Over this period mean daily air temperatures were 19.3°C and 15.3°C, rainfall totals were 9.2 mm and 33.4 mm, and the number of rainy days was 4 and 8 days respectively.

2. The values of such red clover characters as root crown thickness, dry matter content of the rosette and roots, and leaf greenness index, determined after winter 2002, were comparable regardless of variety and sowing rate. Red clover seed yields were dependent on precipitation during flowering to a higher degree than on variety or sowing rate. The yields were as follows: 518.87 kg · ha^{-1} in 2002, 506.29 kg · ha^{-1} in 2003 and 321.94 kg · ha^{-1} in 2004.

3. At a sowing rate exceeding 8 kg · ha^{-1}, red clover seed yields may be negatively correlated with green matter. An increase in green matter yield by 1 t · ha^{-1} is followed by a decrease in seed yield, by about 8 and 16 kg of seeds per ha in diploid and tetraploid varieties respectively. In addition, increased sowing rates may significantly reduce the number of heads per plant; this reduction is higher in diploids than in tetraploids.

4. A sowing rate ranging between from 4 to 16 kg · ha^{-1} had no significant effect on the seed yield in diploid and tetraploid red clover. The yield is approximately 30% higher in diploid varieties than in tetraploid ones.

Translated by authors

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EFFECTS OF SODIUM SACCHARINATE AND VANILLIN ADDED TO DIETS FOR YOUNG GROWING PIGS (20-40 KG OF LIVE WEIGHT) ON THEIR PERFORMANCE

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K e y w o r d s: young growing pigs, sodium saccharinate, vanillin, feed flavors, feed additives, taste, aroma.

A b s t a r c t

Three complete cereal-soybean grower diets with an approximate total protein content of 17.8% ere analyzed: a control diet (1), an experimental diet supplemented with 100 $g \cdot t^{-1}$ of sodium saccharinate (2) and an experimental diet supplemented with 200 $g \cdot t^{-1}$ of vanillin (3). A 28-day growth test was conducted. The experiment was performed on 18 crossbred pigs (♀ Polish Landrace x ♂ Duroc) aged seven weeks, with mean initial body weights of 17.5 kg. The animals were assigned to three feeding groups by the analogue method (taking into account their age, body weight and litter of origin). They were placed in individual flat-deck cages equipped with automatic feeders and nipple drinkers. The pigs were fed friable feed *ad libitum*. Feed intake was monitored daily. The basic growth parameters of the animals were determined. The pigs were weighed individually on day 1, 14 and 28.

It was found that sodium saccharinate and vanillin had no effect on the intake on experimental diets. However, the utilization of the diets containing these additives was highly significantly better, compared with the control diet.

WPŁYW DODATKU SACHARYNIANU SODU I WANILINY DO MIESZANEK STOSOWANYCH W ŻYWIENIU WARCHLAKÓW (20-40 KG MASY CIAŁA) NA WYNIKI ICH ODCHOWU

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S ł o w a k l u c z o w e: warchlaki, sacharynian sodu, wanilina, dodatki aromatyczne, dodatki paszowe.

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Abstrakt

Badano 3 zbożowo-sojowe mieszanki pełnoporcjowe typu grower o zakładanej zawartości białka ogólnego 17,8%: mieszankę kontrolną (1), doświadczalną (2) z dodatkiem 100 $g \cdot t^{-1}$ sacharynianu sodu oraz mieszankę (3) zawierającą 200 $g \cdot t^{-1}$ waniliny. Przeprowadzono test wzrostowy trwający 28 dni. Materiał zwierzęcy stanowiło 18 warchlaków mieszańców (♀ polska biała zwisłoucha x ♂ duroc), w wieku 7. tygodni i średniej początkowej masie ciała 17,5 kg. Zwierzęta dobrano do trzech grup żywieniowych metodą analogów (biorąc pod uwagę ich wiek, masę ciała i pochodzenie z miotu). Warchlaki umieszczono indywidualnie w klatkach typu flat-deck, wyposażonych w autokarmniki i poidła smoczkowe. Mieszanki w formie sypkiej zadawano *ad libitum*. Codziennie kontrolowano ilość pobranej paszy. Określono podstawowe parametry wzrostu warchlaków. Ważenia kontrolne wykonano w 1., 14. i 28. dniu doświadczenia.

Nie stwierdzono wpływu sacharynianu sodu i waniliny na wzrost spożycia diet doświadczalnych. Stwierdzono jednak, że mieszanki z udziałem badanych dodatków były przez zwierzęta wykorzystywane wysoko istotnie lepiej w porównaniu z dietą kontrolną.

Introduction

In order to make pigs consume more feed, complete diets are sometimes supplemented with additives improving their taste and aroma. Diet palatability is of primary importance when newly-weaned piglets are getting used to larger amounts of solid feed. In older animals it may facilitate changing from one kind of feed to another. The daily intake of feed, as well as production results, are also directly related to diet palatability. Feed additives flavors, like sodium saccharinate and vanillin, are also added to diets whose components (e.g. oilseed rape derivatives, pulses), although valuable, may reduce palatability (BUGNACKA et al. 2005, DURAN et al. 2002). According to MCLAUGHLIN et al. (1983), piglets show a decided preference for a sweet, fruity and cheesy taste. However, the results of studies on the use of feed flavors in pig nutrition are inconsistent (ALDINGER et al. 1961, ALBRECHT, MULLER 1972, WAHLSTROM et al. 1974, OCHETIM, ODUR 1983, SHKUNKOWA, KRASKO 1984, SLESAREV et al. 1986, DURAN et al. 2002). SHKUNKOWA and KRASKO (1984) reported a positive effect of saccharine added in the amount of 0.05 $g \cdot kg^{-1}$ to diets for piglets. The animals consumed by 8.1% more sweetened feed, as compared with the control diet, without this additive. In a free-choice feed preference test carried out by WAHLSTROM et al. (1974), weaners preferred a cereal-soybean mixture containing 10% dried whey supplemented with 5% saccharose to the control diet. Its proportion in the total daily feed intake was as high as 80.7%. However, these results were not confirmed by another palatability test performed by these authors on the same kind of feed, by the single stimulus method – the intake of diets with and without saccharose was at the same level. Also, a free-choice feed preference test carried out by ALBRECHT and MULLER (1972) on young growing pigs fed a diet with 0.05% saccharine showed that this additive had no effect on the mean daily feed intake. However, the studies conducted in Spain

(ROURA, FONTANILLAS 2002, 2003, TORRALLARDONA et al. 2000) indicated high efficiency of feed flavor and aroma additives used in diets for suckling piglets and weaners (i.e. wild fruits, strawberry, wild strawberry and cherry-honey flavors).

The aim of the present study was to determine the effects of sodium saccharinate and vanillin added to diets for young growing pigs on their production results.

Materials and Methods

Three complete cereal-soybean grower diets with an approximate total protein content of 17.8% were analyzed: a control diet (1), an experimental diet supplemented with 100 $g \cdot t^{-1}$ of sodium saccharinate (2) and an experimental diet supplemented with 200 $g \cdot t^{-1}$ of vanillin (3) – Table 1. All diets were supplemented with synthetic lysine and methionine, according to *Nutrient Requirements of Pigs* (1993). Nutrient contents in the diets were analysed according to the Weende method (Table 1).

The 28-day experiment was performed on the experimental farm of the Department of Pig Breeding, University of Warmia and Mazury in Olsztyn, on 18 crossbred (♀ Polish Landrace x ♂Duroc) pigs aged seven weeks, with mean initial body weights of 17.47 kg. The animals were allocated to the three feeding groups according to the analogue method (taking into account their age, body weight and litter of origin). They were placed in individual 0.7 m x 1.2 m flat-deck cages with a slatted floor 1 m over a concrete floor, equipped with automatic feeders and nipple drinkers. The pigs were fed friable feed *ad libitum*. Feed intake was monitored daily. The basic growth parameters of the animals were determined. The pigs were weighed individually on day 1, 14 and 28.

The results were verified statistically by one-factor analysis of variance. The calculations were performed using the Statistica for Windows software.

Results and Discussion

The chemical composition of diets is given in Table 1. Nutrient levels satisfied the requirements concerning such mixtures. The total protein content of diets amounted to 18.2% and was close to the assumed level (Table 1).

The initial body weights of pigs of three feeding groups were 17.7, 17.3 and 17.5 kg respectively (Table 2). The growth rate of animals was very high over

Table 1

Composition and nutritive value of the experimental diets for young pigs

Indices	Control diet (1)*
Ingredients, %	
ground wheat	33.00
ground barley	40.00
soybean meal	21.00
rapeseed oil	1.00
dicalcium phosphate	1.50
limestone	1.50
PP – grower premix	1.50
salt (NaCl)	0.30
L – lysine (99%)	0.20
Chemical composition, %	
dry matter	86.77
crude protein	18.2
crude fat	1.12
crude fibre	4.07
crude ash	5.79
N-free extractives	57.59
organic matter	80.98
Metabolizable energy, MJ · kg^{-1} (calculated)	12.60

* in diet (2) – 0.01 of sodium saccharinate was added
in diet (3) – 0.02 of vanillin was added

Table 2

Growth performance of experimental pigs

Specification		Period of experiment (days)		Diets		
				1	2	3
Average initial body weight	(kg)	1	x	17.7	17.3	17.5
			s	1.97	1.85	1.96
Average daily gain	(g)	1-14	x	698^{B}	757^{B}	899^{A}
			s	125.1	81.2	114.7
		15-28	x	961^{A}	830^{B}	901
			s	75.4	77.6	92.9
		1-28	x	829	795	900
			s	113.9	71.5	86.5
Average daily feed intake	(kg)	1-14	x	1.57^{A}	1.45^{B}	1.63^{A}
			s	0.334	0.297	0.347
		15-28	x	2.18^{A}	$1.78^{B,D}$	$2.04^{B,C}$
			s	0.308	0.239	0.293
		1-28	x	1.87^{A}	1.61^{B}	1.84^{A}
			s	0.443	0.316	0.385
Feed/gain ratio	(kg · kg^{-1})	1-14	x	2.25^{B}	1.92^{A}	1.81^{A}
			s	0.446	0.519	0.559
		15-28	x	2.27^{B}	2.14^{A}	2.26^{B}
			s	0.249	0.331	0.364
		1-28	x	2.26^{B}	2.03^{A}	2.05^{A}
			s	0.430	0.513	0.575

A, B – α = 0.01

the entire experimental period, which was reflected by high mean daily gains – from 795 g in group 2 to 900 g in group 3. Our results correspond to those obtained previously in the same experimental pig house (BUGNACKA, FALKOWSKI 2001). There were statistically significant differences in mean daily gains between the groups. At the first stage of the experiment (1 to 14 days) highly significantly higher mean daily gains (899 g) were recorded in group 3, fed a vanillin-supplemented diet. At the second stage of the study (15 to 28 days) the highest growth rate was observed in the control group. However, these differences had no effect on the level of this parameter during the entire experimental period.

High statistical differences were also found in mean daily feed intake and conversion (Table 2). The pigs consumed considerably larger amounts (by about 13%) of diet 1 (control) and diet 3 (containing vanillin), in comparison with diet 2 (containing sodium saccharinate). Figure 1 presents mean daily feed intake per pig on successive days of the experiment. At the beginning of the study (1 to 6 days) a tendency was recorded towards higher intake of the vanillin-supplemented diet (3), whereas the intake of the control diet (1) and the diet with sodium saccharinate (2) was at a similar level. The intake of diet 2 decreased on day 7, and remained at a reduced level until the end of the experiment. The intake of the vanillin-supplemented diet and the control diet was comparable for the first two weeks of the study. From day 15 the highest feed intake was recorded in the control group.

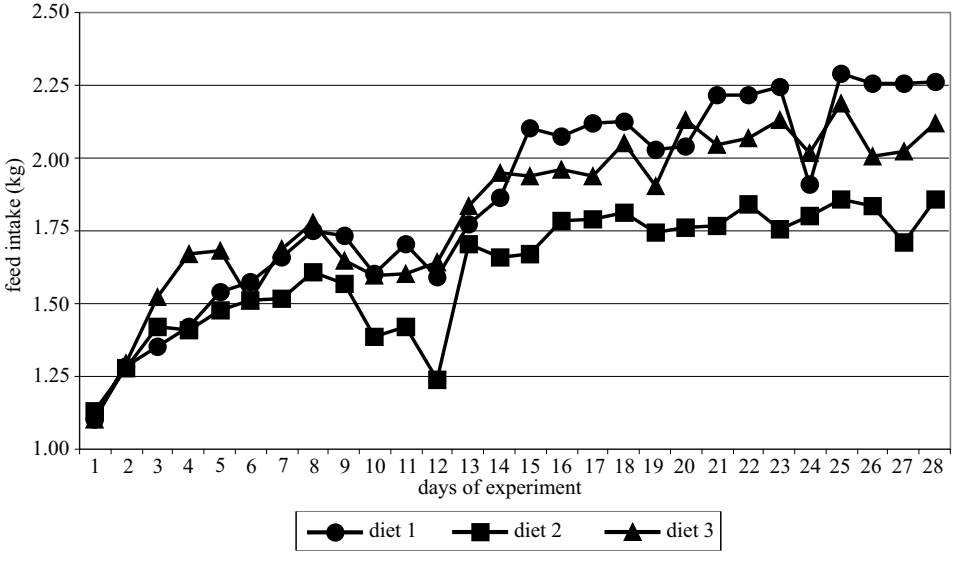

Fig. 1. Average daily feed intake per 1. pig (kg day^{-1})

The fact that sodium saccharinate has no positive effect on feed intake and palatability was confirmed by a single-stimulus preference test (BUGNACKA et al. 2005). The results of our previous studies on the palatability of diets containing sodium saccharinate, fed to weaners and to young growing pigs (FALKOWSKI et al. 2004a,b), showed that this additive is the most effective in the first two weeks. After that time the intake of diets supplemented with sweetener and control diets is similar. This tendency was also observed by other authors, who suggested that sweeteners are the most effective when applied for a short period of time – several or between ten and twenty days (ALBRECHT, MULLER 1972, MCLAUGHLIN et al. 1983, ROURA, FONTANILLAS 2003). ALBRECHT and MILLER (1972) found that young growing pigs (20-30 kg b.w.) displayed a preference for a diet supplemented with 0.05% saccharin only for the first ten (and especially 1 to 4) days of the experiment. This suggests that sweet feed components may be successfully used over shorter period of time, to accustom suckling piglets or weaners to solid feed, or to make it easier for older animals to accept a new kind of feed. At these stages of pig raising each method that contributes to increased feed intake is worth recommending. As demonstrated by MCCRACKEN et al. (1999), the height of small intestinal villous in piglets decreases by 66% on the first two days following weaning if they consume less solid feed. Unfortunately, this happens quite often, and such changes are irreversible and lead to a reduced growth rate and a lower feed conversion ratio at the next stages of development. The positive effects of increased feed intake on the structure and functions of the small intestinal epithelium in newly-weaned piglets have been already documented (MCCRACKEN, KELLY 1984, MCCRACKEN et al. 1999, PLUSKE et al. 1997).

TORRALLARDONA et al. (2000, 2001) did not observe a positive influence of sodium saccharinate on feed intake in weaners, either. However, increased feed intake was recorded when fruit flavors were added to a diet containing sodium saccharinate. The best results were achieved in the case of synthetic cherry-honey and wild strawberry flavors. This shows that the optimum solution is to combine aroma and taste (sweet) enhancers. The addition of a sweetener alone to complete diets does not bring the expected results in feed intake, as confirmed by own studies. In pigs (especially piglets and weaners) feed aroma is the first stimulus that may arise interest in a given diet, since feed taste can be recognized only after the animals had began consumption (FREDERICK, VAN HEUGTEN 2004, ROURA 2004). Therefore, the latter has a lower effect on feed perception by the senses. In addition, an intensive aroma stimulates saliva production in animals immediately before and during feed consumption, in this way enhancing digestion. Moreover, a pleasant taste and aroma stimulates the appetite and the secretion of digestive juices. In consequence, animals can consume more feed and digest it thoroughly (ZAŁUCKI

1998). These physiological mechanisms seem to have a profound influence on feed conversion in animals fed diets supplemented with aroma and flavor additives.

Over the entire experimental period (from 1 to 28 days) the conversion ratio of the control diet per kg of body weight gain was highly significantly lower (by about 10%), compared with the other two diets with aroma and taste enhancers. The values of this parameter in groups 1, 2 and 3 were 2.26, 2.03 and 2.05 $kg \cdot kg^{-1}$ respectively. The results of this experiment prove that the response to feed flavors, manifested by a more favorable feed conversion ratio, is stronger in younger animals. This is consistent with the findings of TORRALLARDONA et al. (2000, 2001), who pays attention to fact, that effectiveness of use of feed flavors it is not only increasing of feed intake, but first of all it is a faster growth rate and better feed conversion ratio in animals fed diets with taste and aroma enhancers. ROURA and FONTANILLAS (2003) suggested clearly that higher daily gains of animals fed diets with these additives are a more reliable indicator of their value than increased feed intake, which is a less objective parameter due to the problems with accurate estimation of the actual amount of feed consumed. Moreover, our study confirmed the hypothesis that feed flavors can stimulate digestive processes and increase their efficiency.

The problem discussed in the paper requires further investigations.

Conclusions

It was found that over the entire experimental period (from 1 to 28 days) none of the feed additives tested had a positive influence on feed intake. Sodium saccharinate negatively affected the amount of feed consumed by young growing pigs, and vanillin had no significant effect on feed intake, in comparison with the control diet. However, the diets containing feed flavors were highly significantly better utilized by animals, as compared with the control diet.

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APPARENT ILEAL DIGESTIBILITY OF FAT AND FATTY ACIDS IN POLAR FOXES FED DIETS USED OVER THE NON-MATING PERIOD*

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K e y w o r d s: polar fox, ileal digestibility, fat, fatty acids.

A b s t r a c t

The objective of the study was to evaluate apparent digestibility of fat and fatty acids in "end-to-end" ileorectal anastomosed blue foxes fed diets used over the non-mating period in the nutrition of parent stock on two farms (A and B). The experimental diets on farm A (A1 and A2) were mainly composed of poultry and fish offals, while diets B (B1 and B2) contained mostly beef offals. Diets B1 and B2 demonstrated a higher content of crude fat (232.1 and 298.1 $g \cdot kg^{-1}$) and saturated fatty acids – SFA (120.7 and 159.6 $g \cdot kg^{-1}$), while diets A1 and A2 contained more monounsaturated fatty acids – MUFA (90.9 and 103.0 $g \cdot kg^{-1}$) and polyunsaturated fatty acids – PUFA (28.6 and 19.8 $g \cdot kg^{-1}$). Higher values of fat digestibility coefficients (95.63 and 96.88%), SFA (95.66 and 96.86%) and MUFA (97.35 and 98.15%) were found in diets B. An excessively high level of ash in diets A1 and A2 could have resulted in a lower digestibility of fat and fatty acids.

POZORNA STRAWNOŚĆ JELITOWA TŁUSZCZU I KWASÓW TŁUSZCZOWYCH U LISÓW POLARNYCH ŻYWIONYCH DIETAMI STOSOWANYMI W OKRESIE SPOKOJU PŁCIOWEGO

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S ł o w a k l u c z o w e: lis polarny, strawność jelitowa, tłuszcz, kwasy tłuszczowe.

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Abstrakt

Celem badań było określenie pozornej strawności jelitowej tłuszczu i kwasów tłuszczowych w dietach stosowanych w okresie spokoju płciowego w żywieniu stada reprodukcyjnego lisów polarnych na dwóch fermach (A i B). Doświadczenie przeprowadzono na lisach z operacyjnie wykonanymi zespoleniami jelitowo-rektalnymi „koniec do końca". Diety z fermy A (A1 i A2) składały się głównie z odpadów drobiowych i rybnych. W dietach z fermy B (B1 i B2) przeważały odpady wołowe. W dietach B1 i B2 stwierdzono większą zawartość tłuszczu surowego (232,1 i 298,1 $g \cdot kg^{-1}$) i kwasów nasyconych – SFA (120,7 i 159,6 $g \cdot kg^{-1}$), natomiast diety A1 i A2 zawierały więcej kwasów jednonienasyconych – MUFA (90,9 i 103,0 $g \cdot kg^{-1}$) i wielonienasyconych – PUFA (28,6 i 19,8 $g \cdot kg^{-1}$). Wyższe współczynniki strawności tłuszczu (95,63 i 96,88%), SFA (95,66 i 96,86%) i MUFA (97,35 i 98,15%) stwierdzono w dietach B. Zbyt wysoki poziom popiołu w dietach A1 i A2 mógł być przyczyną uzyskania niższej strawności tłuszczu i kwasów tłuszczowych.

Introduction

Polar foxes *(Alopex lagopus)*, with a short gastrointestinal tract and a fast digesta passage require diets of a high concentration of energy and protein (NRC 1982, AHLSTRØM and SKREDE 1998, SZYMECZKO 2001). The most efficient source of energy for these animals are fats which provide different fatty acids, including essential unsaturated fatty acids – EFA (NRC 1982).

Based on the reports available so far, it was demonstrated that the main factors determining the fat digestibility in carnivorous fur animals include: the amount and composition of the diet, kind of fat used, the composition of fatty acids and a number of their physicochemical properties, e.g. carbon chain length, number of double bonds (AUSTRENG et al. 1979, ROUVINEN et al. 1988, ROUVINEN 1990, BURLIKOWSKA et al. 2003). The traditional fecal analysis method used commonly in carnivorous animals informs only of the final digestion effect, however it does not factor in transformations of a given nutrient which occur in large intestine as a result of its bacterial microflora (WALKER et al. 1994).

The objective of the present study was to determine the apparent ileal digestibility of fat and fatty acids in the diets used over the non-mating period in the nutrition of parent stock of polar foxes on two domestic farms (A and B).

Material and Methods

Experimental animals. The study included 5, from the same litter, one--year-old male polar foxes of a similar body weight (6.18 ± 0.15 kg). Foxes were premedicated with tranquilizer (0.08 ml $\cdot kg^{-1}$ of the body weight) and atropine (0.03 mg $\cdot kg^{-1}$ of the body weight) and general anaesthesia was induced with ketamine (10 mg $\cdot kg^{-1}$ of the body weight) and operated on to remove large

intestine to make "end-to-end" ileorectal anastomosis surgically following the method developed and used in digestibility experiments with foxes by SZYMECZKO (2001). Over the first five days following the operation, liquids (0.9% NaCl; 5% glucose) were intravenously supplemented and the antibiotic was intramuscularly administered and tranquilizers, antihaemorrhage and anti-swelling medications were subcutaneously supplied to the foxes with dosages recommended by the producers.

Between the 2nd and 10th day after the operation the animals were fed semi-liquid diet and had an unlimited access to water. Starting on the 10th day they were fed once a day with feed of a slightly higher than recommended content of metabolizable energy – ME (NRC 1982). Based on the hematological and veterinary examinations in the 3rd week after the surgery, the foxes were considered clinically healthy and they were admited to the digestibility experiments. They were housed individually in metabolic cages in the climate-controlled room. The experiments were approved by the Local Ethical Committee in Bydgoszcz.

Experimental diets. The digestibility experiment evaluated diets (A1, A2 and B1, B2) used in feeding of reproductive polar foxes on farms A and B in two successive breeding and feeding periods (15.07-15.09 and 15.09-01.12), which covered the so-called non-mating period. The batches of complete diets were delivered frozen from the farms, mixed with Cr_2O_3 at the amount of 5 $g \cdot kg^{-1}$ (SZYMECZKO et al. 2005), homogenized, divided into daily rations (90 kcal ME $\cdot kg^{-1}$ of the body weight) (NRC 1982) and kept frozen (-25°C) until the beginning of the digestibility experiment. Throughout the experiment the animals were fed once a day at 8:00 a.m and they had an unlimited access to water.

Experimental scheme. Four 8-day digestibility experiments were carried out. The examination of each diet was divided into two 4-day periods: adaptation (making the animals get used to the diets) and the digesta collection period (96-hour digesta collection). Having completed the collections of digesta once it was excreted, the containers were stored at -25°C. Frozen experimental diets and digesta were then freeze-dried, following the removal of hairs and grinding.

Chemical analysis. Chemical analyses of diets and digesta samples were carried out to determine the content of nutrients according to the conventional methods. Crude fat was analyzed using Soxhlet method, according to application notes for Soxtec System HT6 apparatus.

Prior to determining fat, diets and digesta samples were hydrolyzed using TECATOR Soxtec Hydrolizing Unit. The composition of fatty acids was analyzed with Hewlett Packard 6890 gas chromatograph (PN-EN ISO 5508, 1996). The analyses were performed at the pre-programmed temperature

of 140°C (1 min) – 1.5°/min – 210°C (8 min). Other parameters were as follows: carrier gas helium (200 kPa), detector 220°C, feeder 210°C, splitting dosage, splitter 30:1. Fatty acids were identified with the analyses of matrixes of a known composition of fatty acids (reference materials) and standards. Fat and fatty acids analyses were performed in duplicate.

Statistical analysis. The results obtained were verified statistically using student's t-test for dependent samples and the level of significance was set for $P < 0.05$.

Results and Discussion

The composition of diets used on farms A and B from 15.07 to 15.09 and from 15.09 to 01.12 (diets A1 and A2, B1 and B2, respectively) is given in Table 1. Diets A1 and A2 were mainly consisted of poultry and

Table 1
Composition of diets fed to polar foxes on farm A (diet A1 and A2) and B (diet B1 and B2) over the non-mating period (15.07-01.12), $g \cdot kg^{-1}$ fresh matter

Ingredient	Feeding period			
	15.07-15.09		15.09-01.12	
	A1	B1	A2	B2
Beef offal	–	699	–	783
Poultry offal	420	–	440	–
Fish offal	299	–	294	–
Meat meal 55%	70	–	74	–
Blood and feathers poultry meal	–	–	29	–
Milk powder 26%	20	–	10	–
Rapeseed oil	–	–	12	–
Excruded cereals	150	–	89	–
Precooked barley	–	300	–	216
Norpol additive (lucerne meal, wheat bran)	40	–	–	–
Wheat bran	–	–	22	–
Apples + cabbage	–	–	29	–
Vit.-min. mixture (Ewomix Fur)*	–	0.5	–	0.5
Vit.-min. mixture (Polfamix LN)**	1.0	–	1.0	–
Iron preparation (Taiga Fur Iron)***	–	0.5	–	0.5

* concentration per 1 g: vit. A 7200 j.m; vit. D3 720 j.m; vit. E 82 mg; vit. B1 30.8 mg; vit. B2 12 mg; vit H 0.1 mg; vit. B6 6 mg; vit. B12 0.04 mg; niacin 20.4 mg; folic acid 0.6 mg; calcium panthotenate 8 mg; choline chloride 50 mg; Mg 66.8 mg; Mn 6 mg; Zn 8.6 mg; Cu 1 mg; Fe 9.2 mg; J 0.3 mg

** concentration per 1 g: vit. A 3500 j.m; vit. D3 500 j.m; vit. E 28 mg; vit. K3 0.2 mg; vit. B1 1.5 mg; vit. B2 2.8 mg; wit. B6 2.8 mg; wit. B12 0.02 mg; wit. H 0.2 mg; kwas foliowy 0.2 mg; niacin 10 mg; D-calcium panthotenate 7 mg; methonine 200 mg; choline chloride 50 mg; Fe 17 mg; Zn 2 mg; Cu 1 mg; Mn 1 mg; Co 1 mg; J 0.1 mg; Se 0,6 mg

*** concentration per 1 ml of preparation: ferrous gluconate 205 mg; ferric sulphate 97.5 mg; cupric sulphate 5.9 mg; cobalt sulphate 1.76 mg

Table 2
Chemical composition of diets ($g \cdot kg^{-1}$) fed to reproductive polar foxes on farm A (diet A1 and A2) and B (diet B1 and B2) over the non-mating period (15.07-01.12)

Ingredient	Feeding period			
	15.07-15.09		15.09-01.12	
	A1	B1	A2	B2
Dry matter, $g \cdot kg^{-1}$	941.9	958.5	959.5	962.9
	$g \cdot kg^{-1}$ dry matter			
Crude protein	351.3	338.8	380.0	414.4
Crude fat	209.0	232.1	213.4	298.1
Crude fibre	14.3	39.5	16.7	17.8
N-free extractives[a]	203.6	296.6	190.1	178.3
Crude ash	163.7	51.5	159.3	54.3
GE, $kcal \cdot g^{-1}$	5.33	5.81	5.34	6.32
ME, $kcal \cdot g^{-1}$	3.66	4.23	3.71	4.93
Fatty acids				
C14 : 0	5.9	6.7	5.7	10.3
C16 : 0	52.1	61.7	51.8	80.6
C16 : 1	11.8	5.3	11.8	5.7
C17 : 0	0.4	3.1	0.4	4.0
C18 : 0	13.0	47.0	15.1	41.0
C18 : 1 trans	1.8	4.9	0.6	6.8
C18 : 1 C9	68.7	65.5	78.9	80.4
C18 : 1 C11	5.4	2.2	6.9	2.8
C18 : 2, n-6	23.4	7.8	15.9	8.0
C18 : 3, n-3	2.2	1.5	1.4	2.0
C20 : 1	2.0	0.7	2.2	0.6
The other fatty acids	8.80	12.2	8.6	17.5
TFA[b]	194.8	218.5	199.5	279.6
SFA[c]	72.3	120.7	75.1	159.6
MUFA[d]	90.9	79.0	103.0	97.2
PUFA[e]	28.6	10.9	19.8	12.2

[a] by difference
[b] total fatty acids
[c] saturated fatty acids
[d] monounsaturated fatty acids
[e] polyunsaturated fatty acids

fish offals, while in diets B1 and B2 beef offals, containing high amounts of fat, predominated. The greatest differences between the diets used on farms A and B were found in the content of crude ash; its amount was almost 3-fold higher in diets A1 and A2 than in diets B1 and B2 (Table 2). Proportions of the metabolizable energy obtained from protein, fat and carbohydrates in the experimental diets (A1: 34, 50, 16; B1: 31, 50, 19; A2: 34, 51, 15; B2: 34, 56, 10%) were similar to nutrient requirements for polar foxes over the non-mating period (NRC 1982, JAROSZ 1994).

In diets B there was found a greater content of fat and fatty acids (TFA), as compared with diets A (Table 2). Fatty acids in the fat present in minor

amount (< 10 g · kg^{-1}) were omitted (AUSTRENG et al. 1979). In diets B, based on beef offals, saturated fatty acids (SFA) prevailed, with the highest content of palmitic acid (C16:0). The diets used on farm A, however, appeared to be richer in monounsaturated fatty acids (MUFA), most represented by oleic acid (C18:1 C9) and polyunsaturated fatty acids (PUFA), represented by linoleic acid (C18:2) and linolenic acid (C18:3). It has to be pointed out that C18:2 and C18:3 acids are essential unsaturated fatty acids for foxes (NRC 1982).

The experiments demonstrated higher values of apparent ileal digestibility of fat in beef offal diets, as compared with the diets containing poultry and fish offals (Table 3). A lower, as compared with the present results, digestibility of beef tallow measured over the entire gastrointestinal tract in polar foxes was reported by ROUVINEN et al. (1988). However, very high values of the ileal digestibility coefficient of beef fat (> 99%) were recorded by HILL et al. (2001) in dogs fed diets with a high content of this nutrient. The fat digestibility coefficients for diets on farm A were similar to the results obtained in dogs by MURRAY et al. (1997).

Table 3

Apparent ileal digestibility of fat and fatty acids (%) in diets fed to reproductive polar foxes on farm A (diet A1 and A2) and B (diet B1 and B2) over the non-mating period (15.07-01.12)

Ingredient	Feeding period									
	15.07-15.09				P	15.09-01.12				P
	A1		B1			A1		B1		
	$\bar{x}$	SD	$\bar{x}$	SD		$\bar{x}$	SD	$\bar{x}$	SD	
Fat	92.88	1.75	95.63	0.72	0.060	93.67	1.11	96.88*	0.49	0.001
C14:0	93.59	1.06	98.15*	0.62	0.004	96.26	0.90	98.41*	0.27	0.007
C16:0	89.22	1.08	95.22*	0.87	0.002	93.47	1.53	96.58*	0.68	0.008
C16:1	98.11	2.03	99.10	0.43	0.384	97.65	0.74	99.16*	0.26	0.005
C17:0	84.11	2.84	96.62*	1.13	0.001	83.51	3.90	97.52*	1.06	0.001
C18:0	85.87	1.20	95.96*	1.19	0.000	89.10	2.82	97.06*	1.19	0.002
C18:1 trans	91.49	2.77	97.94*	0.93	0.015	79.84	10.87	98.52*	0.68	0.016
C18:1 C9	95.82	2.07	97.55	0.40	0.186	95.74	1.05	98.27*	0.23	0.003
C18:1 C11	93.95	2.79	94.62	0.68	0.678	95.29	1.06	95.67	0.36	0.371
C18:2, n-6	94.12	2.63	76.87*	5.41	0.002	87.37	1.92	81.72*	3.00	0.002
C18:3, n-3	93.85	2.66	89.69	3.20	0.102	82.91	4.52	93.29*	1.25	0.005
C20:1	96.42	1.63	88.02*	1.11	0.001	94.54	1.52	87.98*	1.46	0.000
TFA[a]	92.85	1.76	95.66	0.70	0.057	93.63	1.13	96.89*	0.49	0.001
SFA[b]	88.88	1.11	95.66*	0.98	0.001	92.59	1.76	96.86*	0.84	0.004
MUFA[c]	95.85	1.98	97.35	0.43	0.220	95.91	1.00	98.15*	0.22	0.003
PUFA[d]	93.21	3.29	81.09*	4.27	0.006	86.34	2.49	86.19	2.14	0.870

[a] total fatty acids
[b] saturated fatty acids
[c] monounsaturated fatty acids
[d] polyunsaturated fatty acids
* $P < 0.05$

One of the factors limiting the fat digestibility in carnivorous fur animals is an excessive content of crude ash and its main component, calcium, in diets (LOREK et al. 1991, ROUVINEN and KIISKINEN 1991). The ash content in diets on farm A exceeding the nutrition requirements for foxes (NRC 1982) must have accounted for low, as compared with diets B, values of fat digestibility coefficients. Differences in fat digestibility of diets A and B could have been also connected with a different calorific value of ileal digesta. The digesta collected after diets B1 and B2 demonstrated a significantly higher ($P < 0.05$) concentration of energy, as compared with digesta obtained after diets A1 and A2 (B1:4.24; B2:4.07 and A1:2.77; A2:2.77 kcal $\cdot$ g^{-1}), which could have slowed down its passage through the alimentary canal and, as a result, could have resulted in higher values of fat digestibility coefficients (SCHEMANN and EHRLEIN 1986).

All the saturated fatty acids of diets B1 and B2 showed a significantly higher ($P < 0.05$) digestibility, as compared with SFA diet A1 and A2 (Table 3). The higher the content of beef tallow in diets on farm B, the greater the SFA digestibility, which coincides with the results reported in foxes by ROUVINEN et al. (1988). The best absorption of the saturated acid of the experimental diets was found for myristic acid (C14:0), with the shortest carbon chain of all the SFAs discussed. Experiments with foxes (BURLIKOWSKA et al. 2003) and rats (AL-OTHMAN 2000) show a decrease in the digestibility of saturated acids with an increase in carbon chain length. The palmitic acid, most represented in the diets, was the least-absorbed saturated acid in diets B, containing beef fat. Studies on foxes and mink show higher values of the digestibility coefficient of this fatty acid in diets with fish oil than with beef tallow (ROUVINEN et al. 1988, ROUVINEN 1990).The second highest content of saturated acid in diets was stearic acid (C18:0). Its digestibility was similar to the results reported earlier by the present authors investigating foxes (BURLIKOWSKA et al. 2003). However the reports on mink and foxes (AUSTRENG et al. 1979, ROUVINEN et al. 1988, ROUVINEN 1990) show lower, especially as compared with diets B, values of the digestibility coefficient of this fatty acid determined for the whole digestive tract, which could have been due to the using of different digestibility methods (total and ileal) (AUSTRENG et al. 1979).

Monounsaturated fatty acids were better absorbed from diets B, as compared with diets A; significant differences ($P < 0.05$) were observed from 15.09 to 01.12. ROUVINEN et al. (1988), investigating the total digestibility of MUFA, including C18:1 C9 acid, recorded in foxes their lowest absorption from diets with beef tallow. In the present research the highest values of digestibility coefficient of all MUFA were reported by palmitoleic acid (C16:1), which was better absorbed (> 99%) from diets used on farm B. ROUVINEN et al. (1988), investigating foxes, recorded the highest digestibility of C16:1 acid in diets

with fish oil, a lower digestibility with beef tallow and definitely lowest – in the diets with rapeseed oil. In the present study there was found a higher ileal digestibility of monounsaturated acids, as compared with their saturated counterparts, which coincides with the results of earlier reports on foxes (BURLIKOWSKA et al. 2003), mink (AUSTRENG et al. 1979) and piglets (REIS DE SOUZA et al. 1995).

Polyunsaturated fatty acids were significantly better ($P < 0.05$) absorbed from diet A1, as compared with diet B1. At the same time there were found no differences in the digestibility of PUFAs between diets A2 and B2. Linoleic acid (C18:2) was significantly better ($P < 0.05$) absorbed from diets A, as compared with diets B, whereas in diets on farm B it showed the lowest values of digestibility coefficient of all TFAs, (76.87 and 81.72%). ROUVINEN et al. (1988) demonstrated the highest total digestibility of C18:2 acid in diets with rapeseed oil, a lower digestibility – with beef tallow and definitely lowest with fish oil. As for the second EFA, namely linolenic acid (C18:3), there were found no significant differences in its digestibility between diets A1 and B1, while from 15.09 to 01.12 it was significantly better-absorbed from diet B2. Neither did the composition of the diet demonstrate a clear effect on the apparent ileal digestibility of C18:3 acid in foxes fed diets with different animal offals (BURLIKOWSKA et al. 2003). ROUVINEN et al. (1988) in the experiment with foxes fed diets with beef tallow, report on a lower, as compared with diets on farm B, digestibility of linolenic acid, which decreased along with an increase in the content of tallow in the ration.

Conclusions

1. In polar fox diets beef tallow demonstrates a higher apparent ileal digestibility as compared with fat from poultry and fish offals.

2. In the small intestine of polar foxes monounsaturated fatty acids are absorbed more effectively, as compared with their saturated counterparts.

3. A high content of crude ash in the dry matter of diets used in the nutrition of reproductive polar foxes could have decreased the apparent ileal digestibility of fat and fatty acids.

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EFFECT OF HUSBANDRY CONDITIONS AND GENOTYPE OF YOUNG SLAUGHTER TURKEYS ON PRODUCTION AND SLAUGHTER TRAITS

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K e y w o r d s: slaughter turkeys, genotype, shelter, run, meat quality traits.

A b s t r a c t

Young slaughter turkeys, Big 6 (136 birds) and BUT 9 (149 birds), were divided into a control and experimental group. From 7 to 22 weeks of age (July to November) birds of the control group were raised conventionally, i.e. in a house, whereas birds of the experimental group were kept under a shelter with free access to runs. Stocking density was 35 kg BW per m^2 of floor in the house and under the shelter. The turkeys were fed standard diets. The feeding program comprised 7 periods, and the diet composition was changed every 3 weeks. Meat quality traits were evaluated at the completion of the study.

The health condition of birds was generally good, only during the last two weeks increased mortality was recorded in the experimental group, due to ground frost at night.

At 22 weeks of age the body weights of Big 6 and BUT 9 turkey-toms were within normal ranges, i.e. 19.9 kg and 17.9 kg respectively. Feed intake per kg weight gain was 2.93 (Big 6) and 2.87 kg (BUT 9). The body weights of turkeys, the physicochemical and chemical properties of meat and the majority of slaughter quality traits were not related to husbandry conditions. Significant differences between turkeys raised in a house and under a shelter were noted only in the breast muscle content of a carcass (27.6% vs. 28.5%). The birds kept under a shelter had enlarged stomachs and livers.

WPŁYW WARUNKÓW UTRZYMANIA I TYPU UŻYTKOWEGO MŁODYCH INDORÓW RZEŹNYCH NA KSZTAŁTOWANIE SIĘ CECH PRODUKCYJNYCH I RZEŹNYCH

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S ł o w a k l u c z o w e: indyki rzeźne, typ użytkowy, wiata, wybiegi, cechy mięsne.

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Abstrakt

Młode indory rzeźne, Big 6 (136 szt.) i BUT 9 (149 szt.), od 7. do 22. tyg. życia odchowywano tradycyjnie w budynku–wychowalni (ptaki kontrolne) oraz w wiacie z dostępem do wybiegów (ptaki doświadczalne). Chów trwał od lipca do listopada. Przyjęto obsadę 35 kg masy ciała na 1 m^2 podłogi budynków i wiaty. Program żywienia mieszankami standardowymi obejmował 7 okresów (zmiana mieszanki co 3 tyg.). Po zakończeniu badań dokonano oceny cech mięsnych indyków.

Stan zdrowotny indyków nie budził uwag, jedynie w ostatnich 2 tyg., kiedy nocną wystąpiły przymrozki, odnotowano pod wiatą zwiększone upadki ptaków.

W 22. tyg. masa ciała indorów Big 6 i BUT 9 była w granicach normy i wynosiła odpowiednio 19,9 kg i 17,9 kg. Na 1 kg masy ciała ptaki Big 6 spożywały 2,93 kg, BUT 9 – 2,87 kg. Nie odnotowano różnic masy ciała zależnych od warunków odchowu. Podobnie jak w przypadku cech fizykochemicznych i chemicznych mięsa oraz większości wskaźników oceny rzeźnej, nie odnotowano różnic zależnych od warunków chowu. Istotne różnice odnotowano jedynie w przypadku zawartości mięśni piersiowych: u indorów z wychowalni – 27,6%, z wiaty – 28,5%. U indorów z wiaty stwierdzono też powiększone żołądki i wątroby.

Introduction

In Poland the intensive poultry production sector provides as much as 90% of meat, while small family-run farms only 10%. The structure of farm poultry production is as follows: broiler chickens – approx. 78%, turkeys – 17%, geese, hens and ducks – approx. 5% (ADAMOWICZ 2002).

In order to ensure the welfare of farmed birds, as well as to obtain prime-quality raw material, the deep-litter system of poultry production should be further modified. One of the alternatives to the conventional poultry production technology is the free-range system, including access to runs and keeping birds under a shelter with access to fresh air. The guidelines for organic farming are also laid down in the EU Directive 1804/99 of July 19, 1999. According to this Directive, stocking density should be reduced to 21 kg BW per m^2 of floor. The EU requirements (FARUGA 2000) specify also the standards for the following systems of raising slaughter turkeys: extensive deep-litter system (25 kg BW $\cdot$ m^{-2}), free-range farming, conventional farming system with access to a fenced run, conventional farming system with unlimited access to an open-air area, and ecological farming.

Extensive lines of turkeys, characterized by a slower growth rate, are best suited for alternative and ecological farming systems (TYBURSKI 1996). However, such turkeys are not present on the domestic market. Attempts to raise colored turkeys in accordance with the principles of ecological farming have been made in Germany (SCHÖNE 1993). In this system turkeys leave the brooder house relatively early and stay outdoors all year round, if weather permits (WALTER 1999). In Poland studies on husbandry conditions of farms have been limited to testing different types of floor, i.e. slatted floor, wire mesh floor and sand (SOBCZAK cited in FARUGA and JANKOWSKI 1996, SOWIŃSKA 2002).

The aim of the present study was to determine the production efficiency and carcass dressing percentage in medium-heavy and heavy-type turkeys raised in a house on deep litter to 6 weeks of age, and then under a shelter with free access to runs.

Materials and Methods

The experiment was conducted on an experimental farm of the Department of Poultry Science, University of Warmia and Mazury in Olsztyn, from May to November 2001. The experimental materials comprised day-old toms of heavy type Big 6 and medium-heavy type BUT 9, placed on deep litter in a windowless house and raised as recommended by the State Turkey Testing Station. The poults were randomly divided into two groups by genotype, each of 8 replications (subgroups). Stocking density was comparable in both groups.

At 6 weeks of age turkeys of both types were again randomly allocated to two groups, control and experimental. Birds of the control group stayed in a house, whereas birds of the experimental group were kept under a shelter with access to a fenced run sowed with oat (Phot. 1).

Phot. 1. Turkey-toms aged 7 weeks. First week of using the run

The experimental design was as follows:

Age (weeks)	Type – husbandry system			
	Big 6		BUT 9	
1-6	house			
	136 birds (8 replications)		149 birds (8 replications)	
7-22	House	Shelter	House	Shelter
	60 birds (4 replications)	69 birds (3 replications)	67 birds (4 replications)	78 birds (3 replications)

Stocking density was 35 kg BW per m^2 of floor. All turkeys were kept on straw litter, both indoors and outdoors. The birds kept under a shelter could use runs between 7 a.m. and 8 p.m. (Phot. 1). The ratio between the shelter floor area and the run area was 1:2. All turkeys were fed the same complete pelleted commercial diets *ad libitum*. The feeding program comprised 7 periods, and the diet composition was changed every 3 weeks. The flock was under constant veterinary supervision.

The health condition of birds (cases of and reasons for culling) was monitored over the entire experimental period. Feed intake was recorded in subgroups every 7 days. The turkeys were weighed individually at 6, 10, 14, 18 and 22 weeks of age. At the completion of the experimental period (at 22 weeks) 6 birds with average body weights (determined for each subgroup/replication) were selected of 4 experimental groups (i.e. 2 birds of each replication). They were fasted for 12 hours and sacrificed. A simplified slaughter analysis of carcasses was performed by universally accepted methods. The carcasses were chilled for 12 hours and next the weights of breast, thigh and drumstick muscles were determined. A chemical, physicochemical and organoleptic evaluation was performed on samples of breast and thigh muscles, as described by ZNANIECKI (1983).

Results were verified statistically by one- and two-factorial analysis of variance and the Duncan's test, using Statistica 6.0 software.

Results and Discussion

The proper experimental period began in July, when the turkeys were 6 weeks of age. The weather was sunny, as usual in summer (mean weekly temperatures of 20 to 27°C). However, precipitation at the end of July and in the first week of August resulted in lower temperatures and higher air humidity. The second half of August and September were again sunny, but October brought frequent rainfalls, followed by a strong wind and ground frost

at the end of October and at the beginning of November. This resulted in low temperatures (about zero during the day and below zero at night) and high relative air humidity.

Over the entire experimental period cases of fighting and aggressive behaviors were much less frequent in turkey-toms kept under a shelter, as compared with those raised in a house. The former willingly stayed in the run (between 8 a.m. to 8 p.m.), where they could eat green forage (oat) as well as pick up gastroliths. These small stones were later on found in their stomachs (Phot. 2).

Phot. 2. Gastric contents of turkeys slaughtered at 22 weeks of age (on the left: gastric contents of a tom raised conventionally in a house, on the right: gastric contents of a tom kept under a shelter with access to runs. visible gastroliths)

The general health condition of birds was good. During the first 6 weeks the mortality rates were 2.7% (BUT 9) and 2.8% (Big 6). The reasons for culling were yolk sac inflammation and clogging up of the digestive tract with straw. The stress caused by wattle development (between 6 and 12 weeks of age), moving the birds under the shelter and summer heat waves resulted in increased mortality in the group kept outdoors, as compared with the group raised indoors (22 birds vs. 8 birds). Another reason for culling were injuries the birds received while establishing the social hierarchy in a flock. In the case of older turkeys (19 to 22 weeks of age) kept under a shelter, culling was also

related to cold (changes in the lungs and fibrins around the heart) caused by considerable temperature fluctuations.

The mean body weights of turkeys recorded at one day, 6, 10, 14, 18 and 22 weeks of age are presented in Table 1. Differences in the body weights of day-old poults resulted from the fact they hatched from eggs collected at 21 (BUT 9) or 10 (Big 6) weeks of the laying season. These differences could be also affected by the time that passed between the poults were taken out of incubators and weighed on the farm. A relationship between the age of hens and the body weights of poults was also observed by Jankowski (1985), who demonstrated an 8% increase in the body weights of poults that hatched from eggs collected at 18 weeks of the laying season or later. This author found also a correlation between the time that passed since hatching and body weight loss – 2.2 g to 5.3 g within 16 hours following hatching. All these factors could also contribute to differences in the body weights of poults in this study. However, due to compensatory growth that took place in subsequent weeks, 6-week-old Big 6 turkeys were highly significantly (by 8.8%) heavier than BUT 9 ones. As expected, Big 6 toms remained heavier ($P \leq 0.01$) than BUT 9 toms until the end of the experiment. At 22 weeks the difference between these two types was as high as 11% (1.99 kg BW).

The body weights of toms achieved in the present study can be considered satisfactory. The body weights of Big 6 turkeys were comparable with the standards established by worldwide suppliers of turkey breeding stock – Brit-

Table 1

Body weights of turkey-toms (kg)

Age (weeks)	Statistical measures	Husbandry system		Genotype	
		house	shelter	heavy	medium-heavy
1-day	*x* *v*%	*X*	*X*	52.98^{B} 9.76	64.64^{A} 6.58
6	*x* *v*%	2.49 10.30	2.50 9.30	2.60^{A} 8.81	2.39^{B} 8.77
10	*x* *v*%	6.02 10.81	5.86 10.30	6.23^{A} 10.17	5.68^{B} 8.91
14	*x* *v*%	10.85 9.94	10.54 9.65	11.24^{A} 8.27	10.22^{B} 9.08
18	*x* *v*%	14.97 10.38	15.14 9.31	15.88^{A} 8.05	14.34^{B} 8.71
22	*x* *v*%	18.84 10.76	18.85 10.40	19.93^{A} 8.73	17.94^{B} 9.59

Note:
Big 6 – heavy-type
BUT 9 – medium-heavy type
Means followed by different letters differ significantly: *A, B* at $P \leq 0.01$, *a, b* at $P \leq 0.05$.

ish United Turkeys (2002), i.e. 20.70 kg for Big 6 and 17.85 kg for BUT 9 at 22 weeks of age. MAJEWSKA et al. (2000) reported that the final body weights of heavy-type Big 6 turkeys varied from 18.3 kg to 18.9 kg. In the experiment conducted by BURS et al. (2001) the average body weights of medium-heavy But 9 toms ranged between 16.87 kg to 19.67 kg.

At the beginning of the proper experimental period, i.e. at 7 weeks of age, all birds had similar body weights (Table 2). The genotype-environment interaction had no effect on the body weights of turkeys. Table 1 shows that the body weights of turkeys, determined at 6, 10, 14, 18 and 22 weeks of age, were comparable, regardless of the farming system. Since ecological outdoor rearing conditions complied with animal welfare standards, turkeys kept under a shelter were expected to have higher body weights.

Table 2

Feed intake per kg weight gain (kg)

Age (weeks)	Statistical measures	Husbandry system		Genotype	
		house	shelter	heavy	medium-heavy
1-6	x	1.68	X	1.67	1.69
	$v\%$	3.38		4.27	2.66
7-18	x	2.84	2.82	2.85	2.81
	$v\%$	3.44	4.23	4.62	2.53
1-18	x	2.69	2.64	2.62	2.56
	$v\%$	3.21	3.43	3.52	2.40
7-22	x	3.33^{B}	3.46^{A}	3.40	3.37
	$v\%$	2.17	1.77	3.31	2.02
1-22	x	2.86^{b}	2.95^{a}	2.93	2.87
	$v\%$	4.21	2.91	3.28	4.01

Feed intake by heavy and medium-heavy turkeys was at a similar level over the entire experimental period (Table 2). Feed conversion efficiency was good, but slightly higher than recommended by B.U.T. No differences in feed consumption levels, related to husbandry conditions, were noted for the first 18 weeks (Table 2). However, between 19 and 22 weeks feed intake was substantially higher (by 1.03 kg) in the group kept under a shelter, which most probably resulted from deterioration in environmental conditions (ground frost). Thus, it may be assumed that if turkeys were raised earlier in the year, when temperatures are higher, feed intake would be comparable in the groups kept indoor and outdoor.

According to the standards set by British United Turkeys (2002), feed conversion efficiency in medium-heavy turkeys (BUT 9) aged 1 to 18 and 1 to

22 weeks should be respectively 2.31 to 2.57 kg and 2.68 to 3.01 kg feed/kg weight gain, whereas in heavy-type turkeys (Big 6) – 2.34 to 2.61 kg and 2.71 to 3.04 kg feed/kg weight gain over respective periods. These differences result from the concentrations of protein and energy in the diet. FARUGA and PUCHAJDA (2000) reported that heavy-type turkeys aged 1 to 19 and 1 to 22 weeks consumed 2.69 to 2.73 and 2.90 to 2.95 kg feed/kg weight gain respectively. In the experiment performed by MIKULSKI et al. (2000) feed intake per kg weight gain was 2.79 to 2.90 kg.

The carcass dressing percentage (Table 3) was at a good level in turkeys of both genotypes, i.e. 81.7% in medium-heavy and 81.9% in heavy ones. The farming system had a considerable effect on the carcass dressing percentage, which was by 1.8% higher in birds raised conventionally in a house (82.7%), as compared with birds kept under a shelter (80.9%). Our results are comparable to those obtained by other authors for turkeys at a similar age (MAJEWSKA 1995, MAJEWSKA et al., 1999). However, the percentage of breast muscles with

Table 3

Results of slaughter analysis (%) of young turkey-toms (live body weight = 100%)

Trait		Statistical measures	Husbandry system		Genotype	
			house	shelter	heavy	medium-heavy
Live body weight (kg)		*X*	19.01	19.15	19.96^{A}	18.20^{B}
		v%	4.08	6.26	2.24	2.11
Cold carcass weight		*X*	82.7^{a}	80.7^{b}	81.9	81.7
		v%	1.80	2.01	1.40	1.67
Breast muscles including fillet		*X*	27.6^{b}	28.5^{a}	27.8	28.3
		v%	3.41	2.57	2.49	3.01
		X	4.5	4.8	4.6	4.8
		v%	1.72	2.37	2.54	2.06
Thigh muscles		*X*	9.7	10.7	10.7	9.7
		v%	2.40	1.83	2.01	2.26
Drumstick muscles		*x*	6.8	7.7	7.6	7.1
		v%	2.00	1.84	2.86	1.98
Edible giblets including:		*x*	1.8^{b}	2.0^{a}	1.7^{b}	2.2^{a}
		v%	2.68	3.41	2.74	2.36
Liver		*x*	0.78^{b}	1.03^{a}	0.87^{b}	0.95^{a}
		v%	1.58	1.26	2.01	1.98
Heart		*x*	0.31	0.31	0.30	0.30
		v%	3.61	2.42	1.67	2.58
Gizzard		*x*	0.58^{b}	0.82^{a}	0.68	0.73
		v%	2.58	1.98	2.34	2.69
Fat	periintestinal	*x*	0.59	0.60	0.60	0.70
		v%	1.31	1.79	1.58	1.83
	depot	*x*	0.70	0.60	0.60	0.70
		v%	1.93	1.02	1.73	1.47

Table 4

Results of chemical and physicochemical analysis of breast muscles

Trait	Statistical measures	Husbandry system		Genotype	
		house	shelter	heavy	medium-heavy
Juiciness, points	*x*	4.66	4.70	4.66	4.70
	v%	4.37	4.00	4.37	4.00
Tenderness, points	*x*	4.83	4.79	4.87	4.75
	v%	2.67	2.13	2.81	0.0
pH_{24}	*x*	5.55	5.55	5.56	5.53
	v%	1.89	0.99	0.93	1.87
Color (%)	*x*	28.00	28.00	28.33	27.66
	v%	5.53	9.58	10.39	2.95
Water-holding capacity (cm^2)	*x*	4.87	4.34	4.52	4.69
	v%	15.16	20.43	11.83	23.31
Fat (%)	*x*	2.80	2.85	2.79	2.86
	v%	21.77	13.86	22.38	12.91
Protein (%)	*x*	22.83	22.85	22.93	22.75
	v%	2.12	3.48	3.40	2.15
Dry matter (%)	*x*	26.57	27.06	26.68	26.96
	v%	2.81	0.88	2.73	1.55
Ash (%)	*x*	1.07	1.06	1.07	1.06
	v%	1.67	1.69	2.21	0.84

Table 5

Results of chemical and physicochemical analysis of thigh muscles

Trait	Statistical measures	Husbandry system		Genotype	
		house	shelter	heavy	medium-heavy
Juiciness, points	*x*	4.79	4.66	4.66	7.79
	v%	5.13	2.77	4.37	3.93
Tenderness, points	*x*	4.75	4.62	4.62	4.75
	v%	4.71	2.96	4.52	3.33
pH_{24}	*x*	5.66	5.70	5.68	5.68
	v%	1.44	1.57	1.73	1.32
Color (%)	*x*	16.50	17.00	17.50	16.00
	v%	11.97	11.76	13.88	5.59
Water-holding capacity (cm^2)	*x*	5.82	5.59	5.69	5.72
	v%	7.45	15.08	12.10	11.77
Fat (%)	*x*	2.98	2.49	3.01	2.46
	v%	48.27	19.89	35.81	43.00
Protein (%)	*x*	20.81	20.40	21.01	20.20
	v%	2.18	4.72	2.65	3.60
Dry matter (%)	*x*	24.59	24.55	24.87	24.27
	v%	3.60	1.81	3.20	1.48
Ash (%)	*x*	1.04	1.01	1.02	1.03
	v%	0.49	2.12	2.66	1.45

fillet was much higher (by 0.9%) in turkeys raised outdoors (28.5%, Table 3). Particular attention should be paid to the higher than expected breast muscle percentage in medium-heavy turkeys (28.3%), in comparison with heavy-type ones (27.8%). Both these values are also higher than the standards adopted by B.U.T (2002). The analysis of the content of edible giblets (gizzards, livers, hearts) shows that birds kept under a shelter had larger gizzards and livers, since they swallowed small stones (gastroliths) found in the run (Phot. 2). Medium-heavy turkeys were also characterized by a higher percentage of livers in their bodies (0.95%). Contrary to expectations, the depot fat content of a carcass was comparable in all birds, regardless of their genotype and despite the fact that turkeys kept outdoors could take more exercise.

No differences related to genotype or husbandry conditions were noted in the chemical composition and physicochemical properties of breast and thigh muscles (Tables 4 and 5). Our results correspond to those reported by other authors (FILUŚ et al. 1995, JANKOWSKI et al. 1989, MAJEWSKA 1995).

The general assessment of meat quality showed that it met the relevant standards set by poultry breeders and production technologists. All coefficients of variation calculated for groups remained within normal ranges, except for the fat content of a carcass.

Conclusions

1. Raising 7-week-old turkeys according to different husbandry systems did not result in significant changes in the basic production parameters, carcass dressing percentage and meat quality traits. The only exception was the breast muscle percentage, which was found to be higher in birds kept under a shelter with free access to runs.

2. Subzero temperatures recorded at night during the last two weeks of the experimental period caused an increase in feed intake per kg weight gain in birds raised outdoors. The health conditions of turkeys kept under a shelter deteriorated as well.

3. As expected, there were considerable differences between heavy and medium-heavy turkeys in the majority of production traits, particularly in body weight. Birds kept outdoors showed a tendency towards higher body weights until the occurrence of ground frost at night.

4. The results of this study indicate that it is advisable to raise older turkeys outdoors, under a shelter with free access to runs, as long as temperatures in a given area do not fall below zero at night.

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THE ANALYSIS OF REPRODUCTION INDEXES OF COLD-BLOODED STALLIONS FROM THE KĘTRZYN STATE STUD

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K e y w o r d s: cold-blooded stallions, use for reproduction, quality estimation.

A b s t r a c t

The aim of this study was to determine the indexes for cold-blooded stallions, used for reproduction in the area covered by the activities of the State Stud in Kętrzyn. The mating performance of 494 studs was analysed in the study, both those used for reproduction in the past and at present, recorded at the Kętrzyn State Stud from 1 January 1970 to 31 December 2000.

The following facts have been ascertained by the study:

– a variable number of studs and the mares served by them, with an increase in the effectiveness of mating service,

– a significantly larger number of Ardennes from the Polish breeding used in reproduction, which served a statistically larger number of mares (73.9 in a season) than those from other breeds (52.0-56.7),

– an increase in the time a stallion was used and in the number of mares served, with an improvement in quality expressed as the quality estimation, which is corroborated by correlation coefficients,

– cold blooded stallions used for reproduction from the 4^{th} to the 6^{th} mating season served significantly more mares as compared to those used in a smaller or larger number of seasons; they were also more effective (2.01 jumps per one mating).

ANALIZA WSKAŹNIKÓW WYKORZYSTANIA ROZPŁODOWEGO OGIERÓW ZIMNOKRWISTYCH PSO W KĘTRZYNIE

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Abstrakt

Celem badań było określenie wskaźników charakteryzujących ogiery zimnokrwiste używane w rozrodzie na terenie oddziaływania Państwowego Stada Ogierów (PSO) w Kętrzynie. Badaniami objęto 494 reproduktory, tzw. archiwalne i aktualnie użytkowane w rozrodzie, znajdujące się w ewidencji PSO Kętrzyn w okresie od 1.01.1970 do 31.12.2000 r. W badanych latach stwierdzono:

– zmienną liczbę ogierów kryjących oraz stanowionych przez nie klaczy oraz wzrost skuteczności krycia;

– znaczącą przewagę w wykorzystaniu rozpłodowym ogierów ardeńskich pochodzących z hodowli krajowej, które pokryły statystycznie więcej klaczy (73,9 szt. w sezonie) niż osobniki z pozostałych grup rasowych (52,0-56,7 szt.);

– wzrost długości okresu użytkowania rozpłodników z hodowli państwowej oraz liczby pokrytych przez nie klaczy, a jednocześnie poprawę jakości wyrażonej bonitacją, co potwierdzają współczynniki korelacji;

– lepszą skuteczność krycia (2,01 skoków na pokrycie klaczy) ogierów zimnokrwistych użytkowanych rozpłodowo od 4. do 6. sezonu kopulacyjnego w porównaniu z osobnikami użytkowanymi w mniejszej lub większej liczbie sezonów kopulacyjnych.

Introduction

Breeding and raising cold-blooded horses is becoming increasingly popular in various areas of Poland, driving noble breeds out of the areas where they have been traditionally bred. The herd of cold-blooded horses in Poland consists of about 180 thousand animals, which accounts for about 50% of all the horses kept in the country (Rocznik 2004).

The current model of cold-blooded horse has been developed through the work of state reproduction farms, especially stud farms with excellent stallions (BUDZYŃSKI et al. 2000). One of the major centres of breeding cold-blooded stallions which has contributed significantly to the evolution and consolidation of the genetic pool of the breed is the State Stud in Kętrzyn.

It is a common assumption in horse breeding that the population is improved mainly by stallions. Due to the increasing costs of raising and maintaining stallions, they should be utilised in the optimum manner in State Stud (FABIANI 1984).

The period when a stallion is used is important both from the breeding point of view and in terms of its cost-effectiveness (BRZESKI, LESZCZYŃSKI 1969). During a long period of intensive use in reproduction, a stallion can be evaluated by the quality of its offspring, and at the same time, the cost of raising a stallion is spread over a larger number of foals (FEDORSKI, KILIŃSKI 1987).

The studies of reproduction use of stallions conducted to date have covered an earlier and, usually, shorter period. Therefore, it seemed appropri-

ate to determine indexes characterising cold-blooded stallions used for reproduction in the area of activities of the State Stud in Kętrzyn in the years 1970-2000.

Materials and Methods

Cold-blooded stallions were covered by the study, both those used for reproduction at present and in the past – all recorded at the State Stud in Kętrzyn from 1 January 1970 to 31 December 2000. Materials were gathered from sources owned by Kętrzyn State Stud, the Provincial Association of Horse Breeders in Białystok (WZHK) and the Association of Horse Breeders of Warmia and Mazury in Olsztyn (W-MZHK). The analytical data were taken from: stallions' records, protocols of reproduction seasons, registers of state-owned and licensed stallions and stud books (Acta... 1970-2000*a,b,c*; Stud Book... 1964, 1972, 1978, 1986, 1993).

The following reproduction indexes of stallions at the Kętrzyn State Stud were calculated based on the source information:

– the number of mating stallions (breakdown by a breed and breeding sector);

– the average age of stallions when incorporated into the State Stud;

– time period of a stallion at the State Stud, expressed as the number of mating seasons;

– quality characteristics of the stallions used for reproduction, divided into three quality groups;

– the number of served mares: total and the number of mares served by a stallion in a season;

– participation of licensed mares in mating;

– effectiveness of mating by stallions, expressed as the number of jumps needed for mating.

The accumulated data are shown in tables and in a graphic form. Figures were analysed statistically with the use of tests included in the Statistica (Statsoft) software pack; the following were calculated:

– weighted average number of mares served by a stallion in a season and the number of jumps made by a stallion to serve a mare,

– coefficients of variation of the average number of mares served by a stallion in a mating season,

– coefficients of linear correlation between the number of quality points for stallions, broken down by a breeding sector, and the reproduction indexes (number of mares served, number of mating seasons, average number of mares served).

The study also included an analysis of variance for the average number of mares served by a stallion in the breeds, taking into account such factors as a breeding sector, quality ranges, years and mating seasons.

Results and Discussion

1. Breeding parameters for stallions

Table 1 shows the characteristics of stallions in the Kętrzyn State Stud in the years 1970-2000. In order to assess the breeding environments, the stallions from Kętrzyn State Stud were broken down by a breeding sector. The Kętrzyn stud population consisted of 19 Ardennes stallions from state stud farms and 249 cold-blooded horses. 215 cold blooded stallions used at the State Stud were purchased from privately owned farms. There were 11 imported stallions.

The stallions bred in Poland were purchased to the Kętrzyn State Stud with an average age of 3.1-3.9 years (Table 1). The older group was represented by imported stallions, which were incorporated into the

Table 1
The characteristic of reproduction indexes the cold-blooded stallions from the Kętrzyn State Stud

Specification	Sector of breeding				Total
	private	state-owned		import	
		breed			
	cold-blooded	ardennes	cold-blooded	*	
Number of breeding stallions	215	19	249	11	494
The average stallions age incorporated to State Stud (years)	3.1	3.7	3.9	5.3	3.6
The average number of reproduction seasons	7.8	10.8	8.0	9.9	8.0
Number of covered mares	87 130	5617	95 365	2600	190 712
The average number of covered mares in season by one stallion	55.0^{A}	73.9^{B}	56.7^{A}	52.0^{A}	52.3
Coefficient of variation (the average number of covered mares)	47.5	32.2	45.1	50.4	53.9

* the breton, arden and cold-blooded stallions imported from France, Sweden, Republics of Russian Federation

AB – values in columns with different superscripts are statistically different, capital letter denote significance at a level $\alpha = 0.01$

stud at the age of 5.3 which, in a number of cases, may have had economic reasons.

The time of stallion use at the Kętrzyn State Stud during the period under study varied. The longest used were the Ardennes stallions bred at state stud – 10.8 seasons, and the shortest – the cold blooded stallions bred in privately owned ones. The period of use for all the stallions in Kętrzyn was 8.0 seasons (Table1).

The length of period of use of Ardennes horses at the Łobez State Stud was corroborated in a study by PIKUŁA et al. (1994), who calculated the value to be 15 years. A shorter period of use of stallions of various breeds – 2.6 seasons – was found by ZAŁUSKA et al. (1988) in a study conducted at the Kadyny Horse Stud, and by JANISZEWSKA et al. (1993) (6.4 seasons) in a study of cold-blooded stallions used at the Klikowa State Stud. A longer times of use was recorded for stallions in the type of cold-blooded horses at the State Stud Łobez – 8.5 (PIKUŁA et al. 1994) and the elite stallions of various breeds in Poland – 10.6 seasons (ZWOLIŃSKI, ROSZKOWSKA-KĘDZIA 1982).

The stallions used at the Kętrzyn State Stud (494 horses) served 190,712 mares altogether in the period 1970-2000 (Table 1), which makes 52.3 mares per stallion. In the earlier period, i.e. in the years 1978-1982, a higher reproduction index of 61 served mares was found by KAPROŃ and ZIAREK-ZAKASZEWSKA (1985), in a study of the reproduction use of licensed stallions in the Province of Lublin. Lower results were obtained by JANISZEWSKA et al. (1993) – 36.8 mares – in a study of stallions at the Klikowa State Stud, and by FEDORSKI and KILIŃSKI (1987), who analysed the intensity of stallions use at the Łobez State Stud – about 39.0 mares served in the years 1948-1983 – and PIKUŁA et al. (1994) – 18.1 mares in a later period 1984-1991.

In this study, Ardennes stallions from state owned stud farms served significantly more mares during a season (73.9) as compared to those from other groups (Table 1); the variance of the characteristic under study in the group of Ardennes stallions was the lowest (32.2%).

Surprisingly, despite the longest period of use of imported stallions (9.9 seasons) and the prevailing opinion of the high value of "foreign" stallions, their mating results were the poorest, with only 52.0 served mares. This indicates their insufficient use, caused by some unknown reasons. One of the probable factors affecting the mating result was the fact that they were mainly used after 1988, when the number of mares served by stallions gradually decreased. Another may have been the breeders; reluctance to have mares served by imported stallions.

2. Quality characteristics of the used stallions

The results of qualitative analysis the stallions used for reproduction are shown in Table 2.

The data presented in it indicate that the largest number of mares were served by group II (80-82 points) stallions, which accounted for 53.6% of those used at the Kętrzyn State Stud. However, those stallions served significantly less mares in a mating season (49.8) than the stallions from the other two quality classes.

Table 2

Bonitation scores of the cold-blooded stallions used in reproduction in the Kętrzyn State Stud

Bonitation		Breeding stalions		Number of breeding seasons							The average number of mares covered by a stallion in a season
Scores	group	no	%	1-3	4-6	7-9	10-12	13-15	16-18	average	
≤ 79	I	135	27.3	43	20	24	27	12	9	7.3	55.6^{A}
80-82	II	265	53.6	61	60	43	51	30	20	7.9	49.8^{B}
≥ 83	III	94	19.1	10	24	15	21	9	15	9.2	55.4^{A}
Total		494	100.0	114	104	82	99	51	44	8.0	52.3

The largest number of stallions – 163 – were used for reproduction with the highest intensity during the first 4 seasons, and the number of stallions used for mating in seasons 14 and 18 was lower – 13 (Figure 1).

ZWOLIŃSKI and DRZEWIECKI (1965) found a similar relationship in a study of elite stallions in the Poznań area; the majority of them were used during the first three seasons.

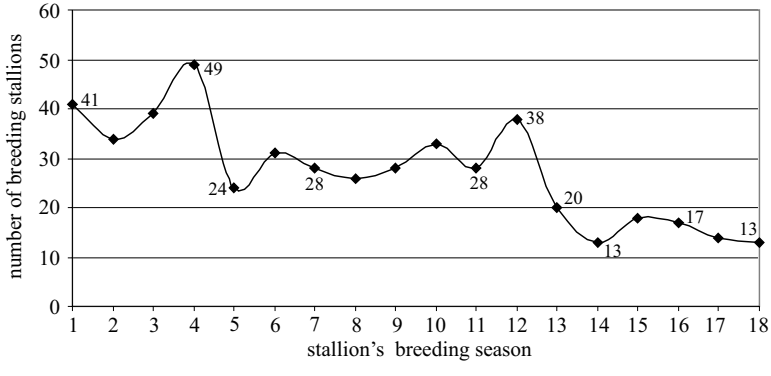

Fig. 1. The length of the cold-blooded stallions used in breeding from the Kętrzyn State Stud

Stallions with 83 and more quality points were used for the longest period of time: 9.2 seasons; during that time they covered 55.4 mares on average. With an increase in the quality, the length of use of stallions from state stud farms increased, which is corroborated by the correlation coefficients (Table 3).

Table 3

The line correlation coefficients between bonitation scores the cold-blooded stallions from the Kętrzyn State Stud and reproduction indexes with taking into consideration the sector of breeding stallions

The feature correlated	Stallion breeder		
	state-owned	private	import
Bonitaton scores – the number of covered mares	0.16**	0.20**	0.57
Bonitation scores – the number of breeding seasons	0.16**	0.10	-0.33
Bonitation scores – the average number of covered mares by stallion in a season	-0.04	0.17*	-0.12
Number of analysed stallions	268	215	11

* – significance at a level of $\alpha = 0.05$, ** $\alpha = 0.01$

An analysis of the quality points of home stud farms and the number of mares covered by them indicates the existence of a significant positive correlation. However, despite the high value of the correlation coefficient in the group of imported stallions – 0.57 – no effect of the number of the quality points on the number of mares served by them was found to exist (Table 3).

In an analysis of the relationship between the number of quality estimation points and the number of mares served in a season, a negative value was found for the stallions from state owned farms and imported ones, whereas the cold-blooded stallions from privately owned breeding farms served a significantly larger number of mares in a season, with an improved quality of stallions (Table 3).

The observed trends concerning the use of stallions for reproduction have been substantiated by studies by other researchers. BRZESKI and LESZCZYŃSKI (1969) recorded a various periods of stallion use at the Klikowa State Stud (13.9 seasons for the best stallions and only 5.3 seasons for the poorest). BRZESKI and MOZDYNIEWICZ (1974) examined the stallions at the Kętrzyn and Łąck stud and an increase in the number of mares served in the years 1947-1971, with a higher number of quality estimation points of the stallions. GERINGER and DOBROWOLSKI (1997) observed a negative correlation between the number of quality estimation points and the use for reproduction of Silesian horses from state stud farms which, in their view, implies the need to use the best stallions more intensely.

3. The use of stallions for reproduction in the years 1970-2000

The use of cold-blooded stallions from the Kętrzyn State Stud for reproduction is shown in Table 4. The whole period was divided into three stages, with the numbers of stallions and the mares served by them.

In the years 1970-1980, 226 stallions were used for reproduction. During that time, they covered the significantly largest number of mares in a season – 64.3 on average per stallion, as compared to the other study periods (Table 4). The number of jumps, indirectly linked to the number of covered mares, was the highest during the period; each stallion made an average of 2.16 jumps to effectively cover a mare, which may be a proof of the highly intensive exploitation of the stallions under study. At the stage under study, the

Table 4

Reproduction efficiency of the cold-blooded stallions from the Kętrzyn State Stud in the years 1970-2000

Year	Number of breeding stallions	Number of covered mares				Number of mounts on mating mare
		total	licensed (%)	average by a stallion in a season	coefficient of variation (%)	
1970	127	7888	11.8	60.2	40.2	2.20
1972	129	9390	10.1	67.6	41.9	2.13
1974	115	8735	7.2	76.0	27.5	2.10
1976	100	5968	14.4	59.7	41.5	2.10
1978	96	5532	11.8	57.6	38.0	2.20
1980	86	5203	15.4	58.5	34.2	2.00
1970-1980	226	78 861	10.6	64.3^{A}	36.9	2.16
1982	87	5583	12.9	63.4	34.2	2.11
1984	98	4000	13.9	39.2	51.5	2.04
1986	101	4635	11.3	43.7	47.4	1.95
1988	109	9199	8.4	78.0	49.2	2.14
1990	114	6324	11.5	50.0	63.4	2.12
1981-1990	205	56 851	11.3	53.7^{B}	55.1	2.06
1992	109	5921	14.0	49.3	53.1	2.04
1994	113	6063	13.5	48.9	56.0	2.06
1996	117	5402	19.0	39.1	64.2	2.04
1998	133	5325	22.0	33.9	62.2	2.15
2000	111	4121	29.5	29.4	66.0	1.83
1991-2000	240	55 000	17.5	40.5^{C}	64.4	2.04

AB – values in columns with different superscripts are statistically different, capital letter denote significance at a level $\alpha = 0.01$

average number of mares served by a stallion in a season was the least variable (36.9%), which indicates the stability of cold-blooded horse breeding in the years 1970-1980.

Much less intensive use of stallions for reproduction, with an average number of 50 covered mares per stallion, was recorded by GRUSZKO (1979), who studied the population of horses in the Province of Olsztyn in the years 1971-1978, and by CHODKOWSKI (1985) who analysed the population in the same area in 1977-1979 (41 mares).

During the second of the analysed decades, covering the years 1981-1990, a sharp decrease in the number of served mares was observed – 56,851 in total (Table 4), which was especially evident in 1984. The corrective actions taken by the Ministry of Agriculture and the "Animex" company, aiming at protecting the breeding intensity, brought about its revival as early as 1986 (KARPETA 1998). This resulted in 1988 in the high number of 78 mares covered by one stallion, with a simultaneous increase in the number of licensed mares (11.3%). A similar increasing trend for the average number of mares covered by a stallion, with a peak in 1988, was observed by PIKUŁA et al. (1994), in a study of the use of stallions in the Łobez State Stud; however, the stallions there only covered 18.7 mares, on average.

At the third stage of observation (1991-2000), the average number of mares covered by the stallions from the Kętrzyn State Stud in a mating season was the lowest – 40.5 (Table 4). That may have been caused by the withdrawal of subsidies for each described foal. At the same time, the proportion of licensed mares increased to 17.5%. This is linked to the progress in horse breeding expressed, for example, by a higher number of mares entered in stud books. During the stage under study, an improvement of the effectiveness of stallions was observed, and the average number of jumps (2.04) per mating was the lowest.

An analysis of use of stallions at the Kętrzyn State Stud in the years 1970-2000 reveals a decrease in the number of covered mares, except for 1988, when the highest average number of mares covered by one stallion – 78.0 – was recorded. After 1996, stallions covered an average of 50.0 mares in a season, which indicates a slump in horse breeding, persisting in the area serviced by the Kętrzyn State Stud. For a comparison, BYSZEWSKI (1999) analysed the use of stallions from the Kętrzyn State Stud and observed slightly poorer mating results: in 1994 – 51.9 covered mares, and w 1998 – 36.0. On the other hand, KARPETA (1998) compared the intensity of use of state- and privately-owned stallions used for mating at the Białystok Association of Horse Breeders in 1985-1995 and observed better results of mating (by about 27%), achieved by stallions from private stud farms. The author's explanation of the findings is that private breeders often keep stallions of higher quality than those kept

at the Kętrzyn State Stud. However, GRZYBOWSKI (1993) disagrees with the opinion; in his view, the material gathered at state stud is of the best quality. According to the information obtained from the chief breeder at the Kętrzyn State Stud, breeders are more often willing to have their mares covered by licensed stallions from state stud, which they consider to be of higher quality.

4. The use of stallions in consecutive mating seasons

When analysing the list of mating results for the stallions from the Kętrzyn State Stud in consecutive mating seasons, the mating activity of the stallions was divided into three-year periods to make interpretation easier (Table 5).

It was found that the highest number of mares (56866) were covered by stallions (at the average age of 5 years) used for 1 to 3 seasons, during which time they covered an average of 48.1 mares in a season. According to ZWOLIŃSKI (1976), stallions should not serve more than 30 mares in the first mating season. This study found that the stallions at the Kętrzyn State Stud were over-exploited during the period under study (Table 5).

Table 5

Results of mating service the cold-blooded stallions from the Kętrzyn State Stud in successive breeding seasons

Breeding season	Breeding stallions		Number of covered mares			Number of mounts on mating mare
	number of stallion	average age (years)	total	average on stallion in a season	coefficient of variation (%)	
1-3	415	5.0	56 866	48.1^{AC}	57.0	2.10
4-6	332	7.5	52 522	56.8^{B}	50.4	2.01
7-9	259	10.2	37 881	53.4^{AB}	53.6	2.06
10-12	191	13.1	26 804	53.3^{AB}	54.0	2.15
13-15	95	16.1	12 660	54.1^{AB}	48.6	2.16
16-18	44	18.9	3 979	43.7^{C}	59.5	2.23

AB – values in columns with different superscripts are statistically different, capital letter denote significance at a level $\alpha = 0.01$

In the second of the periods under study (seasons 4-6), the stallions covered the highest average number of mares – 56.8 in a season. The average age of stallions (7.5 years) indicates their breeding maturity, which was reflected in their effectiveness; they needed 2.01 jumps to cover a mare.

The lowest number of mares were covered by stallions during seasons 16-18; the number was significantly lower than the result achieved in seasons 4 to 15 (Table 5). The average number of mares covered by stallions from the Kętrzyn State Stud (43.7) was all the same higher than that suggested by ZWOLIŃSKI (1976) for older stallions. This may indicate high reproduction capabilities of the stallions (average age – 18.9 years) and breeders' interest in obtaining offspring by these stallions.

The results of this study show that young stallions (4.2 years), covering mares in the first mating season, and the oldest ones, made a higher number of jumps during seasons from 15 to 18 than the stallions in their peak fitness condition from seasons 3 to 8. Horses' mating capabilities are known to be affected by numerous factors, both on the mare's and on the stallion's part. A study by DAVIES MOREL and GUNNARSSON (2000) conducted on a group of Irish stallions did not confirm the effect of stallions; age on mares; reproduction capacity. The authors found a statistically higher fertility index in trained stallions than in others. BRINSKO et al. (1995), CARNAVELE et al. (1993) as well as DAVIES MOREL and GUNNARSSON (2000) observed the effect of a mare's age and fitness on its fertility. Therefore, except for physiological disorders which are beyond the breeder's control, handling a horse in a rational manner will maximize its reproduction potential.

Conclusions

The analysis of the reproduction indexes of stallions from the Kętrzyn State Stud in the years 1970-2000 revealed the following:

1. A variable number of stallions and the number of served mares, with an increase in the mating effectiveness.

2. A significant majority of Ardennes stallions from state stud farms, used for breeding; those served statistically more mares (73.9 in a season) than the stallions from other breeds (52.0-56.7).

3. An increase in the length of period when stallions from state studs were used for reproduction and of the number of mares covered by them, with an improvement in the quality expressed as quality estimation points, which is confirmed by correlation coefficients.

4. Cold-blooded stallions used for reproduction from mating season 4 to 6 covered significantly more mares than those used in a smaller or larger number of mating seasons, at the same time showing a better mating effectiveness (2.01 jumps for effective mating).

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ULTRASTRUCTURE OF THE TURKEY HATCHING EGG SHELL

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K e y w o r d s: turkeys, egg shell, inner shell membrane.

A b s t r a c t

The aim of the present study was to evaluate the structure of shell layers and inner shell membrane fibers in turkey eggs that differed in shell surface characteristics.

A total of 180 eggs were examined, including 60 eggs with good-quality shells, 60 rough-shelled eggs and 60 eggs without shell surface pigmentation. The structure characteristics common to each of the groups were determined based on microscopic images. Then all eggshell layers were photographed at three positions.

Good-quality shells were characterized by tiny cracks in the cuticle, the crystal layer present at some places of the shell, the thickest palisade layer and rectangular mammillae. Inner shell membrane fibers formed a web. Rough shells and shells without surface pigmentation differed from good-quality shells in terms of the following traits: the cuticle had slab-like structure or no cuticle was present, the crystal layer covered all or the major part of the palisade layer, the palisade layer and the mammillary layer were thinner. There was no clear boundary between the palisade layer and the mammillary layer. In the shells without surface pigmentation the palisade layer had long cracks. In these shells the mammillae were cone-shaped and had curved walls, and the spaces between them were larger. Inner shell membrane fibers were thicker and curving.

ULTRASTRUKTURA SKORUPY INDYCZYCH JAJ WYLĘGOWYCH

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S ł o w a k l u c z o w e: indyki, skorupa jaja, błona podskorupowa.

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Abstrakt

Celem pracy była ocena budowy warstw skorupy i włókien błony podskorupowej w jajach indyczych różniących się powierzchnią skorupy.

Do badań użyto po 60 szt. jaj o powierzchni wzorcowej, ziarnistej i bez pigmentu, i po obróbce próbek skorupy określono w obrazie mikroskopowym cechy wspólne dla danej grupy, następnie fotografowano wszystkie warstwy skorupy w trzech miejscach jaja.

Skorupa wzorcowa charakteryzowała się drobnymi pęknięciami kutykuli, warstwą krystaliczną w niektórych miejscach, najgrubszą warstwą palisadową, brodawkami o kształtach prostokątnych. Włókna błony podskorupowej tworzyły sieć. Skorupy ziarnista i bez pigmentu różniły się od skorupy wzorcowej następującymi cechami: kutykula miała postać płytek lub nie występowała, warstwa krystaliczna pokrywała całą lub większą powierzchnię warstwy palisadowej, warstwy palisadowa i brodawkowa były węższe i nie było wyraźnych granic między nimi. W skorupie bez pigmentu warstwa palisadowa miała długie pęknięcia. Brodawki tych skorup miały kształt stożków o wygiętych ścianach, a powierzchnie i przestrzenie między brodawkami były większe. Włókna błony podskorupowej były grubsze i powyginane.

Introduction

The chemical composition and structure of eggshells may vary widely, depending on biological, environmental and pathogenic factors (SOLOMON WATT 1990, SZCZERBIŃSKA 1995, ROBERTS and NOLAN 1997, CHRISTENSEN et al. 2000, MALEC 1999, MALEC et al. 2002). The multilayer structure of the eggshell provides information about shell quality as well as about the shell formation process. Certain traits distinguished in the eggshells of some bird species may indicate structural faults. These are:

– bodies A and B in the mammillary layer having no connection to the inner shell membrane,

– thickened areas on the walls of mammillae, decreasing the spaces between them,

– tightly packed mammillae with no spaces between them,

– shapes of mammillae considered untypical of a given bird species,

– slab-like structure of the cuticle or no cuticle present,

– extra deposits of eggshell mass,

– layer separation and cracking (SOLOMON 1991, ARIAS and FERNANDEZ 1995, MALEC 1999, MALEC et al. 1999, SZCZERBIŃSKA 2002, KRYSTIANIAK 2002). The vast majority of faults appear within the layers at the initial and at the final stage of shell formation.

Irregularities and bumps in the shell surface as well as a complete or partial lack of pigmentation decrease the hatchability of turkey and chicken eggs. They are also associated with lower fertilization rates and increased embryo mortality (MRÓZ 1998, MALEC 1999, MALEC et al. 2002., MRÓZ et al. 2002a, b). Disturbances in the shell formation process in turkeys at the peak and at the end of the laying season are quite common. This is confirmed by a high number

of rough-shelled eggs and eggs without shell surface pigmentation (MRÓZ et al. 1997, MRÓZ 1998).

The aim of the present study was to determine whether in turkey eggs with shell surface defects the structure of deeper layers differs from that found in eggs with normal-quality shells.

Materials and Methods

The experimental materials comprised hatching eggs of broad-breasted white turkeys of the heavy Big-6 type. At the peak of the laying season the eggs were divided into three groups by the method developed by MRÓZ (1998), i.e. group 1 – eggs with good-quality shells, group 2 – rough-shelled eggs, group 3 – eggs without shell surface pigmentation. Good-quality shells were light-brown or cream-colored, with uniformly distributed brown spots. Their surfaces were smooth and shiny. Rough shells had granules or pimples on the surface, dispersed or in clusters. Shells without pigmentation were white, and their surfaces were smooth and glossy.

A total of 180 eggs were examined (60 of each group). The eggs were broken and the contents emptied. The shells were rinsed thoroughly to remove the remains of the albumen. Samples with membranes were taken from the sharp, equatorial and blunt parts of the shell. Part of the inner shell membrane was removed and the rest was etched with 0.5 normal HCl for 15 minutes. Etching time was the same for all samples. Next the outer surface, the inner surface and the area perpendicular to the breaking line were attached to the stabilizers so as to obtain the image of all shell layers. Then the samples were gold-coated with a JEOL Fine Coater, JCF-1200, and viewed under a scanning electron microscope (JSM-5310LV, JEOL, 25 kv). The structure characteristics common to each of the groups were determined based on microscopic images. Images of the surface area, cross-sectional area and the mammillary layer, representative of each of the groups, were photographed at 350 x magnification, whereas those of the inner shell membrane were photographed at 1500 x magnification. The photographs of microscopic images obtained for particular eggs groups are presented in the Results and Discussion Section.

Results and Discussion

The cuticle with numerous tiny cracks covers the entire surface of good-quality eggshells. The white surface (a high water content) around the cracks looks like a torn-up net. The cracks are wide at the blunt end of the egg and

narrow at its sharp end (Phot. 1). On rough shells the cuticle is distributed over some places like on good-quality shells, whereas at other places it exhibits slab-like structure with cracks (Phot. 2). Shell mass deposits are visible on large slabs (Phot. 3). The shells without surface pigmentation have only trace amounts of the cuticle at the sharp end of the egg, while no cuticle is present on the other parts. Deeper layers of the shell are visible on all egg parts, with cracks at the sharp end of the egg (Phot. 4).

The crystal layer covers deeper layers of good-quality shells at some places only (Phot. 5). The palisade layer has spongy structure and is distinctly separated from the mammillary layer. The mammillary layer is composed of rectangular structures, separated from one another at the base. The largest mammillae can be found at the sharp end of the egg. In rough-shelled eggs the crystal layer covers the entire palisade layer. The thickness of the palisade layer is difficult to determine since at many places there are no clear-cut boundaries between this layer and the mammillary layer (Phot. 6). Mammillae of unspecified shapes or cone-shaped ones can be seen in the equatorial and sharp part of the shell. The walls of these mammillae are curved. Both good-quality and rough shells are the thickest at the sharp end of the egg. Shells without surface pigmentation are generally thinner than the other ones, and the thinnest at the sharp end of the egg. The crystal layer covers the whole egg and is very thick at some places, while the palisade layer is significantly narrower in this group of shells, as compared with good-quality and rough ones. The palisade layer is hardly distinguishable in the equatorial part of the egg, and the mammillary layer – at the sharp end. Well-developed, cone-shaped mammillae, separated by large spaces, are visible in the equatorial part of the shell only (Phot. 7).

In eggs with good-quality shells the surface areas of mammillae are similar in shape and separated from one another (Phot. 8). The smallest surface areas of mammillae can be observed in the equatorial part of the shell, while the largest – at the sharp end of the egg. In the central part of the mammillae there are cores with radially extending shell mass. The surfaces of mammillae differ in size and shape to a higher degree in rough shells than in good-quality ones (Phot. 9). Some mammillae have no connection to the inner shell membrane, which suggests that the uterine glands did not function simultaneously during the first 6 to 8 hours of shell formation. In shells without surface pigmentation mammillae have large flat surface areas without clearly visible cores, which distinguishes them from the other groups of eggshells (Phot. 10).

In eggs with good-quality shells the inner shell membrane consists of thick fibers in the equatorial and sharp parts of the egg, and thinner at the blunt end (Phot. 11). Inner shell membrane fibers form a web. Fiber thickness in rough shells and in good-quality shells is comparable. However, in rough-shelled eggs

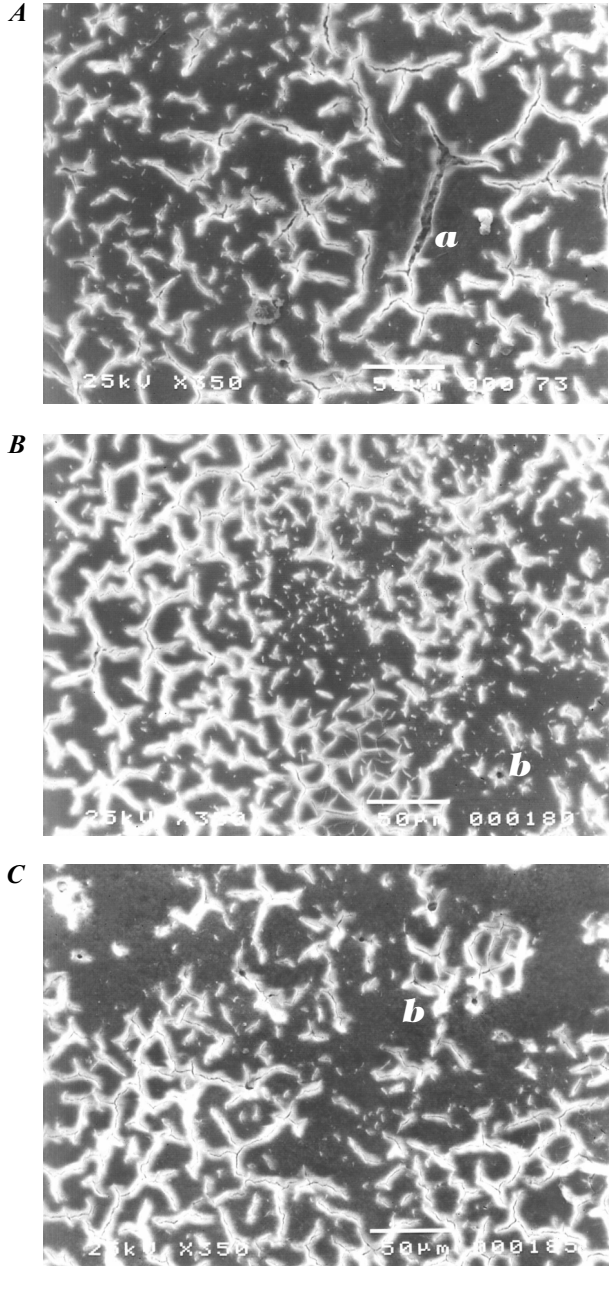

Fig. 1. The cuticle on the surface of good-quality egg shell: *A* – blunt part, *B* – middle part, *C* – sharp part, *a* – cuticle crack, *b* – pores

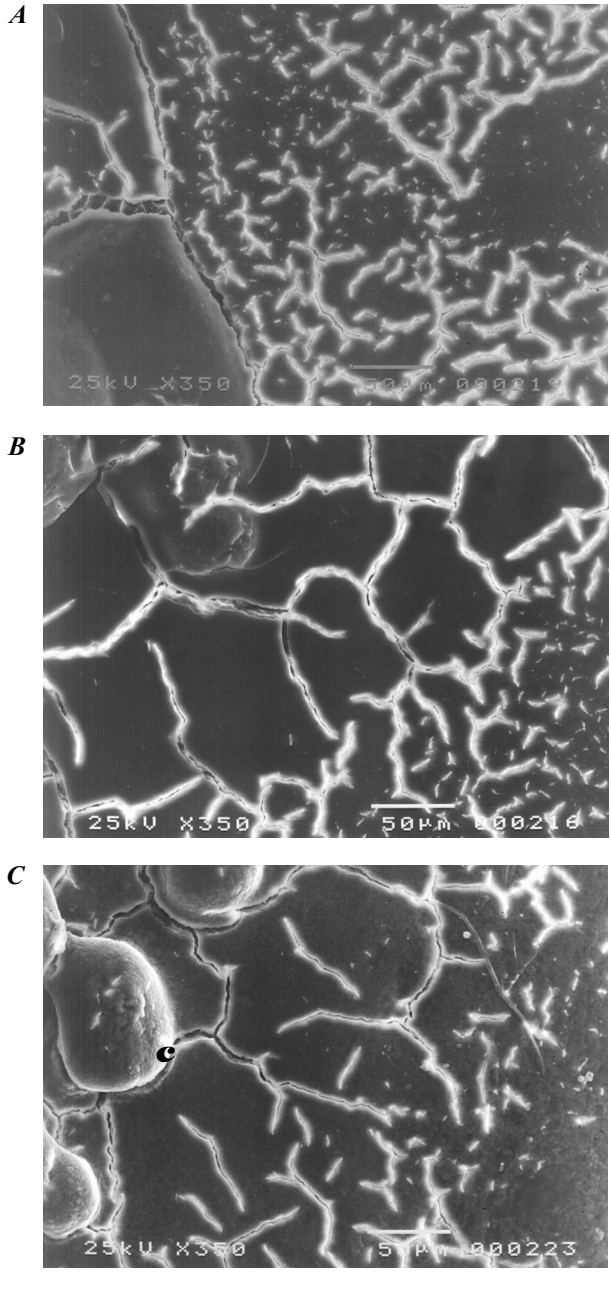

Fig. 2. The cuticle on the surface of rough egg shell: *A, B, C* – see Fig. 1, *c* – additional egg shell mass

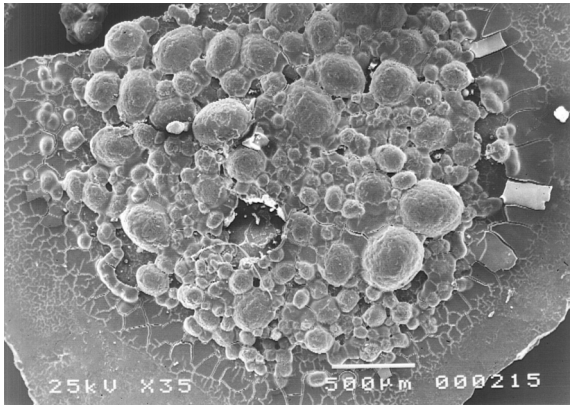

Fig. 3. Swelling of egg shell mass on the rough surface

the fibers are arranged in a parallel pattern in the equatorial part (Phot. 12). In eggs whose shells have no surface pigmentation inner shell membrane fibers are very thick in the equatorial and blunt part of the egg. Curved fibers form large enclosed spaces in these parts of the shell (Phot. 13).

Shell surface defects are related to the shape of cracks as well as to the absence or considerable loss of the cuticle, which was confirmed by studies on other bird species (SOLOMON 1991, MALEC 1999, KRYSTIANIAK 2002). Puchajda et al. (1997) demonstrated differences in cuticle distribution patterns resulting from the origin of turkeys.

The crystal layer can be found only in some parts of the shell in turkey, chicken and emu eggs (SOLOMON 1991, SZCZERBIŃSKA 2002). In pheasants this layer is visible in all shell parts, while in some other bird species it is hardly observable (PARSONS 1982, KRYSTIANIAK 2002). The thickness of this layer increases in shells with structural defects.

Cracks in the palisade layer are the evidence of shell damage in the oviduct. Thinning of the palisade layer as well as the lack of differences between this layer and the mammillary layer indicate a poorer quality of the egg (PARSONS 1982, SOLOMON 1996, MALEC 1999, RICHARDS and DEEMING 2001, KRYSTIANIAK 2002, SZCZERBIŃSKA 2002). Small differences in the structure of the palisade and mammillary layers are observed in eggs characterized by lower hatchability (MRÓZ 1998, KRYSTIANIAK 2002).

Previous research shows that the shape of mammillae is dependent on the origin of birds and shell quality (SOLOMON 1991, PANHELEUX et al. 1997). Mammillae similar in shape and size, and separated from one another, are considered normal. Mammillae with large bases are found

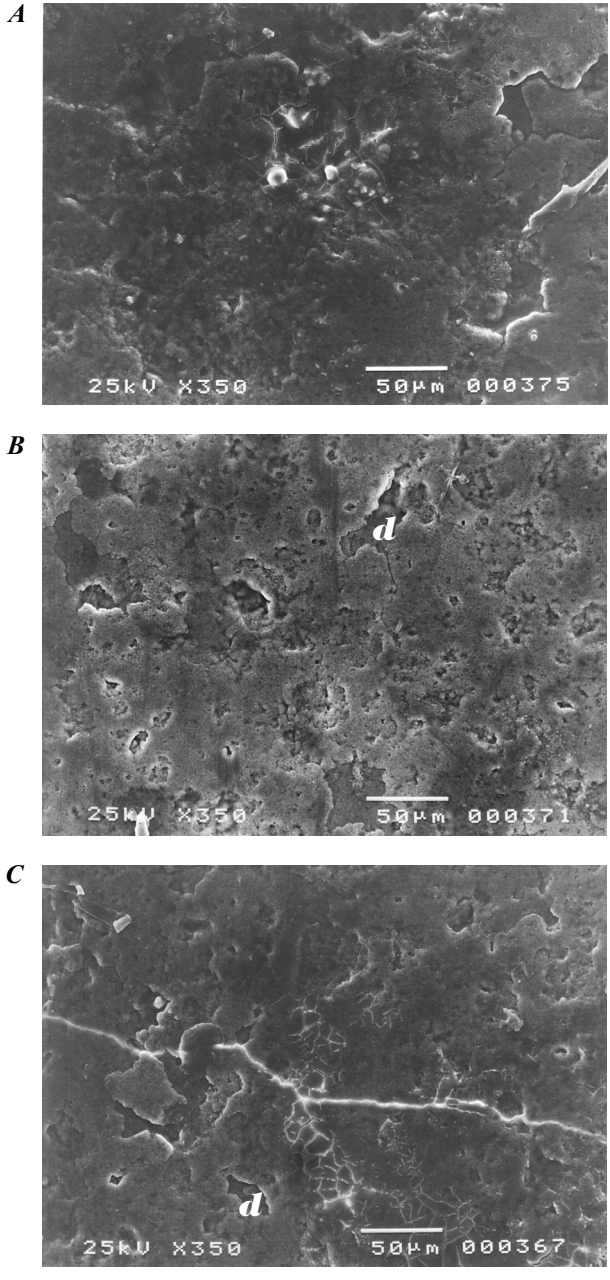

Fig. 4. The surface of egg shell without pigment: *A, B, C* – see Fig. 1, *d* – hollows on the surface of egg shell

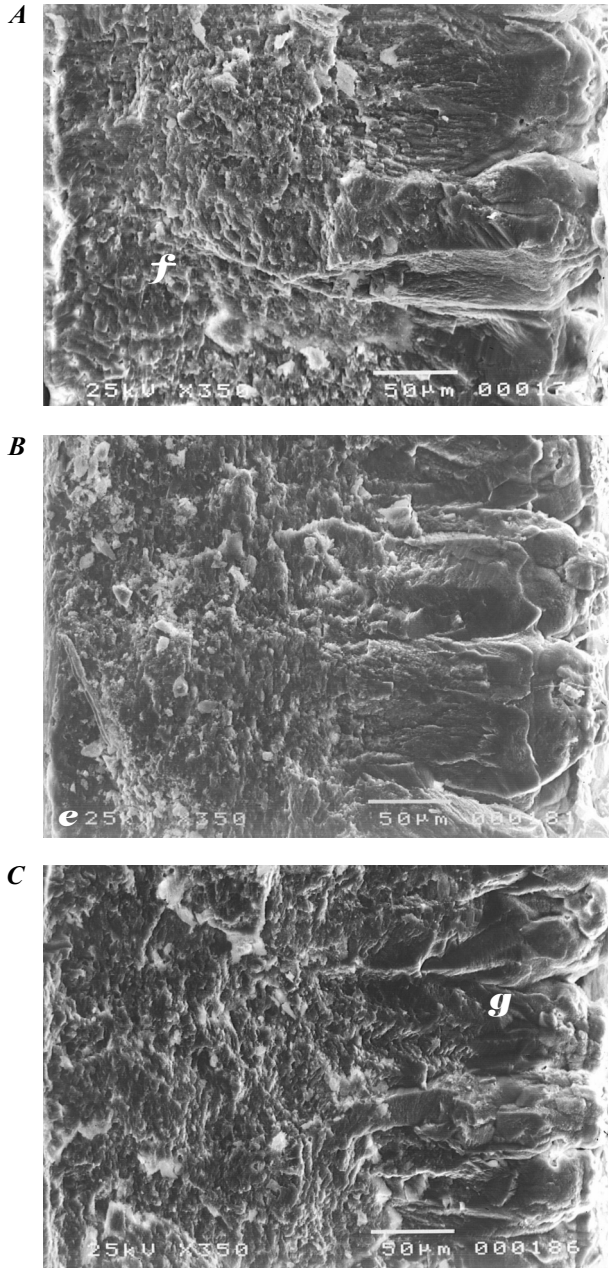

Fig. 5. The cross section of good-quality egg shell: *A, B, C* – see Fig. 1, *e* – crystal layer, *f* palisade layer, *g* – mammillary layer

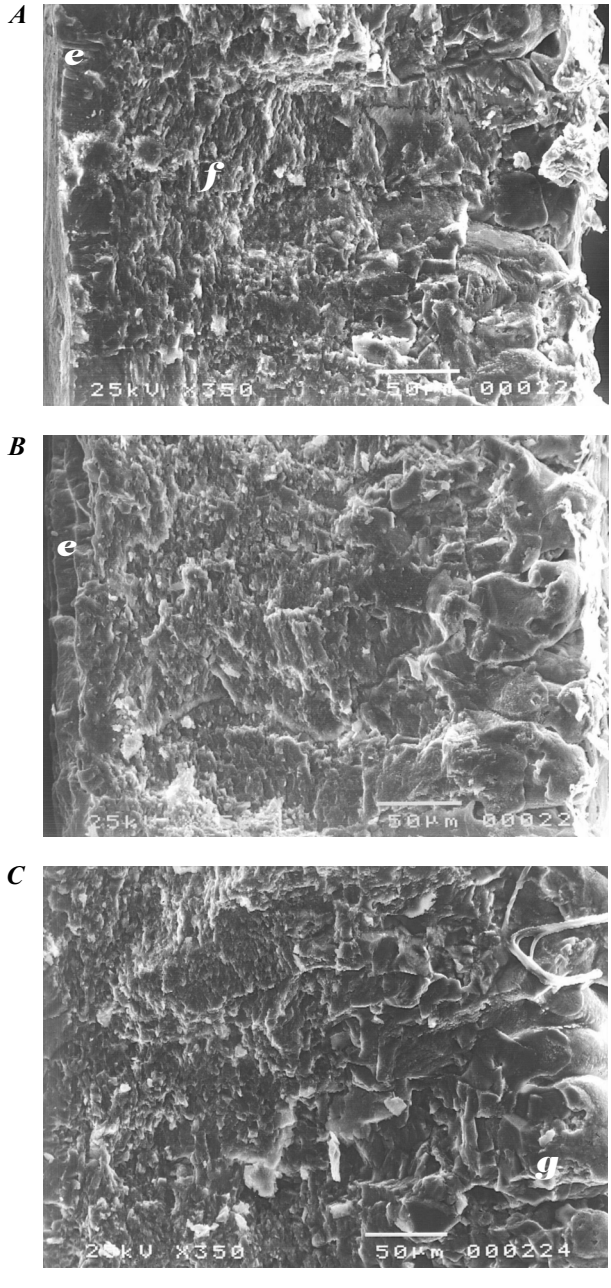

Fig. 6. The cross section of rough egg shell: *A, B, C* – see Fig. 1, *e*, *f*, *g* – see Fig 5

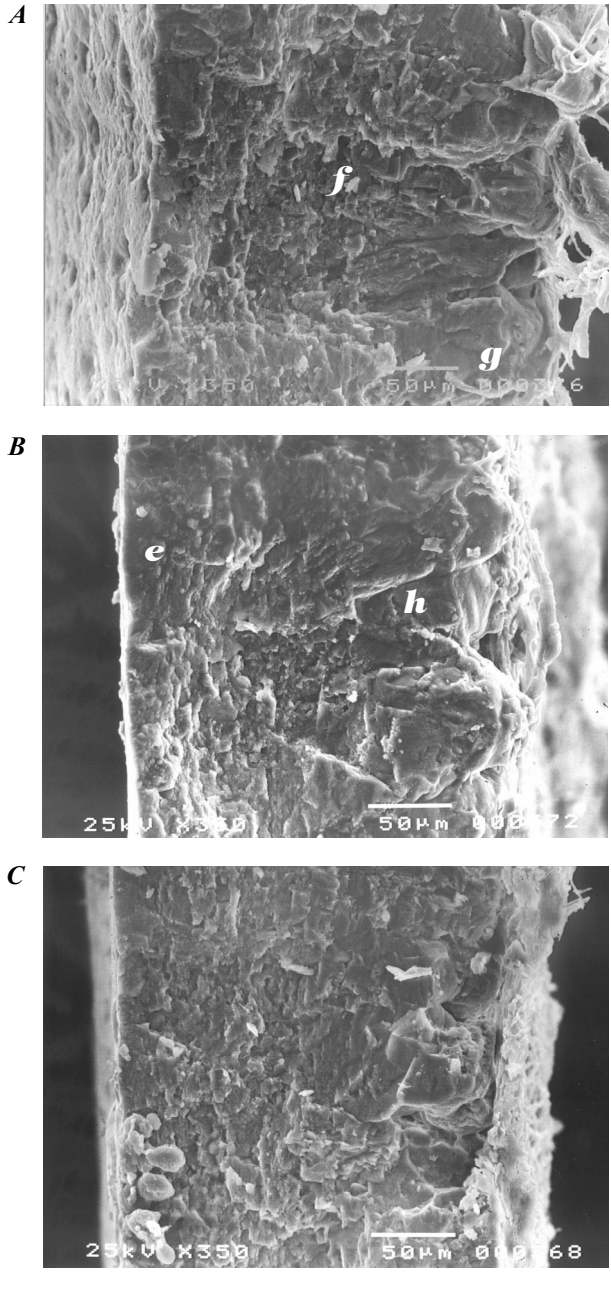

Fig. 7. The cross section of egg shell without pigment: *A, B, C* - see Fig. 1, *e*, *f*, *g* – see Fig 5, *h* – space between mammillary bodies

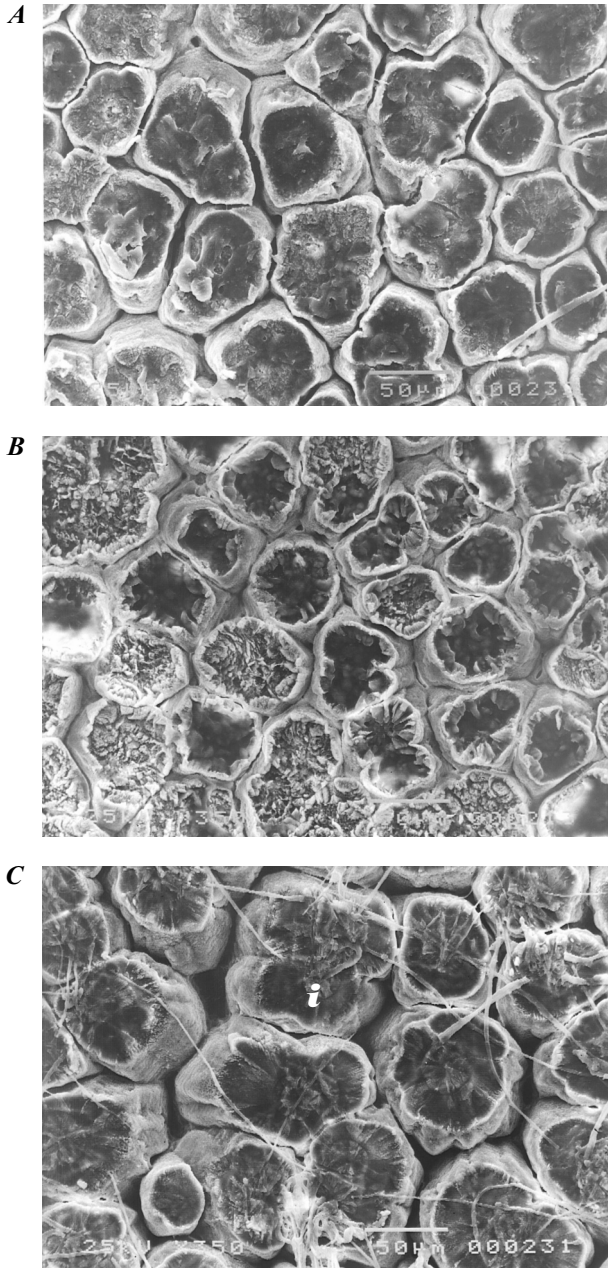

Fig. 8. The mammillary layer of good-quality egg shell: *A, B, C* – see Fig. 1, *i* – mammillary bodies fusion

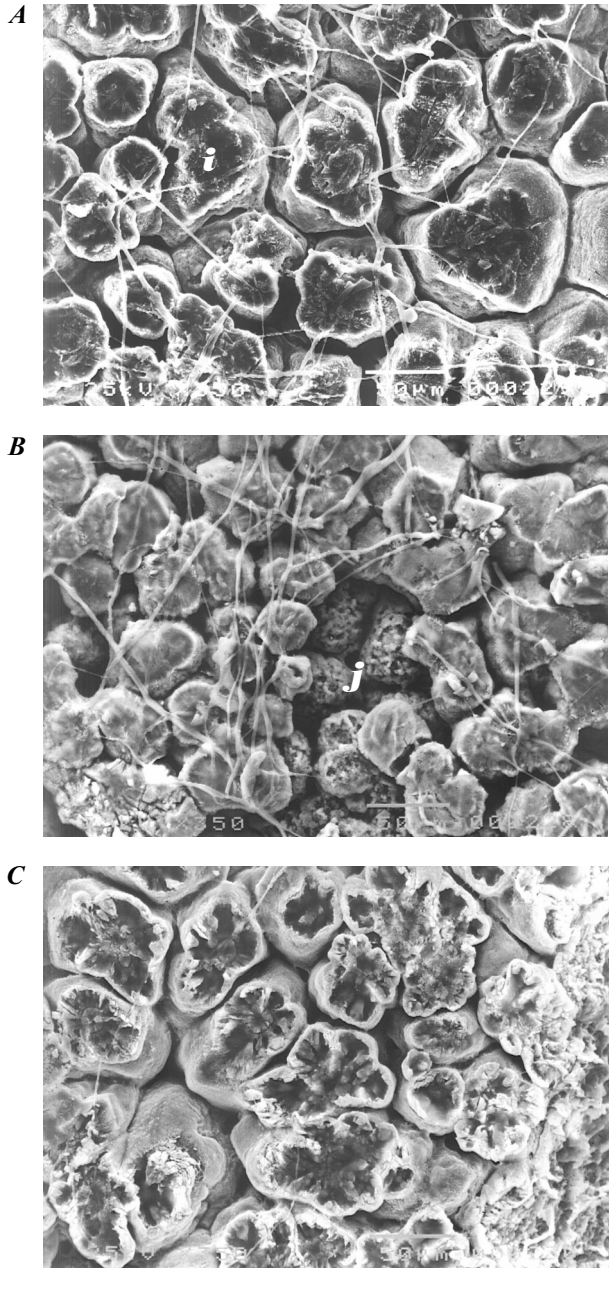

Fig. 9. The mammillary layer of rough egg shell: *A, B, C* – see Fig. 1, Fig. 8, *j* – mammillary bodies unconnected with membrane

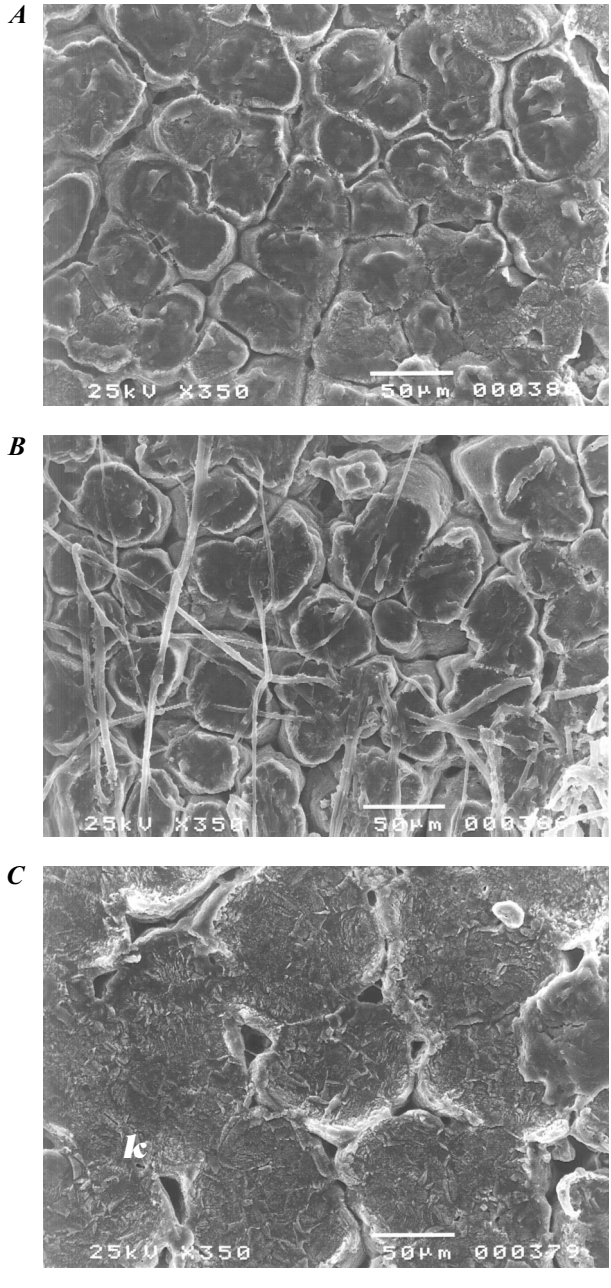

Fig. 10. The mammillary layer of egg shell without pigment: *A*, *B*, *C* – see Fig. 1, *k* – very big surfaces of mammillary bodies

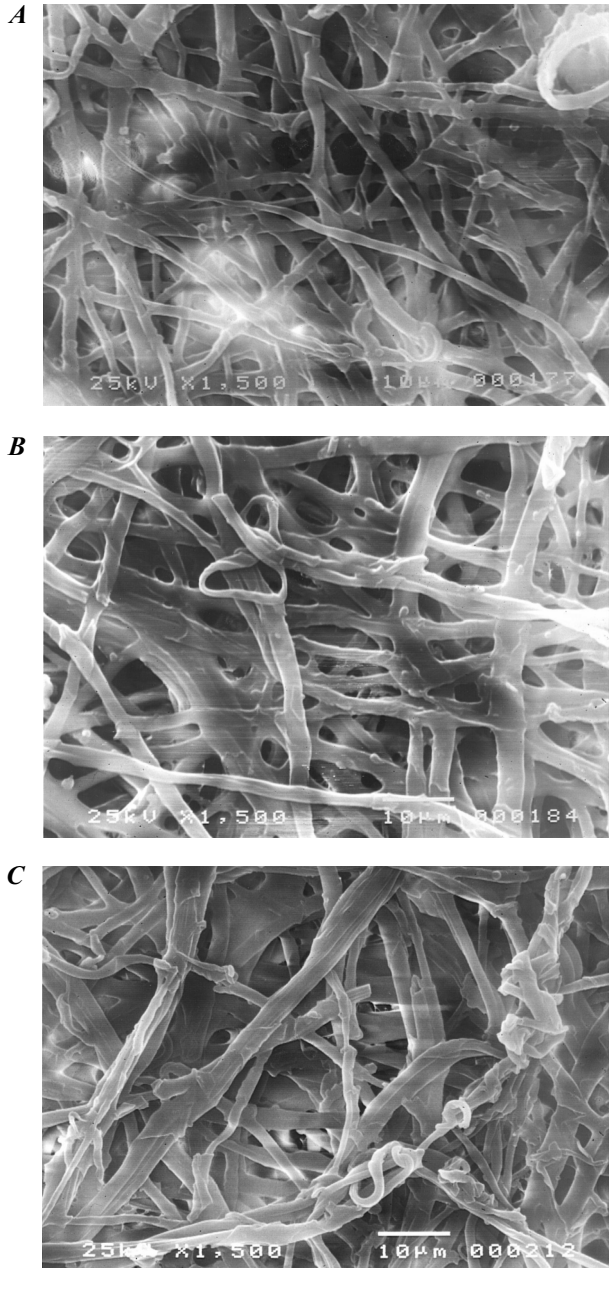

Fig. 11. The fibres of undershell membrane in eggs with good- quality egg shell: *A, B, C* – see Fig. 1

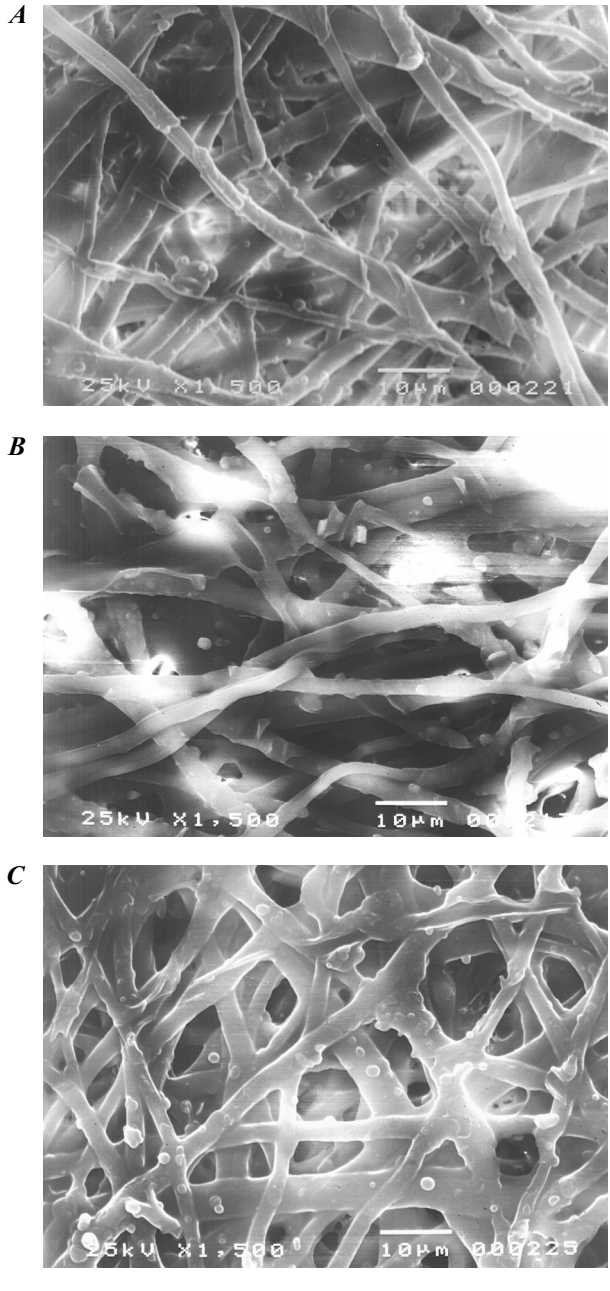

Fig. 12. The fibres of undershell membrane in eggs with rough egg shell: *A, B, C* – see Fig. 1

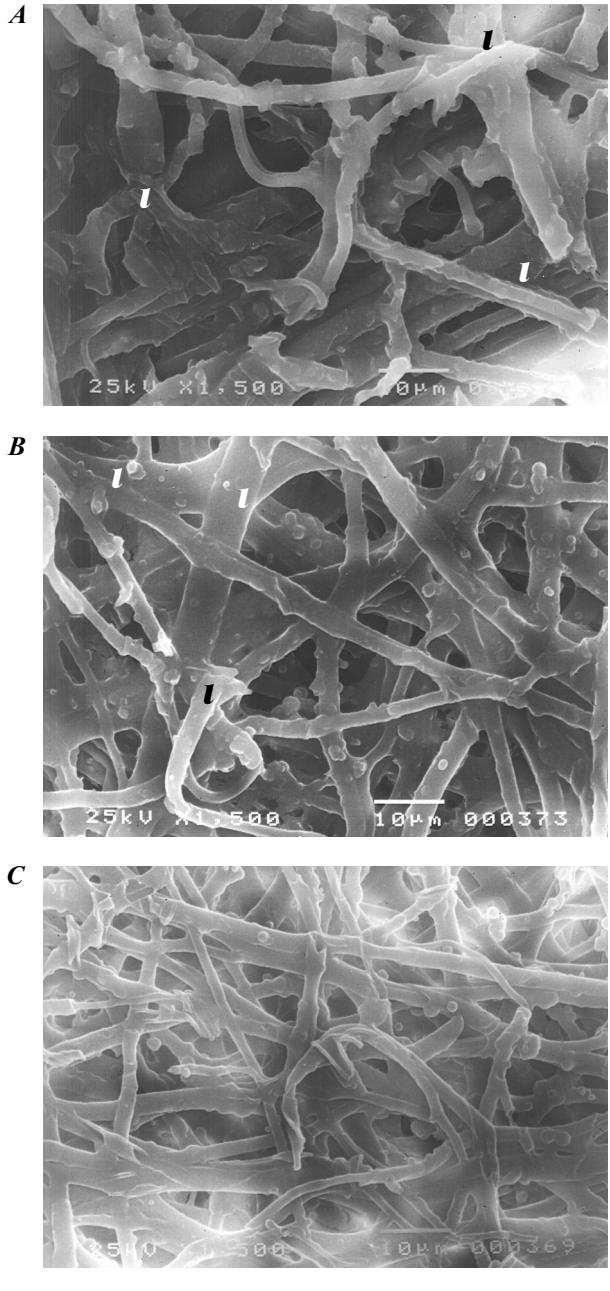

Fig. 13. The fibres of undershell membrane in eggs with egg shell without pigment: *A, B, C* – see Fig. 1, *l* – fibres fusion

in thin eggshells, which was also confirmed by other authors (SOLOMON, and WATT 1990, SOLOMON 1991).

The thickness of inner shell membrane fibers is related to shell surface pigmentation, which was observed in the present experiment as well as in studies on pheasant eggs (KRYSTIANIAK 2002). The thinnest inner shell membrane fibers are typical of eggs with the highest biological value (PUCHAJDA et al. 2000).

Eggs with surface defects and a thin palisade layer with cracks indicate that the uterine glands and the vaginal glands responsible for cuticle deposition, did not function normally during shell formation. The defective structure of this layer could be a result of rapid oviduct contractions. The presence of large amounts of mass forming the crystal layer resulted from increased activity of the glands at the final stage of shell formation. Rough shells and shells without surface pigmentation may be also a consequence of isthmus function disorders. These disorders lead to the thickening and bending of inner shell membrane fibers.

Conclusions

Good-quality shells of turkey hatching eggs are characterized by tiny cracks in the cuticle, the crystal layer present at some places of the shell, the thickest palisade layer and rectangular mammillae of comparable size. Inner shell membrane fibers form a web. Rough shells and shells without surface pigmentation differ from good-quality shells in terms of the following traits:

– the cuticle has slab-like structure or no cuticle is present,

– the crystal layer covers the major part of the palisade layer,

– there are cracks in the palisade layer,

– there is no clear boundary between the palisade layer and the mammillary layer,

– the mammillae are cone-shaped and have curved walls, and the spaces between them are larger,

– inner shell membrane fibers are thicker and curving.

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EFFECTS OF EGG STORAGE TIME ON EMBRYO MORTALITY RATES AND POULT QUALITY

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K e y w o r d s: eggs, storage period, hatchability, turkeys.

A b s t r a c t

The aim of the study was to determine the effects of an extended egg storage period on the time of embryo death during incubation and poult quality. Hatching eggs a total of 2268 of heavy turkeys between 12 and 18 weeks of the laying period were used in the experiment. They were stored for 7, 8, 9, 10, 11 and 12 days and next each of them were weighted. Three hatchings were made for each period of storing and elementary hatching rate were monitored.

The weight of hatching eggs ranged from 93.0 g to 95.0 g and showed low variation. The embryo mortality rate in eggs stored to 9 days was 10.2 to 10.3%, and in eggs stored longer – 11.4 to 13.8%. Unchatched embryos accounted for 1.9 to 4.1% in eggs stored to 9 days, and for 5.5 to 6.5% in eggs stored longer. Hatchability varied from 85.6 to 87.6% in eggs stored to 9 days, and to 79.9 to 82.9% in those stored for 10-12 days.

A prolonged storage period (over 9 days) increased early embryo mortality rates. The highest embryo mortality rate was recorded in the phase of early vasculogenesis. Between 11 and 24 days the embryo mortality rate was much lower in eggs stored for 10 to 12 days, compared with those stored for a shorter time.

The results of the study did not confirm explicitly the effects of egg storage time on poult weight. The body weights of newly hatched poults ranged from 64.6 g to 67.0 g. The poults that hatched from eggs stored for 11 and 7 days were the heaviest, and those which hatched from eggs stored for 10 and 8 days – the lightest. The number of poults with anatomic defects was high in all groups. General weakness and leg weakness were more frequent in poults that hatched from eggs stored for 11 and 12 days, in comparison with those which hatched from eggs stored for 7 to 10 days.

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CZAS PRZECHOWYWANIA JAJ A ŚMIERTELNOŚĆ ZARODKÓW I JAKOŚĆ INDYCZĄT

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S ł o w a k l u c z o w e: jaja, czas przechowywania, wylęgowość, indyczęta.

A b s t r a k t

Określono wpływ wydłużonego czasu przechowywania jaj indyczych na wiek zamierania zarodków w czasie inkubacji i jakość indyczą po wylęgu.

Do badań użyto 2268 jaj wylęgowych pochodzących od indyków typu ciężkiego między 12. a 18. tygodniem nieśności. Magazynowano je przez 7, 8, 9, 10, 11 i 12 dni, i następnie każde z nich zważono. Wykonano 3 lęgi z uwzględnieniem każdego okresu magazynowania i skontrolowano podstawowe wskaźniki wylęgowości.

Masa jaj wylęgowych wahała się od 93,0 g do 95,0 g i była mało zmienna. Śmiertelność zarodków w jajach magazynowanych do 9 dni wynosiła 10,2 – 10,3%, w jajach magazynowanych dłużej – 11,4 – 13,8%. Nie wyklutych indyczą w jajach magazynowanych do 9 dni było 1,9 – 4,1%, a w jajach magazynowanych dłużej – 5,5 – 6,5%. Wyląg indyczą wahał się od 85,6 do 87,6% z jaj magazynowanych do 9 dni, a z jaj przechowywanych 10 – 12 dni – od 79,9 do 82,9%.

Wydłużony okres magazynowania jaj (> 9 dni) wpłynął na zwiększenie pierwszego szczytu śmiertelności zarodków. Największa śmiertelność wystąpiła w fazie wczesnej waskulogenezy. Między 11. a 24. dobą śmiertelność zarodków była znacznie mniejsza w jajach przechowywanych 10-12 dni niż krócej.

Nie wykazano jednoznacznie wpływu czasu magazynowania jaj na masę piskląt. Masa ciała wylęgłych indyczą wahała się od 64,6 g do 67,0 g. Najcięższe indyczęta pochodziły z jaj przechowywanych 11 i 7 dni, najlżejsze z jaj przechowywanych 10 i 8 dni. Liczba indyczą z wadami budowy ciała była duża we wszystkich grupach. Ogólna słabość i wady nóg częściej występowały u indyczą wyklutych z jaj przechowywanych 11 i 12 dni niż 7-10 dni.

Introduction

On the ground of many of study it was demonstrated that long egg storage results in incubation losses (CHRISTENSEN et al. 2003a,b). Leading turkey breeders share the opinion that the best hatching results can be achieved when newly-laid eggs are stored for 2 to 4 days. Eggs storage to 12 days reduces the hatching rate from 1.00 to 0.929 (ANON 1999). According to the Polish Standards, the optimum storage time for turkey hatching eggs is up to 7 days, and 8 to 14 days is considered a permissible period.

An extended storage time alters the biological value of eggs, including changes in the content of water, fatty acids and amino acids, stability of amino acid complexes, enzymatic activity and pH (SMOLIŃSKA et al. 1983, ŚWIERCZEWSKA et al. 1997). In addition, the permeability of the vitelline membrane and shell increases, whereas chalaza loses some strength (ACKER and TERNES 1994, CHRISTENSEN et al. 2003a). Such modifications in the egg content affect the

processes of yolk utilization, the weights of heart and muscle and the concentrations of glucose and glycogen in these organs (CHRISTENSEN et al. 2003b). Egg aging results in a higher embryo mortality rate during the first week of incubation as well as prolonged internal – pierce subshell membranes and external pipping – pierce shell (CHRISTENSEN 2001, CHRISTENSEN et al. 2003a, FASENKO et al. 1992). Available literature on the subject provides no detailed data on the developmental stages at which embryos die during long-term egg storage.

Breeding methods and feed also affect changes in the biological quality of eggs (CROUCH et al. 2002). Breeding work effects of egg weight, chemical composition and modify the structure of some egg parts (FARUGA et al. 1996, JANKOWSKI 1989, MRÓZ 1996, 1998). Due to these changes, it seems that egg storage methods will have to be improved in the nearest future.

Despite a continuous improvement in the breeding value of pedigree flocks as well as in incubation technologies, poult quality remains unsatisfactory. Thus, studies are conducted to determine the reasons for physical defects of hatchlings. Professional literature provides scant information on the effects of egg storage time on poult quality. However, it was demonstrated that the duration of hatching in eggs stored for 3 and 15 days varied and could affect the quality of hatchlings (CHRISTENSEN et al. 2003a). Publications dealing with the methods for evaluation of newly-hatched poults are also few, and the majority of them are based on the results of chick evaluation (MRÓZ and PUDYSZAK 1997, TONA et al. 2003).

The aim of the study was to determine the effects of an extended egg storage period on the time of embryo death during incubation and poult quality.

Materials and Methods

The experimental materials comprised hatching eggs obtained from heavy Nicholas turkeys during 12 to 18 weeks of the laying season. 2020 turkey-hens were farmed in accordance with the relevant technological standards for this species. The eggs considered unsuitable for incubation were eliminated. Each day the eggs were disinfected and transported from the farm to the hatchery. At the hatchery the eggs were disinfected again and placed in a storeroom, where they were stored at a constant temperature (15°C) and air humidity (86%). Air change and movement were controlled automatically and adjusted, depending on the number of eggs.

126 eggs stored for 7, 8, 9, 10, 11 and 12 days (a total of 2268 eggs = 126 eggs x 6 periods x 3 hatchings) were selected randomly for analysis. Egg weight

was determined before incubation, exact to 0.1 g. The eggs were incubated in Petersime incubators, always in the same setting unit and hatching unit, in accordance with the technology recommended for this fowl species (FARUGA and JANKOWSKI 1996). The eggs were candled at 10 days of incubation, to eliminate unfertilized eggs and eggs containing dead embryos. The number of eggs with embryos that died between 11 and 28 days of incubation, and the number of eggs with unhatched poults were determined at the completion of incubation. The eggs with embryos that died were analyzed to estimate the time of embryo death and on the ground were determined the distribution of embryo mortality during the entire incubation period, which was divided intro three stages: stage I – embryos that died during the first 10 days, stage II – embryos that died between 11 and 24 days, stage III – embryos that died between 25 and 28 days (CHERMS 1980, DZIACZKOWSKA and FARUGA 1983). The number of embryos that died on consecutive days of incubation was calculated as a percentage in relation to all dead and unhatched embryos. The days when the number of dead embryos exceeded 10% were referred to as peaks of embryonic mortality. The days when the number of dead embryos ranged between 5% and 10% were referred to as periods of increased embryonic mortality. The days when the number of dead embryos did not exceed 5% were referred to as inter-peak periods. The following hatching indices were determined: fertilization rate, the percentages of dead and unhatched embryos and hatching rate of fertilized eggs.

All hatchlings were weighed exact to 0.1 g and their body conformation was evaluated by the method proposed by TONA et al. (2003), modified by MRÓZ and MICHALAK (2004). The percentage of poults with external defects was calculated. Some poults had several physical defects, so the total percentage of poults with body abnormalities was lower than the sum of defects.

The numerical data were analyzed statistically by one-factor analysis of variance in an orthogonal and non-orthogonal design, and compared with the Duncan's test. The results are presented as means and coefficients of variation.

Results and Discussion

The eggs weight between 12 and 18 weeks of the laying season was comparable in all groups, which proves low variation of this trait (Table 1). Egg weight recorded in the present study was higher than reported by other authors, which resulted from the descent and age of turkey-hens (APPLEGATE and LILBURN 1996, CROUCH et al. 2002, FARUGA et al. 1996, MRÓZ et al. 2002b).

Table 1

Biological value of turkey eggs stored for 7 to 12 days before incubation (x, v%)

Egg traits	Egg storage period in days					
	7	8	9	10	11	12
Egg weight (g)	94.16^{B} 6.83	93.46^{B} 6.87	95.03^{A} 6.85	93.30^{B} 10.09	93.24^{B} 6.82	93.05^{B} 6.64
Fertilization rate (%)	95.77 2.91	96.56 2.64	95.24 1.44	94.18 5.48	94.71 2.42	94.70 3.78
Dead embryo* (%)	10.20 45.40	10.36 23.18	10.27 32.87	13.87 38.22	12.48 40.08	11.46 9.24
Unhatched poults* (%)	4.19 54.34	1.94 50.06	3.35 50.65	6.15 15.32	6.58 41.70	5.54 34.34
Hatched poults* (%)	85.60^{ab} 4.78	87.69^{a} 2.17	86.37^{ab} 3.21	79.97^{b} 7.78	80.93^{ab} 5.43	82.99^{ab} 2.36

* – % in relation to fertilized eggs
Values in lines denoted with:
AB – are significantly different at $p \leq 0.01$
ab – are significantly different at $p \leq 0.05$

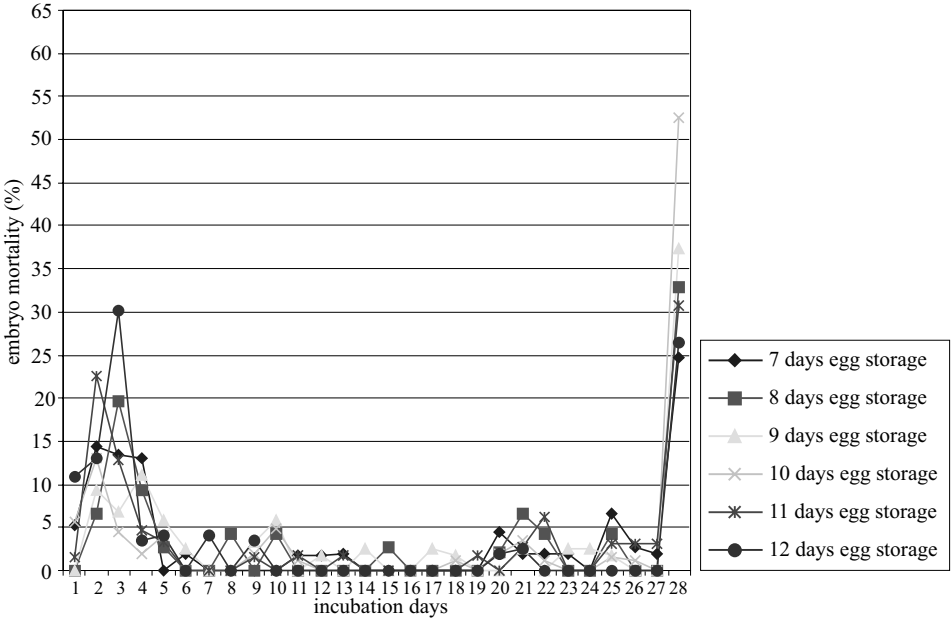

Fig. 1. Embryo mortality decomposition in eggs stored in period from 7 to 12 days

In our experiment, fertilization rates indicates, that between 12 and 18 weeks of the laying season the reproduction capacity of turkeys was high (Table 1). The lowest hatching rate of fertilized eggs was recorded in the group

of eggs stored for 10 days in comparison to other groups ($p \leq 0.05$). In spite of the absency statistical differences results of hatching rate in the groups of eggs stored 7, 8 and 9 days was high, which suggests that the time of egg storage should be shorter. The variation in this trait was low (Table 1). In our experiment, fertilization rates were comparable to those observed by other authors, whereas hatching rates were higher as compared with those achieved during the last 20 years (FARUGA et al. 1996, JANKOWSKI 1989, MRÓZ and PUDYSZAK 1997, MRÓZ et al. 2002a,b). CHRISTENSEN et al. (2003a) demonstrated that longer egg storage caused an increase (by 8.2%) in the number of dead embryos, which is consistent with our results.

The first peak of embryonic mortality reached the highest level in eggs stored for 12, 11 and 8 days, followed by those stored for 10, 9 and 8 days. Increased mortality rates were noted at 10, 21, 22 and 25 days of incubation in all egg groups except for those stored for 10 and 12 days. The length of the inter-peak period was different in particular egg groups. It was the longest (25 and 24 days) in eggs stored for 10 and 12 days, and somewhat shorter (20, 17 and 16) in eggs stored for 7, 8, 9 and 11 days. An analysis of the distribution of embryonic mortality over the entire incubation period confirmed that it was affected by egg storage time. The percentage of dead embryos at successive incubation stages was similar in eggs stored for 7, 8 and 9 days, and widely varied in the groups of eggs stored longer (Table 2). It is difficult to determine the effects of egg storage time on the percentage of dead embryos based on the distribution of embryonic mortality at successive stages of incubation. According to reference data, during long-term egg storage more embryos die at the beginning of incubation than towards the end of this period (CHRISTENSEN 2001, CHRISTENSEN et al. 2003a). The first mortality peak more evidently indicated the correlation between egg storage time and embryo mortality rates.

Table 2
Turkey embryo mortality in dependent for egg storage period before incubation (% in relation to the total number of embryo mortality)

Incubation period	Egg storage period in days					
	7	8	9	10	11	12
To 10 day	48.18	46.93	44.86	35.81	46.44	69.20
11 – 24 day	15.52	15.83	16.15	8.81	13.26	4.41
25 – 28 day	36.20	37.23	38.99	55.34	40.30	26.38

The heaviest poults were obtained from eggs stored for 7 and 11 days, and the lightest – from those stored for 8 and 10 days ($p \leq 0.01$). The differences in the mean body weights of hatchlings did not exceed ± 2.42 g (Table 3).

The mean body weights of poults were high. The variation in this trait was low, which was a consequence of low variation in egg weight. The body weights of hatchlings obtained in this study are comparable with the results presented by MRÓZ and PUDYSZAK (1997), and higher than those reported by APPLEGATE & LILBURN (1996) and MRÓZ & MICHALAK (2004).

Table 3

Quality of hatched turkey poults in dependent for egg storage period (x, v%)

Poults traits	Egg storage period in days					
	7	8	9	10	11	12
Body weight (g)	66.59^{BCcd} 8.30	64.61^{Aad} 7.88	66.17^{BCbc} 8.13	65.63^{ABbd} 7.79	67.03^{Cd} 7.42	66.13^{BC} 7.56
On properly built (%)	43.31 17.45	31.51 31.99	43.00 26.57	41.59 26.46	41.16 58.16	37.18 72.08
With physical body defects (%) in this:	56.68 13.33	68.49 14.72	56.99 20.05	58.40 18.84	58.83 40.70	62.81 42.67
– weak	6.28	3.65	2.25	4.84	8.62	10.10
– with umbilicus defects	94.85	95.89	88.13	97.57	94.85	93.61
– with plumage defects	9.71	8.21	11.86	13.33	9.19	10.63
– with leg defects	1.14	2.28	0.56	0.00	4.59	4.25

Values in lines denoted with:
ABC – are significantly different at $p \leq 0.01$
abcd – are significantly different at $p \leq 0.05$

Egg storage time had no effect on the percentage of poults with structural defects. In all groups the proportion of poults showing no body abnormalities was below 43.3% (Table 3). Navel lesion – navel with bloody crust and umbilical vessel was the most common defect in newly-hatched birds. The percentages of weak poults and poults with plumage and leg defects increased as the time of egg storage was extended (Table 3).

The most common defect in poults hatching under artificial conditions was navel lesion, which was confirmed by the present results. The reasons for navel lesions are infections of the yolk sac and respiration disorders in embryos (BORZEMSKA 1978, 1992). In our experiment embryo infections can be excluded, since turkey-hens, eggs and incubators were under constant veterinary and sanitary control. A higher percentage of weak poults and poults with leg defects obtained from long-stored eggs suggests that embryo development in these eggs was disturbed not only in the initial phase of embryogenesis, but also during leg formation and at the final stage of hatching (BORZEMSKA 1978, 1992, BORZEMSKA and KOSOWSKA 1997).

Conclusions

It was found that an extended egg storage time reduced the hatching rate of fertilized eggs and intensified the first peak of embryonic mortality. In eggs stored for 11 and 12 days the highest embryo mortality rate was recorded during vasculogenesis. Embryo mortality rates between 11 and 24 days of incubation were lower in eggs stored for 10 to 12 days than in eggs stored for a shorter period of time. It cannot be concluded that egg storage time affected the birth weights of hatchlings. Significant differences in the mean body weights of poults did not exceed 2.42 g. The percentage of birds with body abnormalities was similar in all groups of eggs, regardless of storage time. The number of weak poults and poults with leg defects was higher in the case of eggs stored for 11 and 12 days than in that of eggs stored for 7 to 9 days.

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ANALYSIS OF ZOOMETRIC PARAMETERS OF CONTEMPORARY SPORT-CARRIAGE HORSES IN POLAND

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K e y w o r d s: carriage horses, limb and trunk build, limb joint angles, zoometric parameters.

A b s t r a c t

The leading carriage horses in Poland were used for the study. In order to characterise their exterior, a series of zoometric measurements of their trunks and front and hind legs were taken. The study employed a photozoometeric method, developed by the authors; this allowed for estimating the length and joint angle of particular sections of the front and hind legs. The measurements results allowed for the calculation of: 7 indices of the horse body structure, 10 indicators which characterise the proportions of the front and hind leg fragments in relation to the height at withers, as well as the angles between them and estimation of the horse body weight. The average zoometric parameters of the horses included in the study as well as the build indices based on them, adopted the values typical of light breeds of carriage horses. The photozoometric method was found to be usable in estimating the zoometric parameters of horses.

CHARAKTERYSTYKA PARAMETRÓW ZOOMETRYCZNYCH WSPÓŁCZESNEGO KONIA W TYPIE SPORTOWO-ZAPRZĘGOWYM W POLSCE

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S ł o w a k l u c z o w e: konie sportowo-zaprzęgowe, budowa kończyn i kłody, parametry zoometryczne.

A b s t r a k t

Badaniami objęto czołowe konie wykorzystywane w sporcie zaprzęgowym w Polsce. W celu scharakteryzowania ich pokroju dokonano pomiarów zoometrycznych kłody, kończyny przedniej i tylnej. W badaniach zastosowano własną metodę fotozoometryczną, dzięki której oszacowano

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długość oraz kąty między poszczególnymi odcinkami kończyny przedniej i tylnej. Wyniki badań umożliwiły ustalenie: 7 indeksów budowy ciała koni, 10 wskaźników charakteryzujących proporcje fragmentów kończyny przedniej i tylnej w stosunku do wysokości w kłębie, kątów między nimi oraz oszacowanie masy ciała koni. Średnie parametry zoometryczne badanych koni oraz obliczone na ich podstawie indeksy budowy przyjmowały wartości charakterystyczne dla koni ras lekkich w typie zaprzęgowym. Stwierdzono także przydatność metody fotozoometrycznej do przyżyciowego oszacowywania parametrów zoometrycznych koni.

Introduction

Analysis of a horse's exterior and evaluation of its motor characteristics is a basic selection criterion in horse breeding. As horse breeders' and users' observations indicate, the build parameters of a horse's front and hind legs as well as the trunk significantly affect its usability (PRUSKI, 1960 BACK et al. 1996, KAPROŃ 1999, PIETRZAK et al. 2003). This view is reflected in scientific research, which indicate that there is a relationship between the zoometric and biomechanical parameters of a horse, on the one hand, and its usability on the other (HOLMSTRÖM et al. 1990, HODGSON and ROSE 1994, DEUEL 1995, KAPROŃ et al. 2000, PIETRZAK et al. 2001). Currently, selection of horses is largely based on the knowledge and experience of breeders who evaluate their external appearance and usability. However, this is a subjective method. Therefore, research is being carried out aimed at developing objective methods of early selection of horses based on computer analysis of – filmed – gait and manner of obstacle jumping (BACK 1994, LEWCZUK 1999).

One of the objective, and yet effective, methods of horse selection is the speed selection of thoroughbred horses. In the breeding process of these horses, the qualification of horses for reproduction is based on their performance in races: the winners – sometimes with faulty conformation, but best suited for fast running – are qualified. This study is an attempt at an analysis of zoometric parameters of elite carriage horses, which are best suited for this type of use.

Materials and Methods

The material for the study was supplied by horses from Polish and foreign stud farms, taking part in carriage driving competitions in Poland in the 2004 season. The analysis covered 53 horses (16 stallions, 34 geldings, 3 mares) of the following breeds: Noble half-bred – sp, Oldenburger – old, Silesian – sl, Wielkopolska breed – wlkp, Małopolska breed – m, Czech warmblood – CT, of unknown breed – nn.

In order to characterise the conformation of the contemporary carriage horse, the following measurements were taken (according to ZWOLIŃSKI 1983):

1) with a measuring stick: height at withers, oblique trunk length, chest depth;

2) with a measuring tape: chest circumference, front shank circumference;

3) with a compass: chest width, width at hips, croup length.

The following indices of the body build were calculated using the measurement results (according to ZWOLIŃSKI 1983): chest circumference, boniness, trunk length, depth, eurysomia, massiveness and strength.

The horses' body weight was also estimated, using the MEYER'S (1992) formula:

$$\text{body weight} = \frac{(\text{chest circumference})^2 \cdot \text{trunk length}}{11\,900}.$$

The subsequent stage of study consisted in making estimations, employing the author's photozoometric method, of the length of the front and hind legs sections and the angles between them. For the shoulder blade and fetlock, the angle between them and the horizontal plane was measured. To achieve this, the following points were marked on each horse with special markers:

– in the front leg: the furthermost point of the shoulder blade marked by the line of the shoulder blade crest, shoulder joint, elbow joint, wrist joint, fetlock, point on the hoof crown marked by the pastern-hoof axis

– in the hind leg: point of hip, hip joint, knee joint, hock, fetlock, point on the hoof crown marked by the pastern-hoof axis.

The horses were then photographed positioned in the way which made the front and hind legs cover the other pair of legs. The photographs thus obtained were analysed with Microstation computer software. The points (markers) linked by lines made sections, the angles between which were subjected to computer analysis.

Thanks to the method employed in the study it was possible to determine the ratio of limb sections to the height at withers. According to this principle, the following indices were calculated (%): length of shoulder blade (WDŁ), length of arm (WDR), length of forearm (WDPR), length of front shank (WDNP), length of front pastern (WDPP), length of croup (WDZ), length of thigh (WDU), length of second thigh (WDPU), length of hind shank (WDNT), length of hind pastern (WDPT).

The data were analysed statistically by calculating the mean values and standard deviation, taking into account the division into breeds.

Fig. 1. The diagram showing the positions of the points-markers

The significance of differences between horse groups was determined by the single-factor analysis of variance and the Duncan test, using the Statistica 6.0 software, model Anova.

Results

Trunk

Table 1 contains the results of body build parameters analysis for the 53 horses under study, which belonged to the group of elite carriage horses. The average height at withers was 166.4 cm. The other trunk parameters were: trunk length – 178.3 cm; chest width – 30.3 cm, chest depth – 70.7 cm; and its circumference – 199.8 cm. The width at hip and the horses' body weight were 44.3 cm and 597.5 kg, respectively.

Statistically significant differences concerning the height at withers, trunk length, chest circumference and body weight were find in the group of horses under study.

Table 2 contains, calculated from the measurement results, indices of horse body build and their boniness index. According to the data, the indices for a contemporary carriage horse are: trunk length – 107.0%; chest depth – 42.6%; eurysomia – 113.0%; chest circumference – 120.0%; massiveness

Table 1

Zoometric parameters of the trunk and body weight in carriage horses of various breeds

Groups		N	Zoometric dimensions (cm)												Body weight (kg)	
			height at withers		trunk length		chest depth		chest width		width at hips		chest circumference			
			x	s	x	s	x	s	x	s	x	s	x	s	x	s
Breed	sp	17	166.3	3.0	180.5	6.8	70.6	3.7	31.2	3.9	44.0	4.3	200.9	6.4	607.2	48.7
	old	2	166.0	4.2	183.5	6.4	71.0	1.4	30.5	4.9	47.0	0.0	199.5^{b}	6.4	613.5	17.7
	śl	18	166.0^{B}	2.9	174.6^{B}	7.2	69.8	4.2	31.0	2.9	45.0	4.8	199.3^{b}	5.4	583.7^{bd}	39.7
	wlkp	3	170.3^{B}	1.5	179.7	7.1	72.3	0.6	29.7	2.3	45.3	4.6	209.0^{Aa}	5.0	660.0^{Ba}	48.5
	m	6	166.0	5.9	176.5^{b}	10.1	71.3	7.2	26.7	2.2	41.8	5.2	195.7^{B}	7.5	569.7^{bd}	69.4
	CT	4	170.0^{B}	2.9	190.0^{Aa}	6.9	74.0	2.6	29.7	0.5	45.0	3.6	201.2	5.2	647.2^{Bc}	44.5
	nn	3	161.3^{A}	1.5	172.0^{B}	4.6	68.3	4.0	29.3	1.5	42.3	4.2	193.7^{B}	4.5	542.0^{A}	25.4
Total		**53**	**166.4**	**3.6**	**178.3**	**8.3**	**70.7**	**4.2**	**30.3**	**3.3**	**44.3**	**4.4**	**199.8**	**6.4**	**597.5**	**52.3**

AB differences significant at $p \leq 0.01$, *ab* differences significant at $p \leq 0.05$

Table 2

Body build indices of carriage horses of various breeds

Groups		N	Indices (%)												Index strength	
			chest circumference		boniness		trunk length		depth		eurysomia		massiveness			
			x	s	x	s	x	s	x	s	x	s	x	s	x	s
Breed	sp	17	119.4	2.7	13.0	0.5	105.7	3.8	41.8	2.7	113.0	5.4	3.5b	0.2	237.0	12.2
	old	2	122.0	4.2	13.6^{BD}	0.5	110.0	4.2	41.5	2.1	111.0	8.5	3.7	0.1	246.0	15.6
	śl	18	121.4	2.6	13.7^{BD}	0.6	106.5	4.2	43.3	4.1	116.5	13.7	3.6	0.3	246.4	11.3
	wlkp	3	121.0	1.7	13.2	0.2	112.0	4.4	44.0	1.0	107.7	5.5	3.9^{Aa}	0.0	245.0	8.7
	m	6	117.2	5.2	12.7^{C}	0.4	107.2	6.0	42.2	3.5	109.0	5.4	3.4^{Bd}	0.4	228.0	15.7
	CT	4	119.0	5.5	13.0	0.0	110.0	2.8	43.5	0.6	108.2	6.2	3.8^{c}	0.3	241.7	19.8
	nn	3	119.7	1.5	12.5^{A}	0.5	106.7	3.5	42.3	2.3	112.7	4.6	3.4^{Bd}	0.2	232.3	8.6
Total		**53**	**120,0**	**3.4**	**13.2**	**0.6**	**107.0**	**4.3**	**42.6**	**3.1**	**113.0**	**9.4**	**3.6**	**0.3**	**240.0**	**13.6**

AB differences significant at $p \leq 0.01$, *ab* differences significant at $p \leq 0.05$

– 3,6; strength – 240. Significant statistical differences were found between horses from the breed groups under study only in the case of the massiveness and boniness indices.

The index of trunk length for the examined population (107%) lay within the range from 104 to 108%, which, according to ZWOLIŃSKI (1983) is typical of carriage horses. The highest ratio of trunk length/height at withers was found for the Wielkopolska breed horses (112.0%), followed by the Czech warmblood (110.0%) and Oldenburg breed (110.0%). The relatively shortest trunk was recorded for noble half-bred horses (105.7%).

Neither for the chest depth (Table 1) nor for its index (Table 2), were there any differences found between particular groups of horses. The mean values for the features were situated around the level typical of light breed horses (ZWOLIŃSKI 1983).

The chest circumference index, calculated for the examined horses (120% – Table 2) classifies them, according to ZWOLIŃSKI (1983), as belonging to the carriage type. These values are close to the mean value given by BUDZYŃSKI et al. (1996) for Małopolska (120.40%) and Wielkopolska breed horses (119.45%), and only slightly lower than for Silesian horses (122,76%).

The value of a horse chest circumference allows for calculating the eurosomia and strength indices (Table 2). The mean value of the first one in the examined horses was 113.0%. The highest value, typical of heavy breed horses, was found in the Silesian horses (116,5%).

The strength index (Baron's) indicates the usability of a horse for work, provided it is taller than 220 (ZWOLIŃSKI 1983). Its average value in the examined population (240.0%) highly exceeded the above value, which confirms their predisposition to this type of usage and determines their draught force.

The indices calculated from the measurement results are close to those given by NOWICKA-POSŁUSZNA and DOMAGALSKA (2001), who studied the biometric parameters of stallions used in the Gniezno Stud Farm in 1998. Only the index of trunk length of the carriage horses under study was higher by 6 to 12% than that found for the Gniezno stallions.

Another feature, important for a carriage horse use, is its weight, which according to SAPUŁA (after KAPROŃ 1999) affects its draught force, but also has a negative influence on its speed (BUDZYŃSKI 1973). Consequently, for a horse used in carriage driving competitions, the body weight should have a certain specific value, which gives it a sufficient draught force and optimum speed. Based on the measurements, the average weight of carriage horses currently in use in Poland was estimated as 597.5 kg.

Front leg

The results of analyses of the front leg biometric parameters, the angles between its sections and their ratio to the height at withers are shown in Tables 3, 4 and 5, respectively.

The data analysis showed that only the pastern length did not reveal any differences between the examined horse breeds. For the other analysed features, the statistically confirmed differences between the average values were found.

One of the important features in a horse use is the length and angles of the shoulder blade. The average shoulder blade length, its ratio to the height at withers and its incination to the horizontal plane was found to be 57.4 cm; 34.5% and 54.7°, respectively (Tables 3, 4, 5). The study showed highly significant differences between the average length of this limb section in horses of unknown breed (46.5 cm), in which the shoulder blade was the shortest, and the average shoulder blade length in the horses of the other breeds under study (Table 3). The shoulder blade in the horses of unknown breed was the shortest, but the most inclined (51.7° – the statistically significant difference) – Table 4, which in common view positively affects the length of a horse's pace. The average value of the shoulder blade inclination to the horizontal plane for all the horses under study (54.7°) is close to the value observed by PRUSKI (1960) in typical thoroughbred horses.

An analysis of the ratio of the shoulder blade length to the height at withers (Table 5) shows that it is usually close to 1/3.

An analysis of the angle between the shoulder blade and the upper arm shows that its value in the examined population is 96.9° (Table 4). The average arm length in the examined horses – 29.3 cm (Table 3) – accounted for slightly over 50% of the shoulder blade length. The statistically significant differences between the average arm length in horses of various breeds was similar to those found in the shoulder blade length (Table 3).

As can be seen in the average length of the upper arm (29.3 cm), forearm (44.8 cm) and shank (34.8 cm), certain relationships exist within a horse breed. If the upper arm is shorter, the forearm and/or shank is longer and vice versa. The statistical analysis of the results and the WDR, WDPR and WDNP indices, calculated for the breed groups seems to confirm this observation (Table 3 and 5).

The average angle between the upper arm and the forearm in the examined horse population was 135.7° (Table 4). Significant differences between the value of the angle were observed within the groups of horses of one breed, but they do not seem to show any link to the shoulder blade inclination angle.

Table 3

Zoometric dimensions of the front leg of carriage horses of various breeds

Groups		N	Length (cm)											
			shoulder blade		arm		forearm		shank		shank circumference		pastern	
			x	s	x	s	x	s	x	s	x	s	x	s
Breed	sp	17	54.0^{B}	5.5	30.0^{b}	4.9	44.4^{B}	1.3	35.0^{B}	2.1	21.6^{f}	1.0	9.0	1.1
	old	2	60.7^{B}	0.6	30.4^{B}	3.0	43.0^{B}	2.8	35.0^{B}	0.0	22.5^{Bc}	0.7	9.7	0.8
	śl	18	58.6^{B}	2.0	30.4^{B}	2.4	45.1^{b}	1.7	35.4^{B}	3.9	23.0^{Be}	0.7	9.0	0.8
	wlkp	3	58.9^{B}	1.9	26.3	1.1	42.7^{B}	1.5	39.7^{BC}	2.9	22.2^{b}	0.3	9.7	0.8
	m	6	57.9^{B}	2.3	28.9	3.5	45.0^{b}	2.1	32.5^{BD}	5.5	21.0^{Cd}	0.9	8.8	0.6
	CT	4	58.0^{B}	1.5	28.8^{b}	0.9	44.5^{B}	2.4	37.2^{B}	4.0	22.2^{b}	0.6	8.9	0.4
	nn	3	49.5^{A}	4.6	24.1^{Aa}	0.8	48.0^{Aa}	1.0	26.7^{A}	3.8	20.7^{Aa}	1.2	8.7	0.9
Total		**53**	**57,4**	**3.9**	**29.3**	**3.4**	**44.8**	**1.9**	**34.8**	**4.2**	**22.1**	**1.2**	**9.0**	**0.8**

AB differences significant at $p \leq 0.01$, *ab* differences significant at $p \leq 0.05$

Table 4

Analysis of the front leg angles of the examined carriage horses

Groups		N	Angles (°)											
			shoulder blade to horizontal plane		shoulder blade to arm		arm to forearm		forearm to shank		shank to pastern		pastern to horizontal plane	
			x	s	x	s	x	s	x	s	x	s	x	s
Breed	sp	17	56.4^{Bc}	2.7	93.4	8.5	129.8^{c}	10.0	175.4^{b}	1.9	150.3^{b}	7.5	36.8^{A}	9.4
	old	2	54.9^{b}	0.4	99.3	1.0	140.2^{Bd}	1.8	171.9^{Aa}	2.4	151.8^{B}	0.9	41.3^{C}	1.7
	śl	18	55.1^{b}	1.7	97.9	2.7	137.7^{b}	4.9	175.8^{b}	2.3	147.4	2.2	53.1^{BD}	6.0
	wlkp	3	53.1^{d}	1.9	97.4	1.2	141.0^{Bd}	1.6	176.9^{B}	0.4	143.0^{Aa}	1.7	60.0^{BD}	0.1
	m	6	54.1	1.7	95.8	5.7	135.7	6.7	175.3^{b}	1.7	146.8	2.4	57.7^{BD}	1.6
	CT	4	53.4^{d}	0.7	99.5	0.5	140.3^{Bd}	0.8	175.1^{b}	1.8	147.6	2.7	54.7^{BD}	1.8
	nn	3	51.7^{Aa}	0.4	97.3	1.3	127.7^{Aa}	0.7	176.3^{B}	2.1	148.2	2.3	55.6^{BD}	1.5
Total		**53**	**54.7**	**2.1**	**96.9**	**4.9**	**135.7**	**7.1**	**175.5**	**2.1**	**147.9**	**4.2**	**50.7**	**9.6**

AB differences significant at $p \leq 0.01$, *ab* differences significant at $p \leq 0.05$

Table 5

Ratios of limb sections to height at withers (%)

Groups		*N*	Front leg					Hind leg				
			WDŁ *x*	WDR *x*	WDPR *x*	WDNP *x*	WDPT *x*	WDZ *x*	WDU *x*	WDPU *x*	WDNT *x*	WDPT *x*
Breed	sp	17	32.5	18.0	26.7	21.0	5.4	40.1	22.9	26.8	21.8	6.9
	old	2	36.6	18.3	25.9	21.1	5.8	38.6	23.5	27.1	25.1	6.0
	śl	18	35.3	18.3	27.2	21.3	5.4	41.4	23.4	24.6	22.6	6.0
	wlkp	3	34.0	15.4	25.1	23.3	5.7	41.9	21.8	24.8	22.6	5.3
	m	6	34.9	17.4	27.1	19.6	5.3	40.5	23.7	24.2	22.5	6.0
	CT	4	34.1	16.9	26.2	21.9	5.2	42.8	23.9	24.4	23.0	5.8
	nn	3	30.7	14.9	29.8	16.6	5.4	43.6	24.8	25.2	22.8	5.8
Total		**53**	**34.5**	**17.6**	**26.9**	**20.9**	**5.4**	**41.0**	**23.4**	**25.1**	**22.6**	**6.1**

WDŁ – shoulder blade length index
WDR – arm length index
WDPR – forearm length index
WDNP – front shank length index
WDPT – front pastern length index

WDZ – croup length index
WDU – thigh length index
WDPU – second thigh length index
WDNT – hind shank length index
WDPT – hind pastern length index

The average angle between the forearm and front shank in the examined population was 175.5°. The lowest value (171.9°) was observed in the Oldenburg horses. The angle between the forearm and the front shank closest to the optimum value was observed in the horses of unknown breed (176.3°) Wielkopolska breed (176.9°).

Highly significant differences were also found between the shank length, which according to NOWICKIA (2000) should account for about 2/3 of the forearm length. The ratio observed in the authors' study was close to 4/5 (0.77). The WDNP index, calculated for the whole group of horses (20.9%), is not much longer than the upper arm length index (WDR = 17.6%) – Table 5.

The results shown in Table 2 indicate a high diversity of the average shank circumference in the horse breed groups. Its average value was 22.2 cm, and the boniness index in the examined population of carriage horses (13.2%) indicates that the high boniness is among the characteristic features of a sport-and-carriage type horse.

According to PRUSKI (1960), the angle between the shank and the pastern ranges from 140 to 145°. The angle in the horses under study (147.9°) is slightly larger (Table 5).

An analysis of the length of the front pastern did not reveal any significant differences between the horse breed groups. It average length for all the horses was 9 cm (Table 3). Statistically significant differences were found in the value of the angle between the pastern and the horizontal plane, which for the whole analysed population was 50.7° (Table 4). According DUERSTA (1922), the inclination of pastern should range from 53 to 68°. The highest deviation from the value was observed in the noble halfbred (36.8°) and Oldeburg horses (41.3°), in which the pastern inclination was significantly lower than in the other breeds (Table 4). It can be seen from the data in Table 3 that the pastern/shank ratio should be close to 1/4.

Hind leg

The analysis of biometric parameters of a hind leg, the angles between its particular sections and their ratio to the height at withers, are shown in Tables 5-7.

The average parameters of a hind leg in the examined population are: length of croup – 68.3 cm; length of thigh – 38.9 cm; length of second thigh – 41.8 cm; length of shank – 37.6 and the hind pastern – 10.1 cm (Table 6), which in relation to the height at withers was, respectively: 41.0; 23.4; 25.1; 22.6; 6.1% (Table 5). The statistically significant differences found between the groups of horses of one breed concerned the length of croup and pastern and the angles between the bones of the hind leg (Tables 6 and 7).

Table 6

Zoometric dimensions of the hind leg of carriage horses of various breeds

Groups		N	Length (cm)									
			croup		thigh		second thigh		shank		pastern	
			x	s	x	s	x	s	x	s	x	s
Breed	sp	17	66.6	4.7	38.1	4.2	44.5	9.2	36.2	5.9	11.4^{Aa}	1.7
	old	2	64.0^{Aa}	5.7	39.0	0.8	44.9	1.1	41.6	0.6	9.9	0.3
	śl	18	68.8	3.9	38.8	2.4	40.8	3.8	37.5	4.0	10.0	0.8
	wlkp	3	71.3^{b}	4.5	37.2	1.6	42.2	3.2	38.5	2.7	9.1^{B}	0.6
	m	6	67.2	3.0	39.4	1.1	40.2	2.2	37.4	2.6	9.9^{b}	0.7
	CT	4	72.7^{B}	2.2	40.6	1.0	41.4	0.7	39.1	0.8	9.8^{b}	0.3
	nn	3	70.3	5.8	40.0	1.3	40.7	3.4	36.8	2.8	9.4^{b}	0.9
Total		**53**	**68,3**	**4.4**	**38.9**	**2.5**	**41.8**	**5.0**	**37.6**	**3.9**	**10.1**	**1.1**

AB differences significant at $p \leq 0.01$, *ab* differences significant at $p \leq 0.05$

Table 7

Analysis of the hind leg angles of the examined carriage horses

Groups		N	Angles (°)											
			croup to horizontal plane		croup to thigh		thigh to second thigh		second thigh to shank		shank to pastern		pastern to horizontal plane	
			x	s	x	s	x	s	x	s	x	s	x	s
Breed	sp	17	29.2	3.6	90.3^{A}	5.2	136.7^{Aa}	7.2	159.5^{B}	5.1	150.2^{b}	3.5	39.0^{Aa}	10.8
	old	2	27.5	1.5	$81.1B^{Ca}$	2.1	120.9^{Bd}	0.4	151.8^{Aa}	2.6	151.5	2.6	61.0^{B}	1.9
	śl	18	27.2^{b}	2.7	86.0^{d}	4.6	123.1^{Bd}	6.2	156.8	3.2	150.7^{b}	3.8	50.9^{b}	7.5
	wlkp	3	32.6^{Aa}	4.7	93.8^{Dc}	1.2	133.3^{c}	4.3	155.0	2.7	153.4	3.6	53.3^{B}	4.7
	m	6	31.2^{d}	5.0	88.7^{b}	3.4	125.8^{b}	5.7	157.8^{b}	2.6	152.2	5.5	57.3^{B}	3.0
	CT	4	27.7^{d}	2.4	85.8^{d}	2.3	123.2^{Bd}	3.6	157.3^{b}	1.6	148.3^{B}	1.0	54.5^{B}	0.8
	nn	3	25.8^{Bc}	0.5	90.3^{D}	0.8	127.2^{b}	1.6	155.7	1.7	157.1^{Aa}	2.7	60.1^{B}	1.4
Total		**53**	**28.5**	**3.6**	**87.8**	**4.9**	**127.1**	**8.1**	**157.1**	**3.6**	**151.2**	**4.1**	**50.9**	**9.5**

AB differences significant at $p \leq 0.01$, *ab* differences significant at $p \leq 0.05$

The results of the study show that the Oldenburg horses had the shortest croup (64.0 cm, WDZ – 38.6%), while the longest one was found in the horses of unknown breed (70.3 cm, WDZ – 43.6%), Czech warmblood (72.7 cm, WDZ – 42.8%) and Wiekopolska breed horses (71.3 cm, WDZ – 41.9%), which is a favourable feature for a horse usability (Table 5 and 6). The length of pastern was significantly longer only in the noble halfbred horses.

The analysis of the length of thigh (38.9 cm), second thigh (41.8 cm), hind shank (37.6 cm) (Table 6) and their ratio to the height at withers (WDU – 23.4%; WDPU – 25.1%; WDNT – 22.6% – Table 5), shows that the thigh and hind shank have in fact equal length in carriage horses, while the second thigh is slightly longer. It seems interesting whether such relationship would be observed in the leading jumping horses, whose work – and consequently the mechanics of motion – is different than in carriage horses. Since, as was pointed out by HILDEBRABRAND (1987) the shank in riding horses, particularly those selected for their speed, is longer than in carriage horses.

The data shown in Table 7 show that the most differences between the examined horses were found in the angles between the sections of hind legs. Highly significant differences were found between these average values (Table 7):

– the angle between the croup and the horizontal plane – between the Wielkopolska breed horses (32.6°), and those of unknown breed (25.8°);

– the angle between the croup and the thigh – between the Noble halfbred horses (90.3°), and the Oldenburger horses (81.1°), and also between the Oldenburger horses, the Wielkoplska breed (93.8°) and the horses of unknown breed (90.3°);

– the angle between the thigh and second thigh – between the Noble halfbred horses (136.7°), the Oldenburger horses (120.9°), the Silesian horses (123.1°) and the Czech warmblood (123.2°);

– the angle between the second thigh and shank – between the Noble haflbred horses (159.5°), and the Oldenburger horses (151.8°);

– the angle between the shank and the pastern – between the horses of unknown breed (157.1°), and the Czech horses (148.3°);

– the angle between the pastern and a horizontal plane – between the Noble halfbred horses (39.0°), and the other breeds, except for the Silesian horses (50.9°).

According to PRUSKI (1960), the normal angle between a normal croup and a horizontal plane should be 25°. Such inclination was found in the horses of unknown breed (25.8° – Table 7). In most of the other groups, the value ranged from 27 to 28°. In the Wielkoplska and Małoplska breeds, the angle was larger than 30°. This means that the croup in these breeds is moderately truncated, which in PRUSKI'S (1960) view it is a favourable feature in workhorses.

According to the author, the angle between the shank and pastern usually ranges from 140 to 145°, and the shank itself should be perpendicular to the ground. The average estimated value of the angle for all the examined horses (151.2°) was higher by more than 6°. The analysis of the inclination of the hind pastern to a horizontal plane (50,9°) shows that its inclination to a vertical plane was close to 39.1°, which added to the angle between the pastern and shank (151.2°) gives 190.3°. This means a slight deviation from the perpendicular and slight scimitar shaped of the hock in the examined carriage horses.

Conclusion

1. The biometric parameters, build indices and the angles between sections of the front and hind legs, as well as their ratios to the height at withers, are useful in evaluating a carriage horse,

2. The average zoometric parameters in the examined horses and the calculated build indices had values typical of light carriage horses. Their body weight was estimated to be 597.5 kg.

3. The differences found between limb joint angles and in the zoometric parameters for various breeds confirm the differences in their conformation. However, the study should be extended to a greater number of horses.

4. The results of this study shows the usability of the photozoometric method for estimating the biometric parameters of horses. The method can be employed in similar studies of horses used in other horse riding disciplines.

Translated by Joanna Jensen

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FRAMEWORK PRINCIPLES FOR GAME POPULATION MANAGEMENT IN THE WARMIA AND MAZURY PROVINCE

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K e y w o r d s: monitoring of game, hunting management, red deer, roe deer.

A b s t r a c t

The purpose of this study was to lay down the general principles of management of selected deer (red deer and roe deer) populations in the region of Warmia and Mazury as well as to present the measures to be taken within the organizational structures of hunting organizations and associations in Poland, aimed at implementing effective methods for game monitoring and management on particular hunting grounds.

The Annual Hunting Plans for the years 1998/1999 – 2000/2001 and programs of deer male shooting and culling were analyzed in order to develop the above project. A database including information indispensable for the evaluation and scoring of hunting trophies was prepared. Such a database makes it possible to monitor game selection by hunters and to determine the impact of this selection on the health status and quality of animals.

The main objective of rational game management should be to monitor, on a regular basis, the animals and their natural living environment. The monitoring is to be based on reliable data and on constantly improved methods of their acquisition. Large-area planning of wildlife management in Warmia and Mazury should be realized with respect to the division of the Province into hunting grounds. The boundaries of some of these grounds should be changed and adapted to the existing natural conditions. This will enable to follows different breeding principles within individual natural units. The general principles regarding the observed increase in the population size, shooting/culling structure and the limits of population density, were defined so as to adjust breeding policy to the specific character of both a breeding area and the hunting grounds situated there.

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GOSPODARKA ŁOWIECKA I PROJEKT RAMOWYCH ZASAD GOSPODAROWANIA POPULACJAMI ZWIERZYNY GRUBEJ NA WARMII I MAZURACH

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Słowa kluczowe: monitoring, gospodarka łowiecka, jeleń szlachetny, sarna.

Streszczenie

Zaprezentowano projekt ramowych zasad gospodarowania wybranymi populacjami zwierzyny płowej (jelenia szlachetnego i sarny) na Warmii i Mazurach oraz działania, które powinny być realizowane w strukturach organizacyjnych łowiectwa w Polsce. Ich celem jest wdrożenie skutecznych metod monitoringu zwierzyny i jej gospodarowania w obwodach łowieckich. Badano łowiska o powierzchni 2 213 000 ha, w tym do 30% stanowiły lasy.

W celu przygotowania ww. projektu analizowano: Roczne Plany Łowieckie (RPŁ) za lata 1998/1999-2000/2001 oraz oceniono prawidłowość odstrzałów samców zwierzyny płowej. Przygotowano bazę dotyczącą oceny trofeów łowieckich, a pośrednio i ich wyceny. Umożliwia ona monitoring prawidłowości realizowanej przez myśliwych selekcji zwierzyny, a tym samym ocenę jej wpływu na kondycję i jakość zwierząt łownych.

Pierwszoplanowym zadaniem racjonalnej gospodarki łowieckiej powinien być stały monitoring zwierzyny i środowiska jej bytowania, oparty na wiarygodnych danych i stale udoskonalanych metodach ich pozyskiwania. Wielkoobszarowe planowanie gospodarki łowieckiej na terenie woj. warmińsko-mazurskiego nadal musi opierać się na jego podziale na łowieckie rejony hodowlane. Niektóre ich granice należy obecnie zmienić, przystosowując je do realiów przyrodniczych, co umożliwi zróżnicowanie zasad hodowlanych w poszczególnych jednostkach przyrodniczych. Ramowe zasady dotyczące przyrostu zrealizowanego, struktury odstrzałów, pułapu zagęszczenia poszczególnych populacji ustalono w taki sposób, aby realizacja polityki hodowlanej była zależna zarówno od specyfiki łowieckiego rejonu hodowlanego, jak i znajdujących się tam łowisk.

Introduction

A crucial task before implementation of any game population management program, should be putting into effect a constant monitoring of game and environment of its occurrence. Modern hunting game population management requires a creation of such a monitoring system of undertakings related to a realization of game breeding that will make it possible to currently interfere in development of game population and effectively counteract disadvantageous phenomena occurring in these populations. Having a reliable database of game population living in Warmia and Mazury at a disposal, it is possible to control game population consciously and fully professionally to make hunting, as an ecological experiment on a large scale, gain a dimension that lives up to our expectations.

Having established Hunting Breeding Areas in 1997 (Figure 1), it was assumed to implement a realization of a principle of large-area hunting

planning in hunting management. It particularly related to three species of game – a deer, a wild boar and an elk. The idea turned out to be justified with reference to them, thus it is worth continuing and putting it into hunting practice more efficiently. To make these intentions more realistic, such a system of organizing hunting areas should be introduced that would force us to live up to expectations that hunting management in Poland faces.

Adoption of selection principles based exclusively on indicators related to population into planning and realizing hunting management, cannot mean a complete relinquishment an individual selection. It still remains a current problem of a uniform assessment of all trophies, irrespective of a category of a hunting district (Game Breeding Center or a district taken on lease) and a type of realization of shooting (e.g. made by a domestic hunter or a foreigner). It should be only hoped that changes introduced in 2004 will effectively satisfy this principle.

In practice, nowadays game breeding is limited only to taking into account population indicators in game obtainment. Certainly it is a wrong principle.

Work purpose was to present frame principles of management of selected deer population (red deer and roe deer) in Warmia and Mazury and to introduce activities which should be realized within organizational hunting structures in Poland, aiming at implementing efficient methods of running monitoring of game and its management in hunting areas.

Material and Methods

With a purpose of preparing a project of frame principles of game breeding in Warmia and Mazury province, situated in north-eastern Poland, in a first stage, among other things an analysis of Yearly Hunting Plans on years: 1998/1999 – 2000/2001 and an evaluation of shooting of deer males were made. With this purpose, the following breeding indicators were determined:

1) realization of a plan of obtainment of a given game species in a season – as A indicator

$$A = \frac{\text{fulfillment of shooting off in a given season}}{\text{plan of shooting off in a given season}} \cdot 100\%;$$

2) planned obtainment of a given game species in relation to its spring state – as B indicator

$$B = \frac{\text{plan of shooting off for a hunting season 2001/02}}{\text{state on a day 31.03.2001}} \cdot 100\%;$$

3) exploitation of population of a given game species – as C indicator

$$C = \frac{\text{(state before hunting season)} - \text{(state on a day 31.03.2001)}}{\text{plan of shooting off for a hunting season 2001/02}} \cdot 100\%;$$

For a current analysis of, among other things, an individual quality of deer males and indirectly of all populations, a database was prepared in frame of this study, concerning an evaluation, and indirectly an assessment of hunting trophies. It makes possible to currently monitor regularities realized by hunters of game selection, and thus an evaluation of its influence on state and quality of deer (STACHOWIAK 1994, VARIĆAK 2001). Thus, a unique study of breeding data was created, related to game occurring in a province, among other things: deer, roe deer, fallow deer and elks, which is actualized and completed with new elements every year. Following analyses were made for a whole province of Warmia and Mazury and included a division into 11 hunting breeding regions, as well as 40 forestry managements and 374 hunting districts (including those leased by hunting circles and managed by game breeding centers). A carried out analysis concerned totally hunting regions of a general area of 2 213 000 ha, including almost 30% of forest.

The calculations were made in a layout of 17 districts existing in a province, designed for public administration and veterinary service, and submitted to be used during the realization of their tasks in *„Database for implementing monitoring of basic species of game and a beaver in Warmia and Mazury province"* (ZALEWSKI et al. 2001).

Results

With reference to deer species, in a frame of this study, only example-analyses and comparisons were presented, concerning hunting management which should be realized in Warmia and Mazury province as well as in other hunting grounds, mainly related to deer and roe deer that are basic species of deer game in Poland.

The results of studies are, among other things:

1. Comparison of data contained in Yearly Hunting Plan in view of a number of basic herd of particular game species, a level of planning of game obtainment and indirectly presenting also a growth rate accomplished for a discussed game (Tables 1, 2), and defining breeding indicators characterising populations and hunting endeavors there realized (Tables 1, 2).

Table 1

Hunting management realized in populations of deer game in the season of 2000/2001 in Warmia and Mazury province

Species	Shooting off plan. 2000/2001	Realization of shooting off 2000/2001	State on 31.03	State before hunting season	Planned shooting off 2001/2002	Breeding indicators (%)		
						A	B	C
						realization of plan of obtainment on season 2000/2001	planned obtainment with relation to spring state	exploitation of population
	1	2	3	4	5	2/1	5/3	4-3/5
Red deer	4902	4254	13 896	17 553	4775	86.78	34.36	76.59
Roe deer	15 620	15 147	45 747	58 483	15 871	96.97	34.69	80.25
Wild boar	12 817	10 645	12 733	24 665	12 803	83.05	100.55	93.20
Elk	68	46	370	487	65	67.65	17.57	180.00
Fallow deer	237	151	834	1049	207	63.71	24.82	103.86
Mouflon	3	3	35	42	7	100	20	100

Table 1 presents hunting management realized in game populations in a season 2000/2001 in Warmia and Mazury province, taking into consideration all hunting areas. During the year, in the whole province there were obtained: 4254 red deer, 15 147 roe deer, 10 645 wild boars and only 3 mouflons. What can be alarming in this period, is the indicated reduction of basic herd of red deer and roe deer as well as plans to obtain more animals than it is established in standards resulting from game management principles (*Framework rules of game management 1997, Principles of individual and population selection of game in Poland 2005 – resolution of the Main Hunting Council No. 1/2005*).

In the above analysis:

– B indicator exceeds 34.00% for roe deer and red deer,

– C indicator clearly shows reduction among red deer, roe deer and wild boars.

As regards red deer in the structure of Hunting Breeding Areas (Table 2), the above analysis is brings the following results. The highest obtainment of red deer in a season 2000/01 was recorded in Napiwodzko-Ramucki region (3) and in Taborskie Forests (4), at the level of 908 and 952 animals, respectively. The obtainment in the last year, as well as the plan of shooting off for a season 2001/02 in Warmia and Mazury, mainly concerns Hunting Breeding Area 1-7, excluding Hunting Breeding Area No. 5 (North Mazovia). In those regions, A indicator ranges from 80.72% to 89.05%, and at the same time hunting records indicate herd reduction, which in the

Table 2
Hunting management realized in deer population in the season of 2001/2002 in hunting areas designated within Warmia and Mazury province

Breeding area	Deer (individuals)					Breeding indicators (%)		
						A	B	C
	plan of shooting off 2000/2001	realization of shooting off 2000/2001	State on 31.03	state before hunting season	planned shooting off 2001/2002	realization of plan of obtainment on season 2000/2001	planned obtainment with relation to spring state	exploitation of population
	1	2	3	4	5	2/1	5/3	4-3/5
1	658	527	2114	2661	736	80.72	34.82	73.90
2	557	463	1469	1889	541	83.12	36.83	77.63
3	1088	908	3806	4828	1043	83.46	27.40	97.99
4	1147	952	3452	4382	1128	81.28	34.30	76.68
5	15	13	69	88	18	86.67	26.09	105.56
6	569	529	1920	2427	538	92.97	28.02	94.24
7	402	358	1110	1472	403	89.05	36.31	89.83
8	14	11	74	88	20	78.57	27.03	70.00
9 and 10	20	15	53	68	24	75.00	45.28	62.50
11	49	49	137	178	51	100.00	37.23	80.39
Total	**4519**	**3825**	**14204**	**18085**	**4557**	**84.64**	**32.08**	**85.17**

1 – Northern Mazury/Warmińsko-Mazurski
2 – Wipsun
3 – Napiwodzko-Ramucki
4 – The Taborskie Forests
5 – Northern Mazowsze
6 – The Piska Forests
7 – The Great Mazurian Lakes
8 – Biebrzański
9 – Żuławy
10 – The Elbląg Uplands
11 – Brodnicki

regions under analysis, fortunately, is equalized by an indicator of „Realization of a plan...” (A), which was, on average, 84.64% for a Hunting Breeding Region.

2. Properly realized monitoring of game consists in carrying out a shooting off analysis in a field of a realization of game obtainment, among others, in a configuration of gender and age (Figures 1, 2).

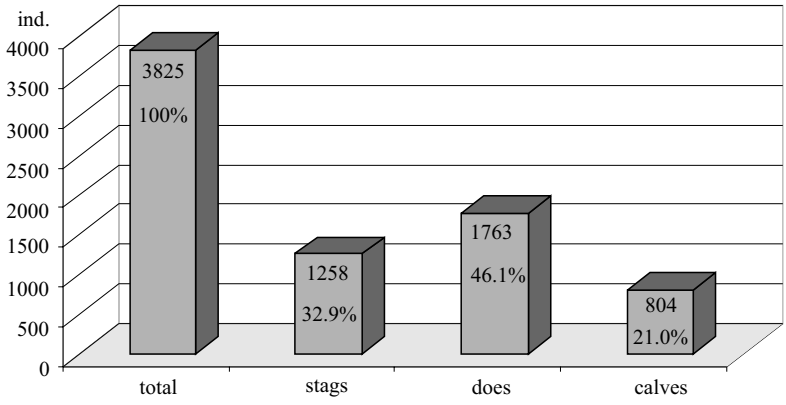

Fig. 1. Average yearly red deer obtainment in gender groups in the hunting season of 2000/2001

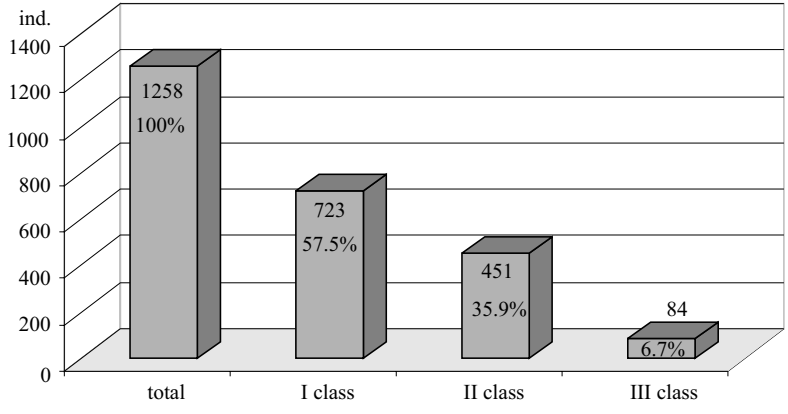

Fig. 2. Average yearly stags obtainment in age classes in the hunting season of 2000/2001

The average annual obtainment of red deer in gender groups for a hunting season 2001/02 was the following: out of 3825 shoot red deer, 32.9% were stags, 46.1% does, and 21% – calves, i.e. 804 animals (Figure 1).

Out of 1258 red deer stags (Figure 2) 84 animals (6.7%) were in age class III, i.e. stags shoot at the age from 11 up, in age class II (6-10 year old) – 451 animals (35.8%), and the number of younger stags, i.e. in age class I, as demonstrated by the data from the assessment of correctness of shooting, amounted to 723 animals (57.5%).

As follows from the conducted analyses, shooting off in age groups was realized in Warmia and Mazury without any major deviations from binding

management principles for a given period. Shooting off in age classes of red deer stags presented an example of obtainment that clearly exceeded standards in age class I, as regards the 2-5 year old animals. Since the assessment of age is done by the Commission that assesses shooting off correctness, such evaluation can be very often erroneous. The error, particularly in class I, consists in overestimating the age by the Commission, therefore this unfavorable image of shooting off in age classes of red deer stags can be even more unfavorable.

3. An important element of properly run hunting management is defining a level of obtainment of individual game species according to binding rules on 1000 ha of forest area or 1000 ha of general area of shooting off (Figures 3, 4)

A sample analysis of red deer obtainment per 1000 ha of forest area inforest divisions (Figure 3) allows us to determine centers of red deer occurrence in hunting grounds under analysis. In the analysed period, the largest number of red deer was obtained in forest divisions of Miłomłyn and Mrągowo, i.e. 10.0-14.4 animals per 1000 ha of forest area in a forest division. The lowest rate of obtainment was recorded for forest divisions of Bartoszyce, Borki, Ełk, Wielbark, Dwukoły, Brodnica and Elbląg, where it ranged between 0.4 and 2.5 animals per 1000 ha of forest area. As regards the structure of Hunting Breeding Regions, the highest obtainment of 9.0-10.0 animals per 1000 ha of forest area in a region was recorded in regions of Wipsun and Taborskie Forests.

4. With the purpose of establishing individual selection principle, it is important to make a profile of mass of carcass, antlers and its forms in particular age groups for males of game animals.

Table 3 presents such characteristics for red deer stags. In individual age groups, the mass of carcass ranged between 86.62 kg (group I) and 140.56 kg (group V); the gross weight of antlers in spike stag was 0.79 kg and in 11 year old stags and older (group VI) – 6.14 kg, at standard deviation of 0.99 kg. At this age, also the average number of tines in deer antlers reaches the highest number of 12.8 tines. This means that a stag at the height of its growth in the region of Warmia i Mazury reaches, on average, a form of irregular fourteen-pointed antlers, at s = 3.35 tines.

As follows from the data included in the analyzed table, stags in hunting grounds of Warmia and Mazury have the following forms of antlers in their respective age groups:

group I (2 years old) – spiked antlers;
group II (3 years old) – irregular eight-pointed antlers;
group III (4-5 years old) – regular eight-pointed antlers;
group IV (6-7 years old) – about 50% have regular ten-pointed antlers and about 50% – irregular ones;
group V (8-10 years old) – irregular twelve-pointed antlers.

Fig. 3. Quantity of deer obtainment over 1000 ha of forestry area of the forestry management

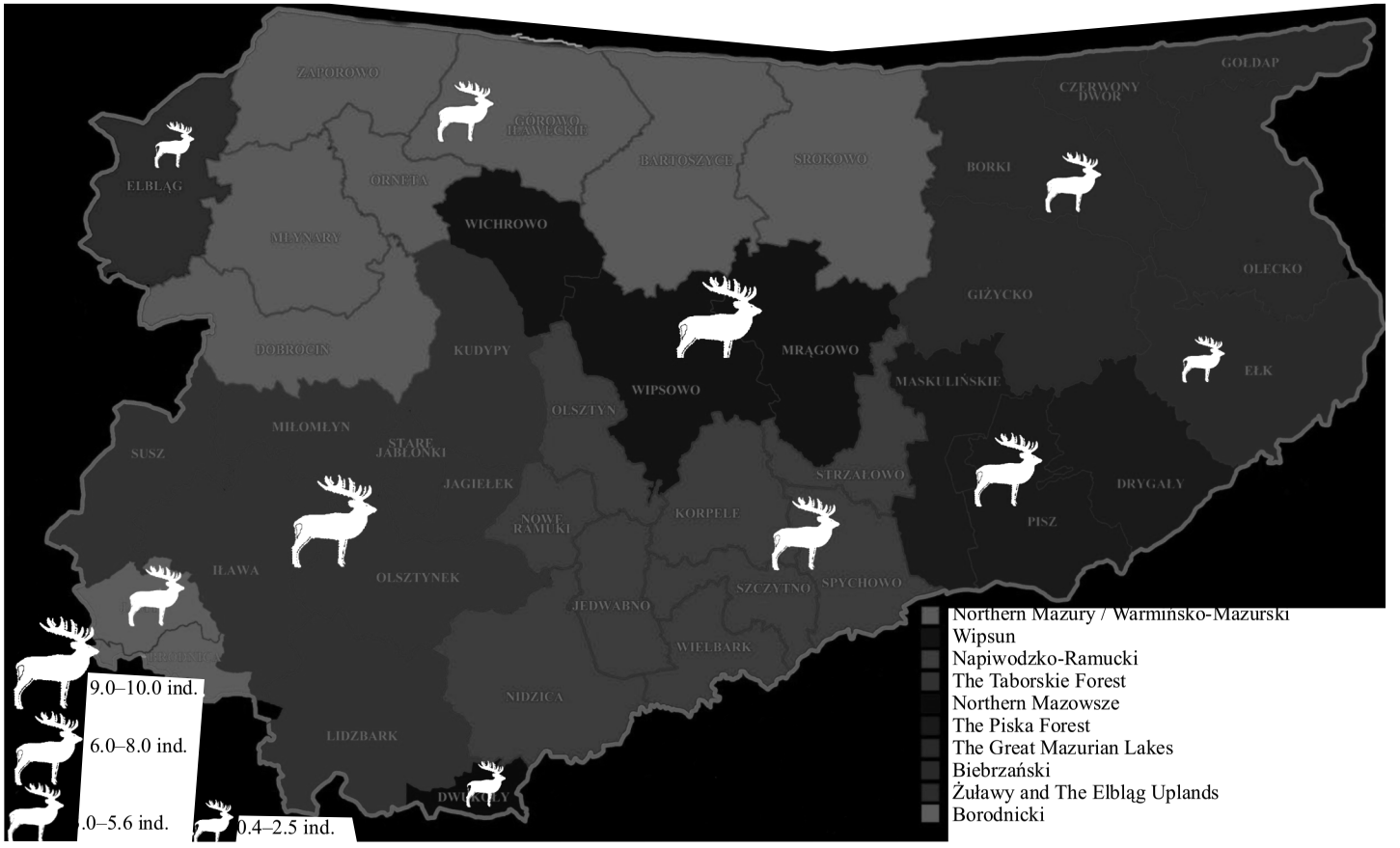

Fig. 4. Quantity of red deer obtainment over 1000 ha of forestry area of the hunting breding area

Table 3
Profile of mass of carcass, antlers and their forms in stags shot off in the season of 2001/2002 in Warmia and Mazury province

Age group	Breeding area	Mass of carcass (kg)		Mass of antlers (kg)		Number of tines (ind)	
		n	*x*	*n*	*x*	*n*	*x*
1	2	3	4	5	6	7	8
2 years of life (I)	1	24	94.37	24	0.9	24	2.00
	2	4	76.00	4	0.76	4	2.00
	3	6	86.33	6	0.67	6	2.00
	4	17	83.71	17	0.8	24	2
	11	7	73.43	7	0.53	7	2
Sum	*n* *x* *s*	58 **86.62** 15.2575		58 **0.79** 0.2109		58 **2** 0	
3 years of life (II)	1	30	111.5	29	1.84	29	6.76
	2	15	102.6	15	1.79	15	7.13
	3	19	97.53	19	1.76	19	7.42
	4	23	101.47	23	1.66	23	6.87
	6	1	75	1	1.01	1	6
	11	6	101.67	6	1.44	6	6.17
Sum	*n* *x* *s*	94 **103.79** 18.39		93 **1.74** 0.35		93 **6.94** 1.19	
4-5 years of life (III)	1	62	122.87	62	2.38	62	8.06
	2	10	121.5	11	2.79	11	8.82
	3	32	112.13	32	2.6	32	8.34
	4	60	109.28	59	2.26	59	7.68
	5	1	130	1	2.53	1	8
	6	2	102	2	2.51	2	9.5
	11	24	117.21	24	2.29	24	8
Sum	*n* *x* *s*	191 **115.84** 19.49		191 **2.39** 0.72		191 **8.04** 1.37	
6-7 years of life (IV)	1	26	140.85	25	3.87	25	9.8
	2	11	122.73	11	3.66	11	9.82
	3	32	112.13	32	2.6	32	8.34
	4	28	125.25	27	3.25	27	9.82
	5	2	157	2	4.47	2	12.5
	6	2	108	2	3.77	2	10.5
	11	7	130	7	3.37	7	10.14
Sum	*n* *x* *s*	108 **125.44** 22.4		106 **3.28** 0.87		106 **9.45** 1.59	

cont. Table 3

1	2	3	4	5	6	7	8
8-10 years of life (V)	1	11	151.73	11	5.22	11	10.73
	2	9	151	9	5.39	9	12.11
	3	19	135.95	20	5.08	20	11.4
	4	25	135.6	25	4.69	25	10.48
	11	2	139	1	4.5	2	8.5
Sum	*n* *x* *s*	66 **140.59** 22.71		66 **4.99** 1.21		67 **10.96** 1.94	
11 years of life and more (VI)	1	5	155.6	5	6.53	5	12.43
	2	1	135	1	6.82	1	13
	3	4	134.75	5	5.87	5	13.4
	4	7	127.71	7	5.73	7	12.43
	6	1	113	1	7.8	1	14
Total	*n* *x* *s*	18 **136.61** 28.37		19 **6.14** 0.99		19 **12.8** 3.35	

A similar characteristic regarding roebucks is presented in Table 4. Totally, this analysis includes over 2800 animals. Particular attention should be given to the gross weight of antlers, which presents diversity of antler growth in roe deer in subsequent years of its life and age groups. A significant diversity of antlers weight, for example between their 2nd and 3rd year of life confirms the correction of criteria established recently by the Main Hunting Council, regarding shooting off of poor quality roe deer to be culled and presenting them separately for animals in their 2nd and 3rd year of life (*Resolution No. 1/2005 of the Main Hunting Council*). This is also reflected in the number of tines developed by a roebuck in its 2nd and 3rd year of life – 0.51 and 1.40 tines, respectively. This means, that roebucks obtained in their 2nd year of life are characterized, on average, by antlers in the form of spikes or in 50% they develop irregular four-pointed antlers, and in their 3rd year of life they are mainly irregular four-pointed roebucks and in 40% – regular four-pointed roebucks.

In age group IV (6th year of life and older), a deer stag has irregular six-pointed antlers (x = 3.0). The average gross weight of antlers at this age is 306.83 g, at standard deviation of 79.12 g. This means that the average net weight of antlers for stags at this age is 216.83 g and as compared, for example, with the buck from Lublin area – over 300.00 g, it indicates that this population is not one of the leading ones in Poland (DZIEDZIC et al. 1999, DZIEDZIC 1991).

Table 4

Profile of mass of carcass, antlers and forms of roe deer antlers shot off in the season of 2000/2001 in Warmia and Mazury province

Age group	Breeding area	Mass of carcass (kg)		Mass of antlers (kg)		Number of tines (ind)	
		n	*x*	*n*	*x*	*n*	*x*
1	2	3	4	5	6	7	8
2 years of life (I)	1	329	15.18	331	146.11	311	2.4*(0.4)
	2	208	15.10	208	147.21	193	2.6*(0.6)
	3	135	14.54	136	139.71	123	2.6*(0.6)
	4	169	15.14	169	149.90	134	2.6*(0.6)
	5	5	12.60	5	167.80	5	2.4*(0.6)
	6	16	14.69	16	168.19	14	2.6*(0.6)
	11	31	13.42	31	107.13	15	2.4*(0.4)
Sum	*n* *x* *s*	893 **14.97** 3.75		896 **145.27** 42.71		795 **2.51*(0.51)** 0.87	
3 years of life (II)	1	273	16.96	273	203.55	266	3.2*(1.2)
	2	122	16.80	122	212.34	122	3.8*(1.8)
	3	87	15.72	87	181.98	86	3.6*(1.6)
	4	106	15.89	106	200.79	102	3.4*(1.4)
	5	1	18.00	1	264.00	1	6.0*(4.0)
	6	15	15.40	15	22.53	14	3.0*(1.0)
	11	20	16.15	20	185.95	14	3.0*(1.0)
Sum	*n* *x* *s*	624 **16.51** 2.21		624 **201.83** 50.35		605 **3.40*(1.40)** 1.26	
4-5 years of life (III)	1	342	18.52	341	279.59	331	4.3*(2.3)
	2	194	18.48	194	266.73	192	4.8*(2.8)
	3	148	17.14	148	243.94	143	4.7*(2.7)
	4	170	16.94	170	266.57	167	4.6*(2.6)
	5	1	19.00	1	285.00	1	6.0*(4.0)
	6	18	16.94	18	304.11	16	5.0*(3.0)
	11	14	17.57	14	258.43	12	3.9*(1.9)
Sum	*n* *x* *s*	887 **17.93** 3.60		886 **268.49** 79.13		862 **4.56*(2.56)** 1.36	
6 years of life and more (IV)	1	172	18.84	172	312.66	167	4.8*(2.8)
	2	75	18.43	75	301.37	73	5.1*(3.1)
	3	69	18.99	69	277.13	68	5.3*(3.3)
	4	105	17.53	106	307.23	105	5.1*(3.1)
	5	2	18.00	2	303	2	6.0*(4.0)
	6	9	17.11	9	312.11	9	5.8*(3.8)
	11	29	18.55	29	354.21	25	5.0*(3.0)
Sum	*n* *x* *s*	461 **18.44** 4.57		462 **306.83** 79.12		449 **501*(3.01)** 1.40	

* While determining the form of antlers in this paper, the sum of the number of tines from both spikes was included. A tine was defined as both the anterior and posterior tines as well as tines end. (...) – the average sum of anterior and posterior tines on both spikes (without tines end) was given in brackets

A carried out analysis showed faults that were noticed in hunting planning. It was the factor that stimulated creation of such a game monitoring system that would be uniform and coherent for a whole province and will consequently allow to currently analyze populations of game in Warmia and Mazury and will make it possible to react earlier to negative effects resulting from a drop or a growth of number of these populations in a micro region of north-eastern Poland.

The very important aspect of hunting management realized in Warmia and Mazury is organizing Hunting Breeding Areas. Hunting Breeding Areas are coordinated by one of the forest managers of forestry managements that it includes. Moreover, there are exclusively employees of Administration of State Forests, who work in a team coordinating an activity of hunting breeding areas. It is hard to imagine that hunting circles and Polish Hunting Association, which are responsible for hunting management and supervising it in more than 90% of districts of a province under Polish law, would not take part in defining main directions of working in a field of game breeding.

Therefore, new principles in a field of organizing and working of Hunting Breeding Areas should be implemented. The basic principle of proposed solutions should be joint coordination and management of working of Hunting Breeding Areas that would be directed by Council of Hunting Area.

In frame of Regional Management of State Forests there are distinguished units called Hunting Breeding Areas. Their work should be organized by coordinators of Hunting Breeding Areas who would also lead Councils of Breeding Areas in their own regions. The council should include:

– forestry managers or deputies from forestry managements situated in an area of Hunting Breeding Area activity,

– representative of Regional Management of State Forests,

– one representative of each Regional Boards of Polish Hunting Association proper for a location of Hunting Breeding Area.

At least once a year, during a settlement of yearly hunting plans for Hunting Breeding Areas, Regional Council should call a plenary meeting with a participation of all leaseholders (huntsmen of circles) and boards of hunting districts, at which issues related to hunting and breeding plans for a hunting area would be analyzed.

Only a complete integration of all surroundings engaged and responsible for a proper hunting management in managed hunting circles, will allow a proper realization of tasks that Polish hunting faces.

An establishment of a lower or higher concentration of individual game species should be a well considered, joint activity of an coordinator, Council of hunting breeding area and users of hunting districts.

Planning of game obtainment in hunting districts of a given hunting area should take into consideration a location of game during a season.

In relation to this, after a motion of a leaseholder or an administrator of a hunting district, it is possible to make amendments of a yearly hunting plan for districts, depending on changes of a game location in an area. Such proposals should receive an opinion of a coordinator of Hunting Breeding Area beforehand.

All Hunting Breeding Areas that are included in Regional Management of State Forests or in a provincial system, should be directed by a Council of Hunting Breeding Areas. It would include:

– coordinators of hunting breeding areas from a region of Regional Management of State Forests,

– regional huntsmen together with chairmen of Commission of Game Breeding of District Hunting Council from an area of Regional Management of State Forests activity,

– employee – inspector for hunting of Regional Management of State Forests,

– it is advisable to include research employees from research institutions engaged in hunting in a given region.

Council's tasks would include an analysis and an evaluation of an accomplishment of Yearly Hunting Plans and Long-term Hunting Breeding Plans for hunting areas and a layout of directions for development of hunting management in an area of Regional Management of State Forests (of a province).

Until now, before elaborating our materials related to game population management in Warmia and Mazury province, Administration of State Forests, has not realized a periodical and all the more a yearly monitoring of hunting management in Warmia and Mazury (ZALEWSKI et al 2001). An establishment of partner principles of fulfilling tasks that Administration of State Forests and Polish Hunting Association face, can produce only good results, which allow to beware of mistakes made so far.

We are also aware of a fact that not only an organization and a realization of tasks that Hunting Breeding Area faces should be changed, but also a methodology of collecting source materials should be constantly improved in order to make them reliable in the most sufficient way.

New methods of game stocktaking should be introduced (among other things, connecting traditional and innovative methods with a use of e.g. thermo visional cameras) – an application for a grant for research in this field is being prepared (GAJDAMOWICZ 2002, ÄLGSTAMMENS ... 2005).

Starting from a season 2004/2005, trainings organized in the District Board of the Polish Hunting Association include the introduction of uniform principles of defining Yearly Hunting Plans, aimed at reconstructing an age structure in population of red deer, basing on monitoring of game condition in

Warmia and Mazury province. It is meant to increase a participation of stags in the second and third age class (from 6 to around 12 years) in the nearest years. For this reason there have been prepared *„Materials from a training of huntsmen of circles"* (2003), for users of hunting districts, which already make it possible to see the changes in the method of running game management in circles.

In order to define and compare the results of individual selection that is realized in our populations, it is essential to standardize rules of defining deer stags; age, also taking into consideration different hunting areas as well as experience and qualification of proper people for making an evaluation of correctness of shooting off of deer stags. A whole number of issues in this field is realized and solved in framework of master's and doctoral theses in our university department at the Faculty of Bioengineering of Animals at Warmia and Mazury University in Olsztyn. Until 2006, an assessment of quality of settlements and their usefulness for game breeding has been elaborated, with the use of organizational data for individual forestry districts. Currently, it is necessary to provide an overall assessment of hunting areas in Warmia and Mazury, which would allow us to widen the knowledge in a field of, among other things, a home base existing in areas and its usefulness for game occurring there. Such study could be also used while establishing possible changes to borders of Hunting Breeding Regions. In previous hunting seasons, 2003/2004 and 2004/2005, a complete monitoring of obtained deer males was implemented. Until now, most of materials relating to deer bucks and roe deer stags, obtained by foreigners in framework of so called foreign exchange shooting off have been collected. In March and April, during a yearly evaluation the remaining materials related to stags and bucks obtained by hunters from Poland were collected. In order to make a complete profile of deer populations living in Warmia and Mazury, documents based on the above evaluations were drawn up, which related to measurements of antlers and assessment of age basing on usage of jaw teeth. With a purpose of verification and definition of a final CIC score and age of a given individual, its antlers and jaw were photographed (Photo. 1, 2), which with an additional use of histological methods of establishing the age of the Cervidae provide fully reliable results of monitoring conducted (KLEVEZAL, KLEINBERG 1967, SZABIK 1973, AZORIT et al. 2002).

The material will complete monitoring that is realized, and at the same time it gives information and photographical documentation for serial trainings that are carried out in our Polish Hunting Association; District for members of a Commission evaluating a correctness of shooting off of deer males.

Photo. 1. The antlers of red deer

Photo. 2. The lower jaw of red deer

Implementation of constant monitoring and mechanisms and procedures presented above aims at running a professional supervision in Warmia and Mazury region and an accomplishment of main tasks that game breeding in our hunting areas faces. This should constitute an example for various hunting structures in Poland of efficient implementation of monitoring of game and its management in hunting grounds.

Project of framework principles for European red deer management in Warmia and Mazury

Deer

Analyses conducted according to the scheme presented in this study allow to formulate general principles of managing selected game species, and at the same time to assess the effects of applied method on ongoing basis.

Gender and age structure of realized shooting off

Hunting management with reference to a population of red deer should be realized mainly within the confines of forestry complex of a minimal area of 1000 ha. At the same time there are cases when it is justified to hold deer in smaller complexes that are an integral part of larger deer mainstays and which together create a uniform acreage of functioning of a population of this species.

A concentration of deer population should be fitted within confines of 10-50 individuals in an area of 1000 ha of forestry district (of hunting area). Defining an upper limit of a deer concentration results from a fact that it is a realistic indicator that can be maintained by some forestry managements of Warmia and Mazury province, where a state of these animals is even higher than a mentioned one, and it does not cause excessive damages to forest management. For example, in Strzałowo Forestry Division, the average concentration of red deer in a season 2005/06 was 76 animals per 1000 ha of forest area.

A completion of food base in forests should be done in a various way, not only by applying different kinds of chopping in wood obtainment or enriching forests in biocenotic additions etc., but also by a cultivation of green arable lands not used so far, as a perfect food base for deer. Not without significance is a principle, which is presently realized by some forestry managements, of carrying out a cut out, a cut-through in a way that in the first place make knocked down trees an abundant food base for deer for a few weeks. Wood removal and tidying an area is then the next action. This element influences

essentially on enlargement of food base in forest and it is conducive to limitation of damages made by deer in forests.

Growth realized in stabilized deer populations is formed, according to the fixed norms, at a level of 10-30% (35%) of spring number state of population with males and females in the ration of 1: 1-1.5.

Within the confines of a fixed growth that is realized, obtainment in hunting areas of Warmia and Mazury in individual gender and age groups should stand for:
stags – 30-40% (approaching upper limit – about 40%);
does – 40-50% (optimal obtainment – about 45%);
calves – 10-20%;

Deer stags obtainment in age classes should be formed at a level of:

I class (2-5 years old) – 40 – max. 50% (assuming saving stags at the age of 2 and a possible elimination from a population only seek and sickly individuals at this age);

II class (6-10 years old) – 30-40%;

III class (11 years old and older) – 10-20% (in this class a level of 20% of obtainment of deer stags should be pursued; in principle almost exclusively warrantable deer stags should be obtained in this age class in future, or quality parameters of antlers, e.g. related to its mass, should be raised systematically).

Warrantable deer stag is an individual at the age of 11 or older. This definition should become a principle in hunting areas of Warmia and Mazury (Table 3). Researches carried out in this field confirm that Mazurian deer stag reaches a peak of development of its antlers at the age of 11-12 (ZALEWSKI, SZCZEPAŃSKI 2004 a-d).

It should be mentioned here that poor quality deer to be culled at the age of 11 is a potential reproductive individual that at the same time passes on undesirable characteristics to its offspring. It is also a fact that presently binding criteria of shooting off of poor quality deer to be culled from the age of 8 were identical in a region of Regional Management of State Forests in Olsztyn for poor quality deer to be culled in classes II and III. From this point of view it would be possible to fix only two age groups and age limit for warrantable deer and obtainment of deer bucks rigorously realized in a proportion:

I class (2.-5. years old) – 40 – max. 50%;

II class (stags at the age of 6 and older) – 50-60% (including warrantable stags – 10-20%).

At present we observe a significant rejuvenation of deer stags in population of Mazurian deer. It is practically impossible to meet a stag at the age over 8.-9. years in majority of our hunting areas. Therefore, decisive steps

have been taken to enable restoration of a suitable age structure of population in the nearest four years. It will cause an „improvement of quality" of deer in Warmia and Mazury in a prospect of a following decade. For this reason in season for years 2003/2004 there were introduced sanctions for circles for shooting off carried out in breach of binding hunting rules, as well as sanctions for hunters for improper shooting off – including taking a gained trophy away from a hunter. Besides the sanctions, there are organized regular trainings for circles in a field of game breeding. It should be only hoped that introduction of *Selection principles*... (2005) by a resolution of the Main Hunting Council will definitely facilitate reaching the final aim – aging of deer stag population in Warmia and Mazury.

Selection of does and calves

Does and calves obtainment should be carried out by shooting off of sickly individuals, leading weak offspring with a mass of carcass lower than an average for an area as well as old does and calves born late. As a strict rule it should be accepted that first we shoot off a calf of a doe, and eventually a doe – never in a different order. Also, the possibility of shooting off does, e.g. in the period 01.08-31.08, followed by a break in September, and another shooting off period from 01.10 to 15.01 should be considered. Therefore, it seems sound to keep a larger disproportion in the amount of obtained does and calves. Otherwise, orphaning many calves must be taken into account, which from the point of view of the population is an unfavourable phenomenon.

Roe deer

Gender and age structure of realized shooting off

A concentration of roe deer in Polish hunting areas, as indicated by principles being in force until the end of a season 2004/2005, should range from 6 to 15 animals per 100 ha of a hunting district, which means to 150 individuals/1000 ha of hunting area (ROZWAŁKA et al. 1997).

In general guidelines it should be pointed that a certain limit of a minimal number should be defined for hunting areas particularly the weakest for roe deer breeding, including hunting areas of Warmia and Mazury. It seems to be justified to define a level of a minimum of roe deer concentration at 3-4 individuals/100 ha of hunting area (of a district). It would also be a goal

that users of weaker hunting districts, who realize hunting management, should pursue, in framework of activities that result from the Long-term Hunting Breeding Plans. At the same time it should be stressed that realization of actual hunting management relating to roe deer population, within the confines of hunting areas, seems to be only a statistical, list and comparative element with reference to other game populations. It is closely connected with territorialism of roe deer and its small, in comparison with other game species, individual acreage. Therefore, possible regionalization should be based on data gathered for hunting districts or forest district as resulting from monitoring as well as on data concerning roe deer, presented in Table 4.

Growth realized in stabilized population, where a proportion of males and females is: 1:1.5-2, should be formed at a level of 20 – max. 25% of spring number of population.

Within the confines of the realized growth, obtainment in gender and age groups should amount to:
roebucks – 40-50%,
does – 40%,
kits – 10% – max. 20% – with pointing at shooting off female kits.

Among roebucks, obtainment in age classes should be formed at a level of:
I class (2.-3. years old) – 40-50%,
II class (at the age of 4. and older) – 50-60%.

An age of a warrantable buck should be fixed at 6 years for hunting areas of Olsztyn District of the Polish Hunting Association, and warrantable stags should amount to about 20% of shot off stags.

At present there is a necessity to differentiate selective criteria of individual in age class I into two subclasses, Ia and Ib, where class Ia will include individuals at the age of 2 and Ib – at the age of 3. It seems to have been preposterous to maintain shooting off criteria in which individuals at the age of 2 and 3 were eliminated according to the same rules. An intense growth of roebucks at this time of their lives and their reaching a full physical development is also connected with large disproportions in antlers development itself, among bucks at the age of 2 and 3, which is presented in Table 4.

It should be hoped that changes to Hunting Law introduced in 2004 and followed by the *Selection principles* ... (2005) introduced by Polish Hunting Council will obligatorily bring about and maintain positive changes concerning individual selection and regulations concerning structural shooting off, and that many parameters and indicators provided above will be reflected in the practice of game management.

Selection of does and kits

Does and kits obtainment should be carried out in the same way as with does and calves. In shooting off of kits, shooting off of female kits should be favored. The population of foxes and other predators that can excessively influence the population of roe deer in the hunting ground should be significantly reduced.

Summary

1. A crucial task in running of rational hunting management in hunting areas of Warmia and Mazury is to continue monitoring of game and its surrounding, which must be based on reliable data and constant improvements of methods for their obtainment.

2. Large-area planning of hunting management in Warmia and Mazury province should still be based on its division into hunting areas. Some of their borders should be now changed adapting them to natural realities. It will allow to differentiate breeding rules within individual natural units. That is why the rules presented above are frame and it is possible to fill them with a concrete content that takes into consideration a specificity of breeding area.

3. In framework of activity of Hunting Breeding Areas, a yearly evaluation of correctness of shooting off of deer males should be carried out that at the same time should be a review of individual quality of game in Hunting Breeding Areas. The evaluation should be carried out with reference to all trophies and districts by Forestry Service together with Polish Hunting Association, irrespective of a category of hunting district (hunting circles, Game Breeding Centres)

4. Medal trophies obtained within the confines of shooting off of poor quality deer to be culled, should be confiscated as a blameworthy shooting off! This rule strongly disciplines hunters.

5. Coordinators of Breeding Areas should be obliged to submit reports on activity of breeding area to Councils of Hunting Breeding Areas. Every three years a collective report should be made, relating to a realization of Long-term Hunting Breeding Plans in Hunting Breeding Areas. It would create a basis of coordinating and supervising hunting management in view of breeding of game population and maintaining stability of ecosystems in framework of Regional Management of State Forests (of provinces).

6. Presented frame rules related to a realized growth, shooting off structure, limit of a concentration of individual populations, were defined in such a way that breeding policy could be realized in an unlimited way, depending on a specificity of a breeding area, as well as hunting areas there situated.

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INFLUENCE OF VFA/TKN RATIO IN WASTEWATER ON THE EFFECTIVENESS OF NITRIFICATION

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Key words: ammonification, autotrophic nitrification, heterotrophic nitrification, activated sludge, volatile fatty acids.

Abstract

The nitrification in a sequencing batch reactor with controlled air supply system, providing oxygen at the concentration of 2 mg $O_2 \cdot l^{-1}$, was studied. Municipal wastewater mixed with 20% of supernatant from digestive tank was used as a medium (reactor influent). Concentration of volatile fatty acids (VFA) in the reactor influent was increased from135.3 mg VFA $\cdot l^{-1}$ (series 1) to 230 mg VFA $\cdot l^{-1}$ (series 2) as a result of an addition of acetate. At the constant total Kjeldahl nitrogen concentration (TKN) – 200 mg TKN $\cdot l^{-1}$, VFA/TKN ratio in a medium increased from 0.66 to 1.1. During the feeding and at the beginning of the aeration time, 7 and 8 h oxygen depletion phase respectively to the series was observed. Depending on VFA/TKN ratio, the contribution of an autotrophic nitrifiers in activated sludge decreased from 16 to 5.3%, but the effectiveness of nitrification increased from 70 to 90%. Independently on VFA/TKN ratio biomass yield coefficient (mass of bacteria formed per mass of COD removed) was about 0.3 g VSS $\cdot g^{-1}$ COD (VSS – volatile suspended solids). Nitrogen concentration used for the biomass growth was about 2.6 mg $N\text{-}NH_4 \cdot l^{-1}$.

WPŁYW STOSUNKU LKT/NK W ŚCIEKACH NA EFEKTYWNOŚĆ NITRYFIKACJI

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Abstrakt

Badano sprawność oraz mechanizm nitryfikacji w reaktorze okresowym, wyposażonym w system kontrolujący poziom doprowadzanego powietrza, zapewniający stężenie tlenu na poziomie 2 mg $O_2 \cdot l^{-1}$. Dopływ do reaktora stanowiły ścieki komunalne z 20% udziałem wód nadosadowych z komór fermentacyjnych. W wyniku dodania octanu zwiększono stężenie lotnych kwasów tłuszczowych (LKT) w ściekach doprowadzanych do reaktora porcjowego z 135,3 mg LKT $\cdot l^{-1}$ (seria 1) do 230 mg LKT $\cdot l^{-1}$ (seria 2). Przy stałym stężeniu azotu Kjeldahl (NK) – 200 mg NK $\cdot l^{-1}$, stosunek LKT/NK w ściekach doprowadzanych do reaktora wzrósł z 0,66 do 1,1. Podczas fazy napełniania oraz na początku fazy reakcji (napowietrzania) obserwowano odpowiednio 7- i 8-godzinną, fazę wyczerpania tlenu w ściekach w reaktorze. W zależności od stosunku LKT/NK w ściekach, udział autotroficznych nitryfikantów w osadzie czynnym zmniejszył się z 16 do 5.3%, jednak sprawność nitryfikacji wzrosła z 70 do 90%. Niezależnie od stosunku LKT/NK współczynnik przyrostu biomasy (masa mikroorganizmów osadu czynnego do masy usuniętego ChZT) wynosił 0,3 g smo $\cdot g^{-1}$ ChZT (smo – sucha masa organiczna). Stężenie azotu usuniętego w wyniku syntezy biomasy wyniosło 2,6 mg N-NH4 $\cdot l^{-1}$.

Introduction

Various microorganisms participate in nitrogen cycle in natural and engineered ecosystems. In wastewater treatment plant the organic forms of nitrogen should be firstly ammonified. Ammonification takes place under both aerobic and anaerobic conditions and the process is carried out by heterotrophic microorganisms (HERBERT 1999). A final product of ammonification – ammonium – is afterwards a substrate for nitrification, assimilation or for generation of new cell structures and enzymes.

The nitrification process has been thought to be carried out mainly by ammonia- and nitrite-oxidizing bacteria which are obligatory aerobic and chemoautotrophic. Nitrification is widely defined as the transformation of ammonium to nitrite with *Nitrosomonas* and nitrite to nitrate with *Nitrobacter* (WAGNER et al. 1996, GINESTET 1998). Autotrophic nitrification is the process that is well known for a long time and is precisely studied in the literature. It takes place in aeration zones or aeration tanks in the wastewater treatment plants.

Nitrification in environment, providing unfavorable conditions for autotrophic nitrifying bacteria, may result from the activity of heterotrophic microorganisms (CASTIGNETTI 1990, KESTER et al. 1997). A number of heterotrophic microorganisms – including bacteria, actinomycetes, and fungi – have been reported to "nitrify". The contribution in the nitrification process has remained unknown in spite of the recent studies on heterotrophic nitrification but it is known that their activities are generally lower than those of authotrophs. However, the sum of their activity may be comparable to those of the authotrophs because the biomass of the heterotrophs is much larger than that of authotrophs.

There is no selective enrichment or isolation method for heterotrophic nitrifying microorganisms. It was proved that media supporting the growth must contain organic carbon in addition to nitrogen (BRIERLEY, WOOD 2001, RHEE et al. 1997). SAKAI et al. (1997) demonstrated that many heterotrophic bacteria including denitrification-positive strains show nitrite oxidation activity when cultured aerobically in nutrient media. The bacteria were tentatively classified into three groups according to their manner of nitrite conversion. Many denitrification-negative strains of *Arthrobacter*, *Bacillus*, and *Micrococcus* consumed nitrite and accumulated similar amounts of nitrate. Many strains of the genera *Corynebacterium*, *Pseudomonas*, and *Bacillus* – many of which are denitrification-positive – consumed nitrite almost without accumulation of nitrate. It means that part of nitrate are denitrified simultaneously. In the third group nitrite was consumed, but quite a small amount of nitrate was accumulated, by enterobacteria strains such as *Escherichia*, *Klebsiella*, *Proteus*. Probably bacteria perform nitrite reduction to ammonia (SAKAI et al. 1996). Despite the diversity of the reactions, the contribution of heterotrophic nitrification remained unclear. Subsequent attention has been paid to the nitrite--oxidizing heterotrophic bacteria, but only a few studies identified a role of heterotrophic nitrification under aerobic conditions. In this study we have focused on the inquiring of the existence of heterotrophic nitrification in activated sludge under aerobic conditions when ammonium and organic nitrogen compounds exist in wastewater.

Aerobic tanks in wastewater treatment plant provides the conditions in which ammonium nitrogen presented in domestic wastewater is easily oxidized by activated sludge. It is common that supernatant released after sludge fermentation is supplied into the aeration tank. The supernatant is an additive source of carbon, ammonium, and organic nitrogen compounds.

At biological treatment the mechanisms of ammonium nitrogen removal by activated sludge can change. The different mechanisms can be the result of the excess of ammonium and organics concentrations in the reactor influent.

The objective of this study was to determine the influence of VFA/TKN in municipal wastewater with addition of supernatant after sludge fermentation on the efficiency of nitrification in activated sludge. In our experiment different mechanisms of ammonium removal were observed under aerobic conditions with oxygen depletion phase in reactor at the beginning of the aeration time. A respirometric method was proposed for the estimation of the contribution of nitrifying biomass in an activated sludge.

Materials and Methods

Organisation of the Experiment

The experiment was carried out in sequencing batch reactor Bioflo 3000 type. Working volume of the reactor was 5 l. Sequencing batch reactor was equipped with controlled air supply system (oxygen supply into the reactor was constant – set value was 2 mg $O_2 \cdot l^{-1}$), and DO probe that showed variations of oxygen concentration in wastewater during the reaction time. Biomass concentration was maintained at about 3 g VSS $\cdot l^{-1}$.

The reactor was operated in a 24-h cycle mode, at 15 minutes for the feed, whereas 2.5 l of wastewater with supernatant was supplied into the reactor, 23 hours for the aeration phase (reaction time), whereas mixture of wastewater and activated sludge was stirred and aerated, 30 minutes for settle of activated sludge, and 15 minutes for decant. Volumetric exchange rate of the reactor was stable – 50%. The amount of the excess sludge allowed to maintain sludge age on the level of 30-40 days. Laboratory SBR reactor was operated at temperature of 20°C. Technological conditions (long aeration phase, and sludge age) were kept in order to favour nitrification (POLLICE et al. 2002).

Two series were performed. Municipal wastewater and supernatant after sludge fermentation were the sources of volatile fatty acids (VFA) and nitrogen in both series. Supernatant after sludge fermentation, taken from open digestive tank of wastewater treatment plant, used in presented experiment contained averagely 2000 mg COD $\cdot l^{-1}$, 676 mg N-$NH_4 \cdot l^{-1}$, and 300 mg $CH_3COOH \cdot l^{-1}$.

In order to determine the influence of VFA/TKN ratio on the effectiveness of nitrification acetate was dosed into wastewater, therefore VFA/TKN ratio increased from 0.66 to 1.1. Characteristic of reactor influent is shown in Table 1.

Table 1

Characteristic of the reactor influent

Parameters		Series 1	Series 2
Supernatant from digestive tank in municipal wastewater	(%)	20	
Ammonium	(mg N-$NH_4 \cdot l^{-1}$)	148.6	150.9
Organic nitrogen	(mg $N_{org} \cdot l^{-1}$)	56.6	50.8
Volatile fatty acids (VFA)	(mg VFA $\cdot l^{-1}$)	135.3	230
VFA/TKN ratio in wastewater		0.66	1.1

After adaptation of activated sludge to the experimental conditions, technological measurements of the parameters were carried out during 30 days for each series. Finally, kinetic measurements were done. Respirometric activity of activated sludge was determined with OxiTop® system. Respirometric measurements were done three times for each series. For calculations the average values were used. The system, operating at temperature of 20°C, registers changes of the pressure in measuring vessel as a result of oxygen uptake by microorganisms. Changes of the pressure were converted on the oxygen uptake expressed as mg $O_2 \cdot l^{-1}$ (PN-EN ISO 9408:2005).

At the beginning of the aeration phase, 291 ml sample was taken from the SBR reactor what resulted in the biomass concentration, organic and nitrogen loading in measuring vessels identical as in the reactor. In OxiTop® vessel oxygen uptake for organics, ammonium oxidation and for the endogenous respiration (A) was defined.

Into the next measuring vessel the same mixture was supplied. In order to determine the oxygen uptake for organics and endogenous respiration (B) autotrophic nitrification inhibitor was also added. Allylthiourea was used as autotrophic nitrification inhibitor. Allylthiourea (86 μM) and azide (24 μM) were show to be strong, selective inhibitors of ammonia and nitrite oxidation without affecting other activity (GINESTET et al. 1998).

The oxygen uptake for endogenous respiration (C) was determined in measuring vessel with activated sludge twice washed with distilled water. Wastewater was replaced by distilled water.

The following equations were used in order to calculate:

– oxygen uptake for organics oxidation L_{0S} (mg $O_2 \cdot l^{-1}$), $L_{0S} = B - C$
– oxygen uptake for ammonium nitrogen oxidationby autotrophic nitrifiers L_{0N} (mg $O_2 \cdot l^{-1}$), $L_{0N} = A - B.$

Analytical Methods

The following parameters were determined in wastewater with the addition of supernatant from digestive tank: organic compounds, expressed as total and dissolved COD (PN-74/C-04578.03), volatile fatty acids (PN-75/C-4616/04), ammonia by the Nesslerization method after distillation (PN-C-04576-4:1994), total Kjeldahl nitrogen (PN-81/C-04527), total suspended solids (TSS) by drying at 103-105°C (dry residue) in the influent and effluent. Concurrently, biomass concentration (volatile suspended solids – VSS) after ignition at 550°C was determined (PN-EN 12879:2004).

Calculation Methods

Nitrogen concentration used for the biomass growth (mg $N\text{-}NH_4 \cdot l^{-1}$) was calculated from the following formula:

$$C_{Nsyn} = Y \cdot (C_{0,COD} - C_{e,COD}) \cdot F_N \quad \text{(i)}$$

Y – biomass yield coefficient (g $VSS \cdot g^{-1}$ COD),
$C_{0,COD}$ – organics concentration in the influent (mg $COD \cdot l^{-1}$),
$C_{e,COD}$ – organics concentration in the effluent (mg $COD \cdot l^{-1}$),
F_N* – nitrogen contents in activated sludge (g $N \cdot g^{-1}$ VSS).

Total ammonium concentration (mg $N\text{-}NH_4 \cdot l^{-1}$) was determined on the assumption that this is a sum of ammonium resultant after ammonification (from t=0 h to t=24 h) and ammonium nitrogen content in wastewater (at t=0 h), and was calculated from the equation:

$$C_{N\text{-}NH_4amon} = C_{0,N\text{-}NH_4} + (C_{0,N_{org}} - C_{e,N_{org}}) \quad \text{(ii)}$$

$C_{0,N\text{-}NH_4}$ – ammonium concentration in the influent (mg $N\text{-}NH_4 \cdot l^{-1}$),
$C_{0,N_{org}}$ – organic nitrogen in the influent (mg $N_{org} \cdot l^{-1}$),
$C_{e,N_{org}}$ – organic nitrogen in the effluentą(mg $N_{org} \cdot l^{-1}$).

The concentration of autotrophic nitrifying biomass (mg $VSS \cdot l^{-1}$) was calculated from the following formula:

$$X_N = X_{org} \cdot \frac{\frac{L_{0N}}{4.6}}{\left(\frac{L_{0S}}{0.7} + \frac{L_{0N}}{4.6}\right)} \quad \text{(iii)}$$

X_{org} – activated sludge concentration (mg $VSS \cdot l^{-1}$);
L_{0N} – maximal oxygen uptake for ammonium oxidation (mg $O_2 \cdot l^{-1}$);
L_{0S} – maximal oxygen uptake for organics oxidation (mg $O_2 \cdot l^{-1}$);
4.6 – the amount of oxygen per 1 g of ammonium oxidation (Eum, Choi 2002) (g $O_2 \cdot g^{-1}$ $N\text{-}NH_4$);
0.7 – the amount of oxygen per 1 g of organics oxidation (Eum, Choi 2002) (g $O_2 \cdot g^{-1}$ COD).

Nitrification efficiency (%) was calculated using the following equation:

$$\eta_N = \frac{C_{N_{OX}}}{C_{0,TKN}} \cdot 100 \quad \text{(iv)}$$

and ammonium oxidized by activated sludge (mg Nox $\cdot$ l^{-1}):

$$C_{N_{OX}} = C_{0,TKN} - C_{e,TKN} - C_{Nsyn} \quad \text{(v)}$$

$C_{0,TKN}$ – Kjeldahl nitrogen concentration in the influent (mg $N_{TKN} \cdot l^{-1}$),
$C_{e,TKN}$ – Kjeldahl nitrogen concentration in the effluent (mg $N\text{-}NH_4 \cdot l^{-1}$),
C_{Nsyn} – nitrogen used for the biomass growth (mg $N\text{-}NH_4 \cdot l^{-1}$).

Ammonification rate was calculated according to the first-order kinetics equation:

$$r_{amon} = (C_{f,N\text{-}NH_{4amon}} - C_{i,N\text{-}NH_4}) \cdot k_{N\text{-}NH_{4amon}} \quad \text{(vi)}$$

r_{amon} – ammonification rate (mg $N\text{-}NH_4 \cdot l^{-1} \cdot h^{-1}$),
$C_{f,N\text{-}NH_4}$ – final ammonium concentration after ammonification (mg $N\text{-}NH_4 \cdot l^{-1}$),
$C_{i,N\text{-}NH_4}$ – ammonium concentration in the influent (mg $N\text{-}NH_4 \cdot l^{-1}$),
$k_{N\text{-}NH_{4amon}}$ – constant rate of ammonification (h^{-1}).

Ammonium removal rate was calculated according to zero-order reaction:

$$r_{N\text{-}H_4r} = -k_{N\text{-}NH_4r} \quad \text{(vii)}$$

$r_{N\text{-}NH_4r}$ – ammonium removal rate (mg $N\text{-}NH_4 \cdot l^{-1} \cdot h^{-1}$),
$k_{N\text{-}NH_4r}$ – constant rate of ammonium removal (mg $N\text{-}NH_4 \cdot l^{-1} \cdot h^{-1}$).

Results

Mechanisms of nitrification was studied in a sequencing batch reactor with controlled air supply system in relation to the concentration of easily biodegradable organic fraction. The average nitrogen loading of activated sludge was about 0.07 g TKN $\cdot$ g^{-1} VSS $\cdot$ d^{-1}, however volatile fatty acids (VFA) loading was on the level 0.046 in series 1, and 0.073 g VFA $\cdot$ g^{-1} VSS $\cdot$ d^{-1} in series 2.

In presented experiment a balance between oxygen supply and dissolved oxygen concentration in the reactor was observed, and DO reflected the

variations of the balance. Controlled air supply system ensured constant oxygen supply into the reactor. Activated sludge used oxygen for organics and ammonium oxidation (carbon and nitrogen concentrations started to decrease) therefore DO concentration in the reactor changed. When oxygen use by the microorganisms surpassed the oxygen supply, DO in the reactor decreased. Figure 1 depicted that at the initial hours of the reaction time DO concentration in wastewater in Bioflo 3000 was nearly zero. It was called oxygen depletion phase. In series 1 and 2 oxygen depletion phase lasted 7 and 8 hours, respectively. Longer oxygen depletion phase in series 2 was a result of higher sludge loading of VFA.

Correspondingly with the decrease in substrates concentrations DO concentration increased (Figure 1).

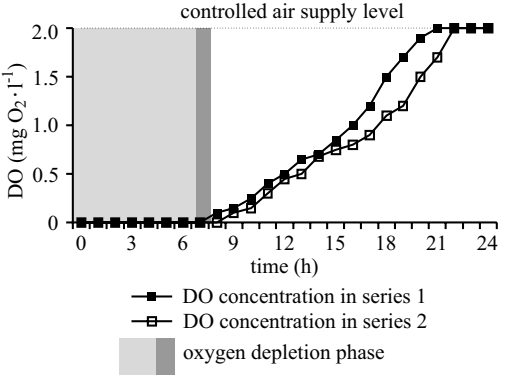


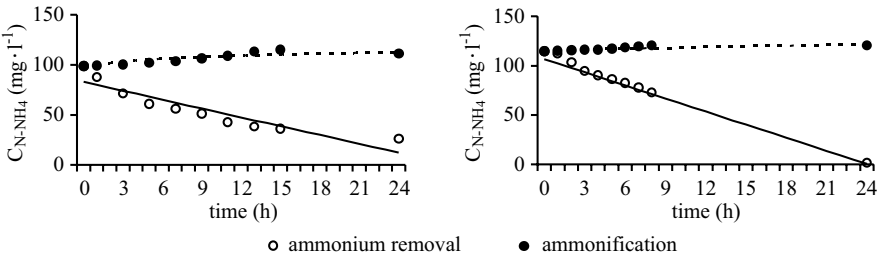
Fig. 1. The duration of oxygen depletion phase and variation of DO concentration in the reactor during the reaction time in series 1 and 2

Under aerated conditions with oxygen depletion phase occurring in wastewater in the reactor, ammonification performed according to the first-order reaction. Constant rates of the reaction in series 1 and 2 was 0.007 h^{-1} and 0.002 h^{-1}, respectively. The ammonification of organic nitrogen content in wastewater ranged from 70 to 80%. Organic nitrogen concentration in the effluent was on the level of about 10.5 mg $N_{org} \cdot l^{-1}$.

It was shown that concurrently with ammonification activated sludge carried out ammonium oxidation and used ammonium for the biomass growth. The rate of ammonium removal in wastewater in series 2 was 4.43 mg $N\text{-}NH_4 \cdot g^{-1}$ VSS $\cdot h^{-1}$, and was 1.5-fold higher in contrary to series 1. It was calculated according to zero-order reaction (Figure 2). Almost all ammonium was oxidized to nitrate, there was no nitrite accumulation. Biomass yield coefficients in both series was about 0.3 g VSS $\cdot g^{-1}$ COD. Ammonium

nitrogen used for the biomass growth was low, and at VFA/TKN ratio 0.66 and 1.1 only 2.4 and 2.8 mg $N\text{-}NH_4 \cdot l^{-1}$ was used for the biomass synthesis.

Despite the similar length of the oxygen depletion phase in SBR reactor in both series, changes in VFA/TKN ratio influenced on various effectiveness of nitrification. At VFA/TKN ratio 0.66 the accumulation of ammonium in the effluent to 26 mg $N\text{-}NH_4 \cdot l^{-1}$ was observed. During the experiment the residual ammonium concentration in the effluent at VFA/TKN 1.1 did not exceed 1 mg $N\text{-}NH_4 \cdot l^{-1}$ (Figure 3).



series		1	2
ammonification	k (h^{-1})	0.007	0.002
	r (mg $N\text{-}NH_4 \cdot g^{-1}$ VSS h^{-1})	0.073	0.0084
ammonium removal	k=r (mg $N\text{-}NH_4 \cdot g^{-1}$ VSS h^{-1})	2.95	4.43

Fig. 2. Variation of ammonium concentration due to ammonification ($C_{N\text{-}NH_4}$amon) and ammonium removal during the reaction time ($C_{N\text{-}NH_4s}$) a) series 1, b) series 2 (in the table the values of the constant rate (k) and the initial rate (r) are shown)

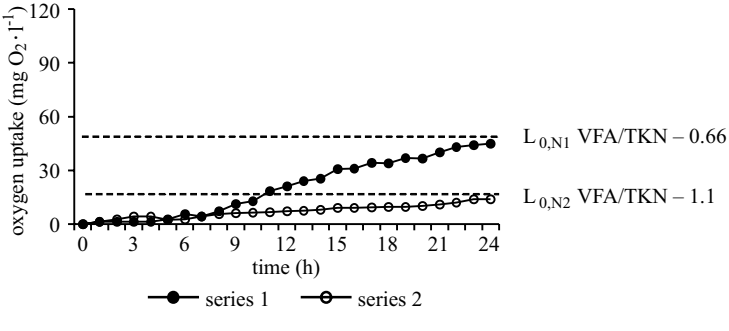
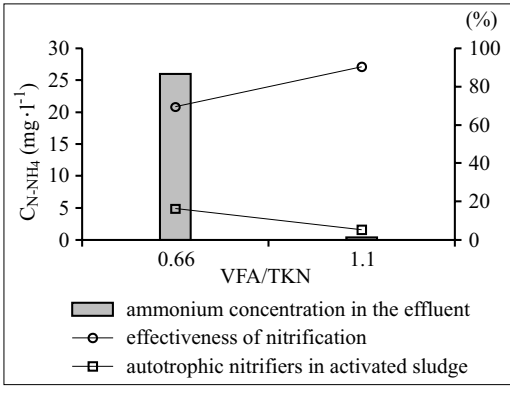


Fig. 3. Oxygen uptake by authotrophs for ammonium oxidation depending on VFA/TKN ratio in wastewater (series 1 and 2)

Using respirometric measurements higher nitrifying activity of activated sludge was shown in series 1. It was reflected by the higher oxygen uptake for autotrophic ammonium oxidation in time from t=0 h to t=24 h. Figure 3

shows that the value of L_{0N} after 24 h of the measurements was 3-fold higher at VFA/TKN 0.66 than at VFA/TKN 1.1.

The contribution of nitrifying biomass in activated sludge, estimated on the basis of the respirometric measurements (calculation methods; iii), in series 1 and 2 were 16.3% and 5.3%, respectively (Figure 4). If in both series nitrification was carried only by autotrophic bacteria, the effectiveness of nitrification at VFA/TKN ratio 1.1 would be lower, because of lower nitrifying biomass content. The effectiveness of ammonium oxidation increased from 70 to 90%, what may indicate on the participation of heterotrophic microorganisms in ammonium oxidation.



series		1	2
activated sludge	(mg VSS l^{-1})	2917.0	3164.0
	(% TSS)	79.1	84.0
nitrifiers	(mg VSS l^{-1})	457.8	169.0
	(% TSS)	16.3	5.3

Fig. 4. The average value of the residual ammonium in the effluent, nitrification effectiveness and the contribution of autotrophic nitrifiers in activated sludge depending on VFA/TKN ratio in wastewater (in the table activated sludge and nitrifiers concentrations are shown)

Discussion

Presented study proved that under aerated conditions, with the initial oxygen depletion phase, nitrification took place. At the ratio VFA/TKN 0.66 in wastewater, the effectiveness of nitrification was on the level 70%. Autotrophic nitrifiers contributed 16% of total biomass concentration. It is generally known that during the oxidation of inorganic N compounds chemoautotrophic bacteria generate the energy for carbon dioxide fixation and growth (HOOPER et al. 1997). It is obvious that chemolithotrophic microorganisms do not need any external organic carbon source but prefer oxygen conditions. Presence of easily biodegradable fraction in wastewater in our experiment did not favour autotrophic nitrification. Despite the high contribution of autotrophs in activated sludge, reactor effluent contained high ammonium concentration and

the nitrification efficiency was low. According to PATUREAU et al. (1997), despite the higher autotrophs affinity in comparison to ammonium of autotrophs, compared to heterotrophs, the nitrifying activity decreased in the presence of organic carbon source, because of higher heterotrophic cell numbers and competition for oxygen and ammonium. Authors have found that the availability of organic carbon in relation to available amount of ammonium were the key factors in the outcome of such competition.

In some cases high C/N ratio do not influence autotrophic nitrification. SEIXO et al. (2004) studied the influence of C_{org}/N ratio ranging from 0.5 to 11 on nitrifying effectiveness. The highest nitrification efficiency (99.6%) was obtained at the C_{org}/N 11. However, the increase at the nitrification rate could be explained through the operating schedule applied from SBR cycle. The feed was carried out in three different steps along whole batch and was followed by a denitrification step. So the heterotrophic biomass responsible for nitrate removal used carbon source added to the system.

Compared to autotrophic nitrifiers, heterotrophic nitrifiers require lower DO concentration, tolerate more acidic environment and prefer higher C/N ratios (ZHAO et al. 1999). In our experiment in SBR reactor specific oxygen conditions was shown. Applying of controlled air supply system resulted in oxygen depletion phase at the initial hours of the experiment. Probably there was also a competition for oxygen in favour of heterotrophs. In both series there was long oxygen depletion phase that lasted 7 and 8 hours of the reaction time. Throughout the addition of acetate as an easily biodegradable organic fraction, VFA/TKN ratio increased to 1.1 and volatile acids concentration increased from 135 to 230 mg VFA $\cdot$ l^{-1}. The results of respirometric measurements showed lower activity of autotrophic biomass and autotrophic nitrifiers contributed 5.3% of total biomass concentration. Interestingly, ammonium nitrogen concentration in the effluent was on the level 0.4 mg N-NH_4 $\cdot$ l^{-1}. The increase in the nitrification efficiency to 90% might be the result of nitrifying activity of heterotrophic organisms of activated sludge. Similarly, ZHAO et al. (1999) observed that nitrification efficiency improved due to the addition of the external carbon source (acetate and methanol to the concentration of 100 and 60 mg COD $\cdot$ l^{-1}, respectively) in a 3-stage Bardenpho process at laboratory scale. It might indicate on the heterotrophic activity in activated sludge. The studies of NISHIO et al. (1998) proved that at higher TOC/TN ratios heterotrophic nitrification took place.

ROBERTSON et al. (1988), DE BOER, KOWALCHUK (2001) reported that in contrary to autotrophic nitrification, heterotrophic ammonium oxidation is not linked to cellular growth and biomass synthesis. In our experiment both at the ratio of VFA/TKN 0.66 and 1.1, biomass yield coefficients were on the level of about 0.3 g VSS $\cdot$ g^{-1} COD, despite the increase in easy accessible organics concentration.

Conclusions

1. Using controlled air supply system in SBR reactor resulted in the initial oxygen depletion phase. Oxygen depletion phase (8 hours of the reaction time) and the concentration of easily biodegradable fraction on the level 230 mg VFA $\cdot$ l^{-1} favoured heterotrophic nitrification.

2. At VFA/TKN ratio 1.1 autotrophic nitrification biomass contributed 5.3% of total biomass concentration, however the increase in nitrification effectiveness to 90% was observed; at 16% of autotrophic nitrifying biomass concentration the efficiency of nitrification was 70%.

3. At VFA/TKN ratios of 0.66 and 1.1, ammonium used for the biomass growth was 3 mg $N\text{-}NH_4 \cdot l^{-1}$. Low biomass yield coefficient at VFA/TKN ratio 1.1 also may confirm heterotrophic nitrification in SBR with the initial oxygen depletion phase in wastewater.

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VARIABILITY OF DISSOLVED ORGANIC MATTER CONTENT IN SMALL RESERVOIRS

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Key words: DOM, DOC, $SUVA_{260}$, small reservoirs, watershed.

Abstract

Quantity (measured as DOC) and quality (measured as $SUVA_{260}$) of the dissolved organic matter in twenty-five small water reservoirs were characterized. The surroundings of the examined reservoirs varied from typically urban, through wasteland or cultivated land, to covered with trees. The max. mean DOC concentrations in spring and autumn were measured in the reservoirs situated in the cultivated areas. Little lower values of DOC were detected in the reservoirs adjacent to wasteland or tree stands, however, the matter was more aromatic. The lowest DOC quantities were observed in spring in the urban reservoirs which contained more aliphatic (lower $SUVA_{260}$) matter, an indicator or its anthropogenic origin. The statistical analysis revealed significant differences in DOC concentrations between the reservoirs surrounded by the built-up land and the cultivated land. With regard to the $SUVA_{260}$ parameter, the land grown with trees markedly varied from the other types of the analysed surroundings.

ZMIENNOŚĆ ZAWARTOŚCI ROZPUSZCZONEJ MATERII ORGANICZNEJ W MAŁYCH ZBIORNIKACH

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Słowa kluczowe: DOM, DOC, $SUVA_{260}$, małe zbiorniki, zlewnia.

Abstrakt

Scharakteryzowano ilościowo (mierzona jako DOC) i jakościowo (mierzona jako parametr $SUVA_{260}$) rozpuszczoną materię organiczną w 25 małych zbiornikach wodnych aglomeracji miejskiej. Otoczenie zbiorników było bardzo zróżnicowane: od obszarów związanych ze ścisłą zabudową

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miejską, poprzez nieużytki i tereny rolnicze, aż po obszary zadrzewione. Największe średnie stężenia DOC, zarówno wiosną, jak i jesienią, stwierdzono w zbiornikach na terenach użytkowanych rolniczo. Nieco niższe wartości DOC oznaczono w zbiornikach występujących na nieużytkach i terenach zadrzewionych, przy czym w materii w nich zawartej wykazano większy udział związków aromatycznych. Najmniejsze stężenia DOC obserwowano wiosną w zbiornikach na terenie zabudowanym. W zawartej w nich materii stwierdzono więcej rodników alifatycznych (niższy parametr $SUVA_{260}$), co świadczy o dopływie materii pochodzenia antropogenicznego. Analiza statystyczna wykazała istotne różnice stężeń DOC w zbiornikach otoczonych terenami zabudowanymi i gruntami rolnymi. W przypadku wskaźnika $SUVA_{260}$, istotne znaczenie miały tereny zadrzewione w stosunku do pozostałych sposobów zagospodarowania zlewni.

Introduction

Water reservoirs always constituted an essential element of man's surroundings. For centuries they were built for economic as well as aesthetic purposes. They play various roles in the environment: store water for economic use or agricultural irrigation, provide water for animals (water holes) or aquaculture (fish ponds), and serve as firewater storage tanks or electric energy source. Small reservoirs create conditions for recreation; they are used for bathing, sport fishing, or as ornamental ponds (MIODUSZEWSKI 1996, TUŹNIK-KOSNO 1998).

Small water reservoirs play also many functions in the natural environment, providing habitats for various fishes, birds and other animals. They create ecological islands and corridors connecting areas with special natural value, and constitute one of the most important elements that secure biological diversity of the landscape (KUCHARSKI, SAMOSIEJ 1993, MATUSIAK 1996).

Reservoirs grown with vegetation function as biological filters treating water running off from cultivated land (including water from melioration systems) and are very effective in removing nutrients (nitrogen, phosphorus) and pesticides from water.

Small reservoirs protect against floods and water erosion. If situated amid arable fields, they make biogeochemical barriers catching up suspensions and chemical compounds (JUSZCZAK 2001). They enhance underground water resources therefore constitute a specific element of the water circulation system. Together with rivers and lakes, small reservoirs regulate the water table of underground and surface waters (KASPRZAK 1985).

Small water reservoirs have also an effect on microclimate, increasing air humidity, reducing temperature fluctuations, or through fogs and horizontal precipitation.

Due to ubiquity and functional variety, small water reservoirs constitute a valuable element of the landscape; their existence should be maintained by preservation of the ecosystems in the most natural or the closest to natural condition. Advanced eutrophication and degradation processes allow classify-

ing the reservoirs as polytrophic i.e. characterized by high biologic production but impoverished biological diversity. Changeability of nutrients' concentrations in the water of small reservoirs is first of all related to the way of the watershed development, the size of the watershed being the secondary factor (KOC et al. 2002).

This paper examines the effect of the surroundings on quantity and quality of the dissolved organic matter in small water reservoirs.

Material and Methods

Water sampling was done once in May 2003 and once in October 2003 from small reservoirs located in four districts of the Olsztyn city: Centrum (C – 5 reservoirs), Gutkowo (G – 5 reservoirs), Kortowo (K – 7 reservoirs) and Redykajny (R – 8 reservoirs), (Table 1, Figure 1). The number of reservoirs examined in October was smaller (18 out of 25) because some dried.

Table 1
The characteristics of the reservoirs of the individual districts: Centrum (C), Gutkowo (G), Kortowo (K) and Redykajny (R)

Symbol of reservoir	Surroundings of reservoir	Description of reservoir shores	Type of reservoir
1	2	3	4
C1	single trees (alder and birch)	small clusters of willow, cattail	afforested land
C2	multi-family houses, general public buildings, trodden and paved paths	reeds, small clusters of willow	built-up land
C3	multi-family houses, general public buildings, trodden and paved paths	reeds, small clusters of willow	built-up land
C4	single- and multi-family houses, busy street, alleys for pedestrians	partly concrete-paved, the reservoir is a receiving water for run-off waters and storm water	built-up land
C5	multi-family houses, city park, alleys for pedestrians	small clusters of willow	built-up land
G1	wasteland, unpaved road, a few single-family houses	steep shores, small clusters of willow and cattail	wasteland
G2	wasteland, unpaved road, littered barren land	steep shores, small clusters of willow and cattail	wasteland
G3	forest, single-family houses under construction	dense clusters of willow and cattail	afforested land
G4	forest	dense clusters of birch and poplar	afforested land
G5	forest, meadows	clusters of willow, single birch trees, dense reeds	afforested land

cont. Table 1

1	2	3	4
K1	single-family houses, general public buildings	steep shores, clusters of larch, oak, alder and birch, small island	built-up land
K2	forest, garden plots	steep shores, single trees, dense bushes and clusters of reeds	afforested land
K3	single-family houses, general public buildings, angling ground, concrete-paved road	dense clusters of reeds, single trees and bushes	built-up land
K4	single-family houses, small forest	dense clusters of reeds and cattail	built-up land
K5	forest, lake, garden plots	clusters of cattail and single alder trees	afforested land
K6	forest, unpaved paths, garden plots	densely grown with reeds	afforested land
K7	forest, unpaved road, railroad	single willow trees, densely grown with reeds and cattail	afforested land
R1	wasteland, a few single-family houses	clusters of reeds	wasteland
R2	forest, wetland, lake	steep shores, single trees, dense bushes and clusters of reeds	afforested land
R3	arable land, meadows, in-field tree stands	grown with reeds and cattail	cultivated land
R4	meadows, in-field tree stands, unpaved road	clusters of willow and birch trees, partly grown with reeds	cultivated land
R5	arable land, meadows, orchard	single willow trees, partly grown with bulrush	cultivated land
R6	arable land, meadows, stud, birch stand, unpaved road	single birch trees, clusters of reeds	cultivated land
R7	private property screened with a concrete fence	steep shores, single clusters of reeds	built-up land
R8	arable land, farm, unpaved road	single clusters of reeds	cultivated land

In the water samples, quantity (measured as dissolved organic carbon, DOC) and quality (measured as specific UV radiation at 260 nm, $SUVA_{260}$) of the dissolved organic matter was determined (DUNALSKA, ZDANOWSKI 2004). In parallel, hydro-chemical examinations were carried out (Standard methods 1980).

To give an overall characteristics of all data and to analyse the data with regard to the individual sampling stations, districts, and year seasons, the following statistical measures were used: arithmetic mean, range, standard variation, minimal and maximal value. Significance of the correlation between two variables was assessed with the Person's linear correlation coefficient.

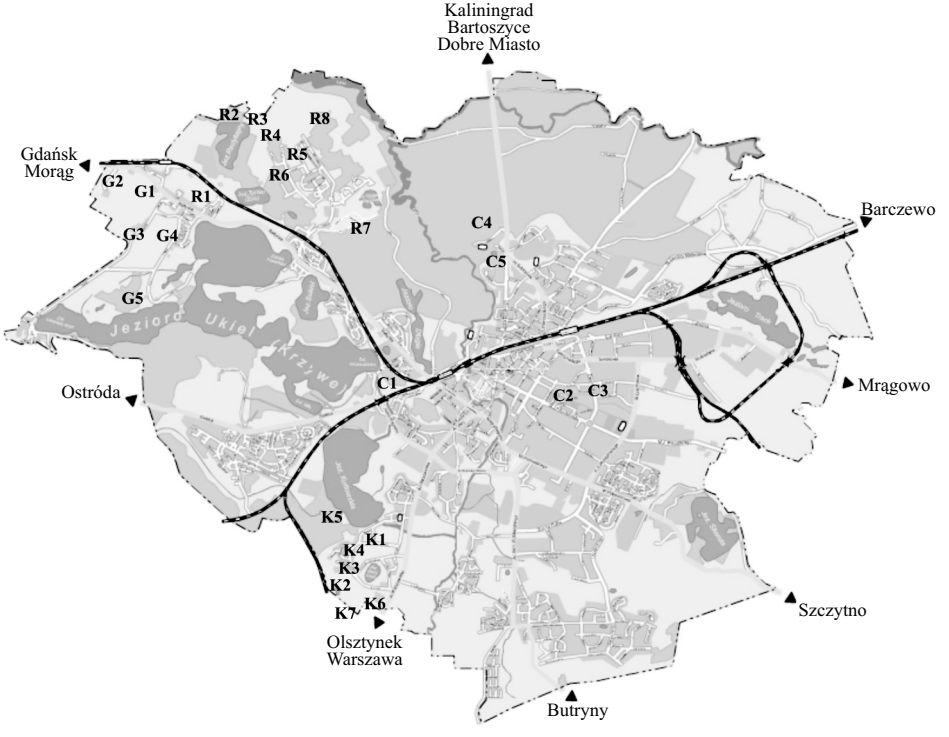

Fig. 1. Location of the studied reservoirs (http://www.mapa.olsztyna.pl)

The whole data set served to verify the hypothesis that there were no differences between the means for districts and groups of reservoirs (built-up areas, cultivated land, wasteland, afforested land). The verification was done with the t-test (assessing statistical difference between two groups of means) and the Mann-Whitney U-test. The districts and the individual types of reservoirs were compared by the analysis of variance (ANOVA) and the non parametric alternative to ANOVA (i.e. Kołomogorow – Smirnow test). The statistically significant results of the ANOVA were further analysed with the Tukey Test (NIR test).

Results and Discussion

The analyses of all mean DOC concentrations (including all examined districts) allowed concluding that the lowest values in the spring and autumn occurred in the most developed Centrum district. They equalled 9.9 mg C · dm^{-3}

and 9.8 mg C · dm^{-3} (respectively) with the parallel very low $SUVA_{260}$ parameter, particularly in the spring (Figure 2).

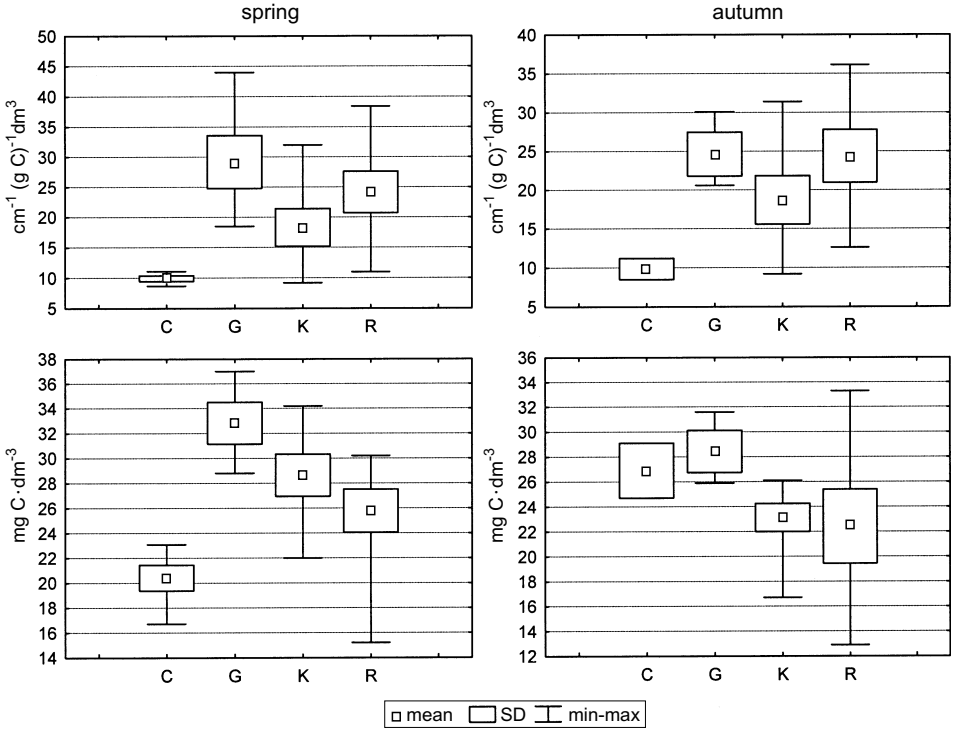

Fig. 2. DOC concentrations and $SUVA_{260}$ values in water of the reservoirs of the individual districts

In Centrum, the lowest DOC concentration was measured in the spring in C1 (8.7 mg C · dm^{-3}). The difference between the lowest DOC value in C1 and the highest in C4 was as small as 2.4 mg C · dm^{-3} and makes the evidence for little difference in the organic matter quantity in the individual reservoirs. The same conclusion can be referred to the seasonal distribution. In the spring and autumn in reservoirs C4 and C5 the differences were minimal and equalled 0.1 mg C · dm^{-3} and 0.5 mg C · dm^{-3}, respectively (Figure 3).

The variable values of $SUVA_{260}$ indicate a different character of organic matter in the Centrum district. The low values of $SUVA_{260}$ in the spring may indicate the occurrence of mainly autochthonous organic matter or the import of anthropogenic biodegradable DOC (Figure 3).

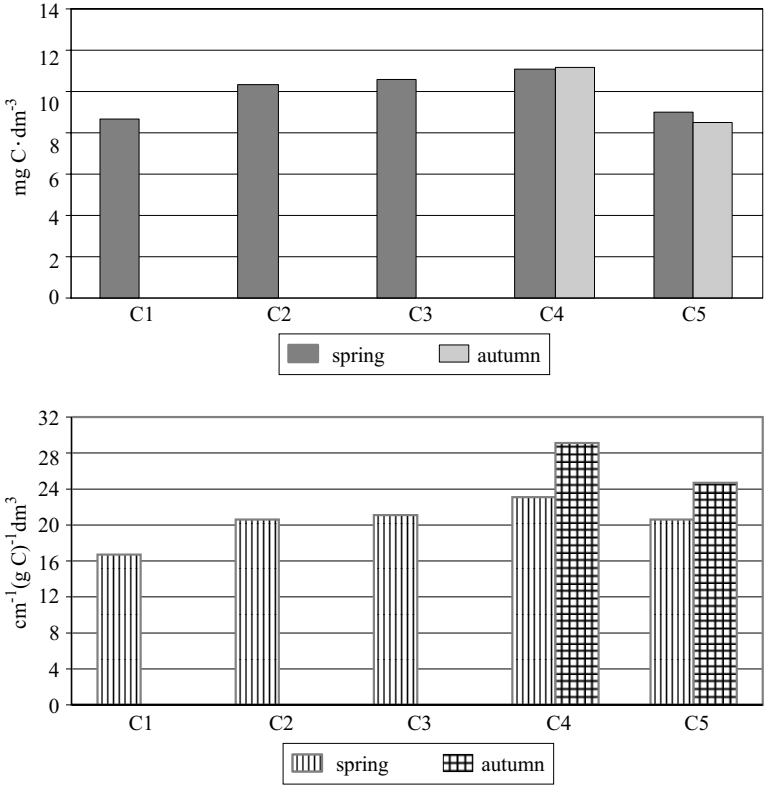

Fig. 3. DOC concentrations and $SUVA_{260}$ values in water of the C reservoirs

Value of the $SUVA_{260}$ parameter reflects the number of the polar functional groups in a molecule, aromatic properties and molecular weight (DUNALSKA, ZDANOWSKI 2004, GŁAŻEWSKI, PARSZUTO 2002, GÓRNIAK, ZIELIŃSKI 1999). Low $SUVA_{260}$ and therefore low number of aromatic rings, indicates that water contains autochthonous organic matter of high bioavailability. The intensive primary production in the Centrum district reservoirs is confirmed by the statistically significant correlation between DOC, and chlorophyll and permanganate value ($r = 0.94$ and $r = 0.86$, $p = 0.05$, respectively).

Important differences in the quantity and quality of the dissolved organic matter were observed in the other surveyed districts (Figure 2). The highest mean concentrations of DOC were measured in the Gutkowo district. In the spring, the mean DOC value equalled 29.2 mg $C \cdot dm^{-3}$ and was by 5.4 mg $C \cdot dm^{-3}$ higher than in the autumn. Gutkowo was also characterized by the highest values of $SUVA_{260}$ (Figure 4). The likely cause is the localisation

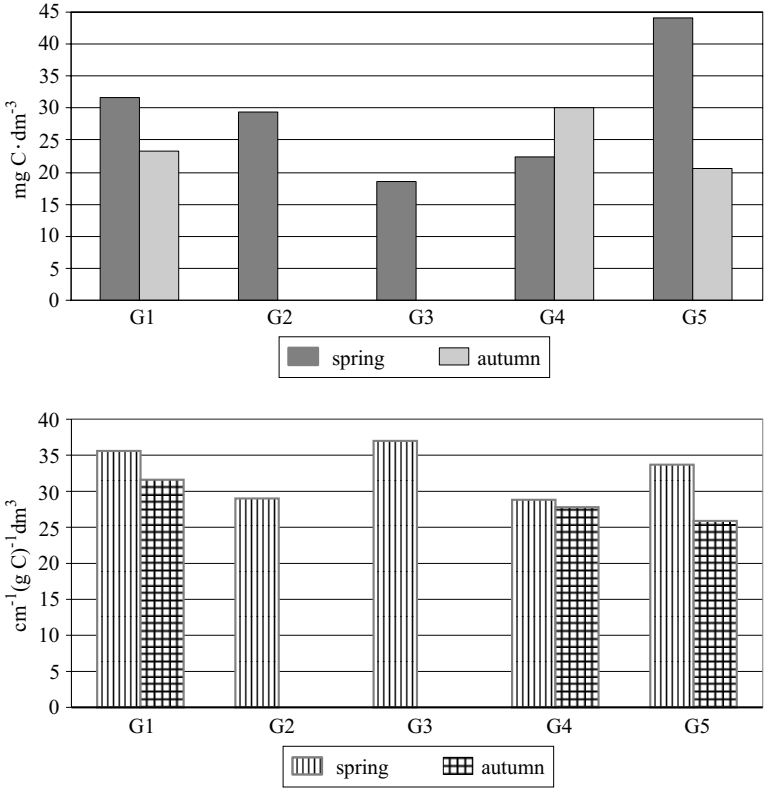

Fig. 4. DOC concentrations and $SUVA_{260}$ values in water of the G reservoirs

of the reservoirs i.e. in the agricultural area or amid forests, both a source of very aromatic organic matter.

Input of the allochthonous organic matter was confirmed by the values of the parameters measured in G5 i.e., max. concentrations of DOC (44 mg C · dm^{-3}), high $SUVA_{260}$ (33.7), and elevated in comparison to other reservoirs values of electric conductivity (467 μS · cm^{-1}), calcium ions (99.25 mg · dm^{-3}), total hardness (5.4 mval · dm^{-3}) and alkalinity (5.3 mval · dm^{-3}).

In the autumn, the concentration of DOC in G5 was by 53% lower than in the spring (Figure 4). Many processes cause the decline of concentrations proceeding together with the growing season. Degradation of organic matter stored in a reservoir in summer is caused by biotic and abiotic factors (UVA and UVB radiation, redox reactions and free radicals reactions). The decomposed matter is then used by bacteria (DE HANN 1993, DUNALSKA et al. 2003, WILLIAMSON et al. 1996). In the autumn, when the external input ceased, the amount of DOC in the water remained at a lower level.

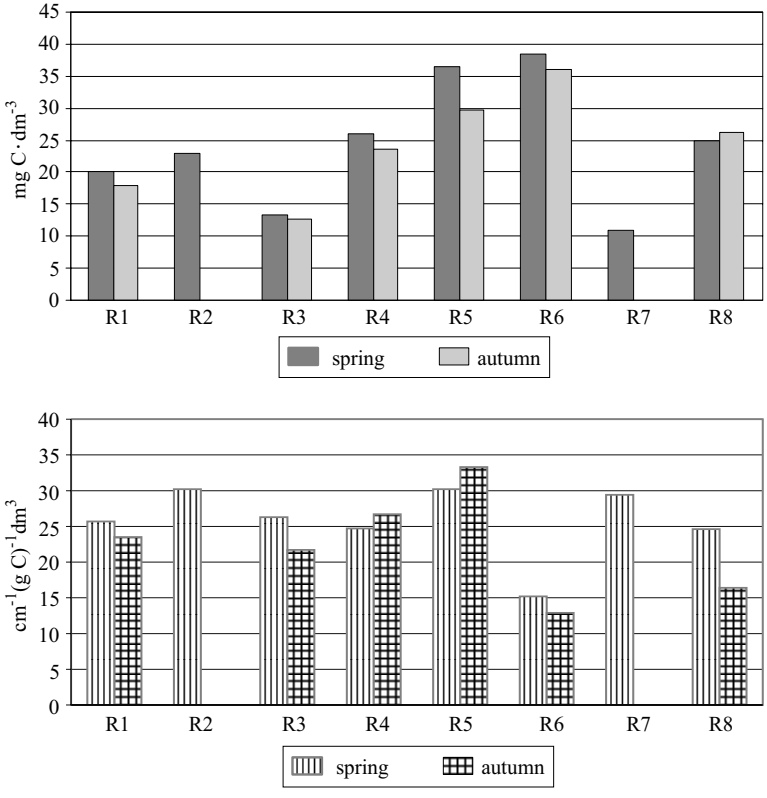

Fig. 5. DOC concentrations and $SUVA_{260}$ values in water of the R reservoirs

In the Redykajny district, the concentrations of DOC ranged from 11 to 38.4 mg C · dm^{-3} in the spring and from 12.6 to 36.1 mg C · dm^{-3} in the autumn. $SUVA_{260}$ values were also quite diverse (Figure 5). In the spring and autumn, the concentrations of dissolved organic matter in R6 were similar but simultaneously the highest of all reservoirs examined in Redykajny. The values equalled 38.4 mg C · dm^{-3} and 36.1 mg C · dm^{-3}, respectively. On the other hand, the $SUVA_{260}$ values were the lowest of all examined reservoirs i.e. in Redykajny and in three other districts. The high DOC in R6 observed in the spring was related to the high concentrations of organic phosphorus (3.8 mg · dm^{-3}) and organic nitrogen (23.4 mg · dm^{-3}), both indicating a high primary production. The latter was additionally displayed by the alkaline water reaction (pH 9.8), high water colour, and elevated temperature (23°C). High alkalinity of water lowers stability of the mineral-organic complexes. Disintegration of the complexes intensifies primary production. In aquatic

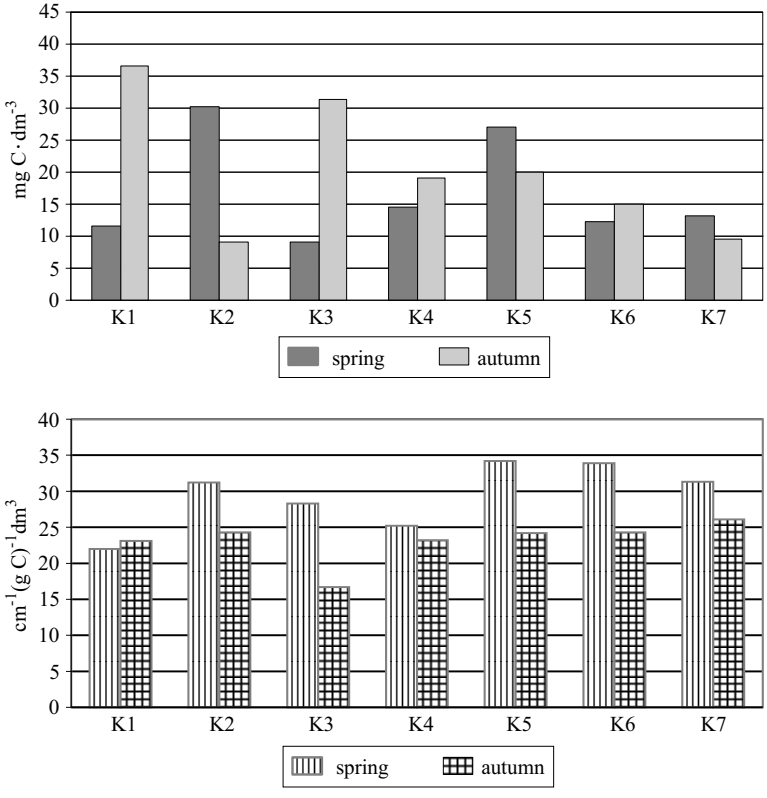

Fig. 6. DOC concentrations and $SUVA_{260}$ values in water of the K reservoirs

ecosystems, very important is the ratio between the charges of active humic substances (HS) and the quantities of mineral substances occurring in the water, responsible for its fertility (KOC et al. 2002, WOJCIECHOWSKI 1999).

Kortowo similarly to Gutkowo and Redykajny, was characterized by a high variability of the organic matter quantity in all reservoirs. In the spring, in K1 and K3 the concentrations of DOC were by approximately 70 % lower than in the autumn, with the opposite condition observed in K2 (Figure 6).

More than 3-fold increase of the DOC concentration measured in K1 in the autumn and simultaneously the high $SUVA_{260}$ value, indicate that most of the DOC comprise aromatic forms. The reason was the large amount of fallen leaves in the K1 watershed.

Similar observation was made in K3, however the 3-fold increase of the DOC value in the autumn was accompanied by a 41% reduction of $SUVA_{260}$. In this case, the examined water may have contained a lot of easily available organic matter as the pond was used for fish breeding.

Lack of clear regularity regarding the quantity and quality of dissolved organic matter in the individual reservoirs and examination seasons illustrates an obvious impact of the watershed development on the water quality in the reservoirs. The surroundings of the reservoirs were very diverse i.e., from densely built-up areas through wasteland and cultivated land, to afforested land. Despite the high variability of the examined parameters, groups of reservoirs with the same type of the surroundings were distinguished.

ANOVA and NIR tests applied to compare the watersheds on the grounds of the mean DOC concentrations showed statistically significant differences between the built-up areas and the cultivated land throughout the study; decisive were the results obtained in the spring. As regards the $SUVA_{260}$ parameter, the Kruskal-Wallis test revealed that the afforested areas varied considerably from the other types of the studied watersheds.

In natural conditions, DOC load depends mainly on the soil-forming processes and the amount of dissolved organic matter in the soil solution and in the underground water. Dominant are humic acids released to the soil solution by humification of plant and animal remains (GÓRNIAK 1996). In the case of small water reservoirs the critical role plays direct watershed. The highest DOC concentrations were determined in both spring and autumn in the reservoirs surrounded by cultivated land (Figure 7). The small depth and volume of the reservoirs and the pulsating input of organic matter from soil fertilization favour the accumulation of organic matter. Temperature, exposure to light, and enzymatic activity of bacteria (the reservoirs are placed in open, free of trees area) are the key factors of the organic matter transformation dynamics.

Lower DOC amounts were measured in the reservoirs surrounded by wasteland although the detected organic matter was more aromatic (Figure 7). In these reservoirs dominated the allochthonous (more aromatic) soil HS. QUINBY (2000) points at the positive correlation between the percentage of meadows in the watershed and the DOC concentration. High percentage of meadow ecosystems is responsible for input of very aromatic organic matter (high $SUVA_{260}$). Cellulose and lignin, containing aromatic rings, are the most common form of biomass in all terrestrial ecosystems. They constitute the structural material in plants and at the same time are the substrates in HS-creation reactions (HESSEN, TRANVIK 1998).

The lowest mean value of DOC was detected in the spring in the reservoirs placed in built-up areas (Figure 7). In comparison to the other categories of reservoirs, the organic matter was more aliphatic (lower $SUVA_{260}$) – the evidence for anthropogenic DOC input. DOC of the anthropogenic origin, due to simple structure, undergoes fast biological utilization (ZIELIŃSKI, GÓRNIAK 1999).

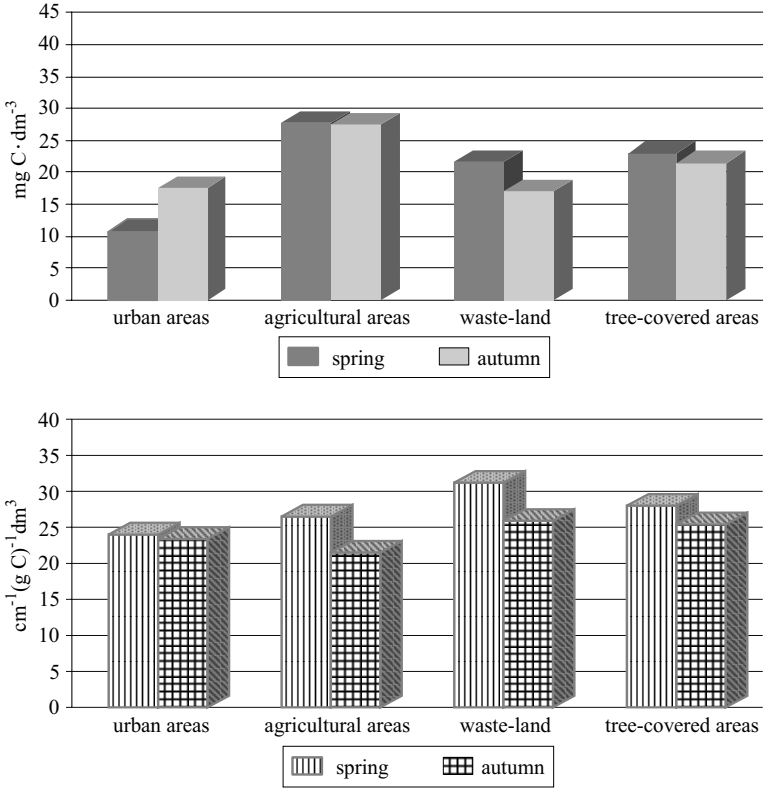

Fig. 7. Mean DOC concentrations and $SUVA_{260}$ parameter relating to the watershed use

Small water reservoirs are very important for organic matter retention. They have the high ability to collect (intercept) and immobilise (at least periodically) the matter migrating from the watershed and they produce (using imported nutrients) large quantities of organic matter, playing the role of stabilizers in the biogeochemical processes. Unfortunately, due to improper and sometimes also purposeful activities of man, especially in urban areas, the number of the small reservoirs declines. Therefore, smooth incorporation of infrastructure into the ecologic system of the landscape, leaving undisturbed the natural area-specific elements of the environment, should become an indispensable element of each city's development.

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THE ECOLOGICAL CHARACTERISTICS OF ANTHROPOGENIC FOREST PHYTOCOENOSES THREATENED BY A PESTICIDE TOMB*

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K e y w o r d s: pesticide tomb, ecological factor, anthropogenic community forest.

A b s t r a c t

Studies of ecological conditions of anthropogenic forest comprised of spruce community represented by *Sambucus racemosa* and *Sambucus nigra* and situated near a pesticide tomb were carried out in 2003-2004 based on 16 phytosociological releves. The analysis was performed with the use of ecological indices. The qualitative and quantitative ratios in the phytocenoses under study indicate a neutral response to continentalism. The luminous index (L) differentiates the habitat from the East to the West. Based on the temperature index, the area under study can be classified as a northern Polish lowland. The edaphic indices were in line with the physico-chemical analyses of the soils covering the studied area which indicated young sandy and loamy soils. The soil humidity (W) and the soil dispersion (D) indices were the lowest within the direct vicinity of the tomb. This situation can form a barrier for the migration of compounds deposited in the tomb with potential reflux. However, a high acidity index (R) indicates that the effect of this barrier is limited by the activity of hydrogen ions. The indices of organic matter content (H), nitrophylity (N) and soil trophism (Tr) had higher values in the direct vicinity of the tomb. This proves the permeation of biogenic compounds from the tomb area.

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The values of the ecological indices are similar and the ecological conditions did not significantly differ between the community of *Sambucus nigra-Picea abies* and the community of *Sambuco racemosi-Pieceetum* within the area under study. Moreover, the results obtained agree with the data available in references. This indicates the necessity for further studies since the lack of differences-suggests the effect of pesticide tomb on the environment. The differences in the floral composition and the quantitative ratios between the species forming the phytocenoses under study were indicated by the numerical analyses which divided the developed releves into two distinctive groups.

CHARAKTERYSTYKA EKOLOGICZNA ANTROPOGENICZNYCH FITOCENOZ LEŚNYCH POD PRESJĄ MOGILNIKA PESTYCYDOWEGO

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S ł o w a k l u c z o w e: mogilnik pestycydowy, wskaźniki ekologiczne, antropogeniczne zbiorowiska leśne.

A b s t r a k t

W 2004 r. w na podstawie 16 zdjęć fitosocjologicznych badano za pomocą ekologicznych liczb wskaźnikowych warunki ekologiczne antropogenicznego lasu budowanego przez zbiorowiska świerkowe z udziałem *Sambucus racemosa* i *Sambucus nigra*. Stosunki jakościowe i ilościowe w badanych fitocenozach wskazują na neutralność wobec kontynentalizmu. Wskaźnik świetlny (L) różnicuje siedlisko w kierunku od wschodu na zachód. Wskaźnik temperaturowy sytuuje badany obszar wśród działu północnego na niżu Polski. Wskaźniki edaficzne potwierdzają badania fizykochemiczne gleb tego obszaru, wskazując na gleby świeże, piaszczysto-gliniaste. Wskaźniki wilgotności gleby (W) i wskaźnik dyspersji gleby (D) były najniższe w bezpośrednim sąsiedztwie mogilnika, co może świadczyć, że potencjalne odcieki mogą stanowić barierę dla migracji związków zdeponowanych w mogilniku. Jednak wysoki wskaźnik kwasowości (R) wskazuje na ograniczenie tej bariery wskutek aktywnej roli jonów wodorowych. Wskaźniki materii organicznej (H), zawartości azotu w glebie (N) i trofizmu gleby (Tr) przybierają wyższe wartości w bezpośrednim sąsiedztwie mogilnika, co może oznaczać zasilanie związkami biogennymi z obszaru mogilnika.

Uzyskane wartości ekologicznych liczb wskaźnikowych są do siebie zbliżone, nie świadczą o występowaniu istotnych różnic w warunkach ekologicznych między zbiorowiskiem z *Sambucus nigra-Picea abies* oraz zespołem *Sambuco racemosi-Pieceetum* na terenie badań. Jednocześnie uzyskane wyniki są zgodne z dostępnymi danymi literaturowymi na ich temat. Wskazuje to na konieczność dalszych badań; być może brak różnic został spowodowany oddziaływaniem mogilnika. Różnicę w składzie florystycznym i stosunki ilościowe między gatunkami budującymi badane fitocenozy wykazuje analiza numeryczne dzieląca wykonane zdjęcia fitosocjologiczne na dwie wyraźne grupy.

Introduction

Environmentally degraded areas have worsened as the result of human activity. Urban, industrial and transportation expansion is constantly extending over increasingly larger areas and causing degradation of the landscape and natural environment. This degradation results from pollutant emissions to the atmosphere, exploitation of natural resources, waste landfills, industrial investments, agrotechnical treatments and tourist activity.

The problem of waste pesticides in Poland has not been resolved. To date, pesticide wastes were mainly disposed of in landfills. Since the early 1970s, this waste has been stored in pesticide tombs. These are primitive landfill sites comprised of concrete rings placed underground with the bottom protected with a bituminous seal. The design of the tombs did not take into account the long-term effect of the external factors on concrete (often of low quality). Over time, the concrete corroded and started leaking. The stored waste contaminated ground water, soil, farm wells and even lakes and rivers. Pesticides are polytoxic and often seriously dangerous for the environment. Therefore, it is necessary to monitor potential changes caused by the effect of these landfills on the environment.

Numerous plant species are good indicators of habitat conditions. A 50-year study identified the ecological indices of vascular plants for different regions, including: Central Europe (ELLENBERG 1974, 1979, ELLENBERG et al. 1991), Great Britain (HILL et al. 1999), Switzerland (LANDOLT 1977), Poland (ZARZYCKI 1984, ZARZYCKI et al. 2002) and Norway (VELVE, AASE 1980). Numerous attempts to adapt the Ellenberg ecological indices to local conditions have been undertaken in Holland (TER BRAAK, GREMMEN 1987, ERTSEN et al. 1998), Sweden (DIEKMANN 1995, DIEKMANN, DUPRÉ 1997) and Estonia (PÄRLER et al. 1996).

The Ellenberg ecological indices were used to interpret the results of studies on flora systemization (PERSSON 1981) since they explain the plant response to environmental changes (PERSSON 1980), to evaluate the humus form or soil quality in forests (MÖLLER 1997), to estimate the probability of occurrence of species (DUPRÉ, DIEKMANN 1998), and to evaluate ecological risks (LATOUR et al. 1994). Numerous papers refer to the use of ecological indices to evaluate the effect of forest ecosystem on the neighboring habitats (FRAVER 1994, KINNIBURGH, TRAFFORD 1996) with particular attention to anthropogenic effect (KIVELL 1993, GOUDIE 1994, DIEKMANN, DUPRÉ 1997, SZIBALSKI, FELIX-HENNINGSEN 1999, GOLDEFROID, KOEDAM 2003) and microclimatic changes (GEHLHAUSEN et al. 2000). Good results can be obtained when using ecological indices supported by phytosociological research in the evaluation of climatic and edaphic conditions of habitats (GRZYBOWSKI et al. 2004, HILL et al. 2000,

ZARZYCKI 1984, ROO-ZIELIŃSKA, SOLON 1988, VAN DER MAAREL 1993, VELVE, AASE 1980). Indices of vascular plants are a good indicator used in many studies on flora and vegetation of different ecosystems.

The aim of this paper was to determine ecological conditions in the area of anthropogenic forest phytocenoses threatened by a pesticide tomb. Since the syntaxonomic position of anthropogenic forest communities represented by *Sambucus nigra* and *Sambucus racemosa* has not been completely established and has provoked much discussion (ENDLER 1987, ENDLER 1990, MATUSZKIEWICZ 2001, SOKOŁOWSKI 1980), this paper is a contribution to the establishment of the habitat conditions in which they occur.

Characteristics of the study area

The forest area under study is situated within the Warlity Fishing Farm, approx. 800 m from the nearby village of Warlity Wielkie (Figure 1). The area covers 6.5 ha. The habitat type is young, mixed coniferous forest. The former farmland was artificially planted with trees. On the eastern side, the forest is adjoined by a hill, on top of which, between 1968-2004 a pesticide tomb was utilized. The tomb covered an area of 0.7 ha and comprised 9 wells of which 7.5 wells were filled. Approximately 50 tons of pesticides, packages and chemical reagents were buried at this site. The pesticide tomb under study was situated in sandy formations and even at slight leakages, the tomb is a threat to contamination of ground water and the neighboring ecosystems, including the forest (Figure 1).

Near the hill is a cavity from which sand was excavated in the past. Approximately 5 m from the former pesticide site is a steep slope on which one can see animal bones remaining on the site of a former fox farm. The western edge of the forest borders three fish ponds and beyond that, Lake Szeląg Wielki (599 ha, h_{max} = 35.5 m) – the largest lake in the basin of the Drwęca Warmińska River (GRZYBOWSKI et al. 2004). Along the north-western edge of the forest runs a drainage canal for draining the pond water. In the north-western part of the forest is a bog covering an area of 0.23 ha. In the northern part there is another 0.08 ha bog. South of the forest, the terrain suddenly lowers. The presence of species representing the class of *Phragmitetea* is dominated by common reed (*Phragmites australis*) which indicates a final succession phase after a former water body.

Based on the geobotanical map of Poland, the area under study is part of the Elbląg – Ostróda District. This district and the Districts of Kartuzy and Wałcz – Drawsko situated on the banks of the Vistula River form the Pomerania Lake District, belonging to the Baltic Division and to the Subdivi-

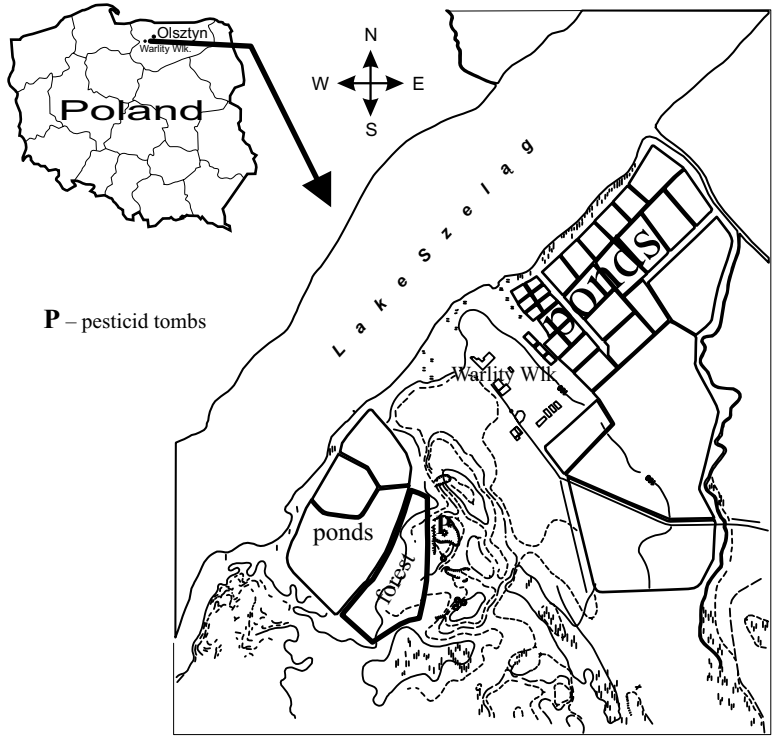

Fig. 1. Location of the study area

sion of the strip of Pomeranian Planes and Pomeranian Uplands (SZAFER, ZARZYCKI 1977). The mixed coniferous forests in this region have a complex character because they combine features of sub-Atlantic communities of acidophilus oaks from the association of *Quercion roboris – petraea* and features of subcontinental communities of mixed coniferous forests of *Pino – Quercetum*, always with considerable representation of species typical of bright oak forests and dry ground forests. It is likely that the naturally unclear geographical picture of these communities was changed by the intense forest management in this area.

According to the physico-geographical regionalization map of Poland, the forest in Warlity Wielkie is part of the Olsztyn Lake District, which forms the western part of the Mazurian Lake District. The latter is a macroregion of the Eastern European area, the province of the Western Russian Lowland and a subprovince of the Eastern Baltic Lake Districts.

Methods

Field studies were carried out in 2003-2004 on 16 phytosociological releves sing the Braun-Blanquet method (BRAUN-BLANQUET 1964). The relevés covered an area of 400-600 m^2. The nomenclature of vascular plants was adopted according RUTKOWSKI (2004). The names of bryophyte species were given according to *Die Moos – und Farnpflanzen Europas* (FREY, FRAHM 1995).

This paper used the division of spruce forests proposed by SOKOŁOWSKI (1980) and ENDLER (1990). The remaining syntactic units were adopted according to the common nomenclature of MATUSZKIEWICZ (2001).

In this paper, a computerized elaboration of phytosociological data was used. It comprised the following phases:

1. Development of a database with Turboveg software (HENNEKENS 1995). The version of Turboveg for Windows 1.99 q. was used.

2. Export of phytosociological data from TURBOVEG to NCLAS software included in the software package of Syn-Tax 5.0 to analyze the internal differentiation at the level of communities. Use was made of the *Complete Link* method belonging to the hierarchic agglomeration methods group. To calculate the matrix of distances between sites, an Euclidian measure was used (PODANI 1993). They were also used to establish the sequence of the phytosociological releves in tables.

To calculate the quantitative degrees on the Braun-Blanquet scale to the average percentage of the particular species, the modified average degrees of coverage were used (BRAUN-BLANQUET 1964).

The releves provided material for further studies on the basis of index numbers characterizing edaphic conditions: humidity (W), trophism (Tr), acidity (R), mechanical soil content (D), organic matter content (H), nitrophylity (N), and climatic indicators: light (L), thermal (T) continental (K), were also taken under consideration (ELLENBERG 1974, ZARZYCKI 1984, ZARZYCKI et al. 2002).

The results were set in a table in Table 1. For each of the observed species, a corresponding indicatory value was given. The average values of coefficients for a particular surface were calculated according to the following formula:

$$X = \frac{\Sigma X_n \cdot i_n}{\Sigma i_n},$$

where:

X – stands for coefficients W, Tr, R, D, H, N, F, L, T, K respectively, for the entire research surface;

X_n – assumes values of coefficients W, Tr, R, D, H, N, F, L, T, K individually for each taxon n;

i – the quantity of each species on the studied surface ac-cording to an average percentage of species.

Furthermore, average values for the whole research area were calculated (Tables 2 and 3).

Table 1

Ecological indices of the forest phytocenoses under study

Number of releve	L	T	K	W	Tr	R	D	H	N
1	3.69	3.19	2.99	3.29	3.58	3.69	3.9	3.1	6.56
2	3.8	3.15	3.00	3.33	3.35	3.38	3.86	3.11	6.27
3	3.92	3.17	2.99	3.30	3.63	3.64	3.79	3.11	6.81
4	3.77	3.37	2.96	3.25	3.70	3.72	3.90	3.09	7.12
5	3.4	3.09	2.99	3.23	3.50	3.54	3.64	3.16	7.21
6	3.57	3.10	2.99	3.34	3.48	3.48	3.76	3.20	7.13
7	3.97	3.33	2.99	3.35	3.59	3.72	3.87	3.07	6.25
8	3.64	3.20	2.99	3.25	3.49	3.53	3.86	3.15	6.25
9	3.96	3.09	2.97	3.45	3.27	3.25	3.72	3.21	6.63
10	3.92	3.97	2.99	3.36	3.37	3.42	3.88	3.14	5.76
11	3.79	3.35	3.00	3.46	3.35	3.43	3.97	3.20	5.7
12	4.04	3.30	3.00	3.16	3.62	3.58	3.83	3.11	7.47
13	4.06	3.14	2.99	3.22	3.58	3.47	3.75	3.14	7.19
14	3.57	3.38	2.99	3.15	3.82	3.82	3.88	3.09	7.07
15	3.86	3.33	3.00	3.32	3.53	3.57	3.71	3.07	6.6
16	3.63	3.29	2.99	3.30	3.85	3.71	3.82	3.13	7.41
Average	**3.79**	**3.28**	**2.99**	**3.30**	**3.54**	**3.56**	**3.82**	**3.13**	**6.71**

Indices L, T, K, W, Tr, R, D, H – acc. ZARZYCKI (1984, 2001)
Index N acc. ELLENBERG (1974)

Sambucus nigra-Picea abies

Sambuco racemosi-Piceetum Jut.-Trzeb. 1980

Table 2

Association *Sambuco racemosi-Piceetum* Jut.-Trzeb. 1980

Succesive number		1	2	3	4	5	6	7	8	9	10	11	12	
Number of releve		1	7	11	2	3	9	10	4	5	6	8	16	
Date		06. 2003	06. 2003	06. 2004	06. 2003	06. 2003	06. 2004	06. 2004	06. 2003	06. 2003	06. 2003	06. 2004	06. 2004	
Area of releve (m^2)		200	600	200	200	300	400	400	300	200	200	300	500	
Cover of plants layer (%) a		100	100	100	100	100	100	100	100	100	100	100	100	constancy
b		70	60	60	70	70	60	40	60	60	50	60	40	
c		90	90	80	90	90	80	80	90	80	90	90	80	
d		30	30	30	20	30	40	50	30	20	40	40	40	
Total number of species in 1 releve		73	80	42	67	75	40	39	67	52	61	59	39	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ch. *Sambuco racemosi-Piceetum*														
Sambucus racemosa	b	3	1	1	3	3	2	2	2	4	3	2	2	V
Ch. *Vaccinio-Piceion*														
Picea abies	a	4	4	3	5	5	5	5	5	5	5	5	5	V
Picea abies	c	·	+	1	+	·	2	1	·	1	+	+	+	IV
Mycelis muralis	c	1	·	·	+	+	·	1	2	2	1	1	3	IV
Plagiomnium affine	d	2	+	·	1	1	1	+	1	+	·	1	·	IV
Ch. *Vaccinio-Picetea*														
Sorbus aucuparia	b	+	+	1	2	+	1	2	·	1	1	1	·	V
Pleurozium schreberi	d	2	3	·	3	2	2	2	·	2	3	3	·	IV
Calamagrostis arundinacea	c	·	·	3	2	·	1	·	1	1	1	2	·	III
Betula pendula	a	·	·	4	·	+	1	·	·	+	·	·	·	II
Betula pendula	b	·	1	·	·	·	1	1	·	·	·	1	·	II
Betula pendula	c	·	·	·	·	·	1	1	·	·	·	·	·	I
Betula pubescens	a	·	·	+	·	·	·	·	·	·	·	·	·	I
Betula pubescens	c	+	·	·	·	1	·	·	·	1	1	·	·	II
Populus tremula	a	·	·	·	·	+	·	·	·	·	·	·	·	I
Populus tremula	b	+	·	·	·	·	·	·	·	·	·	·	·	I
Populus tremula	c	·	·	1	·	·	·	·	·	·	·	·	·	I
Trientalis europaea	c	·	·	·	+	+	·	·	·	·	·	·	·	I
Hylocomium splendens	d	·	+	·	·	·	·	·	·	·	·	·	·	I

cont. Table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ch. *Dicrano-Pinion*														
Pinus sylvestris	a	·	·	·	2	2	·	·	·	·	+	·	·	II
Dicranum polysetum	d	·	·	·	·	·	·	·	·	·	2	·	·	I
Ch. *Pino-Quercion*														
Veronica officinalis	c	1	1	+	+	+	+	+	·	1	1	1	·	V
Polytrichum formosum	d	1	·	·	·	·	1	·	·	·	·	2	·	II
Hieracium vulgatum	c	·	·	·	+	·	·	·	·	·	·	·	·	I
Ch. *Alnetea Glutinosae*														
Solanum dulcamara	c	+	·	·	·	+	·	·	·	+	+	·	·	II
Alnus glutinosa	a	·	·	1	·	·	·	·	·	·	·	·	·	I
Salix cinerea	b	·	·	2	·	·	·	·	·	·	·	·	·	I
Ribes nigrum	b	·	1	1	·	·	·	·	·	·	·	·	·	I
Ch. *Tilio-Piceion*														
Dryopteris filix-mas	c	1	1	1	1	·	·	1	1	1	·	1	1	IV
Milium effusum	c	·	·	·	1	·	·	·	1	·	1	·	2	II
Aegopodium podagraria	c	·	·	·	·	·	·	·	2	·	·	·	1	I
Ch. *Querco-Fagetea*														
Acer platanoides	a	·	·	·	·	·	·	5	·	·	·	·	·	I
Acer platanoides	b	·	·	·	·	·	·	2	·	·	1	2	·	II
Acer platanoides	c	+	+	+	1	+	1	1	2	·	1	2	·	V
Tilia cordata	b	·	+	·	·	·	·	·	3	·	·	1	·	II
Tilia cordata	c	·	·	·	·	·	·	·	·	·	·	1	·	I
Corylus avellana	b	·	·	·	·	+	·	·	3	·	·	2	3	II
Corylus avellana	c	+	·	·	·	·	·	·	2	·	·	·	·	I
Fagus sylvatica	b	·	+	·	·	·	1	1	2	+	+	1	·	III
Fagus sylvatica	c	1	+	·	·	+	1	·	1	1	·	+	·	III
Ranunculus ficaria	c	1	1	1	1	1	1	1	1	+	+	+	1	V
Epilobium montanum	c	1	·	+	1	1	+	+	·	+	+	·	·	IV
Poa nemoralis	c	2	2	·	1	1	·	·	1	1	·	1	·	III
Moehringia trinervia	c	1	1	·	·	·	·	·	·	2	·	1	·	II
Ulmus glabra	a	·	·	·	·	·	·	·	·	·	·	2	2	I
Ulmus glabra	b	·	·	·	·	·	·	·	·	·	·	·	1	I
Viburnum opulus	c	·	·	·	·	·	·	·	·	·	·	+	·	I
Scrophularia nodosa	c	·	·	·	·	+	·	·	·	·	·	1	·	I

cont. Table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acer pseudoplatanus	c	·	1	·	·	·	·	·	·	·	·	·	·	I
Euonymus europaeus	b	·	·	·	·	·	·	·	·	·	·	·	1	I
Circaea alpina	c	·	·	·	+	·	·	·	·	·	·	·	·	I
Accompanying species														
Sambucus nigra	b	1	+	+	1	1	2	+	1	1	1	+	+	V
Rubus idaeus	b	3	3	3	3	3	3	·	2	1	3	3	2	V
Pteridium aquilinum	c	1	1	2	1	1	·	2	+	+	1	1	1	V
Quercus robur	a	·	·	1	·	·	·	·	·	·	·	·	·	I
Quercus robur	b	·	·	1	·	·	+	·	·	·	·	·	·	I
Quercus robur	c	+	+	·	+	+	2	1	1	·	+	1	·	IV
Stellaria media	c	3	2	·	·	3	1	2	2	2	1	3	2	V
Galium aparine	c	1	3	2	+	1	1	2	4	3	3	2	3	V
Dactylis glomerata	c	1	1	+	+	1	·	+	+	+	+	1	·	V
Holcus mollis	c	2	2	2	1	1	2	2	·	1	1	1	·	V
Saponaria officinalis	c	+	+	·	+	+	+	·	+	+	+	+	+	V
Frangula alnus	c	·	1	1	1	+	2	2	1	1	1	1	·	V
Fragaria vesca	c	1	2	1	1	1	1	·	1	2	3	3	+	V
Chelidonium majus	c	·	+	·	1	4	·	·	2	+	1	+	3	IV
Poa trivialis	c	2	2	·	1	1	2	2	1	1	·	·	·	IV
Geum urbanum	c	2	+	·	·	1	+	1	2	·	+	1	2	IV
Arrhenatherum elatius	c	2	2	2	·	1	2	·	·	2	2	1	·	IV
Artemisia vulgaris	c	1	1	+	+	1	·	·	·	+	+	+	+	IV
Athyrium filix-femina	c	+	+	·	+	·	·	·	+	+	+	+	+	IV
Equisetum pratense	c	+	+	·	+	+	+	1	+	·	+	+	·	IV
Cardamine pratensis	c	+	+	+	+	+	+	·	·	+	+	+	·	IV
Berula erecta	c	+	+	·	+	+	+	·	·	+	+	+	·	IV
Urtica dioica	c	1	2	·	1	2	·	·	3	3	3	3	3	IV
Frangula alnus	b	+	+	2	·	+	·	·	·	+	1	2	2	IV
Veronica chamaedrys	c	+	1	2	+	+	·	·	+	1	1	2	·	IV
Oxalis acetosella	c	·	+	·	1	·	1	·	·	3	3	2	·	III
Epilobium angustifolium	c	·	·	2	·	·	1	+	+	1	1	·	·	III
Galeopsis pubescens	c	1	·	1	4	3	·	2	·	·	·	·	·	III
Anthriscus sylvestris	c	·	1	·	·	1	·	·	1	1	1	·	2	III

cont. Table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Avenula pubescens	c	2	·	·	·	1	·	·	1	1	·	·	2	III
Cirsium vulgare	c	·	·	·	+	+	·	·	+	1	·	1	·	III
Glechoma hederacea	c	1	1	·	·+	·	+	+	·	·	·	+	·	III
Filipendula ulmaria	b	1	·	1	1	1	·	1	·	+	1	1	·	III
Silene latifolia	c	1	1	1	1	+	·	·	+	·	+	·	·	III
Myosotis sylvatica	c	1	+	·	·	·	·	1	+	+	+	·	+	III
Alliaria petiolata	c	+	·	·	·	+	·	·	+	·	·	+	2	III
Medicago lupulina	c	+	+	·	·	+	·	·	·	+	+	·	·	III
Calamagrostis stricta	c	·	2	·	1	1	·	·	·	·	1	·	·	II
Galeopsis tetrahit	c	·	·	2	2	·	·	2	4	·	·	·	·	II
Carex hirta	c	2	2	2	·	·	·	·	·	·	·	·	·	II
Poa palustris	c	2	2	·	·	1	·	·	·	·	·	·	·	II
Achillea millefolium	c	1	1	·	·	·	·	·	1	·	1	·	·	II
Senecio sylvaticus	c	+	1	·	1	·	·	·	·	·	·	·	·	II
Anthoxanthum odoratum	c	2	2	·	·	1	·	·	·	·	·	1	·	II
Poa pratensis	c	·	2	·	·	·	2	·	1	·	·	·	2	II
Convolvulus arvensis	c	·	+	·	·	+	·	·	·	·	·	·	+	II
Viola arvensis	c	+	·	·	+	+	·	·	·	·	·	·	·	II
Geranium robertianum	c	·	·	·	·	1	·	·	·	2	1	·	·	II
Agrostis gigantea	c	·	·	·	·	1	·	·	·	·	1	1	1	II
Rumex acetosella	c	·	·	·	+	+	·	·	·	·	+	·	·	II
Plantago major	c	·	·	3	·	·	+	3	·	·	·	·	1	II
Eupatorium cannabinum	c	1	·	·	1	·	·	·	·	1	1	·	·	II
Circaea lutetiana	c	·	·	·	·	+	·	·	·	+	+	·	·	II
Rumex sanguineus	c	·	·	·	+	+	·	·	·	+	·	·	+	II
Crataegus monogyna	b	·	+	·	·	+	·	·	·	·	+	·	·	II
Crataegus monogyna	c	1	+	·	·	·	·	·	1	·	·	·	·	II
Rosa canina	b	·	3	·	·	+	·	·	·	·	·	2	·	II
Myosotis arvensis	c	+	·	·	·	·	·	·	+	·	·	+	·	II
Phalaris arundinacea	c	·	·	2	1	·	2	·	·	·	·	·	·	II
Cirsium arvense	c	·	·	·	+	·	·	·	+	·	·	1	·	II
Agrimonia eupatoria	c	·	1	·	·	·	·	·	+	·	+	·	·	II
Potentilla argentea	c	·	1	·	·	·	·	·	·	·	+	+	·	II

cont. Table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Knautia arvensis	c	·	+	·	·	·	·	·	+	·	+	1	·	II
Ajuga reptans	c	+	·	·	1	·	·	·	+	·	·	·	·	II
Avena fatua	c	·	2	2	·	·	·	·	·	·	·	·	·	I
Festuca pratensis	c	·	2	·	1	·	·	·	·	·	·	·	·	I
Festuca gigantea	c	·	·	·	1	·	·	·	·	·	1	·	·	I
Ranunculus repens	c	1	1	·	·	·	·	·	·	·	·	·	·	I
Matricaria indora	c	1	1	·	·	·	·	·	·	·	·	·	·	I
Deschampsia caespitosa	c	·	·	·	·	1	·	·	1	·	·	·	·	I
Trifolium arvense	c	·	·	·	·	1	·	·	1	·	·	·	·	I
Campanula rotundifolia	c	+	1	·	·	·	·	·	·	·	·	·	·	I
Hypericum perforatum	c	·	1	·	·	·	·	·	·	·	+	·	·	I
Vicia cracca	c	·	+	·	·	·	·	1	·	·	·	·	·	I
Galium mollugo	c	·	·	·	·	1	·	·	+	·	·	·	·	I
Cirsium oleraceum	c	·	·	·	·	+	·	·	·	·	·	1	·	I
Valeriana officinalis	c	+	+	·	·	·	·	·	·	·	·	·	·	I
Trifolium pratense	c	+	+	·	·	·	·	·	·	·	·	·	·	I
Trifolium repens	c	+	+	·	·	·	·	·	·	·	·	·	·	I
Chenopodium polyspermum	c	+	·	·	+	·	·	·	·	·	·	·	·	I
Hieracium pilosella	c	·	+	·	+	·	·	·	·	·	·	·	·	I
Lapsana communis	c	·	·	·	·	+	·	·	+	·				I
Arctium tomentosum	c	·	·	·	·	+	·	·	·	+	·	·	·	I
Syringa vulgaris	b	·	·	·	·	·	·	·	3	·	·	·	·	I
Elymus repens	c	·	2	·	·	·	·	·	·	·	·	·	·	I
Trisetum flavescens	c	·	2	·	·	·	·	·	·	·	·	·	·	I
Alopecurus pratensis	c	·	2	·	·	·	·	·	·	·	·	·	·	I
Lolium multiflorum	c	·	·	2	·	·	·	·	·	·	·	·	·	I
Syringa vulgaris	c	·	·	·	·	·	·	·	2	·	·	·	·	I
Bromus hordaceus ssp. *hordaceus*	c	·	·	·	·	·	·	·	2	·	·	·	·	I
Ulmus minor	a	·	·	·	·	·	·	·	·	·	·	·	2	I
Ulmus minor	b	·	·	·	·	·	·	·	·	·	·	·	1	I
Hieracium murorum	c	·	·	1	·	·	·	·	·	·	·	·	·	I
Galeopsis bifida	c	·	·	·	1	·	·	·	·	·	·	·	·	I
Sisymbrium altissimum	c	·	·	·	1	·	·	·	·	·	·	·	·	I

cont. Table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Festuca ovina	c	·	·	·	1	·	·	·	·	·	·	·	·	I
Agrostis stolonifera	c	·	·	·	·	1	·	·	·	·	·	·	·	I
Vicia hirsuta	c	·	·	·	·	·	·	1	·	·	·	·	·	I
Lamium album	c	·	·	·	·	·	·	·	1	·	·	·	·	I
Galeopsis speciosa	c	·	·	·	·	·	·	·	·	1	·	·	·	I
Heracleum sphondylium ssp. *sphondylium*	c	·	·	·	·	·	·	·	·	·	·	·	1	I
Crataegus laevigata	b	+	·	·	·	·	·	·	·	·	·	·	·	I
Chenopodium album	c	+	·	·	·	·	·	·	·	·	·	·	·	I
Ranunculus acris	c	+	·	·	·	·	·	·	·	·	·	·	·	I
Vicia tetrasperma	c	+	·	·	·	·	·	·	·	·	·	·	·	I
Lathyrus pratensis	c	·	+	·	·	·	·	·	·	·	·	·	·	I
Plantago lanceolata	c	·	+	·	·	·	·	·	·	·	·	·	·	I
Coronilla varia	c	·	+	·	·	·	·	·	·	·	·	·	·	I
Juncus effusus	c	·	·	·	+	·	·	·	·	·	·	·	·	I
Hypericum maculatum	c	·	·	·	+	·	·	·	·	·	·	·	·	I
Vicia grandiflora	c	·	·	·	+	·	·	·	·	·	·	·	·	I
Succisella inflexa	c	·	·	·	·	+	·	·	·	·	·	·	·	I
Sium latifolium	c	·	·	·	·	+	·	·	·	·	·	·	·	I
Solidago graminifolia	c	·	·	·	·	+	·	·	·	·	·	·	·	I
Linaria vulgaris	c	·	·	·	·	·	+	·	·	·	·	·	·	I
Rhamnus catharticus	b	·	·	·	·	·	·	·	+	·	·	·	·	I
Rumex acetosa	c	·	·	·	·		·	·	+	·	·	·	·	I
Euphorbia cyparissias	c	·	·	·	·	·	·	·	+	·	·	·	·	I
Verbascum thapsus	c	·	·	·	·	·	·	·	+	·	·	·	·	I
Atriplex patula	c	·	·	·	·	·	·	·	+	·	·	·	·	I
Conyza canadensis	c	·	·	·	·	·	·	·	+	·	·	·	·	I
Plantago major ssp· intermedia	c	·	·	·	·	·	·	·	+	·	·	·	·	I
Polygonum minus	c	·	·	·	·	·	·	·	+	·	·	·	·	I
Scabiosa canescens	c	·	·	·	·	·	·	·	+	·	·	·	·	I
Bryophytina														
Brachythecium rutabulum	d	1	·	2	2	1	2	+	2	2	·	2	1	V
Rhytidiadelphus squarrosus	d	2	2	1	1	+	1	2	·	·	·	1	·	IV

cont. Table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Brachythecium oedipodium	d	·	·	·	·	2	·	3	·	·	+	·	·	II
Scleropodium purum	d	·	1	·	·	2	·	·	·	·	+	·	·	II
Eurhynchium hians	d	·	·	·	·	·	·	·	1	·	·	·	2	II
Plagiomnium undulatum	d	+	·	·	·	·	·	·	1	+	1	·	·	II
Plagiothecium denticulatum	d	1	·	+	·	·	+	·	·	·	·	·	·	II
Brachythecium starkei	d	·	·	·	+	·	·	·	·	·	1	·	·	II
Plagiomnium cuspidatum	d	·	·	·	·	·	·	·	·	·	·	·	1	I
Cirriphyllum piliferum	d	·	·	·	·	·	·	+	·	·	·	+	·	I
Mnium stellare	d	·	·	·	·	·	·	+	·	·	·	·	·	I
Eurhynchium angustirete	d	·	·	·	+	·	·	·	·	·	·	·	·	I
Ditrichum pusillum	d	·	·	·	·	·	+	·	·	·	·	·	·	I

Table 3

Community *Sambucus nigra-Picea abies*

Succesive number		1	2	3	4
Number of releve		12	13	14	15
Date		06. 2003	06. 2003	06. 2004	06. 2003
Area of releve (m^2)		200	600	400	400
Cover of plants layer (%) a		100	100	100	100
b		80	60	80	80
c		60	60	60	60
d		20	20	10	20
Total number of species in 1 releve		38	27	46	53
1	2	3	4	5	6
Ch. *Sambucus nigra-Picea abies*					
Picea abies	a	5	5	3	5
Picea abies	c	·	·	+	+
Sambucus nigra	b	3	4	3	1
Ch. *Myceli-Piceion*					
Mycelis muralis	c	1	·	2	1
Plagiomnium affine	d	2	1	1	·
Ch. *Vaccinio-Piceetea*					
Sorbus aucuparia	b	·	·	1	+
Calamagrostis arundinacea	c	·	·	·	1
Betula pendula	a	·	·	·	1
Ch. *Tilio-Piceion*					
Aegopodium podagraria	c	3	2	2	4
Dryopteris filix-mas	c	·	·	1	1
Milium effusum	c	·	·	1	·
Ch. *Querco-Fagetea*					
Acer platanoides	a	1	·	3	2
Acer platanoides	b	·	·	2	·
Acer platanoides	c	1	·	1	1
Corylus avellana	b	2	1	2	2
Corylus avellana	c	·	·	1	1
Tilia cordata	a	·	·	1	·
Fagus sylvatica	c	·	·	+	+
Poa nemoralis	c	1	2	·	1
Epilobium montanum	c	+	·	·	1
Ranunculus ficaria	c	·	·	·	+
Scrophularia nodosa	c	·	·	+	·
Moehriniga trinervia	c	·	·	+	·
Accompanying species					
Sambucus racemosa	b	1	1	1	+
Rubus idaeus	b	2	·	2	2
Frangula alnus	b	·	·	+	2
Frangula alnus	c	·	1	2	+
Quercus robur	a	·	·	2	2
Quercus obur	b	·	·	1	·
Quercus robur	c	1	·	+	1
Chelidonium majus	c	2	+	2	+
Equisetum pratense	c	+	1	1	1
Veronica chamaedrys	c	·	1	3	1

cont. Table 3

1	2	3	4	5	6
Galeopsis tetrahit	c	2	·	2	2
Galium aparine	c	2	·	2	2
Poa pratensis	c	1	1	·	1
Geum urbanum	c	1	·	3	2
Anthriscus sylvestris	c	·	+	2	1
Arrhenatherum elatius	c	·	2	1	2
Avenula pubescens	c	1	·	1	2
Saponaria officinalis	c	+	+	+	·
Convolvulus arvensis	c	+	+	+	·
Lapsana communis	c	1	·	+	1
Melilotus officinalis	c	1	·	+	1
Plantago major	c	3	3	·	·
Urtica dioica	c	·	·	3	2
Stellaria media	c	·	·	2	2
Pteridium aquilinum	c	·	·	2	1
Fragaria vesca	c	1	·	2	·
Holcus mollis	c	1	·	·	2
Agrostis gigantea	c	·	1	·	1
Poa trivialis	c	1	1	·	·
Calamagrostis stricta	c	1	·	·	1
Dactylis glomerata	c	·	·	1	1
Galeopsis bifida	c	·	·	1	+
Leonurus cardiaca	c	+	+	·	·
Artemisia vulgaris	c	+	·	·	+
Myosotis sylvatica	c	·	·	+	+
Filipendula ulmaria	c	·	·	+	+
Maianthemum bifolium	c	+	+	·	·
Cirsium vulgare	c	·	·	2	·
Geranium robertianum	c	·	·	2	·
Verbascum nigrum	c	·	·	·	2
Silene latifolia	c	1	·	·	·
Festuca arundinacea	c	1	·	·	·
Cirsium arvense	c	1	·	·	·
Galeopsis speciosa	c	·	1	·	·
Vicia sativa	c	·	1	·	·
Valeriana officinalis	c	·	·	1	·
Verbascum thapsus	c	·	·	·	1
Alliaria petiolata	c	·	·	·	1
Rumex crispus	c	·	·	·	1
Agrimonia eupatoria	c	·	·	·	1
Conyza canadensis	c	·	·	·	1
Rumex acetosa	c	·	+	·	·
Rumex sanguineus	c	·	+	·	·
Ranunculus acris	c	·	·	+	·
Hypericum perforatum	c	·	·	+	·
Levisticum officinale	c	·	·	+	·
Epilobium angustifolium	c	·	·	·	+
Senecio sylvaticus	c	·	·	·	+
Euphorbia cyparissias	c	·	·	·	+
Bryophytina					
Brachythecium rutabulum	d	2	2	2	+
Eurhynchium hians	d	·	·	·	3

cont. Table 3

1	2	3	4	5	6
Plagiomnium undulatum	d	1	·	·	·
Eurhynchium praelongum	d	·	1	·	·
Plagiothecium succulentum	d	·	·	·	1
Eurhynchium striatum	d	+	·	·	·
Eurhynchium schleicheri	d	+	·	·	·

Results and Discussion

Within the area under study, 161 species of vascular plants and 24 species of bryophytes were identified. The presence of 159 species of vascular plants representing 49 families and 23 bryophyte species from 8 families was identified. Among the vascular plants, three species were protected (*Ribes nigrum*, *Frangula alnus*, *Viburnum opulus*), while among the byrophyte species, five were subject to protection (*Eurhynchium angustirete*, *Eurhynchium striatum*, *Pleurozium schreberi*, *Hylocomium splendens*, *Rhytidiadelphus squarrosus*). The following families were most commonly represented: *Poaceae*, *Asteraceae*, *Fabaceae*, *Rosaceae* and *Lamiaceae*. An increased percentage of the meadow species is a common phenomenon in the early developmental phases of substitute forest phytocenoses.

The studied forest was partitioned into two phytosociological syntaxonomic units: an association with *Sambucus nigra-Picea abies* and a community *Sambuco racemosi-Pieceetum* Jut.-Trzeb. 1980 (Tables 2 and 3). Both communities have an anthropogenic character. A considerable part of the species which build the identified phytocenoses are anthropophytes growing in the vicinity of human settlements and sites such as waste landfills, along fences and roads, etc.

Species associated with *Sambucus nigra-Picea abies* often grow on fertile, humic, humid and moderately insolated habitats (ENDLER 1987). The soils they grow in are artificially processed and have different degrees of soil process formation or have a changed profile like the habitat under study (ZMYSŁOWSKA et al. 2004).

Sambuco racemosi-Pieceetum most often grows along forest roads and logged areas (SOKOŁOWSKI 1980). In addition, within the studied area they occurred near a forest road connecting the tomb to the ponds (Figure 2). Their soil and humidity requirements are moderate and they often grow on young, fertile and loamy soils (SZAFER, ZARZYCKI 1977).

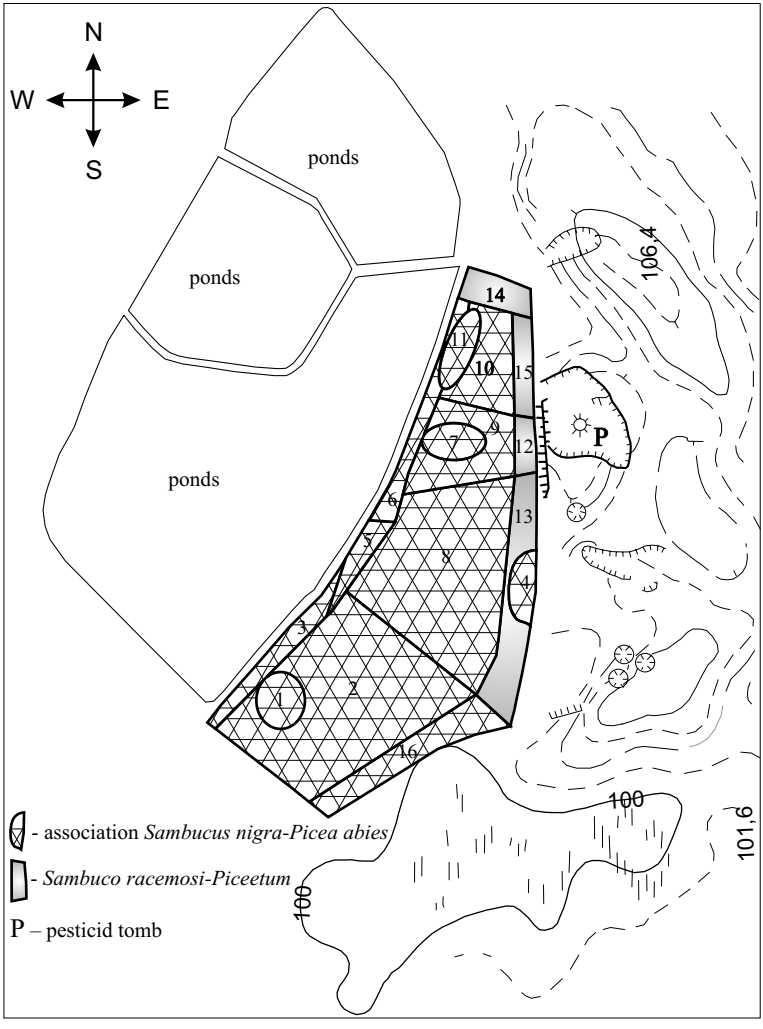

Fig. 2. Location of the phytosociological relevès within the study area in the vicinity of the pesticide tomb

The spatial structure of the communities under study indicates a clear functional difference between the external strip (comprised of *Sambuco racemosi-Pieceetum*), which plays the role of a buffer zone between the tomb area and the internal zone (including the area adjacent to the ponds) which is comprised of the association of *Sambucus nigra-Picea abies*.

Within the area of the forest under study, a climatic effect was not observed. The average values of the continentalism indicator (K) – Table 1 and Figure 3 indicate the presence of species neutral to continentalism throughout

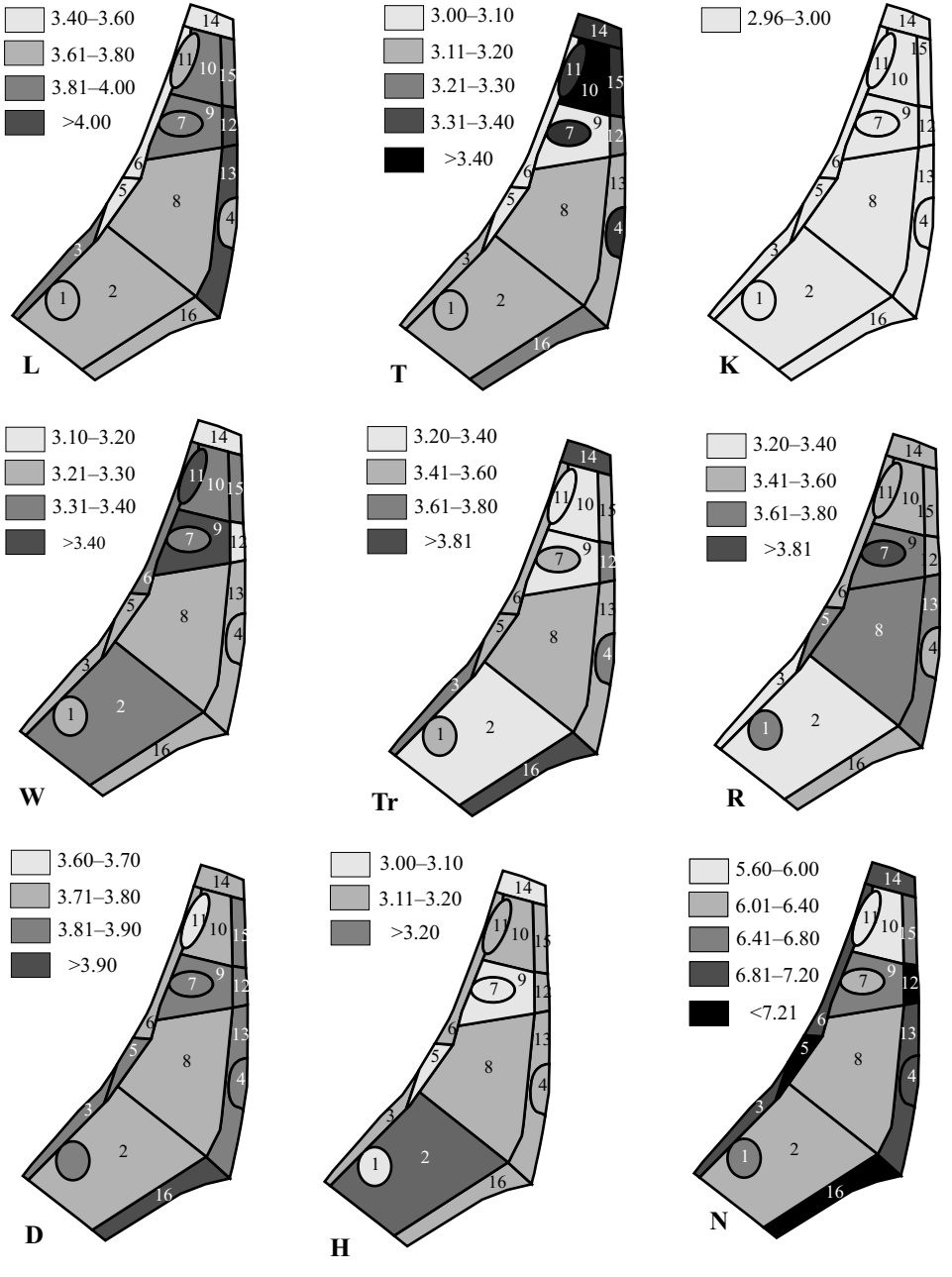

Fig. 3. Ecologic indices within the studied forest areas near the pesticide tomb

the entire area under study. The reference data indicates that both distinguished syntaxonomic units have a boreal character (ENDLER 1987, SOKOŁOWSKI 1980) occurring within the limits of spruce. The thermal indicator T ranges from 3.09 to 3.38 (Table 1) and indicates moderately cool climatic conditions typical of the northern lowland. Only one area (Relevé 10 in Table 2 and Figure 2), due to its numerous thermophilous species, is associated with a warm climate, typical of the southern lowland.

The light values (L), Figure 3, change from east to west from semi-shade in the western part (Relevé 5, 6, 11, 14 in Table 2) to semi-insolation in the eastern part of the forest (Relevé 13, 12, 15 in Table 2). This is typical and agrees with literature data indicating the greater insolation demands of *Sabuco racemosi-Picetum* than of *Sambucus nigra-Picea abies* (ENDLER 1987).

Moisture relations affect the productivity of forest habitats and the distribution of soil compounds. The humidity indicator (W) calculated for particular forest complexes indicates the presence of young soil. The lowest values of the W indicator were recorded in the direct vicinity of the pesticide tomb (Figure 3), which can constitute a barrier to permeation and diffusion of chemical compounds from potential reflux from the pesticide tomb into the soil within the studied area. This is also confirmed by a soil dispersion indicator (D), whose values are greater in the eastern part of the studied area. This indicates the presence of sandy-loamy soils which become loamy-sandy with low water capacity.

The differences between the values of organic matter content (H) are small. The H index indicates the presence of mineral-humus soils, which become increasingly fertile towards the south (Relevé 2 in Table 2 and Figure 3). An increase occurs where the forest has direct contact with the tomb and leads up to the center of the studied forest. The values of the nitrogen content index N and the trophism index Tr (Table 2) exhibit a similar pattern. Both indices had the highest values in the vicinity of the tomb. This can indicate the immediate effect of biogenic compounds from the tomb area, which also deposits organic matter in the waste landfill remaining from the former fox farm. The obtained results are confirmed by the soil analyses (ZMYSŁOWSKA et al. 2004).

The acidity index R indicates acid soils of pH 4.5 – 5.5 (Table 2), which also agrees with the soil analyses (ZMYSŁOWSKA et al. 2004).

Recapitulation and Conclusion

The results of the evaluation of the climatic conditions of the phytocenoses understudy indicate their neutral character to continentalism. Next to the

oceanic character species (e.g. *Fagus sylvatica*, *Mycelis muralis*, *Ajuga reptans*, *Holcus mollis*) the presence of subcontinental boreal species (e.g. *Picea abies*, *Pinus sylvestris*, *Equisetum pratense*, *Elymus repens*, *Trientalis europaea*, *Brachythecium oedipodium*, *Rhytidiadelphus squarrosus*, *Dicranum polysetum*, *Hylocomium splendens*) was recorded. The mean K index indicates a transient climate characteristic of north-eastern Poland. The light level index (L) indicates good light conditions in the habitat under study. Moreover, this index differentiates the habitat from East to West. The temperature index classifies the area under study in the northern division of the Polish lowland. The mean thermal indices (T) confirm the moderately cool climatic conditions typical of a northern lowland.

The evaluation of edaphic conditions confirmed the physico-chemical analyses of soils within the area under study and indicated fresh and sand-loamy soils. The soil humidity (W) and dispersion (D) had the lowest values in the direct vicinity of the tomb, which may be a barrier for the migration of compounds deposited in the tomb with potential reflux. However, a high acidity index (R) indicates the inhibition of this barrier by the active role of hydrogen ions, which favor the migration of numerous compounds.

The indices of organic matter (H), nitrogen content in the soil (N) and soil trophism (Tr) have higher values in direct vicinity of the tomb. This indicates the permeation of biogenic compounds from the tomb area.

The obtained values of the ecological indices are similar for the distinguished phytocenoses and agree with the data available in references, however, they indicate significant differences in ecological conditions between the association with *Sambucus nigra-Picea abies* and the community of *Sambuco racemosi-Pieceetum*. This fact indicates the necessity for further studies. It is likely that the lack of differences was caused by the effect of the pesticide tomb. The differences in the floral composition and in the quantitative ratios between the species constituting the phytocenoses under study were highlighted by the numerical analyses which divided the photosociological releves into two clear groups.

Translated by JOANNA JENSEN

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PROCESS DAIRY WASTEWATER AS EXTERNAL SOURCE OF VOLATILE FATTY ACIDS (VFA)*

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K e y w o r d s: dairy wastewater, potential for nutrients removal, process dairy wastewater characteristics, VFA production.

A b s t r a c t

The goal of the study was to characterize wastewater from selected production processes in a dairy plant with regard to their potential for nutrients removal and applicability in volatile fatty acids (VFA) production. Biological oxygen demand without nitrification (BOD_5), chemical oxygen demand (COD), total nitrogen (TKN), total phosphorus (TP) and VFA, were done in wastewater, sour whey and sweet whey. Additionally, quality of the wastewater from the same sources was analyzed, after prior 24-hour fermentation at 15°C.

The results of the study have revealed that process dairy wastewater, irrespective of the origin, is characteristic of very favourable values of the following ratios: COD:TKN, COD:TP, BOD_5:TKN and BOD_5:TP. They indicate that the processes of denitrification and biological phosphorus removal should not be limited by the availability of organic substrate.

Moreover, VFA concentrations and the consequent values of the VFA:(TKN + TP) ratios in the wastewater subjected to fermentation, indicate that in most types of the examined wastewater, the amount of organic substrate may be insufficient to remove phosphorus to the level below 1 mg $P \cdot dm^{-3}$. Only in the fermented wastewater from the pumping station was the VFA:(TKN + TP) ratio higher than 5 mg $CH_3COOH \cdot (mg^{-1}$ TKN + mg^{-1} TP) while the wastewater from other production processes did not exceed 3,5 mg $CH_3COOH \cdot (mg^{-1}$ TKN + mg^{-1} TP).

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ŚCIEKI MLECZARSKIE JAKO ZEWNĘTRZNE ŹRÓDŁO LOTNYCH KWASÓW TŁUSZCZOWYCH (LKT)

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S ł o w a k l u c z o w e: ścieki mleczarskie, możliwość usuwania związków biogennych, charakterystyka ścieków produkcyjnych, wytwarzanie lotnych kwasów tłuszczowych (LKT).

A b s t r a k t

Celem badań było scharakteryzowanie ścieków mleczarskich z wybranych procesów produkcyjnych pod kątem możliwości usuwania z nich związków biogennych i przydatności do wytwarzania LKT. Analizy fizykochemiczne (BZT_5, ChZT, N_{og}, P_{og}, LKT) wykonywano w ściekach oraz w serwatce z produkcji sera żółtego i serwatce z produkcji twarogu. Dodatkowo analizowano jakość ścieków z tych samych źródeł, po wcześniejszym poddaniu ich 24-godzinnej fermentacji w temp. 15°C.

Wyniki badań dowodzą, że ścieki mleczarskie, bez względu na źródło pochodzenia, mają bardzo korzystne wartości stosunków ChZT:N_{og}, ChZT:P_{og}, BZT_5:N_{og} i BZT_5:P_{og}. Można z tego wnioskować, że procesy denitryfikacji i biologicznego usuwania fosforu nie powinny być ograniczane dostępnością biochemicznie rozkładalnego substratu organicznego.

Jednocześnie stężenie LKT i wynikające z niego wartości stosunku LKT:(N_{og} + P_{og}) dla ścieków poddanych procesowi fermentacji pokazują, iż w większości z przebadanych ścieków ilość substratu organicznego może okazać się niewystarczająca do usunięcia fosforu do poziomu poniżej 1 mg $P \cdot dm^{-3}$. Tylko w przypadku przefermentowanych ścieków z pompowni wartość stosunku LKT:(N_{og} + P_{og}) była wyższa od 5 mg $CH_3COOH \cdot (mg^{-1}\ N_{og} + mg^{-1}\ P_{og})$, natomiast w przypadku ścieków z aparatowni nie przekroczyła 3,0 mg $CH_3COOH \cdot (mg^{-1}\ N_{og} + mg^{-1}\ P_{og})$. Blisko tego poziomu były ścieki z punktu odbioru mleka, a nieznacznie powyżej ścieki z masłowni, około 3,5 mg $CH_3COOH \cdot (mg^{-1}\ N_{og} + mg^{-1}\ P_{og})$.

Introduction

The effectiveness of denitrification and enhanced biological phosphorus removal (EBPR) depends on the availability of easily degradable organic compounds. Organic compounds used in both processes are usually taken up directly from treated wastewater (so-called internal carbon source). However, there are cases when treated wastewater contains insufficient amount of easily degradable organic substrate, and it is necessary to provide it from the outside (so-called external carbon source). The latter may be methanol, sodium acetate, anaerobically stabilized sludge from a municipal WWTP or VFA as technical products. Many surveys have confirmed the applicability of VFA for this purpose (SATOH et al. 1992, TAM et al. 1992, RANDALL et al. 1997).

In the reference literature, much attention is paid to the fact that the process wastewater from food industry often contains organic substrate in

amounts higher than needed for both complete denitrification and P removal to the level below 1 mg $P \cdot dm^{-3}$. Likewise, it is reported that due to the very favourable relationships between the quantity of easily degradable organic substances and the quantity of nutrients contained in the effluents from food industry, they can be a source of VFA used in the treatment of wastewater deficient in organic compounds after prior fermentation (BERNET et al. 1996, LEE et al. 1997).

The potential to produce VFA from dairy wastewater was confirmed in the detailed studies carried out at 15 dairies in the USA (DANALEWICH et al. 1998). The total VFA concentration oscillated between 1 and 431 mg $CH_3COOH \cdot dm^{-3}$. The mean value equalled 147 mg $CH_3COOH \cdot dm^{-3}$. The wastewater from cheese production was regarded as the most suitable for VFA production. However, such thesis outcome will not necessarily be confirmed in Polish conditions as the dairy wastewater in Poland lacks sour whey obtained in cottage cheese production.

VFA production from wastewater is related to transformation of fats, proteins and hydrocarbons into acetate acid and other fatty acids. At the same time, the quantity of nutrients contained in the wastewater is only to a small degree changed during the fermentation. That is the reason when making analysis of a given process wastewater applicability in VFA production, the load of nutrients put to the fermenters should be considered as eventually it will be found in the effluent from the fermenters.

When making analyses of the organic substrate reserves, COD and BOD_5 are the most frequently studied indexes. Moreover, the ratios of COD: P and BOD_5:P, reflecting the potential to remove P to less than 1 mg $P \cdot dm^{-3}$, are quoted (BARNARD 2000). The reference literature provides also another way to express substrate concentration: i.e., the VFA index. It is followed by the reports indicating the VFA:P ratio in the beginning of the anaerobic phase as suitable to remove P below the level of 1 mg $P \cdot dm^{-3}$ (VAN MUNCH et al. 1996, BARNARD 2000). The VFA index seems the most representative, as it reflects the content of organic compounds easily available to the denitrification bacteria and the polyphosphates accumulating bacteria.

Transformation of organic compounds contained in wastewater fed to biological reactors occurs in the anaerobic phase, in an effect of fermentation. However, as the mix phase is usually limited to only a few hours, mainly the easily degradable compounds are transformed while the compounds of a more complicated structure are broken down during the aerobic phase.

The processes of anaerobic degradation of organic contaminants and of the assimilation of simple organic compounds by the polyphosphates accumulating bacteria occur in parallel yet at a different rate. The rate of the latter is much higher. In effect, despite favourable ratios of COD:P, BOD_5:P, COD:N and

BOD_5:P in the treated wastewater, the substrate in the form non-available to the organisms, reduces the effectiveness of denitrification and phosphates removal to the level often lower than expected. The problem should be resolved by a prior fermentation of the wastewater.

The goal of this study was to characterize selected types of process wastewater from a dairy plant with regard to nutrients removal potential and applicability in VFA production and eventually with regard to P removal potential from dairy wastewater (after prior fermentation) or from any other wastewater requiring application of external VFA source.

Materials and Methods

Raw dairy wastewater, typical for small Polish dairy plants, was examined. Samples were taken in monthly intervals, in September 2003 through August 2004 (12 times). The wastewater was sampled from the milk reception point, apparatus room (milk distribution in centrifuges, thermal processing, homogenization, densification in evaporators), butter section, cottage cheese section, cheese section, sewage pumping station (mixture of all plant's wastewater types). Additionally, whey was sampled from cheese production (sweet whey) and from cottage cheese production (sour whey).

Wastewater and both types of whey were fermented in anaerobic biological reactors with the following technological parameters: retention time 24 h, biomass concentration 3-4 g d.w. $\cdot$ dm^{-3}, fermentation temperature 15°C.

The analyses in raw and digested wastewater, conducted in accordance with the current Polish Standards, included BOD_5, COD, TKN, TP and VFA.

Results and Discussion

With regard the amount of organic substances comprising the potential source of VFA during their fermentation, the most suitable could be both whey types (sweet and sour), with the COD over 69.000 and 56.000 mg $O_2 \cdot dm^{-3}$, respectively (Table 1). The highest COD values in the other types of the wastewater were measured in the effluent from the cottage cheese section and from the apparatus room (COD > 15.000 mg $O_2 \cdot dm^{-3}$) and from the cheese section (COD > 13.000 mg $O_2 \cdot dm^{-3}$). Their obvious dominance over the other types of wastewater is additionally confirmed by the high BOD_5 values (Table 1).

The highest concentration of TKN was detected in whey from the cheese production (> 1.300 mg $N \cdot dm^{-3}$) and in whey from the cottage cheese produc-

Table 1

Chemical characteristics of process dairy wastewater

Process/section	Measur-ment	BOD_5 (mg $O_2 \cdot dm^{-3}$)	COD (mg $O_2 \cdot dm^{-3}$)	BOD_5:COD	TKN (mg TKN $\cdot dm^{-3}$)	TP (mg P $\cdot dm^{-3}$)	VFA mg $CH_3COOH \cdot dm^{-3}$
1	2	3	4	5	6	7	8
Milk reception point	mean	850.08	2019.93	0.46	59.97	13.88	52.80
	min	217.00	784.00	0.15	9.10	1.20	2.60
	max	2150.00	5000.00	0.87	188.80	44.00	106.30
	SD	651.78	1388.12	0.24	47.09	13.07	33.97
Apparatus room	mean	4001.08	15228.57	0.29	279.63	76.35	109.88
	min	2540.00	6272.00	0.19	37.40	7.20	42.00
	max	8768.00	34089.60	0.55	725.60	131.70	267.60
	SD	1710.21	7952.17	0.12	203.34	40.28	62.90
Butter section	mean	1854.42	7058.64	0.34	217.09	45.59	61.23
	min	213.00	940.00	0.07	17.60	1.10	24.60
	max	2776.00	20720.60	0.59	812.60	169.20	179.20
	SD	938.16	5656.05	0.17	258.36	58.69	41.69
Cottage cheese section	mean	2713.42	15877.20	0.21	409.78	210.08	245.88
	min	1186.00	3488.00	0.05	107.80	58.00	96.30
	max	4234.00	23915.00	0.42	670.50	378.00	422.00
	SD	868.38	6422.50	0.10	186.62	97.72	98.93
Cheese section	mean	2922.92	13760.17	0.21	431.34	112.13	236.33
	min	1086.00	7208.00	0.14	186.10	42.70	86.30
	max	5030.00	20044.80	0.35	689.20	187.80	474.80
	SD	1150.18	3900.57	0.07	143.85	45.04	117.13

cont. Table 1

1	2	3	4	5	6	7	8
Cottage cheese whey (sour whey)	mean	28586.00	56337.67	0.51	995.50	710.05	792.13
	min	13200.00	45198.00	0.21	438.10	460.90	448.80
	max	41890.00	70502.00	0.74	1507.00	862.00	1196.00
	SD	8294.57	7140.37	0.15	293.02	119.97	247.17
Cheese whey (sweet whey)	mean	28911.50	69948.58	0.41	1382.31	507.98	299.91
	min	18967.00	53000.00	0.30	1264.00	431.00	53.60
	max	49000.00	87659.00	0.62	1502.00	546.20	623.10
	SD	8744.39	9514.09	0.09	77.98	39.31	157.03
Pumping station	mean	1622.42	3762.93	0.43	59.43	32.92	92.04
	min	283.00	1044.20	0.24	9.60	21.20	38.30
	max	2790.00	6737.30	0.74	121.20	60.20	181.50
	SD	851.79	1769.56	0.18	31.03	11.57	39.17

tion (nearly 1.000 mg N · dm^{-3}). Regarding the different wastewater types, the highest amounts of N were detected in the effluent from the cheese section (> 430 mg N · dm^{-3}), from the cottage cheese section (> 400 mg N · dm^{-3}) and from the apparatus room (> 270 mg N · dm^{-3}).

The highest values of TP were found in the sour whey: i.e., more than 700 mg P · dm^{-3} and in the sweet whey: i.e., more than 500 mg P · dm^{-3}. The maximum content of P was determined in the wastewater from the cottage cheese section (> 210 mg P · dm^{-3}), from the cheese section (> 110 mg P · dm^{-3}) and from the apparatus room (> 75 mg P · dm^{-3}) – Table 1.

The most favourable ratios between organic substance quantity (expressed as COD) and nutritive elements (expressed as a sum of TKN and TP) were found in the wastewater from the apparatus room (67.6 mg COD · (mg^{-1} TKN + mg^{-1} TP)) and from the butter section (59.5 mg COD · (mg^{-1} TKN + mg^{-1} TP)); (Figure 1). The ratios in the wastewater from the milk reception point and from the pumping station were approximately 43 mg COD · (mg^{-1} TKN + mg^{-1} TP). In the other types of wastewater and in both types of whey, the ratio ranged between 26.3 and 37.0 mg COD · (mg^{-1} TKN + mg^{-1} TP) for the cottage cheese section and the sweet whey, respectively.

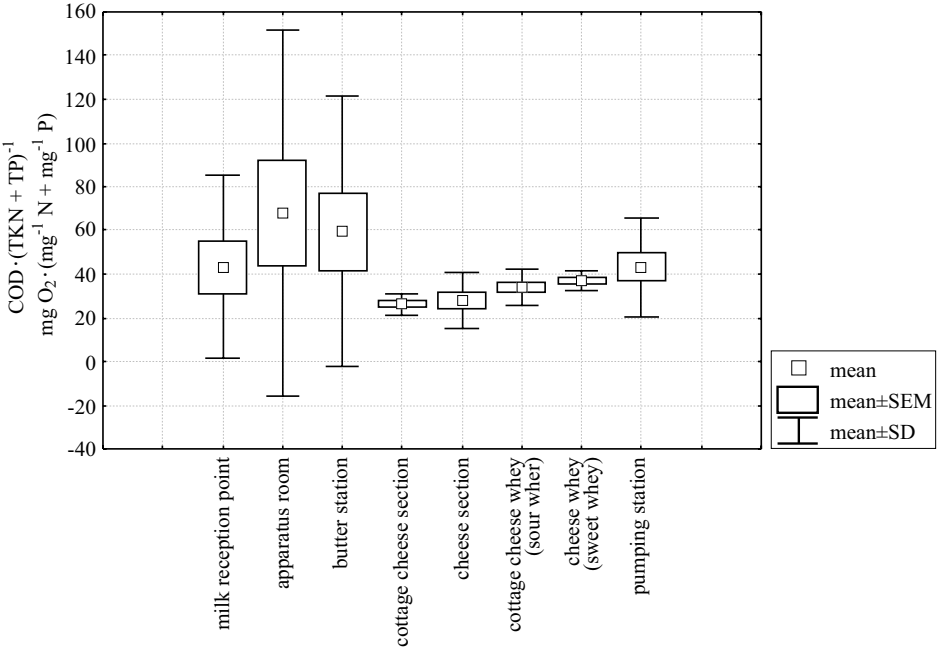

Fig. 1. COD/(TKN + TP) ratio

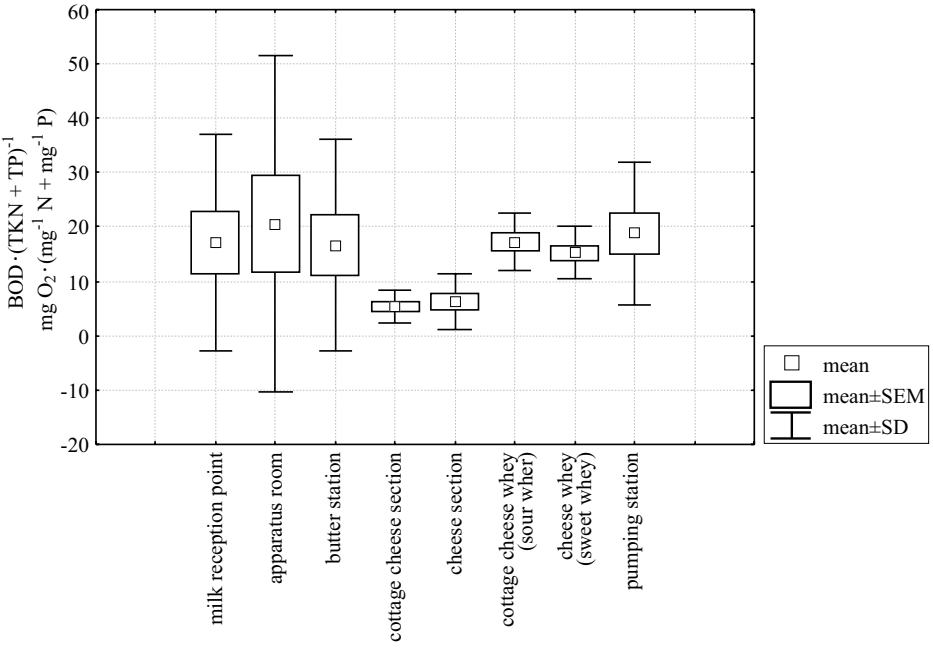

Fig. 2. BOD/(TKN + TP) ratio

Regarding the ratios between the amount of organic substance (expressed as BOD_5) and the sum of TKN and TP (Figure 2), the most favourable values were found in the wastewater from the apparatus room (20.59 mg $BOD_5 \cdot (mg^{-1}$ TKN + mg^{-1} TP)), from the pumping station (18.82 mg $BOD_5 \cdot (mg^{-1}$ TKN + mg^{-1} TP)), in the sour whey (17.72 mg $BOD_5 \cdot (mg^{-1}$ TKN+ + mg^{-1} TP)), in the wastewater from the milk reception point (17.22 mg $BOD_5 \cdot (mg^{-1}$ TKN + mg^{-1} TP)) and from the butter section (16.64 mg $BOD_5 \cdot (mg^{-1}$ TKN + mg^{-1} TP)).

The data show that irrespective of the wastewater origin, the mean values of the COD:TKN ratios were much above 9. At the same time, in all analysed wastewater types, the mean values of the COD:TP ratios were clearly higher than 40:1 (from 80.7 to 1.061 g COD $\cdot$ g^{-1} P). Only in the case of the BOD_5:TP ratio in the wastewater from the cottage cheese section, the mean value was lower than 20:1. In other types of the wastewater, the values of the BOD_5:TP ratios were much above 20 (from 31 to 156 g $BOD_5 \cdot g^{-1}$ P). The study concludes that in all examined wastewater types, denitrification should not be limited by the availability of organic substrate. Only in the wastewater from the cottage cheese section, the EBPR process may be less effective due to insufficient quantity of degradable carbon compounds (GRADY et al. 1999).

When making the analysis of organic substance in the form of VFA (Figure 3), attention should be paid to the value of the VFA:(TKN + TP) ratio in the raw wastewater that, irrespective of the source, was too low and did not exceed 1.5 mg $CH_3COOH \cdot (mg^{-1}$ TKN + mg^{-1} TP). BARNARD (2000) reports that for P removal to less than 1 mg $P \cdot dm^{-3}$ at least 4 mg $CH_3COOH \cdot mg^{-1}$ PPO_4 is necessary while van Munch (VAN MUNCH et al. 1996) quotes the value of 7.0 mg $CH_3COOH \cdot mg^{-1}$ PPO_4 needed to achieve the same treatment goal.

Examinations in the raw wastewater from the individual sources revealed that potentially the best source of VFA could be the wastewater from the apparatus room and the mixture of all the plant's process effluents (wastewater from the pumping station). Their applicability was confirmed by the most favourable ratio between the quantity of organic substance expressed as BOD_5 (the portion of organic contaminants vulnerable to biological degradation) and the sum of TKN and TP.

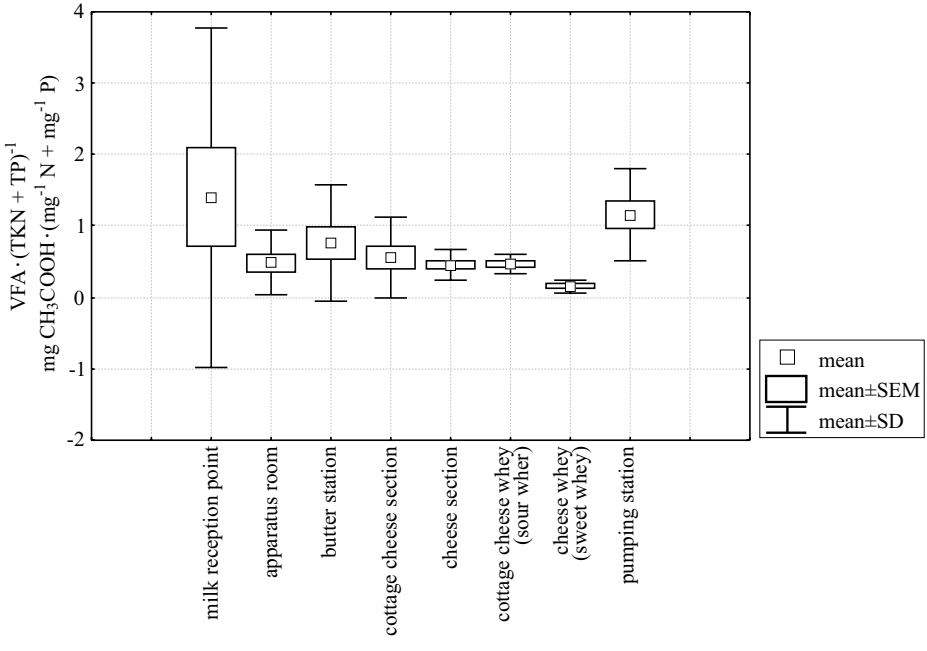

Fig. 3. VFA/(TKN + TP) ratio – raw wastewater

Pollution indicators in the fermented wastewater not fully confirmed the above hypotheses. It was observed (Figure 4) that after fermentation only in the wastewater from the pumping station, the VFA:(TKN + TP) ratio was higher than 5 mg $CH_3COOH \cdot (mg^{-1}$ TKN + mg^{-1} TP) whereas the wastewater

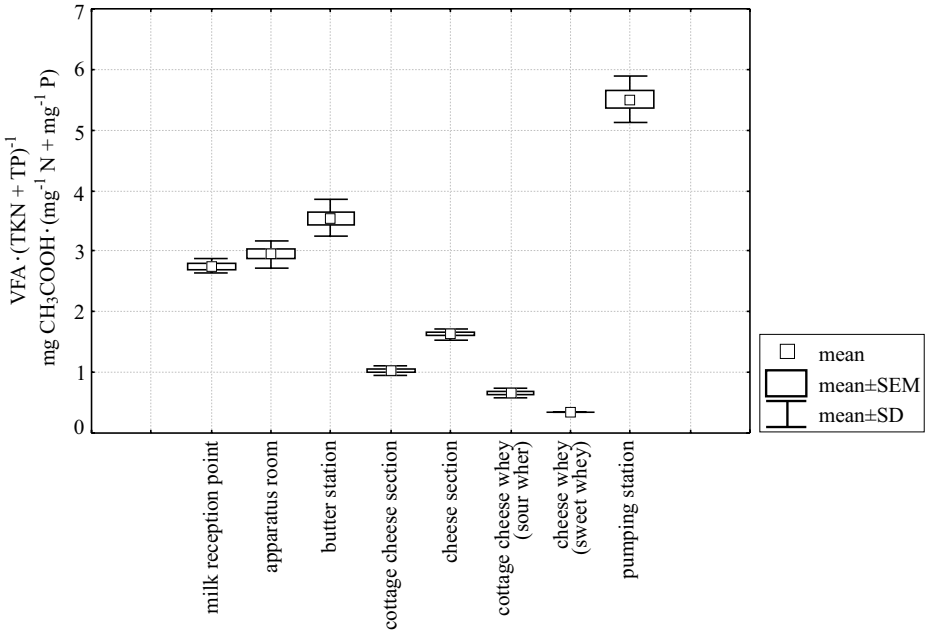

Fig. 4. VF/(TKN + TP) ratio – fermented wastewater

from the apparatus room did not exceed 3.0 mg $CH_3COOH \cdot (mg^{-1}$ TKN + + mg^{-1} TP). The same level approached the ratio in the wastewater from the milk reception point, and it was slightly higher in the wastewater from the butter section (approximately 3.5 mg $CH_3COOH \cdot (mg^{-1}$ TKN + mg^{-1} TP)).

The results discussed above indicate the appropriateness of dairy effluents preliminary fermentation even at 15°C, which means that reactors do not require any heating. Such treatment, preceding the wastewater treatment in an EBPR reactor, makes possible the production of VFA from selected wastewater types in amounts sufficient for effective N and P removal. It was confirmed by the results of the examinations in the mixture of all plant's wastewater types. Hence, it is possible to remove nutrients biologically from dairy wastewater to a very low level, using the so-called internal carbon source.

However, the results of this study do not allow formulating the hypothesis that any type of process dairy wastewater can be used as external VFA source in the treatment of wastewater deficient in organic compounds. Certainly, it does not mean that for example, fermentation conducted at higher temperature would not improve the effectiveness of the VFA production process and thus would make the selected dairy effluents suitable for the above-mentioned purpose.

Conclusions

Process dairy wastewater of various origin is characteristic of very favourable ratios between the following indexes: COD:(TKN + TP), BOD_5:(TKN + + TP) and the resultant values of COD:TKN, COD:TP, BOD_5:TKN and BOD_5:TP. These values show that the processes of denitrification and EBPR should be limited by the availability of the biochemically degradable organic substrate.

In parallel, the VFA values and the resultant values of the VFA:(TKN + + TP) ratios in the wastewater subjected to fermentation at 15°C reveal that in most of the examined wastewater types, quantity of the organic substrate may be insufficient to remove P to a low level because VFA will be used first by the denitrification bacteria and then for P removal. Only in the mixture of all wastewater types (from the pumping station) should there be no problems in removing P to less than 1 mg $P \cdot dm^{-3}$. In the other wastewater types, P removal to such level may not be possible.

Fermentation of the wastewater was conducted at the temperature of 15°C on purpose. In Poland, higher temperatures in autumn and winter should not be expected (JANCZUKOWICZ et al. 2004). Such temperature can be measured at this time of the year in the activated-sludge tanks, treating dairy effluents. If VFA were to be produced for example in the anaerobic phase of an EBPR reactor, due to the phase's duration (usually a few hours), the concentration of the produced VFA would be certainly lower than obtained after a 24-hour process ran in an anaerobic digestion tank. Therefore, if the technological goal of the process dairy wastewater treatment is high efficiency of the biological N and P removal, it should be fermented first. Only then the high quantity of VFA can be expected in the wastewater fed to the anaerobic zone of the reactor performing denitrification and EBPR.

1. Raw dairy wastewaters characterize favourable ratios of COD:(TKN + + TP) and BOD_5:(TKN + TP). It could indicate the potential for highly--effective process of biological N and P removal.

2. Only during fermentation of the dairy wastewater mixture (from the pumping station) are the amounts of produced VFA sufficient to conduct the parallel denitrification and EBPR. In the other types of dairy wastewater, EBPR can be limited by VFA availability.

3. High nutrients concentrations may determine the limited applicability of dairy wastewater as external carbon source.

4. Difference between the values of the BOD_5 and the VFA indexes in the mixture of all wastewater types (1622.4 ± 851.8 mg $O_2 \cdot dm^{-3}$ and 92.0 ± 39.2 mg $CH_3COOH \cdot dm^{-3}$, respectively) indicates the suitability of the activities aimed at transforming biochemically degradable organic substrate into the form

easily available by the organisms responsible for phosphates removal and denitrification (VFA). The proper solution may be use of a fermenter preceding the biological reactor.

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EFFECT OF ROUNDUP 360 SL HERBICIDE ON THE NUMBER OF *AEROMONAS HYDROPHILA* AND *PSEUDOMONAS FLUORESCENS* IN LAKE WATER

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K e y w o r d s: bacteria, *Aeromonas hydrophila*, *Pseudomonas fluorescens*, herbicide, lake water.

A b s t r a c t

As affected by different concentrations of Roundup 360 SL herbicide, both *Aeromonas hydrophila* and *Pseudomonas fluorescens* bacteria either increased or decreased their population numbers over the period of sample exposure. The Roundup 360 SL herbicide applied evoked the greatest changes in the numbers of microorganisms investigated after a longer time of sample exposure (21-28 days). The maximum growth of *Aeromonas hydrophila* strain at a temp. of 5°C and 10°C as well as pH 6, 7 and 8 occurred after 7, whereas that of *Pseudomonas fluorescens* strains, under the same medium conditions, after 21-28 days of sample exposure. At a temperature of 20°C, the maximum growth of both strains was observed after 7 days of exposure. Both the *Aeromonas hydrophila* and *Pseudomonas fluorescens* bacteria used the herbicide as a dietary substrate, even at low reaction (pH 6) and temperature (5°C).

WPŁYW HERBICYDU ROUNDUP 360 SL NA LICZEBNOŚĆ *AEROMONAS HYDROPHILA* I *PSEUDOMONAS FLUORESCENS* W WODZIE JEZIORNEJ

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S ł o w a k l u c z o w e: bakterie, *Aeromonas hydrophila*, *Pseudomonas fluorescens*, herbicyd, woda jeziorna.

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Abstrakt

Wykazano, że zarówno bakterie *Aeromonas hydrophila*, jak i *Pseudomonas fluorescens* pod wpływem różnych stężeń herbicydu Roundup 360 SL zwiększały lub zmniejszały swoją liczebność w okresie ekspozycji prób. Zastosowany herbicyd Roundup 360 SL powodował największe zmiany liczebności badanych drobnoustrojów po dłuższym czasie ekspozycji prób (21-28 dni). Maksymalny rozwój szczepu *Aeromonas hydrophila* w temp. 5°C i 10°C oraz pH 6, 7 i 8 następował po 7 dniach ekspozycji prób, a szczepu *Pseudomonas fluorescens*, w tych samych warunkach środowiska, po 21-28. W temp. 20°C stwierdzono maksymalny rozwój obu szczepów po 7 dniach ekspozycji. Zarówno bakterie *Aeromonas hydrophila*, jak i *Pseudomonas fluorescens* wykorzystywały herbicyd jako substrat pokarmowy, nawet w niskim odczynie (pH 6) i niskiej temperaturze (5°C).

Introduction

Common use of crop protection products, referred to as herbicides, prompts researchers to undertake studies into their effects on microorganisms. Herbicides intentionally administered into environment circulate in soil, water and air, and accumulate at different levels of trophic chains. As chemically-active compounds, the are likely to exert a peculiar effect on bacteria that are highly susceptible to environmental stimuli.

The Roundup 360 SL herbicide applied in the study is a systemic, non-selective herbicide. Its active substance is glyphosate, referred to as N(phosphonomethyl)glycine. Reported concentrations of glyphosate in water range from several hundredths to over a hundred $\mu g \cdot ml^{-1}$ (SATO et al. 2001). This xenobiotic causes enhanced microbiological activity that results from its application as an available carbon substrate (BUSSE et al. 2001).

The study was aimed at determining the effect of various concentrations of Roundup 360 SL herbicide in the number of bacteria strains *Aeromonas hydrophila* and *Pseudomonas fluorescens* in filtered lake water devoid of zoo- and phytoplankton, under diversified conditions of pH and temperature.

Material and Methods

Natural lake water collected from the Lake Kortowskie, was filtered with the use of a subatmospheric pressure device, through membrane filters with a pore size of 0.45 μm and Ø 50 mm by Schleicher & Schuell company. 100 cm^3 samples of filtered lake water were fixed in sterile glass bottles with the volume of 100 cm^3. The bottles were closed with a screwed cap with a hole in which a sterile stopper made of cotton-wool was fixed. In the samples of filtered water, the reaction was adjusted to pH 6, 7 and 8 (±0.2). Various doses of Roundup 360 SL herbicide were added to each group of water samples prepared with different pHs, so that the final concentration of the active

substance reached 0.005 $\mu g \cdot dm^{-3}$ and 5000 $\mu g \cdot dm^{-3}$. Half the samples prepared were inoculated with a 16-h culture of *Aeromonas hydrophila*, whereas the second half were inoculated with *Pseudomonas fluorescens*, thus obtaining a suspension with bacteria counts of 5 cfu $\cdot$ cm^{-3} and 4 cfu $\cdot$ cm^{-3}, respectively. The prepared analytical samples were exposed at temperatures of 5°C, 10°C and 20°C for 28 days.

The number of *Aeromonas hydrophila* and *Pseudomonas fluorescens* bacteria was determined before exposure of the samples (in time t_0) – control sample, and in all variants of samples after 7, 14, 21 and 28 days of exposure. The quantitative analysis was carried out with the culture method (plate) using King B medium (incubation at a temp. of 26°C for 48-72 h) for *Pseudomonas fluorescens* bacteria and mA medium (incubation at a temp. of 37°C for 24-48 h) for *Aeromonas hydrophila* bacteria. The results obtained were converted into colony forming units (cfu) per 1 cm^3 of water.

Results of the quantitative assays were subjected to a statistical analysis by means of the analysis of variance method.

The bacterial strains *Aeromonas hydrophila* and *Pseudomonas fluorescens* applied in the study were isolated from lake water and identified based on morphological, physiological and biochemical traits with the use of API 20NE tests by bioMériux (France).

Results

Changes in the population numbers of *Aeromonas hydrophila* and *Pseudomonas fluorescens* strains, as affected by Roundup 360 SL herbicide, within 28 days of exposure, in lake water with a temp. of 5°C and varying reaction (pH) are shown in Figures 1 *a* and *b*. In water with a reaction of pH 6, herbicide concentration of 5000 $\mu g \cdot dm^{-3}$ caused a slight decrease in the cell count of *Aeromonas hydrophila* bacteria over the entire experimental period, as compared to the control sample. In turn, a herbicide concentration of 0.005 $\mu g \cdot dm^{-3}$ stimulated the growth of these bacteria up to day 21, with the maximum reported in day 14 of exposure. In water with a reaction of pH 7, both concentrations of the herbicide stimulated the growth of bacterial cells up to day 7 of exposure, whereas from day 14 till day 28 their numbers were observed to decline. In water with pH 8, a herbicide concentration of 5000 $\mu g \cdot dm^{-3}$ stimulated the growth of the bacteria, with the highest number on day 7, whereas a herbicide dose of 0.005 $\mu g \cdot dm^{-3}$ appeared to have an inhibiting effect on the bacteria under study.

In water with a reaction of pH 6, a herbicide concentration of 0.005 $\mu g \cdot \cdot dm^{-3}$ caused an increase in the number of *Pseudomonas fluorescens* cells

within the first 14 days of exposure, as compared to the control sample. A herbicide concentration reaching 5000 $\mu g \cdot dm^{-3}$ also had a stimulating impact on the growth of bacteria, yet their maximum growth occurred after 21 days of sample exposure. In water with pH 7, herbicide concentration of 0.005 $\mu g \cdot dm^{-3}$ stimulated the growth of *Pseudomonas fluorescens* cells, proportionally to the time of sample exposure. The concentration of 5000 $\mu g \cdot dm^{-3}$ decreased the number of bacterial cells from day 7 till day 21, as compared to the control sample. In water with pH 8, a herbicide dose of 5000 $\mu g \cdot dm^{-3}$ slightly inhibited the growth of *Pseudomonas fluorescens* bacteria after 21 days of sample exposure, as compared with the control, whereas a herbicide concentration of 0.005 $\mu g \cdot dm^{-3}$ inhibited their growth between day 14 and day 21 of the exposure.

Changes in the numbers of the bacterial strains examined, as affected by two concentrations of the herbicide, in water with a temp. of 10°C and diversified reaction were presented in Figures 2 *a* and *b*. In water with the reaction of pH 6, herbicide concentration of 0.005 $\mu g \cdot dm^{-3}$ distinctly stimulated an increase in the number of *Aeromonas hydrophila* bacteria cells over the entire period of exposure, whereas its concentration of 5000 $\mu g \cdot dm^{-3}$ of water changed the count of the bacteria examined to a negligible extent, as compared to the control sample. In water with medium reaction of pH 7, herbicide concentration of 0.005 $\mu g \cdot dm^{-3}$ increased the count of *Aeromonas hydrophila* bacteria, as compared to the control, up to day 28 of analyses. In turn, a herbicide concentration of 5000 $\mu g \cdot dm^{-3}$ appeared to exert an opposite effect, i.e. it inhibited the growth of these bacteria over the entire period of exposure. In water with a medium reaction of pH 8, a herbicide concentration of 0.005 $\mu g \cdot dm^{-3}$ inhibited the growth of bacterial cells almost throughout the entire period of exposure (except for day 21), whereas that of 5000 $\mu g \cdot dm^{-3}$ had an opposite effect, i.e. it increased the bacterial count, especially on day 7 of exposure.

In water with a reaction of pH 6, a herbicide concentration of 5000 $\mu g \cdot dm^{-3}$ had a strong stimulating effect on the increase in the number of bacterial cells of *Pseudomonas fluorescens*, evoking an increase in their count up to the maximum after 21 days of sample exposure. In turn, a herbicide concentration of 0.005 $\mu g \cdot dm^{-3}$ considerably inhibited the growth of *Pseudomonas fluorescens* bacteria after 14 and 21 days of sample exposure. In water with a medium reaction of pH 7, a herbicide dose of 5000 $\mu g \cdot dm^{-3}$ caused a decrease in the number of bacterial cells of *Pseudomonas fluorescens*, almost throughout the entire period of sample exposure, whereas the dose of 0.005 $\mu g \cdot dm^{-3}$ – only after 7 and 21 days. In water with medium reaction of pH 8, both concentrations of the herbicide stimulated, to a different extent, the growth of bacterial cells after 21 days of sample exposure.

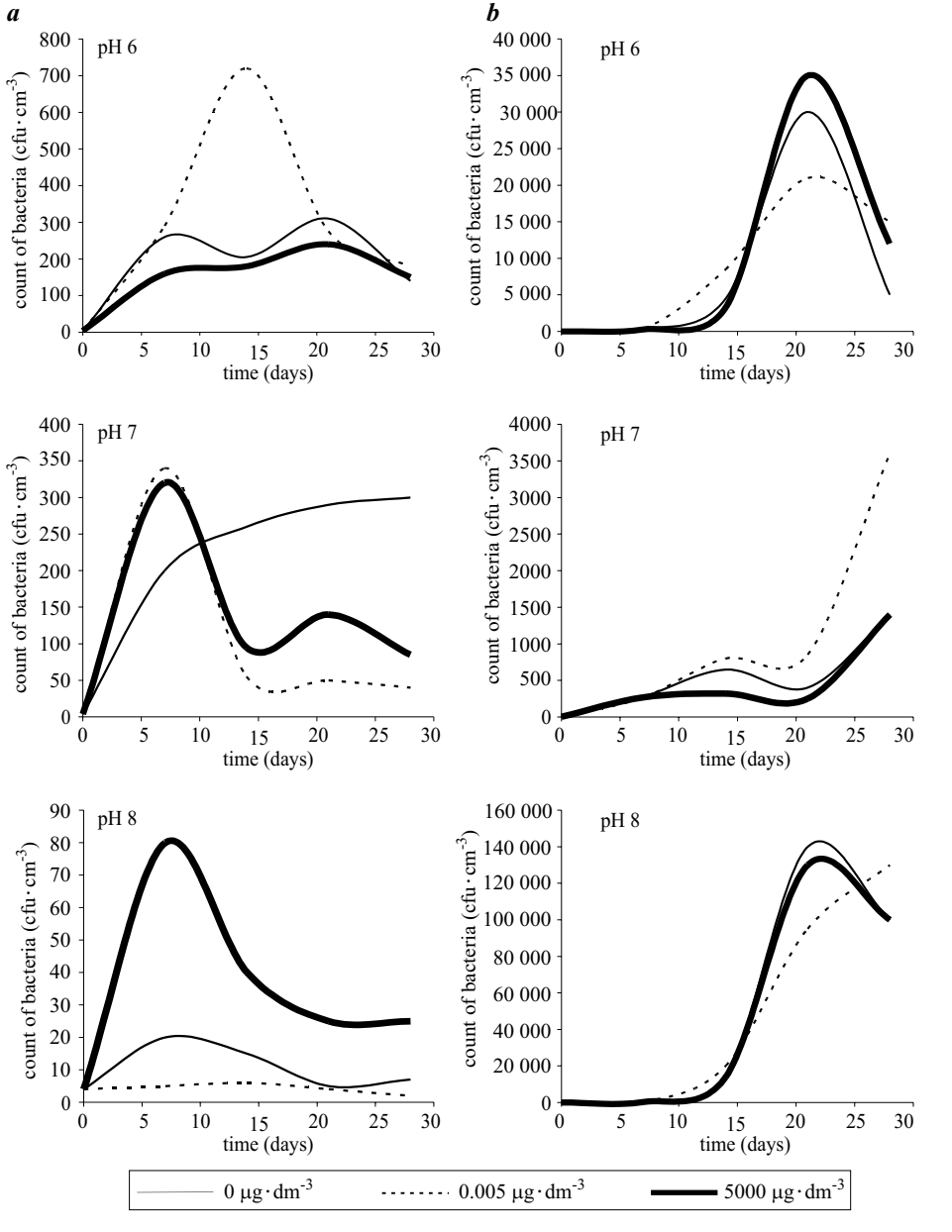

Fig. 1. Changes of counts of *Aeromonas hydrophila* (*a*) and *Pseudomonas fluorescens* (*b*) under the influence of the herbicide Roundup 360 SL in probes of filtrated lake water in temp. 5°C and different reaction pH

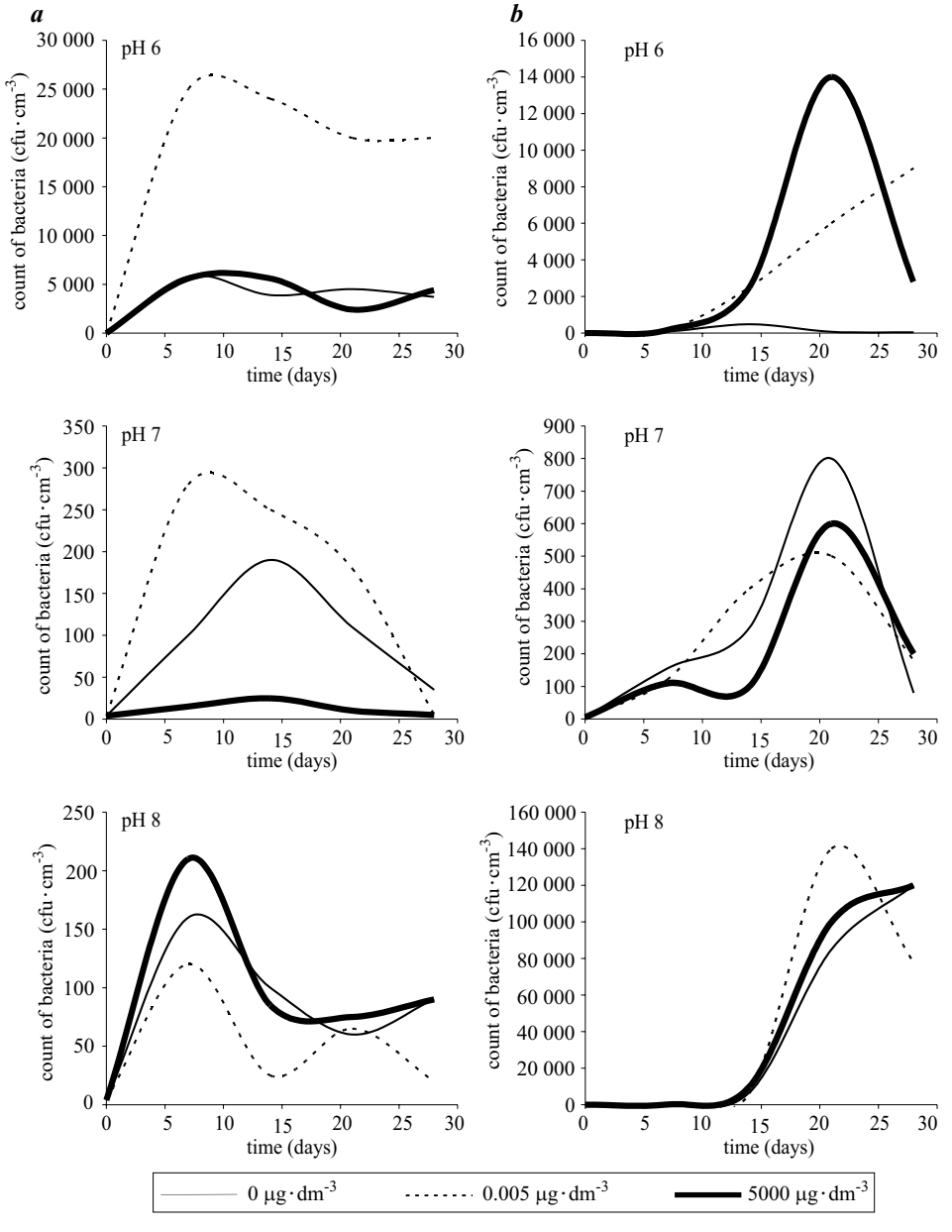

Fig. 2. Changes of counts of *Aeromonas hydrophila* (*a*) and *Pseudomonas fluorescens* (*b*) under the influence of the herbicide Roundup 360 SL in probes of filtrated lake water in temp. 10°C and different reaction pH

Figure 3 depicts changes in the n umbers of the bacterial strains examined in water with a temp. of 20°C and variable pH, as affected by both concentrations of the herbicide. In water with a reaction of pH 6, a herbicide concentration of 0.005 $\mu g \cdot dm^{-3}$ evoked an increase in the number of *Aeromonas hydrophila* bacteria over the entire time of sample exposure, as compared to the control sample. A herbicide concentration of 5000 $\mu g \cdot dm^{-3}$ appeared to have an opposite effect by inhibiting the growth of those bacteria. In water with a medium reaction of pH 7, a herbicide concentration of 5000 $\mu g \cdot dm^{-3}$ had a strong and tangible stimulating impact on the growth of *Aeromonas hydrophila* cells over the entire analytical period. The effect of herbicide applied at a dose of 0.005 $\mu g \cdot dm^{-3}$ was similar but considerably less intense and persisted only up to day 21 of exposure. At the herbicide dose of 5000 $\mu g \cdot dm^{-3}$, in water with medium reaction of pH 8, the number of *Aeromonas hydrophila* cells was subject to a considerable increase over the entire exposure time, as compared with the control. The herbicide applied at the concentration of 0.005 $\mu g \cdot dm^{-3}$ caused a decrease in the number of bacterial cells over the entire analytical period.

In water with pH 6, a herbicide concentration of 0.005 $\mu g \cdot dm^{-3}$ stimulated the growth of *Pseudomonas fluorescens* bacteria by day 14 of exposure, with the maximum growth observed on day 7. In turn, a herbicide concentration of 5000 $\mu g \cdot dm^{-3}$ exerted an opposite effect, substantially inhibiting their growth over the entire period of sample exposure. In water with a medium reaction of pH 7, the herbicide applied at a dose of 5000 $\mu g \cdot dm^{-3}$ evoked an increase in the number of *Pseudomonas fluorescens* bacteria over the entire exposure period, as compared to the control sample. The second dose of the herbicide appeared to have an inhibiting effect, except for day 14 of sample exposure (Figure 3). At a medium reaction of pH 8, a herbicide concentration of 5000 $\mu g \cdot dm^{-3}$ stimulated the growth of *Pseudomonas fluorescens* bacteria over the entire analytical period, as compared to the control, whereas that of 0.005 $\mu g \cdot dm^{-3}$ exerted an inhibiting effect, except for the first 7 days of sample exposure.

Table 1 collates results of the statistical analysis. The analysis indicates that changes in the population number of *Aeromonas hydrophila* bacteria in filtered lake water was statistically significantly affected by the following variables: time, herbicide concentration, combined effects of time and temperature, herbicide concentration and temperature, temperature and pH, time and pH, as well as the combined effects of time, temperature and pH, and herbicide concentration, temperature and pH.

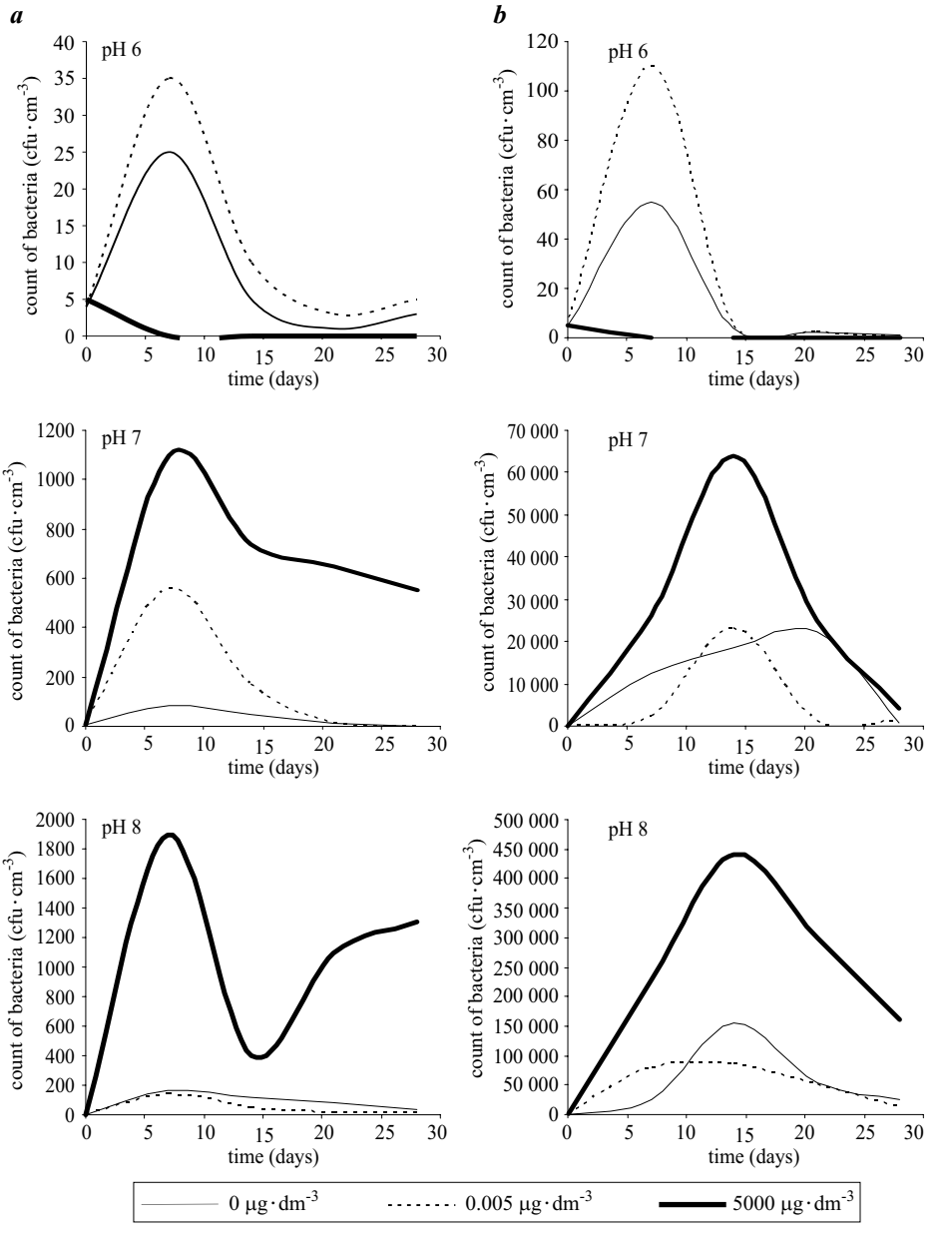

Fig. 3. Changes of counts of *Aeromonas hydrophila* (*a*) and *Pseudomonas fluorescens* (*b*) under the influence of the herbicide Roundup 360 SL in probes of filtrated lake water in temp. 20°C and different reaction pH

Table 1
Levels of substantiality for the analysis of variation of bacteria *Aeromonas hydrophila* and *Pseudomonas fluorescens* cultured in filtrated lake water

Factors occurring in the examination	*Aeromonas hydrophila*	*Pseudomonas fluorescens*
Time	0.000000*	0.000000*
Concentration of the herbicide	0.001361*	0.337251
Temperature	0.000000*	0.000000*
Reaction pH	0.032400	0.000000*
Co-operation of the time and the concentration of the herbicide	0.339356	0.999886
Co-operation of the time and the temperature	0.000000*	0.000000*
Co-operation of the concentration of the herbicide and the temperature	0.000908*	0.449090
Co-operation of the time and the reaction pH	0.002839*	0.000000*
Co-operation of the concentration of the herbicide and the reaction pH	0.000000*	0.001697*
Co-operation of the reaction pH and the temperature	0.000000*	0.000000*
Co-operation of the time, the concentration of the herbicide and the temperature	0.964761	0.977636
Co-operation of the time, the concentration of the herbicide and the reaction pH	0.400263	0.986151
Co-operation of the time, the reaction pH and the temperature	0.000000*	0.000000*
Co-operation of the concentration of the herbicide, the reaction pH and the temperature	0.000000*	0.000008*

* – a factor essentialy influencing the change of the count of bacteria

Changes in the population number of *Pseudomonas fluorescens* bacteria in filtered lake water appeared to be statistically significantly affected by the following variables: time, temperature, pH, as well as combined effects of time and temperature, time and pH, temperature and pH, herbicide concentration and pH, and combined effects of time, temperature and pH, and herbicide concentration, temperature and pH.

Discussion

Studies into the effect of pesticides on microorganisms have been carried out by a number of researchers and in a variety of aspects beginning from their impact on quantitative changes, through mutagenic properties (PAWLISZAK et al. 2000), up to a detailed analysis of enzymes participating in their biode-

gradation (SELVAPANDIYAN and BHATNAGAR RAJ 1994) and finally to molecular assays (KARNS 1999). In the reported study, attention was paid to the susceptibility of two species – *Aeromonas hydrophila* and *Pseudomonas fluorescens*, to the applied Roundup 360 SL herbicide. The monitoring of the quantitative changes in the bacteria examined as affected by the preparation applied enables determination of their tolerance to its activity and observation of the intensity of biodegradation (ŠVIHÁLEK and HOFMAN 2002). This refers especially to glyphosate, an active compound of Roundup 360 SL used in the study, since its biodegradation is determined, to the greatest extent, by the activity of bacteria (RÓŻAŃSKI 1992, ZBOIŃSKA and MALISZEWSKA 1996).

Researchers investigating crop protection products unanimously agree that they exert both a stimulating and an inhibiting effect on the growth and development of microorganisms (GODLEWSKA-LIPOWA 1975, ZMYSŁOWSKA 1987, WĘGOREK et al. 1994, ZMYSŁOWSKA and JANKOWSKA 2003). In the case of the Roundup 360 SL preparation, the results obtained indicated that it was its concentration that affected the bacteria by either increasing or decreasing their counts, which was consistent with findings of other authors (FRANZ et al. 1997, WACHOWSKA and BANASZKIEWICZ 1999).

Not always, however, did the results of the reported study confirm data reported by other authors (ZMYSŁOWSKA 1987, STRZELEC and DEC-PLEWKA 1992, GOTO et al. 1994) who claimed that higher concentrations of pesticides usually exerted bactericidal or bacteriostatic effects on microorganisms. Higher doses of the Roundup 360 SL herbicide (5000 $\mu g \cdot dm^{-3}$), especially in water with higher values of reaction (pH 8) and temperature (20°C), were clearly used as a dietary substrate, thus increasing bacterial counts. In contrast, a lower concentration of the herbicide (0.005 $\mu g \cdot dm^{-3}$), at temp. of 5°C and 10°C and water reaction of pH 8, decreased the number of *Aeromonas hydrophila* bacteria to a considerable extent, as compared with the control, thus indicating the process of growth inhibition. This is likely to be caused by the fact that glyphosate, the active compound of Roundup 360 SL herbicide, may be metabolized on various pathways, which depends on the species of bacteria degrading it (ZBOIŃSKA and MALISZEWSKA 1996). This can also be caused by a greater impact of glyphosate degradation products than the preparation itself.

The greatest changes in bacterial counts under the influence of the herbicide applied were observed even by the end of the exposure period of samples (21-28 days). In the studies of SMITH-GRENIER and ADKINS (1996), the highest quantitative increase of microorganisms capable of degrading a given pesticide occurred within the first few hours of incubation. This indicates that, apart from herbicide concentration, a significant role must be attributed to other environmental factors that are likely to affect the growth rate of bacterial microflora.

Aeromonas hydrophila bacteria, belonging to mesophilic bacteria, were more susceptible to the presence of the herbicide than the *Pseudomonas fluorescens* strain belonging to psychtrotrophic bacteria. This has been confirmed by the data obtained in an earlier study (ZMYSŁOWSKA and JANKOWSKA 2001).

The excessive development of *Pseudomonas fluorescens* and *Aeromonas hydrophila* bacteria, as affected by herbicide residues in water, is unfavorable to humans from an economic point of view since these bacteria are potential pathogens to fish, aquatic animals and humans (LECLERC and MOSSEL 1989). It is of special significance at the water reaction of pH 8 which occurs in inland water bodies, since the number of bacteria increases under the influence of herbicide in water with that reaction, as has been confirmed in this study.

Available literature provides scant data on the pesticide-biodegrading properties of *Aeromonas hydrophila* bacteria. The reported study indicates that in filtered water devoid of phyto- and zooplankton, these bacteria metabolized the herbicide applied in the study, using it as a dietary component. According to other investigations, the *Aeromonas hydrophila* bacteria are the most effective in biodegradation of single pesticides or their mixtures at medium reaction of pH 8 (ZMYSŁOWSKA and JANKOWSKA 2003). Worth emphasizing is the fact that the *Aeromonas hydrophila* bacteria, belonging to mesophilic bacteria, i.e. best developing at a temperature range of 30°C – 40°C, metabolized the Roundup 360 SL herbicide also at a low temperature (5°C) and acidic reaction (pH 6).

Conclusions

1. As affected by various concentrations of the Roundup 360 SL herbicide, both the *Aeromonas hydrophila* and *Pseudomonas fluorescens* bacteria either increased or decreased their counts over the time of sample exposure.

2. The Roundup 360 SL herbicide applied caused the greatest changes in bacterial counts after a longer period of sample exposure (21-28 days).

3. The maximum growth of *Aeromonas hydrophila* strain at a temp. of 5°C and 10°C (pH 6, 7 and 8) occurred after 7, whereas that of *Pseudomonas fluorescens* strain – after 21-28 days of sample exposure; at a temp. of 20°C the maximum growth of both strains was similar and occurred after 7 days.

4. The Roundup 360 SL herbicide applied at the concentration of 5000 $\mu g \cdot dm^{-3}$ inhibited the growth of both strains analyzed, in water with a temp. of 20°C and pH 6.

5. Both the *Aeromonas hydrophila* and *Pseudomonas fluorescens* bacteria were observed to use the herbicide as a dietary substrate, even at a low reaction (pH 6) and temperature (5°C) of lake water.

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ADSORPTION OF CADMIUM (Cd) AND ZINC (Zn) ONTO ALGINATE BIOSORBENTS IN SINGLE AND BINARY COMPONENT SYSTEMS

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K e y w o r d s: cadmium, zinc, immobilized activated sludge, alginate, polyvinyl alcohol.

A b s t r a c t

The adsorption of two metal ions Cd and Zn in a single and binary systems by activated sludge immobilized in alginate 2% and alginate 1.5% with PVA 0.5% was investigated. Ratios of metals concentration ($mg \cdot dm^{-3}$) in adsorbate were 1:1 and 1:2.

Adsorption of cadmium and zinc from single components was evaluated on the basis of constants in Freundlich equation. For activated sludge immobilized in alginate 2% the constants were $k = 0.73$, $1/n = 0.45$ for cadmium and $k = 0.73$, $1/n = 0.37$ for zinc. For activated sludge immobilized in alginate 1.5% with PVA 0.5% values of k and $1/n$ were 0.53 and 0.33 for cadmium and 0.48 and 0.41 for zinc, respectively.

Competitive biosorption of cadmium and zinc was not stated, what proves high average percentage errors between measured equilibrium capacities (q_{exp}) and calculated (q_{cal}) from the two-component Freundlich isotherm.

Efficiency of removal both metals onto two tested biosorbents was lower for cadmium and negligible higher for zinc in comparison with the removal of single metals from binary mixtures.

ADSORPCJA KADMU (Cd) I CYNKU (Zn) NA BIOSORBENTACH ALGINIANOWYCH Z ROZTWORÓW WODNYCH ZAWIERAJĄCYCH POJEDYNCZE METALE ORAZ ICH MIESZANINĘ

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S ł o w a k l u c z o w e: kadm, cynk, osad czynny immobilizowany, alginian, poliwinylowy alkohol.

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Abstrakt

Badano adsorpcję kadmu i cynku z roztworów zawierających pojedyncze metale oraz mieszaninę metali w proporcji Cd:Zn 1:1 i 1:2. Jako biosorbenty zastosowano osad czynny immobilizowany w alginianie 2% i osad czynny immobilizowany w mieszaninie alginianiu 1,5% i 0,5% alkoholu poliwinylowego (APV).

Adsorpcję kadmu i cynku z roztworów zawierających pojedyncze metale oceniano na podstawie stałych wyznaczonych z równania Freundlicha. W przypadku osadu czynnego immobilizowanego w alginianie 2%, wynosiły one dla kadmu k = 0,73 i $1/n$ = 0,45, a dla cynku k = 0,73 i $1/n$ = 0,37, w przypadku osadu czynnego immobilizowanego w alginianie 1,5% z APV 0,5%, wartości k *i* $1/n$ wynosiły odpowiednio 0,53 i 0,33 dla kadmu oraz 0,48 0,41 dla cynku.

Nie stwierdzono kompetycji między kadmem i cynkiem, czego potwierdzeniem jest wysoki błąd średni między pojemnością adsorpcyjną wyznaczoną z eksperymentu (q_{exp}) a pojemnością adsorpcyjną wyznaczoną z modelu Freundlicha z kompetycją (q_{cal}).

Efektywność usuwania kadmu i cynku (jako suma) z obu testowanych biosorbentów, w porównaniu z efektywnością usuwania pojedynczych metali z roztworów zawierających ich mieszaninę, była niższa w przypadku kadmu i nieznacznie wyższa w przypadku cynku.

Introduction

Many industries, including metal plating, mining and battery manufacturing contain heavy metals. For reducing their concentration to environmentally acceptable limits many methods can be applied such as precipitation or complexation, coagulation, chemical oxidation or reduction, membrane processes, ion exchange and adsorption. Among them adsorption has shown many advantages. It is quite selective process and effective in heavy metals removal, even at their low concentrations from solution.

The newly discovered, considerable attention is the ability of biological materials to metal ions sequestration. It is considered that biosorbents are commonly used in development of an efficient technologies to clean-up heavy metals pollution. The biosorption process offers the advantages of low operating costs, the volume reduction of chemicals and/or biological sludge to be disposed, high efficiency in detoxification of very dilute effluents (KRATOCHVIL, VOLESKY 1998). Several materials in the categories like waste biomass of algae, moss, fungi, bacteria or activated sludge have been successfully applied for metal biosorption. Quantitative information on biosorption performance were obtained from batch equilibrium studies. As a result of studying behaviour of different biosorbent materials, simple sorption equation was used to estimate adsorption capacity.

Whereas numerous studies have reported on the metals biosorption by materials of various biological origin, they have remained limited to single species of heavy metal ions. Then, the main challenge for the industrial application of biosorption technology is biosorbents efficiency in real wastewater treatment. Wastewater commonly contain several metal ions.

The adsorption of two or more metals onto biosorbents could be more complex. If the preference of one ion for a ligand is similar to that of another metal ion, a competition effect can be observed. TSEZOS et al. (1996) improved that metals belonging to either the hard or soft classes exhibited competitive effects among ions of their class. However, for metals occurring in different classes, their biosorptive uptake was not significantly affected by the presence of another metal.

Until now, comparative studies of simultaneous biosorption of Cr(VI) and Fe(III) by *Chlorella vulgaris* and *Rhizopus arrhizus* (SAĞ et al. 1998a), Cu(II) and Zn by *Rhizopus arrhizus* (SAĞ et al. 1998b), Cd(II) and Pb(II) by *Phanerochaete chrysoporium* (LI et al. 2004), Cr(VI), Ni(II) onto dried activated sludge (AKSU et al. 2002) were explored. HAMMAINI et al. (2003) improved a competitive uptake of three metal ions in bicomponent mixture systems of Cu-Cd, Cu-Zn and Cd-Zn by activated sludge.

Use of biomass on large scale generally requires preliminary immobilization in a synthetic polymer matrix enhancing the mechanical strength of the particles. Information for two and multimetal biosorption systems by immobilized biomass are available in smaller amount. For example, biosorption of two metals was performed onto FCAN2 prepared from *Ascophyllum nodosum* seaweed biomass crosslinking with formaldehyde (CHONG, VOLESKY 1995).

The present paper reports on experimental results about the effect of cadmium and zinc removal from binary mixtures by activated sludge immobilized in alginate carriers. The Freundlich model was used to estimation of mono- and two-component equilibrium data.

Materials and Methods

Biosorbents preparation

Activated sludge. Activated sludge used in this study was obtained from standardized culture (PN-87/C-04616/10). The powdered biomass of activated sludge was prepared as follows: the activated sludge was separated by sedimentation and centrifugation. The biomass was rinsed twice with acetone, centrifuged and left in water bath at 50°C for 48 h. Dry sludge was grinded in agate mill, next sieved to 0.1 mm fraction sizes. Thus prepared activated sludge was stored for immobilization purpose.

Preparation of immobilized activated sludge – activated sludge immobilized in alginate. Weighted portions of 2 g activated sludge and 2 g sodium alginate (medium viscosity – produced by Sigma) were dissolved in 96 g deionized water, led to formation homogenous suspension, and then dropped into

0.05 M $CaCl_2$ solution. The beads were 2.8 mm in diameter. Next, they were left for 24 h to form gel and rinsed with deionized water until chloride was not detected.

Preparation of immobilized activated sludge – activated sludge immobilized in alginate and polyvinyl alcohol (PVA) mixture. Weighted portions of 2 g activated sludge and 1.5 g sodium alginate with 0.5 g PVA were mixed and dissolved in 96 g deionized water. Homogenous suspension was dropped into 0.05 M $CaCl_2$ solution saturated in bromic acid solution. Addition of bromic acid was essential to form gel of PVA. Formed beads were left for 24 h to form gel and rinsed with deionized water until chloride was not detected.

Metal solutions

For zinc and cadmium adsorption research, cadmium ($Cd(SO_4)_2 \cdot 8H_2O$) and zinc ($ZnSO_4 \cdot 7H_2O$) aqueous solutions at a ratios of Cd:Zn 1:1.7 and 1:3.4 (mol Cd:mol Zn) were used, what corresponded to 1:1 and 1:2 ratios (mg Cd: :mg Zn), respectively. For single metal sorption research the range of concentrations were from 0.009 to 7.12 $mmol \cdot dm^{-3}$ (1-800 $mg \cdot dm^{-3}$) for cadmium and from 0.015 to 12.23 $mmol \cdot dm^{-3}$ (1-800 $mg \cdot dm^{-3}$) for zinc. The metal concentrations in mixture at a 1:2 ratio corresponded to metals concentration in batch experiments for single components. In case of metals mixture at a 1:1.7 ratio both metals concentrations corresponded to cadmium concentrations.

Metal sorption studies

To determine the adsorption isotherms, the batch sorption experiments for single and binary component systems were carried out. Firstly, 8 g of biosorbent was added to each 200 cm^3 sealed flask, and filled with 100 cm^3 of metal solutions. Thus prepared samples were shaken on the shaker at 200 rpm and 20°C for 2 hours and equilibrium metals concentration in the solution was estimated.

Analytical methods

Adsorption investigations were based on measurements of equilibrium cadmium and zinc in aqueous solution using an atomic absorption spectrophotometer (AA280FS, Varian). Samples of 10 cm^3 were collected, centrifuged for 15 min at 12 000 rpm, acidified with 0.1 cm^3 65% HNO_3 and analyzed.

Results and Discussion

Adsorption of metals from single-component solution. The amount of metals adsorbed in batch experiments was calculated from the following equation of mass balance in system with solution volume (V), dry weight of biosorbent (W), initial (C_0) and equilibrium (C) liquid phase concentration:

$$q = \frac{V}{W}(C_0 - C) \tag{1}$$

To describe the equilibrium adsorption capacitiy the Freundlich equation was used. The Freundlich isotherm model assumes heterogeneous site energies and non-limited levels of adsorption.

Freundlich isotherm is expressed by the following equation:

$$q = k \cdot C^{1/n} \tag{2}$$

where:

q – the amount of cadmium or zinc adsorbed per unit of biosorbent weight ($mmol \cdot g^{-1}$),

C – concentration of cadmium or zinc at equilibrium concentration ($mmol \cdot dm^{-3}$),

k, $1/n$ – constants in Freundlich equation.

Recently, a number of authors consider that in case of biosorbents better fitting gives the Freundlich isotherm model than commonly used Langmuir isotherm model. CAY et al. (2004) investigated the adsorption ability of Turkish tea waste (fibrous) obtained from various tea-processing factories for the removal of Cu(II) nad Cd(II) from single and binary aqueous systems. Authors correlated the experimental data by both linearised Langmuir and Freundlich equations. They showed, that the data fitted better the Freundlich isotherm than Langmuir isotherm.

The experimental data and adsorption isotherms given by the Freundlich equation are shown in Figure 1. The values of Freundlich constants, k and $1/n$ indicate the adsorption capacity and adsorption intensity, respectively. The higher value of the exponent $1/n$, the higher affinity and the heterogeneity of the adsorbent sites. The Freundlich model parameters: k and $1/n$ are listed in Table 1. Those parameters were obtained by non-linear regression using APNIELIN software. This is an iteration method requiring an initial approximation. Corrections of determined parameters are calculated by replacing the increment of the function with its total differential, which

allows a linear problem to be solved in each iteration. Additionally, the reduction of the step was applied to improve the convergence, and the Marquardt method to expand the convergence range (BRANDT 1998). The initial approximation mentioned above is to be obtained either by transforming (e.g. by logarithmical operation) the equation describing the model or by simplifying the model itself. Degree of Freundlich isotherm fitting into experimental data was determined statistically (Statistica 7.1) using least squares estimation, assuming significance level $p < 0.05$.

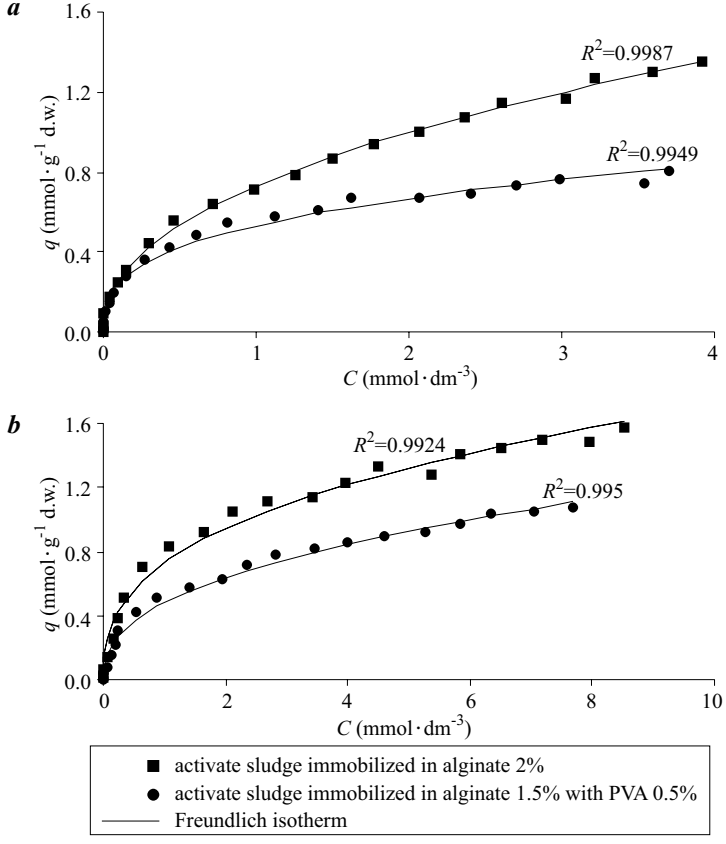

Fig. 1. Mass of cadmium and zinc adsorbed onto activated sludge immobilized in alginate carriers from single metal solutions and Freundlich isotherm: *a* – cadmium, *b* – zinc

From obtained data (Table 1) it results that activated sludge immobilized in alginate 2% shows greater heterogeneity for Cd(II) and Zn(II) than activated sludge immobilized in alginate 1.5% with PVA 0.5%. Higher adsorption

Table 1
Adsorption constants estimated from Freundlich equation for tested biosorbents and single metal solutions (cadmium and zinc)

Biosorbents	Cadmium		Zinc	
	k	$1/n$	k	$1/n$
Activated sludge immobilized in alginate 2%	0.73	0.45	0.73	0.37
Activated sludge immobilized in alginate 1.5% with PVA 0.5%	0.53	0.33	0.48	0.41

capacity (q) for cadmium was observed for activated sludge immobilized in alginate 2%, whereas for activated sludge immobilized in alginate 1.5% with PVA 0.5% there was observed higher q for zinc in comparison with cadmium (Figure 1).

Adsorption of metals from binary mixtures. In present investigations the Freundlich model for two-component isotherm is given as follows:

$$q_1 = \frac{n\left(\frac{k_1}{n_1}\right)^{1/n_1} \cdot C_1}{\left[\left(\frac{k_1}{n_1}\right)^{1/n_1} \cdot C_1 + \left(\frac{k_2}{n_2}\right)^{1/n_2} \cdot C_2\right]^{1-n}} + \Delta F_2 \tag{3}$$

$$\Delta F_2 = (n_1 - n_2) \cdot \frac{\left(\frac{k_1}{n_1}\right)^{1/n_1} \cdot C_1 \cdot \left(\frac{k_2}{n_2}\right)^{1/n_2} \cdot C_2}{\left[\left(\frac{k_1}{n_1}\right)^{1/n_2} \cdot C_1 + \left(\frac{k_2}{n_2}\right)^{1/n_2} \cdot C_2\right]^{2-n}} \cdot \ln\left(\frac{k_1}{n_1}\right)^{1/n_1} \cdot C_1 \cdot \left(\frac{k_2}{n_2}\right)^{1/n_2} \cdot C_2 \tag{4}$$

$$n = \frac{n_1\left(\frac{k_1}{n_1}\right)^{1/n_1} \cdot C_1 + n_2\left(\frac{k_2}{n_2}\right)^{1/n_2} \cdot C_2}{\left(\frac{k_1}{n_1}\right)^{1/n_1} \cdot C_1 + \left(\frac{k_2}{n_2}\right)^{1/n_2} \cdot C_2} \tag{5}$$

where:

q_1 ; q_2 – the amount of cadmium or zinc adsorbed per unit of biosorbent weight ($\text{mmol} \cdot \text{g}^{-1}$),

C_1 ; C_2 – concentration of cadmium or zinc at equilibrium concentration ($\text{mmol} \cdot \text{dm}^{-3}$).

Equation (3) consists of parameters like k_1, n_1, k_2, n_2 estimated from the corresponding individual Freundlich isotherm equations for each metal ion (equation 2). The parameters substituted into equation (3) enable to predict adsorption from the binary systems using the extended Freundlich isotherm.

SRIVASTAVA et al. (2006) investigated competitive adsorption of cadmium and nickel ions onto bagasse fly ash from single components and binary systems. Equilibrium isotherms for the binary adsorption of Cd(II) and Ni(II) ions, the authors analyzed by using non-modified Langmuir, modified Langmuir, extended Langmuir, extended Freundlich and Sheindorf-Rebuhn-Sheintuch models. The research showed that the competitive extended Freundlich model fits the best the binary adsorption equilibrium data.

The relationship between the measured (q_{exp}) and the modified Freundlich model-calculated equilibrium adsorption capacities (q_{cal}) of metal ions onto biosorbent is characterized by linear plot. The slope and intercept straight line indicate compatibility of calculated equilibrium capacities with experimental data. In Figure 2, there were shown calculated (q_{cal}) and measured (q_{exp}) equilibrium capacities from equation 3 for cadmium and zinc as well as straight line indicating the entire competition.

The average percentage errors (ε %) between experimental and calculated from equation 3 values were determined using equation 6, where the subscripts of 'exp' and 'cal' indicate experimental and calculated values, whereas N is the number of measurements (ALIMOHAMADI et al. 2005):

$$\varepsilon\,\% = \frac{\sum_{i=1}^{N} \left| \left(q_{i,\mathrm{exp}} - q_{i,\mathrm{cal}} \right) / q_{i,\mathrm{exp}} \right|}{N} \cdot 100 \tag{6}$$

It was observed, that the entire competition was not stated, what proves high the average percentage errors (8.7-18.5%) – Table 2.

From obtained data, there follows some regularity. For activated sludge immobilized in alginate 2% and cadmium all measured data underlied calculated data, while for zinc they were over them (Figure 2a).

Table 2

The average percentage errors (ε) between the measured equilibrium capacities (q_{exp}) and calculated from two-component Freundlich isotherm (q_{cal}) for tested biosorbents

Biosorbents	ε (%)	
	cadmium	zinc
Activated sludge immobilized in alginate 2%	8.7	12.6
Activated sludge immobilized in alginate 1.5% with APV 0.5%	10.6	18.5

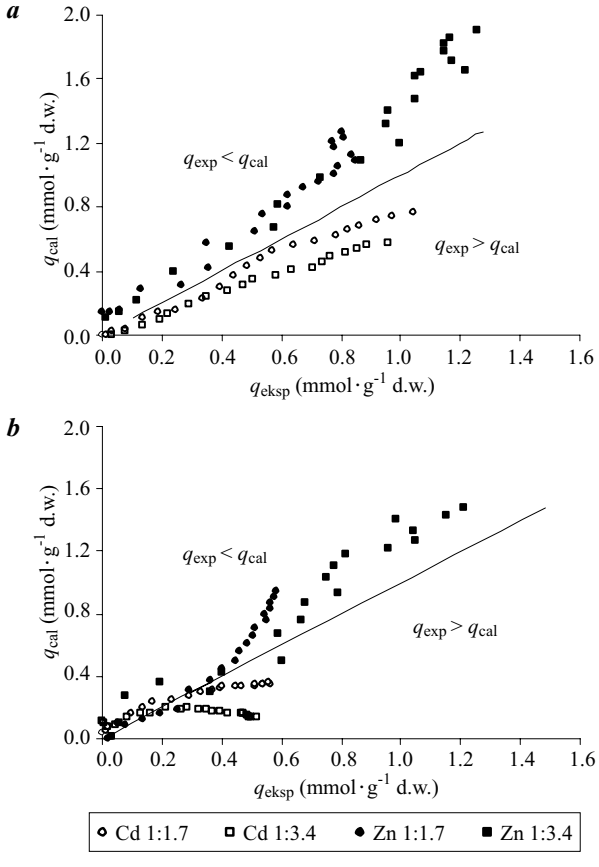

Fig. 2. Mass of cadmium and zinc adsorbed from binary metal mixtures calculated from two component Freundlich equation (q_{cal}) versus of measured equilibrium data (q_{exp}): *a* – activate sludge immobilized in alginate 2%, *b* – activated sludge immobilized in alginate 1.5% with PVA 0.5%

In activated sludge immobilized in alginate 1.5% with PVA 0.5%, this regularity was verified only in specified range of initial metal concentrations (C_0). Cadmium showed lower measured capacities in comparison with calculated ones ($q_{exp} < q_{cal}$) to metal concentrations of 1.33 mmol Cd · dm^{-3} (Cd:Zn 1:1.7) and 0.67 mmol Cd · dm^{-3} (Cd:Zn 1:3.4). However, at concentrations above them $q_{exp} > q_{cal}$ (Figure 2b). For zinc at Cd:Zn 1:1 ratio, $q_{exp} < q_{cal}$ to initial zinc concentrations 1.53 mmol Zn · dm^{-3}, whereas at Cd:Zn 1:3.4 ratio values of q_{exp} were lower in comparison with q_{cal} in the whole concentrations range (Figure 2b).

TOBIN et al. (1988) tested metal uptake by *Rhizopus arrhizus*. In case of Cd(II)-Zn(II) systems they proved direct competition of ions uptake by biosor-

bent sites. However, at saturation below concentration ranges, a certain fraction of binding sites preferentially bounds individual cations. But this regularity was reversed at higher concentration for one of cations.

AKSU and DÖNMEZ (2006) studied the competitive biosorption of cadmium and nickel onto dried *Chlorella vulgaris* from binary metals mixture and the results were compared with single metal ion adsorption. They confirmed that dried *C. vulgaris* was selective for mixed cation components and it had a higher sorption capacity for cadmium than for nickel. Biosorption data in binary systems showed that the adsorbed amount of one metal decreases as the concentration of the other competitive metal simultaneously present in solution increases. This effect was substantial for nickel biosorption that is strongly repressed by increasing cadmium concentration in solution, what suggests that the two metal ions were bound to same sites on the cell.

MCKAY, PORTER (1997) used the competitive Langmuir adsorption isotherm for the sorption of metals ions onto peat. However, the agreement between the experimental data and theoretical prediction was poor. HO, MCKAY (2000) proved that the experimental and calculated values were poor when extended Langmuir equation with a competition term was used to describe data of binary sorption of copper and nickel onto peat. An interaction factor incorporates into the extended Langmuir equation makes a significant improvement in correlation to isotherm equilibrium data.

The research showed, that removal efficiency of both metals (cadmium and zinc) from the binary systems (equation 7), was only negligible lower in comparison with the efficiency of single metals (Figure 3).

$$\eta = \left[\frac{\left(C_{0,1} + C_{0,2}\right) - \left(C_1 + C_2\right)}{C_{0,1} + C_{0,2}} \right] \cdot 100 \tag{7}$$

where:

η – efficiency removal of both cadmium and zinc from the binary systems (%),

$C_{0.1}$; $C_{0.2}$ – initial concentration of cadmium or zinc (mmol · dm^{-3}),

C_1 ; C_2 – concentration of cadmium or zinc at equilibrium concentration (mmol · dm^{-3}).

However, the removal efficiency of each metal from the binary systems (equation 8), was always higher for cadmium than for zinc (Figures 4, 5).

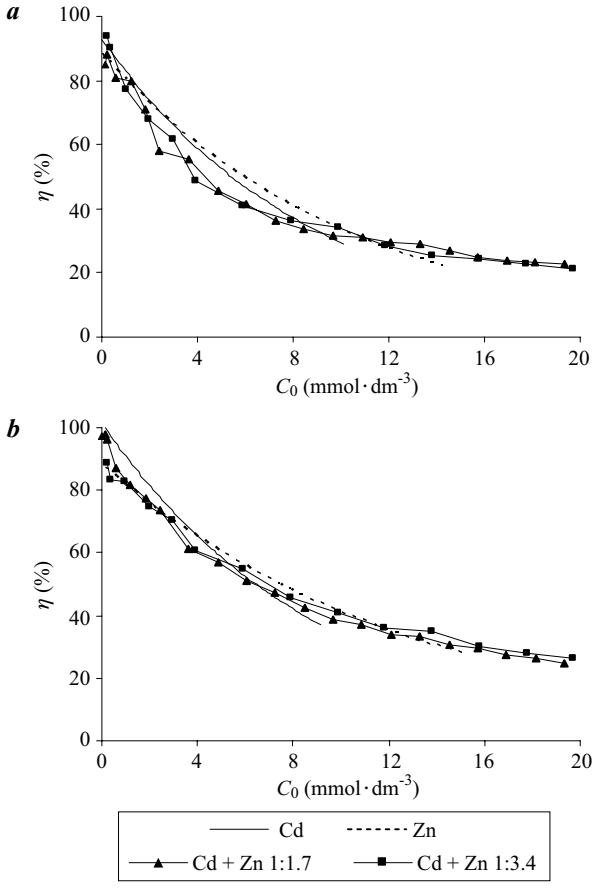

Fig. 3. Removal efficiency of cadmium and zinc from single metal solutions (equation 8) and a sum of metals (equation 7) at ratios Cd:Zn 1:1.7 and 1:3.4: *a* – activated sludge immobilized in alginate 2%, *b* – activated sludge immobilized in alginate 1.5% with PVA 0.5%

$$\eta_1 = \left(\frac{C_{0,1} - C_1}{C_{0,1}} \right) \cdot 100 \tag{8}$$

A mixture of different adsorbates may exhibit synergism (the effect of the mixture is greater than that of each of the individual adsorbates in the mixture), antagonism (the effect of the mixture is less than that of each of the individual adsorbates in the mixture) and noninteraction (the mixture has no effect on the adsorption of each of the adsorbates in the mixture).

From obtained data it results that for both biosorbents and metal concentrations ratios, removal efficiency of cadmium and zinc (sum of metals)

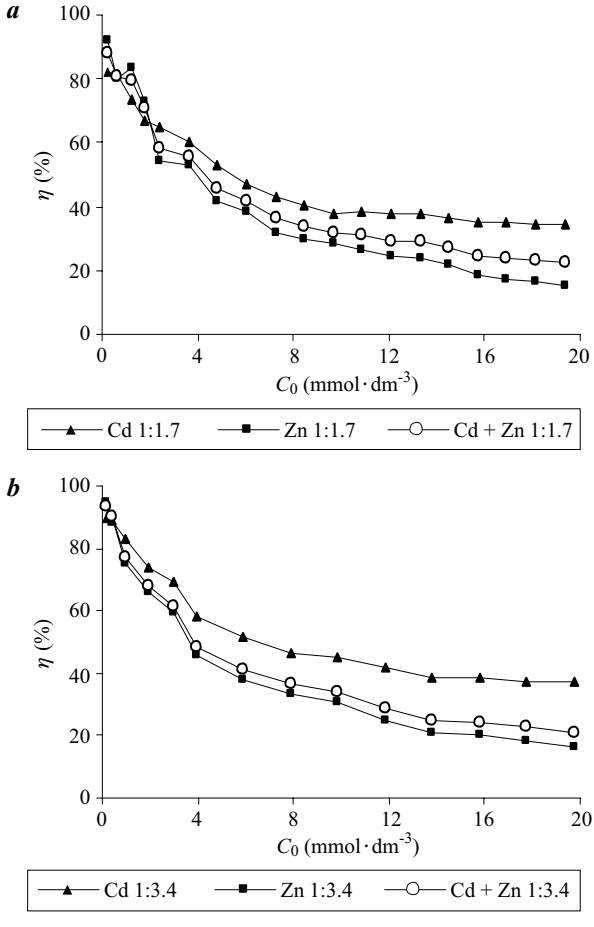

Fig. 4. Removal efficiency of cadmium and zinc from binary metal mixtures by activated sludge immobilized in alginate 2% and sum of metals: *a* – Cd:Zn 1:1.7, *b* – Cd:Zn 1:3.4

from mixture was lower than removal efficiency of single Cd(II) and negligible higher in relation to removal efficiency of single Zn(II) from the mixture. This indicates, that zinc affected inhibitory effect on cadmium uptake. Further, the extent of suppression in cadmium sorption was enhanced according to increasing zinc concentration in the metals mixture.

AL-RUB et al. (2006) investigated competitive biosorption of the binary Cu(II) + Pb(II), Cu(II) + Zn(II) and the ternary Cu(II) + Zn(II) + Pb(II) systems on powdered *Chlorella vulgaris* algal cells. The authors stated, that the presence of lead, zinc or both metals in aqueous solutions suppressed the removal of copper ions. The suppressions in copper uptake in the presence of

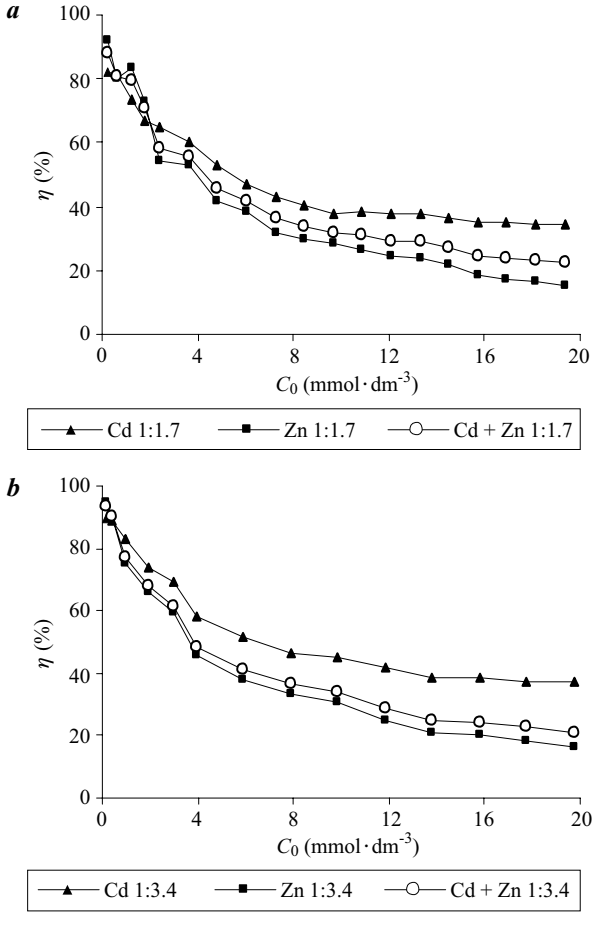

Fig. 5. Removal efficiency of cadmium and zinc from binary metal mixtures by activated sludge immobilized in alginate 1.5% with PVA 0.5% and sum of metals: *a* – Cd:Zn 1:1.7, *b* – Cd:Zn 1:3.4

lead, and lead and zinc were much more significant than those in the presence of zinc ions. In the presence of multimetals in solution, chemical interactions between these metals as well as with the biomass take place, resulting in a competition on the active sites of the sorbent.

LI et al. (2004) investigated the competitive biosorption of Cd(II) and Pb(II) from binary metal solutions by *Phanerochaete chrysosporium*. They found that the interaction between Cd(II) and Pb(II) ions as a result biosorption onto *P. chrysosporium* was generally antagonistic. Pb(II) ions were found to be adsorbed more effectively to *P. chrysosporium* than Cd(II) ions in binary metal solutions.

ALIMOHAMADI et al. (2005) studied the biosorption of Pb(II), Cu(II) ions individually and in binary mixtures by *Rhizopus arrhizus* in batch system. They showed that, the equilibrium uptakes of Pb(II) and Cu(II) in the binary mixture by *R. arrhizus* decreased due to the antagonistic interaction between these components.

Conclusions

In this study, the ability of activated sludge immobilized in alginate 2% and alginate 1.5% with PVA 0.5% to uptake cadmium and zinc in single and binary systems was investigated. The results have been evaluated using the Freundlich model (single systems) and the Freundlich model for two-component isotherm (binary systems). From the research it follows:

1. Activated sludge immobilized in alginate 2% showed higher ability of cadmium uptake than zinc from single metal solutions. It pointed to higher adsorption intensity ($1/n$) for cadmium at comparable k constants. Activated sludge immobilized in alginate 1.5% with PVA 0.5% appeared more efficient in zinc removal (the constant $1/n$ was higher for zinc than cadmium).

2. The usefulness of the Freundlich model for two-component isotherm was verified from relationship $q_{cal} = f(q_{exp})$. It was proved, that the entire competition was not stated. The average percentage errors between measured (q_{exp}) and calculated (q_{cal}) data ranged from 8.7% to 18.5%, depending on Cd:Zn ratio and type of biosorbents.

3. Higher values of q_{exp} for cadmium and lower for zinc in comparison with the calculated equilibrium capacities (q_{cal}) from the two-component Freundlich isotherm for activated sludge immobilized in alginate 2% were obtained. In activated sludge immobilized in alginate 1.5% with PVA 0.5% q_{exp} was higher than q_{cal} for cadmium at initial metal concentrations above 1.33 mmol Cd $\cdot$ dm^{-3} (Cd:Zn 1:1.7) and 0.67 mmol Cd $\cdot$ dm^{-3} (Cd:Zn 1:3.4).

4. The efficiency of removal both tested metals (as a sum) was lower than single cadmium and negligible higher than single zinc from the binary metal mixtures.

5. Owing to potential possibility of application of this process engineering type for industrial wastewater treatment, the activated sludge immobilized in alginate 2% seems more favourable, because of its higher ability of cadmium uptake. The admissible cadmium concentration amounts 0.4 mg Cd $\cdot$ dm^{-3} in treated wastewater and it is 5-fold lower than zinc concentration.

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SURVIVAL OF *PSEUDOMONAS FLUORESCENS* IN CARBONATED AND NONCARBONATED MINERAL WATER

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K e y w o r d s: mineral water, *Ps. fluorescens*, survival.

A b s t r a c t

The study was carried out on the survival of *Pseudomonas fluorescens* in samples of carbonated and noncarbonated mineral waters. Enumeration of the bacteria was performed by spread inoculation of samples (0.1 cm^3) over the surface of selected medium in Petri plates. Sixteen bottles (two bottles of carbonated and two bottles of noncarbonated mineral waters each of the four brands) of mineral waters with different levels of dissolved solids and organic content were chosen to study. Eight bottles of mineral water were stored at 4°C, the other eight were kept at 22°C. The resulting growth curves of *Ps. fluorescens* depended on the time of mineral water storage. The number of *Ps. fluorescens* was at the same level during the first week and decreased during the next days. *Ps. fluorescens* was detected in 88% of water samples after 193 days of storage. The temperature of storage was inessential for growth. The most important factors were the time of storage and the carbonating or uncarbonating of water. The highest numbers of the bacteria analysed were detected in noncarbonated water, irrespective of the water brand and temperature of storage.

PRZEŻYWALNOŚĆ *PSEUDOMONAS FLUORESCENS* W MINERALNEJ WODZIE GAZOWANEJ I NIEGAZOWANEJ

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S ł o w a k l u c z o w e: woda mineralna, *Ps. fluorescens*, przeżywalność.

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Abstrakt

Badano przeżywalność bakterii *Pseudomonas fluorescens* w próbkach wody mineralnej gazowanej i niegazowanej, przechowując ją w różnych warunkach temperaturowych ponad pół roku. W celu oznaczenia liczebności bakterii badaną wodę (0,1 cm^3) wysiewano na powierzchni podłoża różnicującego (Kinga B) w płytkach Petriego. Ogółem przebadano 16 butelek wody mineralnej (po 2 butelki z 4 gatunków wody gazowanej i niegazowanej), zróżnicowanej pod względem zawartości związków mineralnych. Osiem butelek prób wody mineralnej przechowywano w temp. 4°C, kolejne 8 w temp. 22°C. Przeżywalność bakterii *Ps. fluorescens* w głównej mierze zależała od czasu przechowywania wody mineralnej. Liczba *Ps. fluorescens* była wyrównana w pierwszym tygodniu jej przechowywania i malała w kolejnych dniach trwania eksperymentu. *Ps. fluorescens* wykrywano w 88% prób wody po 193 dniach jej przechowywania. Istotnym statystycznie czynnikiem wpływającym na przeżywalność tych bakterii był również stopień nasycenia dwutlenkiem węgla. Najwięcej tych bakterii stwierdzano w wodzie niegazowanej, niezależnie od gatunku wody i temperatury jej przechowywania.

Introduction

In recent years, there has been a considerable increase in consumption of bottled water in the world, and this trend is expected to continue. Moreover, uncarbonated water is now considerably more popular than carbonated, having become a substitute for tap water in some households. This reflects consumer concerns about tap water, since bottled water is often regarded as safer and healthier than tap water. *Allochthonous* bacteria, which are contaminants, usually enter water during bottling. Some studies have shown that populations of these bacteria increase to approximately 10^3-10^6 cfu $\cdot$ cm^{-3} after bottling (HUNTER 1993). Their survival in bottled water is generally poor because of a low nutrient concentration in the water (LECLERC, MOREAU 2002).

Pseudomonas fluorescens is a member of the fluorescent pseudomonad group and commonly colonise soil, water and plants, and is well described as food and milk contaminants (MAHON, MANUSELIS 2000). Contamination by *Ps. fluorescens* is of particular concern in the food industry, since as a psychro-phile it has the capacity to secrete, at low temperatures, a large range of heat-stable proteases and lipases (BURINI et al. 1994). Unlike the well-characterised opportunistic pathogen *Ps. aeruginosa, Ps. fluorescens* has been generally considered as presenting low-level virulence potential and, therefore, of being of little clinical significance. Nevertheless, strains of *Ps. fluorescens* have increasingly been identified as contaminants of human skin and as the causative agents of pseudobacteremia and procedure-related infections in hospital patients (ANDERSON, DAVEY 1994). *Ps. fluorescens* is capable of adhering to the human extracellular matrix protein fibronectin and to A549 pneumocyte cells (MARTINO 2001, MARTINO et al. 2000), consistent with its involvement in nosocomial respiratory infections. Moreover,

recent reports on the ability of *Ps. fluorescens* to adhere and cause alterations to human nerve cells stress the potential of this bacterium as a human pathogen (PICOT et al. 2001).

Because drinking untreated water could be identified as a significant risk factor for *Ps. fluorescens* diseases, it is very important to investigate the survival of these bacteria in the water during storage at different conditions.

Materials and Methods

Bottled water Two bottles (1.5 dm^3), all with the same expiry date, of each of the four brands of carbonated and the same non-carbonated mineral waters in polyethylene terephthalate bottles (PET) with different levels of dissolved solids and organic content (Table 1) were purchased directly from manufacturers.

Table 1
Analytical characteristics of four brands of bottled water (information on label of bottle $mg \cdot dm^{-3}$)

Brand		Saturation CO_2	Total mineral components	HCO_3^-	SO_4^{2-}	Cl^-	F^-	SiO_2	Ca^{2+}	Mg^{2+}	Na^+	K^+
N	C*	6000	700	483.6	–	32	7.1	32	111.9	23.3	12.5	5.1
	N**	–	700	483.6	–	7.1	0.24	32	111.9	23.3	12.5	5.1
Ż	C	6000	309.98	200.4	–	–	–	–	61.24	6.9	7.23	–
	N	–	185.84	109	–	4.6	0.07	–	27.73	8.18	8	–
M	C	6000	2070.6	1525.5	21.2	14.2	–	–	212.8	85.1	171	13.3
	N	–	644.5	439.3	31.6	5.3	–	–	121.4	21.4	3.3	1.3
T	C	4000	263	159.87	–	7.09	0.19	–	43.61	5.83	8	1.6
	N	–	263	159.87	10	7.09	0.19	–	43.61	5.83	8	1.6

* – carbonated water; ** – non–carbonated water

Sampling Genetically-marked *Ps. fluorescens* isolated from potable water in our laboratory were used in this study. *Ps. fluorescens* was inoculated into samples of mineral water at a density of $7.8 \cdot 10^4$ cfu $\cdot$ cm^{-3}. The density of final suspension used for inoculation was measured with quantitative method on the surface of selected medium (viable counts). Four bottles of each brand of water (two bottles of carbonated and two bottles of non-carbonated water) were inoculated with *Ps. fluorescens*. Two bottles (one bottle of carbonated water and one bottle of non-carbonated water) of each type of water inoculated with *Ps. fluorescens* were stored for over six months (193 days) at 4°C,

the other samples were stored at 22°C. Viable counting of tested bacteria was repeated after 2, 7, 21, 35 and 193 days of storage.

Microbiological analyses Total viable count of heterotrophic bacteria at 22 and 37°C in 1 cm^3 (pour plate technique) and presence of *Ps. fluorescens* in 250 cm^3 (membrane filter technique) of each sample of raw mineral water were tested first (KORZENIEWSKA at al. 2005a).

Microbiological analyses involved determination of *Ps. fluorescens* count in 1 cm^3 of mineral water on an King B medium (BURBIANKA, PLISZKA 1983) after 48 and 72 hours incubation at 25°C.

Microbial numbers were estimated by decimal dilution in 1/4 strength Ringer's solution. The water samples were inoculated on the surface of selected medium in Petri plates and incubated at a specified temperature for the desired period of time. After the incubation typical colonies were counted. The occurrence of *Ps. fluorescens* was verified under the light of a Wood UV lamp; colonies which produced fluorescein were counted.

If no colonies were recovered with the surface plating technique, pour plates with 1 cm^3 of water sample were prepared with the same selective medium.

Statistical evaluation In order to obtain information concerning potential differences between bacteria numbers for various brands of water, for carbonated/noncarbonated water, for water stored at different time and temperature, a single factor analysis of variance (ANOVA) was conducted, verifying the hypothesis of the equality of means ($H_0 : x_1 = x_2 = ... = x_5$) at the level of significance $\alpha = 0.05$, assuming that the variance for the numerousness of the bacteria groups under study are uniform. The uniformity of variance was tested with Levene's test. If the test proved significant, the hypothesis was rejected. Next, the Kruskal-Wallis test was applied, which is a non-parametric equivalent of the analysis of variance (STANISZ 2006). Estimation by Spearman' correlation between numbers of studied microorganisms received during whole time of water storage and some chemical compounds in mineral water were used in this study too.

Results

Bacterial numbers The number of *Ps. fluorescens* in 250 cm^3 of raw mineral water was unessential (it was detected only in brand T – 5 cfu in 250 cm^3). Total viable count of heterotrophic bacteria at 22 and 37°C in 1 cm^3 of raw mineral water was the highest for brands T and M and the lowest for brand Ż (detailed results are available in KORZENIEWSKA at al. 2005a).

The viable counts of *Ps. fluorescens* inoculated into bottled mineral water

increased at both temperatures of storage (4 and 22°C) for non-carbonated mineral water and carbonated mineral water stored at 4°C. It decreased for carbonated mineral water stored at room temperature. These bacteria were typically more numerous in noncarbonated water samples stored at both temperatures (Figure 1). In our experiment the number of *Ps. fluorescens* in mineral water stored at room temperature increased during the first week, decreased during the next days (Figures 1) and were detected in 75% of water samples after 193 days of storage. The number of *Ps. fluorescens* in mineral water stored at 4°C increased only during the first two days, decreased during the next days (Figure 1) and was detected in 100% of water samples after whole time of storage.

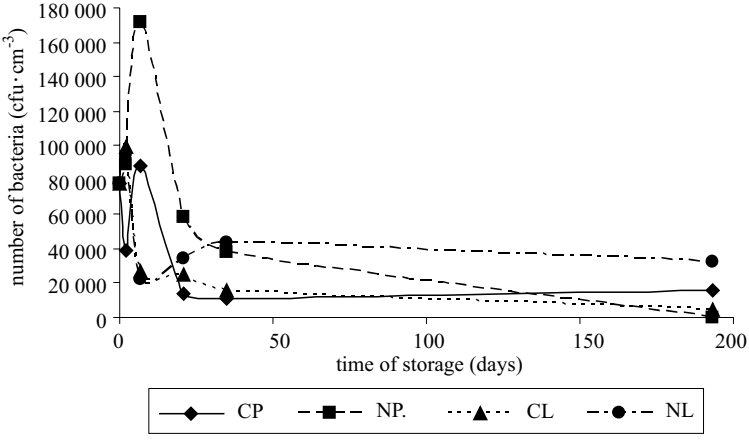

Fig. 1. Survival of *Ps. fluorescens* in carbonated and non-carbonated mineral water during over half-year storage in 4 and 22°C: CP – carbonated water stored at 22°C, NP – non-carbonated water stored at 22°C, CL – carbonated water stored at 4°C, NL – non-carbonated water stored at 4°C

The survival of the bacteria examined in mineral water was the highest in Ż mineral water, which contained low level of dissolved solids and organic content and the lowest microbial contamination. The survival of these bacteria in mineral water was the lowest (except of seven day) in T mineral water, which contained the lowest levels of dissolved solids and organic content and the highest microbial contamination. Enumeration of *Ps. fluorescens* recovered from Ż and T water decreased respectively to $5 \cdot 10^4$ and 10^3 cfu in 1 cm^3 after 193 days of storage (Figure 2).

Statistical evaluation This paper presents only the general statistical relationships; the detailed results can be obtained from the authors.

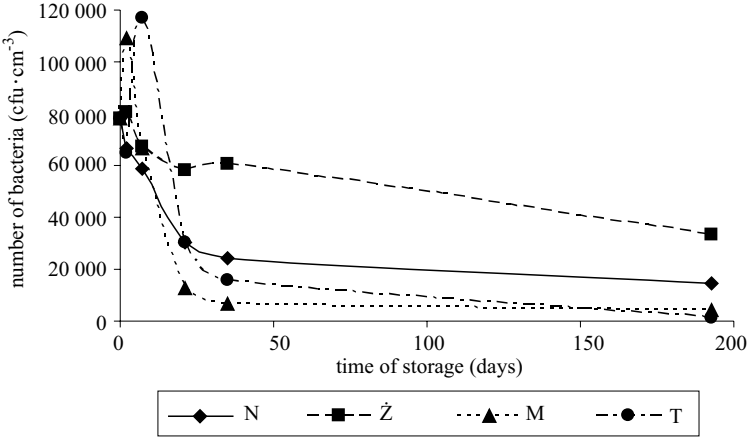

Fig. 2. Survival of *Ps. fluorescens* in different brands of carbonated and noncarbonated mineral water (as in Table 1) during over half-year storage after inoculation

There were significant differences between the number of tested microorganisms initially presented in the water and those after storage for over 6 months (p = 0.000) The mean numbers of the tested bacteria were the highest in water after two days of storage (Figure 3).

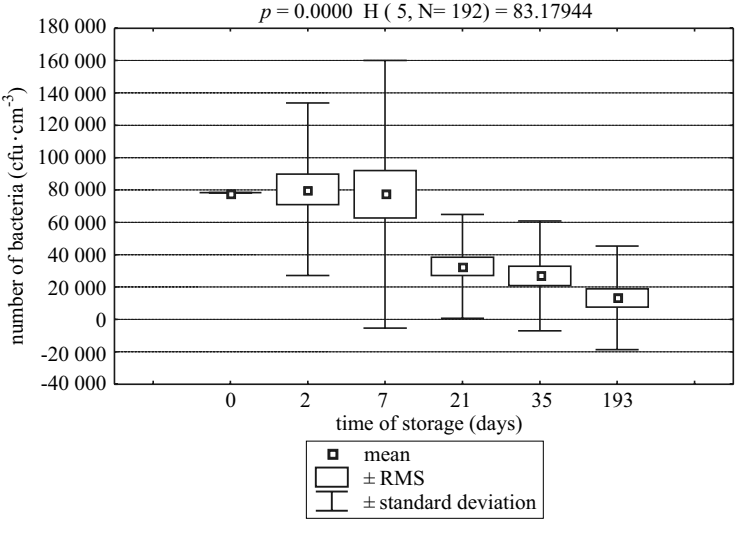

Fig. 3. Averages numbers (± standard deviation and ± random mean square-RMS) of *Ps. fluorescens* inoculated into mineral water during storage – total viable counts (cfu · cm^{-3}). Independent variable (assembling): time. ANOVA test of Kruskal-Wallisa ranges

There were significant differences between the numbers of studied microorganisms recovered from the carbonated and noncarbonated water (p = 0.0134). The mean numbers of the tested bacteria were the highest in noncarbonated water stored at 22°C and 4°C (Figure 4).

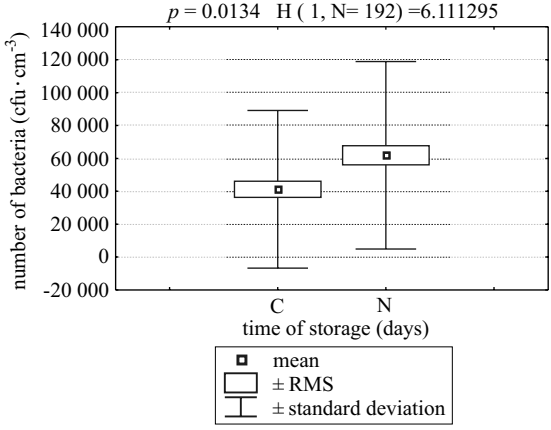

Fig. 4. Averages numbers (± standard deviation and ± random mean square-RMS) of *Ps. fluorescens* inoculated into carbonated/non-carbonated mineral water during over half-year storage at 4 and 22°C – total viable counts (cfu · cm^{-3}). Independent variable (assembling): carbonated/non-carbonated water. ANOVA test of Kruskal-Wallis ranges

Table 2

Statistic estimation by Spearman' correlation between numbers (cfu · cm^{-3}) of studied microorganisms received during whole time of water storage and some chemical compounds (mg · dm^{-3}) in mineral water. BD eliminated in couple. Important correlations (p < 0.05000) marked

Variable	*Ps. fluorescens*
CO_2	*-0.182420*
Total mineral components	-0.129932
HCO_3^-	-0.129932
SO_4^{2-}	-0.089431
Cl^-	-0.149731
F^-	*-0.216409*
Ca^{2+}	*-0.148364*
Mg^{2+}	-0.017569
Na^+	0.032493
K^+	0.010426

There were no significant differences between the numbers of *Ps. fluorescens* recovered from the different brands of bottled water, but the mean numerousness of the tested bacteria was the highest in Ż brand.

There were no significant differences between the numbers of tested microorganisms recovered from the water stored at temperatures 4 and 22°C, but the mean numerousness of the tested bacteria was the highest in water stored at temperatures 22°C.

According statistic estimation by Spearman' correlation the iron and calcium ions and carbon dioxide contents were correlated negative (statistically significant) with number of *Ps. fluorescens* (Table 2).

Discussion

Microbes have evolved longer than any other living organisms, so in all probability, the non-spore-forming heterotrophic bacteria must have developed mechanisms to survive long periods when no energy or nutrients were available (LECLERC, MOREAU 2002). MORITA (1997) described four pattern of starvation-survival. The most frequently pattern noted which might be representative for most environmental bacteria shows an initial increase in cell number due to fragmentation followed by a decline. The starvation pattern with time occurs in three stages. During the first stage lasting 14 days, large fluctuations in plate counts were noted. In the second stage (14-70 days) the colony count decreased by 99.7%. The third stage was marked by a stabilization of viable cells. Microorganisms in bottled water may also multiply and exceed 10^5 cfu $\cdot$ cm^{-3} after storage (HUNTER et. al. 1990). The number of *Ps. fluorescens* was at the same level during the first week and decreased during the next days and was detected in 88% of water samples after 193 days of storage. Usually the maximum bacterial density was observed in samples stored at room temperature. Nevertheless, storage at low temperatures, such as that of refrigeration, does not stop bacterial multiplication. There were no statistically significant differences between the numbers of tested microorganisms recovered from the water stored at temperatures 4 and 22°C. The same results received LECLERC and da COSTA (1998) in their studies of natural mineral waters and BHARATH et al. (2003) who did not find statistically significant differences between prevalence of aerobic bacteria in bottled water stored at room and refrigeration temperature (respectively 25 and 4°C) and air-conditioned environments (18°C).

According statistic estimation by Spearman; correlation the carbon dioxide content was correlated negative with number of *Ps. fluorescens*. Carbonation is known to decrease pH of the water and in turn have a bactericidal effect on the aerobic bacteria (CAROLI 1985).

The survival of *Ps. fluorescens* in mineral water depended on the number of autochtonous microorganisms in the water samples and was the highest in water which contained the lowest microbial contamination. RAMALHO et al. (2001) and KORZENIEWSKA at al. (2005b) implied that autochthonous bacteria of mineral water have been reported to have an inhibitory effect on the survival of *E. coli* and other pathogenic bacteria. The densities of existing (autochtonous) microbiota from nutrient poor waters significantly affect the survival capacity of *Ps. fluorescens*. Relatively low densities of these bacteria found in some types of water are as result of population control by external factors, which may inhibit bacterial population.

Conclusions

1. The growth of *Ps. fluorescens* inoculated into bottled mineral water depended on the time of storage and decreased during storage.

2. The carbonating or noncarbonating of water was the most important factor for the growth of microorganisms.

3. The kind of brand and temperature of storage of mineral water were inessential for survival of *Ps. fluorescens*.

4. The survival of *Ps. fluorescens* in mineral water depended on the number of autochtonous microorganisms in the water samples and was the highest in water which contained the lowest microbial contamination.

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CADMIUM AND ZINC REMOVAL IN THE BINARY SYSTEMS BY IMMOBILIZED ACTIVATED SLUDGE IN MULTIPLE CYCLES OF ADSORPTION/DESORPTION

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K e y w o r d s: cadmium, zinc, adsorption/desorption cycles, immobilized activated sludge, alginate, chitin.

A b s t r a c t

The multiple cadmium and zinc adsorption/desorption in binary mixture by immobilized activated sludge was investigated. Concentration of metals in adsorbate was 10 mg Cd · dm^{-3} and 10 mg Zn · dm^{-3} (1:1 ratio) and 10 mg Cd · dm^{-3} and 20 mg Zn · dm^{-3} (1:2 ratio). Polymer matrices such as sodium alginate, mixture of sodium alginate with PVA and chitin with PVA were used. As desorbing agent 0.1 M H_2SO_4 was employed.

It was observed that metals adsorption diminished with consecutive cycles in comparison with adsorption in the first cycle. The numbers of adsorption/desorption cycles decreased in order: 2% chitin + 0.5% PVA (17 cycles) > 1.5% alginate + 0.5% PVA (7 cycles) > 2% alginate (5 cycles). The cumulative amount of metals adsorbed was the highest for activated sludge immobilized in 2% chitin + 0.5% PVA (28.5 mg metals · g^{-1} d.w.), when metals ratio in adsorbate was 1:1 and for 1.5% alginate + 0.5% PVA (44.45 mg metals · g^{-1} d.w.), when Cd:Zn = 1:2.

Almost complete recovery of the metals was obtained for cadmium (94-99%) and for zinc (97-99%) from alginate biosorbents. The effectiveness of metals desorption from metal-laden activated sludge immobilized in 2% chitin + 0.5% PVA was low and changing from 64% to 69% for cadmium and 72% to 75% for zinc.

Percentage of metals bound in activated sludge immobilized in 2% alginate and released to eluate was similar as metals ratio in adsorbate. However, for activated sludge immobilized in 1.5% alginate + 0.5% PVA and especially for 2% chitin + 0.5% PVA, the percentage of cadmium bound in biosorbent and released to eluate was clearly higher than percentage in adsorbate, what indicates preferences of its uptake in comparison with zinc.

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USUWANIE KADMU I CYNKU Z ROZTWORÓW WODNYCH ZAWIERAJĄCYCH ICH MIESZANINĘ PRZEZ IMMOBILIZOWANY OSAD CZYNNY W CYKLICZNEJ ADSORPCJI/DESORPCJI

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S ł o w a k l u c z o w e: kadm, cynk, cykliczna adsorpcja/desorpcja, osad czynny immobilizowany, alginian, chityna.

A b s t r a k t

Badano cykliczną adsorpcję/desorpcję kadmu i cynku z roztworów zawierających ich mieszaninę. Jako sorbentów użyto immobilizowanego osadu czynnego. Badano roztwory zawierające mieszaninę metali w stężeniu 10 mg Cd · dm^{-3} i 10 mg Zn · dm^{-3} (proporcja 1:1) oraz 10 mg Cd · dm^{-3} i 20 mg Zn · dm^{-3} (proporcja 1:2). Do immobilizacji osadu czynnego wykorzystano następujące nośniki: alginian sodu, mieszaninę alginianu sodu i alkoholu poliwinylowego (APV) oraz mieszaninę chityny i alkoholu poliwinylowego. Czynnikiem desorbującym był 0,1 M H_2SO_4.

Wykazano, że efektywność adsorpcji metali malała w kolejnych cyklach w porównaniu z cyklem pierwszym. Liczba cykli adsorpcji/desorpcji malała w szeregu: chityna 2% + APV 0,5% (17 cykli) > alginian 1,5% + APV 0,5% (7 cykli) > alginian 2% (5 cykli). Największą kumulację metali po adsorpcji stwierdzono w przypadku osadu czynnego immobilizowanego w chitynie 2% + APV 0,5%, gdy Cd:Zn = 1:1, oraz w przypadku osadu czynnego immobilizowanego w alginianie 1,5% + APV 0,5%, gdy Cd:Zn = 1:2. Prawie całkowity odzysk zaadsorbowanego kadmu (94-99%) i cynku (97-99%) uzyskano, stosując biosorbenty alginianowe. Efektywność desorpcji kadmu i cynku z osadu czynnego immobilizowanego w chitynie 2% + 0,5 APV była niska, i zmieniała się odpowiednio od 64% do 69% i od 72% do 75%.

Procent metali związanych w osadzie czynnym immobilizowanym w alginianie 2% i uwolnionych do eluatu był podobny do ich proporcji wagowej w adsorbacie. Jednak w osadzie czynnym immobilizowanym w alginianie 1,5% + APV 0,5%, zwłaszcza w osadzie immobilizowanym w chitynie 2% + 0,5% APV, procent kadmu związanego w biosorbencie i uwolnionego do eluatu był wyraźnie wyższy niż jego procent w adsorbacie, co wskazuje na preferencyjne wiązanie kadmu, w porównaniu z cynkiem.

Introduction

Biosorption is a process that utilizes inexpensive dead biomass to sequester toxic heavy metals and in particularly useful for the removal of contaminants from industrial effluents (KRATOCHVIL, VOLESKY 1998). Many industries, including metal plating, mining and battery manufacturing contain heavy metals. Wastewater from metal finishing plants contain: 10-100 mg Zn · dm^{-3}, 10-50 mg Cu · dm^{-3}, 10-50 mg Ni · dm^{-3}, 5-30 mg Cd · dm^{-3} and 20-200 mg Cr · · dm^{-3} (ŁEBKOWSKA, KARWOWSKA 2003).

Biosorbents are prepared from waste biomass or bacteria. Some type of biomass are pretreated by acid or base washing, drying and granulating.

Another way is biomass immobilization in a synthetic polymer matrix (VOLESKY 2001).

The economical feasibility of biosorption methods depends on the biosorbent capacity to reach metal concentration in legal limits for wastewater treatment, the biosorbents stability and the ability of desorbing agents to release sequestered metal in subsequent recovery. Regeneration of the biosorbents increases the process economy by allowing their reusability in multiple adsorption/desorption cycles. The process of multiply sorption/desorption enables obtaining of effluents containing concentrated metal, which can be easily recovered.

The efficiency of multiple adsorption and desorption processes depends on the metal species, type and concentration of desorbing agent and operational conditions such solid/liquid (S/L) or reaction time. Lowering of pH values causes metals desorption when mineral or organic acids are used. Cu, Zn, Cd or Pb can be easily recovered from biosorbents so that acids can be used as desorbing agent (WILHELMI, DUNCAN 1995, CHU et al. 1997, JEON et al. 2002, GONG et al. 2005). Data for chromium desorption carried out in similar conditions indicate much lower recoveries (FERRAZ et al. 2004, IQBAL et al. 2002). Among the chemicals, strong chelating agents as EDTA and NTA were also successfully tested (JEON et al. 2002, GONG et al. 2005, JEON et al. 2005). However, these agents cause reduction of metal uptake in subsequent cycles of adsorption/desorption. BAI and ABRAHAM (2003) investigated biosorption of Cr(VI) onto immobilized *Rhizopus nigricans*. The biomass was immobilized by various mechanisms using five different polymeric matrices: calcium alginate, polyvinyl alcohol, polyacrylamide, polyisoprene and polysulfone. Biosorption efficiency and mechanical stability to desorbents were compared for the matrices. The authors showed that polysulfone was the most suitable whereas polyacrylamide the least for the Cr(VI) biosorption.

For the industrial application of biosorption technology the efficiency of multiple adsorption/desorption in real wastewater is a key problem. Therefore, some important factors including ionic strength or concentration of organic matter on sorption and desorption efficiency were studied (JEON et al. 2005). Another problem of wastewater treatment is the fact that they commonly contain not single but metals mixture. The adsorption/desorption of two or more metals in adsorbate has not properly investigated till now.

The aim of this study the multiple cadmium and zinc adsorption/desorption in binary mixture by immobilized activated sludge was investigated. It was assumed, that metals ratio was 1:1 and 1:2, what corresponded to metals ratio in metal finishing plants wastewater. Different polymer matrices as sodium alginate, mixtures of sodium alginate with PVA and chitin with PVA were used. The adsorption and desorption efficiency of each tested metals was

investigated in consecutive cycles. Proportion of cadmium and zinc in the biosorbent and eluate was determined.

Materials and Methods

Activated sludge

Activated sludge was obtained from standardized culture according to PN--87/C-04616/10. The powdered biomass of activated sludge was prepared as follows: the activated sludge was separated by sedimentation and centrifugation, the biomass was rinsed with acetone twice, again centrifuged and left in water bath at 50°C for 48 h. Dry sludge was grinded in agate mill, next sieved to 0.1 mm fraction sizes. Activated sludge prepared in this way was stored for immobilization purpose.

Biosorbents preparation

Preparation of immobilized activated sludge – activated sludge immobilized in alginate. Weighted portion of 2 g activated sludge and 2 g of sodium alginate (medium viscosity – produced by Sigma) was dissolved in 96 g deionized water, led to formation of homogenous suspension, and then dropped to 0.05 M $CaCl_2$ solution. The beads were 2.8 mm in diameter. Next, they were left to gel forming for 24 h, and rinsed with deionized water until chloride was not detected.

Preparation of immobilized activated sludge - activated sludge immobilized in mixture of alginate and polyvinyl alcohol (PVA). Weighted portion of 2 g activated sludge and 1.5 g of sodium alginate with 0.5 g PVA mixed and dissolved in 96 g deionized water. Homogenous suspension was dropped into 0.05 M $CaCl_2$ dissolved in saturated boric acid solution. Addition of boric acid was essential to gel forming of PVA. The beads were 2.8 mm in diameter. Next, they were left to gel forming for 24 h, and rinsed with deionized water until chloride was not detected.

Preparation of immobilized activated sludge- activated sludge immobilized in mixture of chitin and polyvinyl alcohol (PVA). Weighted portion of 2 g chitin was dissolved in 48 g of 5% CH_3COOH, weighted portion of 0.5 g PVA was dissolved in 24.5 g deionized water, and 2 g of sludge was mixed in 23 g of deionized water. After that, all ingredients were mixed, led to homogenous mixture, and dropped into 5% NaOH solution. The beads were about 3.0 mm in diameter. Next, they were left to gel forming for 24 h. After that the biosorbent was rinsed with deionized water until a stable pH 7.0 was reached.

Metals solutions

$3CdSO_4 \cdot 8H_2O$ and $ZnSO_4 \cdot 7H_2O$ metals salts were used in aqueous solution preparation. The stock solutions were prepared in concentration of 20 g $Cd \cdot dm^{-3}$ and 40 g $Zn \cdot dm^{-3}$. The mixtures were prepared in concentration of 10 mg $Cd \cdot dm^{-3}$ and 10 mg $Zn \cdot dm^{-3}$ (Cd:Zn ratio = 1:1) and 10 mg $Cd \cdot dm^{-3}$ and 20 mg $Zn \cdot dm^{-3}$ (Cd:Zn ratio = 1:2). Cd and Zn solution was adjusted to pH 7.0 using 1 M NH_4OH with prior to mixing with the biosorbents.

Adsorption/desorption experiments

2 g of biosorbent was pleaced into reaction vessel (500 cm^3 volume), next 250 cm^3 of metals mixture solution in 1:1 or 1:2 ratio was added and then mixed with the magnetic stirrer. Adsorption time was 60 minutes for alginate biosorbents and 6 h for chitin biosorbent at stable value of pH 7.0, kept by 1 M NH_4OH. After sorption experiment measurements of metals concentration in the solution were carried out. Sulfuric acid solution was used as a desorbing agent at concentration of 0.1 M. Desorption time was 60 min. After being gently agitated, the biosorbent suspension was separated, and measurements of metal concentration in eluate were carried out.

In the next stage, biosorbents were rinsed with deionised water to remove residual metals. After rinsing, water excess was removed. The regenerated biosorbents were again suspended in metal-containing solutions for the next adsorption run. Before each subsequent adsorption/desorption cycle the biosorbents were weighed in order to determine the loss of biomass weight. Above procedure was employed for consecutive cycles until biosorbents destruction. All experiments were carried out in triplicate.

Metals concentration analysis

Adsorption/desorption of cadmium and zinc were analyzed by measurements of residual metals concentration in aqueous solution using the atomic absorption method SpectrAA, Varian. 10 cm^3 of each sample was centrifuged for 15 min at 12 000 rpm. The cadmium and zinc concentrations were read out from the standard curve.

Results

The reusability of activated sludge immobilized in alginate and alginate or chitin with PVA was tested in multiple adsorption/desorption cycles of cadmium and zinc from binary mixture. Adsorption of each metals in consecutive cycles and desorption from loaded metals biosorbents were calculated according to Eqs. 1-3:

$$q_{\text{ads}} = \frac{(C_{0\text{i}} - C_{e_i}) \cdot V}{m} \tag{1}$$

$$q_{\text{des}} = \frac{C_{d_i} \cdot V_{\text{el}}}{m} \tag{2}$$

$$q_{\text{ac}_{\text{i}+1}} = q_{\text{ads}_\text{i}} - q_{\text{des}_\text{i}} \tag{3}$$

where:

q_{ads} – metals uptake ($\text{mg} \cdot \text{g}^{-1}$),
C_0 – initial metals concentration ($\text{mg} \cdot \text{dm}^{-3}$),
C_e – final metals concentration after adsorption ($\text{mg} \cdot \text{dm}^{-3}$),
V – solution volume (dm^3),
m – dry weight of biosorbent (g),
q_{des} – metals desorption ($\text{mg} \cdot \text{g}^{-1}$),
C_d – final metals concentration in eluate ($\text{mg} \cdot \text{dm}^{-3}$),
V_{el} – eluate volume (dm^3),
q_{ac} – metals accumulation in biosorbent ($\text{mg} \cdot \text{g}^{-1}$).

Figures 1a-c show the experimental results of cadmium adsorption, desorption and accumulation from binary cadmium and zinc mixture (at a 1:1 ratio in adsorbate) obtained for activated sludge immobilized in: 2% alginate, 1.5% alginate + 0.5% PVA and 2% chitin + 0.5% PVA. From experimental data it results that a limiting factor for alginate biosorbents appeared their low stability (for activated sludge immobilized in 2% alginate – five cycles and 1.5% alginate + 0.5% PVA – seven cycles, respectively), whereas the stability of chitin carrier were higher, but the process proceeded with metals accumulation. Analogical results were obtained for zinc.

Adsorption efficiency. Efficiency of metals adsorption in multiple cycles was estimated by comparison of metals uptake in subsequent cycles with metal uptake by the original adsorbent in the first cycle. This parameter, named reloading efficiency, allows to assess whether metals uptake capacity of the adsorbent remains unchanged in successive cycles (HASHIM et al. 2000).

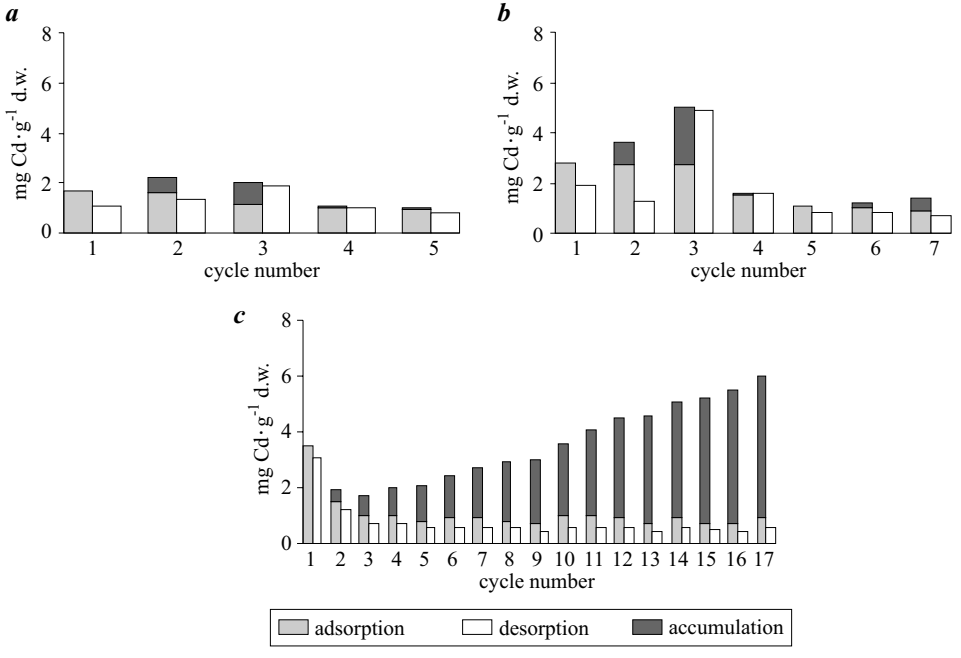

Fig. 1. Amount of adsorbed, desorbed and accumulated cadmium: *a* – activated sludge immobilized in 2% alginate, *b* – activated sludge immobilized in 1.5% alginate + 0.5% PVA, *c* – activated sludge immobilized in 2% chitin + 0.5% PVA

The own data clearly indicate that great efficiency of alginate biosorbents was obtained only in a two or three cycles and later gradual decrease in metals uptake with increasing number of cycles (Figures 2a,b,c,d). Reolading efficiency was higher at 1:2 metals ratio in adsorbate than at 1:1 ratio. Particularly significant decrease of reloading efficiency to approximately 33.4% for Cd and 16.9% for Zn in the last cycle was stated for metals mixture in adsorbate at a 1:1 ratio and for activated sludge immobilized in 1.5% alginate + 0.5% PVA (Figures 2c).

For tested alginate biosorbents, in all series a distinct drop of metals uptake capacity had been followed one cycle earlier before decreasing of biosorbents concentration expressed in mg of dry weight (Figures 2a,b,c,d). Similar relationship was observed also for chitin biosorbent, for which significant decrease of reloading efficiency was already obtained in the second cycle (Figures 2e,f). At a 1:1 Cd:Zn ratio in adsorbate the reloading efficiency in the subsequent 15 cycles was kept at a level of 25.2% for Cd and 13.2% for Zn. At a 1:2 metals ratio in adsorbate the efficiency from the 4th cycle was on average 20.4% for Cd and 12.3% for Zn. It means, that increase in Zn

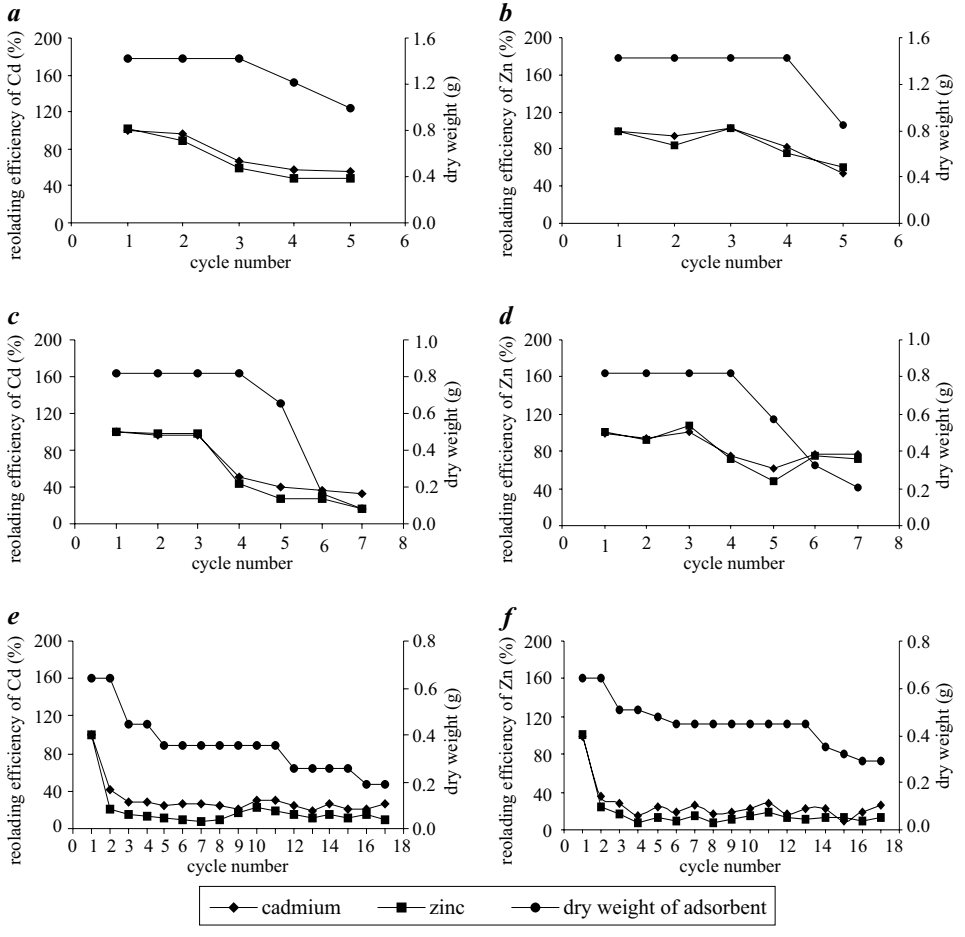

Fig. 2. The reolading efficiency of cadmium and zinc and amount of biosorbents in subsequent adsorption/desorption cycles at a 1:1 Cd:Zn ratio in adsorbate for: *a* – activated sludge immobilized in 2% alginate, *c* – activated sludge immobilized in 1.5% alginate + 0.5% PVA, *e* – activated sludge immobilized in 2% chitin + 0.5% PVA and at a 1:2 Cd:Zn ratio in adsorbate for: *b* – activated sludge immobilized in 2% alginate, *d* – activated sludge immobilized in 1.5% alginate + 0.5% PVA, *f* – activated sludge immobilized in 2% chitin + 0.5% PVA

concentration in adsorbate not caused a increase of reloading efficiency both for Cd and Zn. *Desorption efficiency*. Desorption efficiency was expressed as a ratio between amount of desorbed and loaded metal in each cycle. From the data shown in Figures 3a-f some similarities for alginate biosorbents and variety of chitin behaviour in comparison with alginate were stated. For alginate biosorbents, it was observed that in the first and second cycle the amount of cadmium and zinc desorbed was lower than the amount of metals

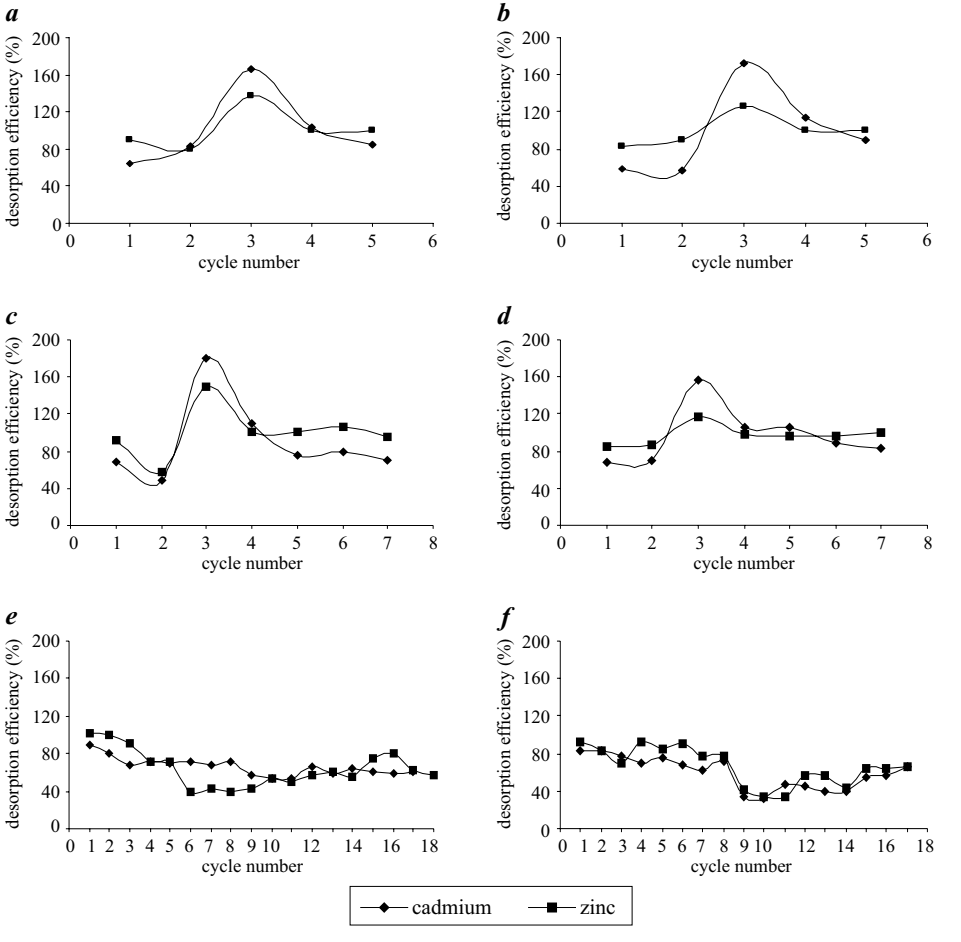

Fig. 3. Desorption efficiency of cadmium and zinc from biosorbents at a 1:1 Cd:Zn ratio in adsorbate for: *a* – activated sludge immobilized in 2% alginate, *c* – activated sludge immobilized in 1.5% alginate + 0.5% PVA, *e* – activated sludge immobilized in 2% chitin + 0.5% PVA and at a 1:2 Cd:Zn ratio in adsorbate for: *b* – activated sludge immobilized in 2% alginate, *d* – activated sludge immobilized in 1.5% alginate + 0.5% PVA, *f* – activated sludge immobilized in 2% chitin + 0.5% PVA

loaded. As a result of it, desorption efficiency did not exceed 100%. However, in the third cycle desorption efficiency increased above 100%. For cadmium, it was changing between 157% and 181%, and for Zn from 117% to 149% (Figures 3a-d). It can be explained by the elution of metals, which were accumulated and not desorbed during the first and second cycle. As a result, almost complete desorption of cadmium and zinc was achieved. In next cycles desorption process was complete or nearly complete. It means, that cadmium and zinc binding by immobilized activated sludge in alginate is a reversible

process with a little accumulation of irreversibly cadmium bound in biosorbent. This accumulation varied from 1-6% for Cd to 1-3% for Zn.

From own experiments it follows that desorption from activated sludge immobilized in chitin carrier run with lower efficiency. For 1:2 Cd:Zn ratio, desorption effectiveness achieved the lowest value between 9th and 11th cycle and was equal about 31% and 33% for Cd and Zn, respectively. When metals ratio in adsorbate was 1:1, starting from the 4th cycle desorption efficiency was maintained in consecutive cycles on unchanged level of 63.2% and 57.5% for Cd and Zn, respectively (Figures 3e,f). As a result, for 1:1 metals ratio about 38.5 % of Cd and 40.8% of Zn were accumulated in biosorbent. However, for 1:2 Cd:Zn ratio in adsorbate, the amounts of accumulated metals were lower (35.8% and 35.7%, respectively).

It was confirmed that cadmium and zinc binding by immobilized activated sludge in chitin carrier partially is an irreversible process. It follow that, accumulation of both metals by the biosorbent was significant.

Percentage of cadmium and zinc in biosorbent and the eluate. In Figure 4a, it was shown the mass of cadmium and zinc bound in biosorbents during consecutive cycles of adsorption. From presented data it follows that depending on metals ratio in adsorbate, the sum of cadmium and zinc bound in all cycles proceeded in the following order:

$$\text{Cd:Zn ratio} = 1{:}1$$
$$2\%\ \text{chitin} + 0.5\%\ \text{PVA} > 1.5\%\ \text{alginate} + 0.5\%\ \text{PVA} \gg 2\%\ \text{alginate},$$
$$\text{Cd:Zn ratio} = 1{:}2$$
$$1.5\%\ \text{alginate} + 0.5\%\ \text{PVA} > 2\%\ \text{chitin} + 0.5\%\ \text{PVA} > 2\%\ \text{alginate}.$$

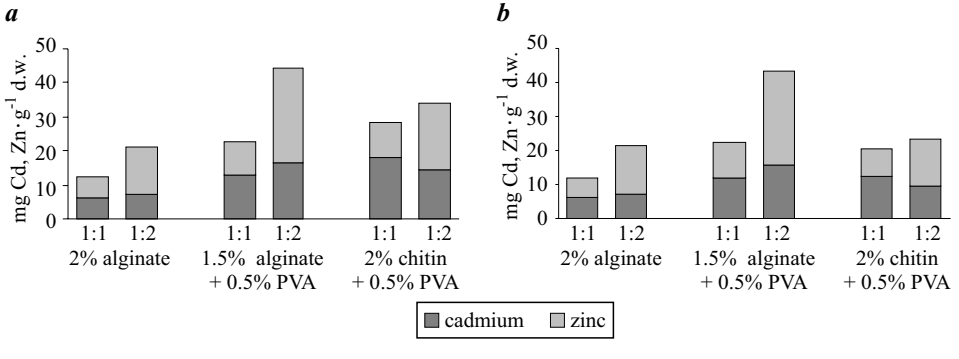

Fig. 4. Cadmium and zinc adsorption and desorption calculated as a sum of metal mass: *a* – binding in immobilized activated sludge in all adsorption cycles, *b* – elucidated from immobilized activated sludge in all desorption cycles

Desorption expressed as the sum of metals concentration in the eluate (Figure 4b) changed as follows:

Cd:Zn ratio = 1:1

1.5% alginate + 0.5% PVA ≥ 2% chitin + 0.5% PVA >> 2% alginate,

Cd:Zn ratio = 1:2

1.5% alginate + 0.5% PVA >> 2% chitin + 0.5% PVA ≥ 2% alginate.

In Figure 5a it was shown the percentage of metals binding in biosorbents and in Figure 5b the percentage of metals in the eluate calculated as arithmetic mean obtained from all cycles. From the data it results that for mixture containing Cd and Zn at a 1:1 ratio similar percentage of metals in biosorbent as well as in the adsorbate was obtained only for 2% alginate. For 1.5% alginate + 0.5%

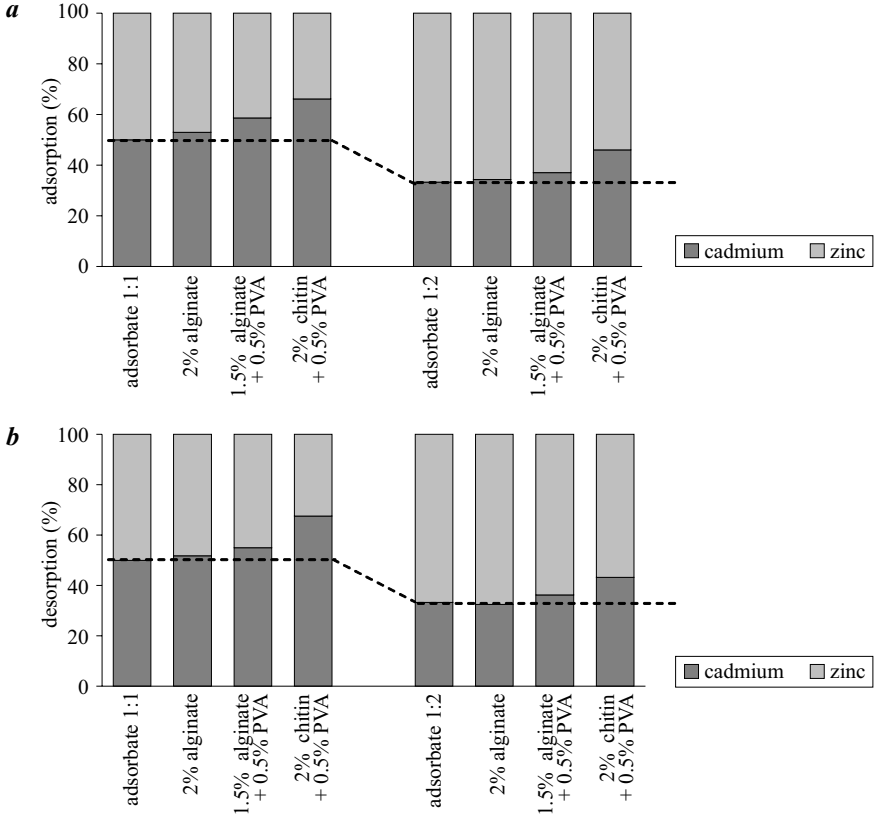

Fig. 5. Percentage of cadmium and zinc in: *a* – biosorbents, *b* – eluate. Some biosorbents possess different ability of metal adsorption in comparison with proportion of Cd and Zn in adsorbate

PVA and especially for chitin carrier it was evident that the percentage of Cd in biosorbents was significantly higher than in adsorbate. Therefore, the results indicate the preferable binding of Cd.

Similary, in the eluate there were noticed alike proportions such as in adsorbate. Distinctly higher concentration of Cd in the eluate was obtained for 2% chitin + 0.5% PVA, whereas it was comparable for 2% alginate in relation to Zn. In other words, proportion of metals in the eluate depends on the percentage of metals bound in biosorbents in adsorption/desorption cycles.

Discussion

The results of experimental data presented above suggest that using of desorbing agent caused reduction of metals uptake capacity for all adsorbents in the multiple cadmium and zinc adsorption/desorption. For activated sludge immobilized in 2% alginate metals uptake measured as reloading efficiency was constant only in 2 or 3 cycles, whereas for activated sludge immobilized in 1.5% alginate + 0.5% PVA in 3 cycles. However, decreasing of metals reloading efficiency for chitin carrier was noticed already in the 2nd cycle. The diminishing of metal uptake could be occurred due to changes in the biosorbents structure (CHU et al. 1997). This hypothesis is supported by the observations that there is a gradual biomass weight loss. FERRAZ et al. (2004) observed loss of biomass with an average value for the second and third cycle of 17.5%. In our study losses of biomass clearly depended on matrix type.

The decrease of metals uptake efficiency appeared always with one cycle earlier than biomass losses. The losses of chitin expressed in mg of dry weight were observed in the 3rd cycle, whereas for alginate carriers they usually followed during the 4th or the 5th cycle. Therefore, another possibility responsible for decreasing of metals uptake need be taken into consideration. Consequently, repeated exposure of the biomass to an acid led to destruction and reduction of metal binding sites. The modification of binding sites chemistry by acid results in decreasing of metal reloading efficiency (CHU et al. 1997).

HASHIM et al. (2000) tested the reusability of immobilized *Sargassum baccularia* in five consecutive cycles of adsorption/desorption using copper as a metal model. After the first cycle, copper uptake by the immobilized biomass in 2-5 cycles with hydrochloric acid as a desorbing agent was reduced to about 50% of the copper uptake observed in the first cycle. A similar reduction was noticed with nondestructive desorbing agent such as EDTA, where yield adsorption efficiency varied from 66% to 71%. The authors suggest that decrease in the metal reloading efficiency could be occur if the desorbing agent

was not completely removed. The release of residual desorbing agent in subsequent cycles during the copper reloading step would hinder copper uptake by the immobilized biomass. In our research for alginate biosorbents clearly decrease of cadmium and zinc reloading efficiency starting from the 3rd cycle, when desorption was significantly high, indicates that for these biosorbents such mechanism was highly probable.

Many authors refer to complete or nearly complete recovery of metals from biosorbent using mineral acids. CHU et al. (1997) proved that cadmium uptake by *S. baccularia* is easily reversible with no accumulation of irreversibly bound cadmium on the biomass. The results obtained in present study for activated sludge immobilized in alginate carriers showed that desorption efficiency changed in subsequent cycles and was the greatest in the 3rd cycle. MACHIDA et al. (2004) examined adsorption and desorption of Pb(II) from aqueous solution on activated carbon. Based on the experimental results, a two-site adsorption model was proposed for the adsorption and desorption of Pb(II). The difference in the equilibrium Pb(II) concentrations between adsorption and desorption was considered to be corresponding to Pb(II) desorbed from the weak adsorption site, whereas the equilibrium amount of Pb(II) in the desorption would be attributed to the Pb(II) remained mostly in the strong adsorption site.

For chitin carrier, it was observed that metal was not completely desorbed and its accumulated amount in biosorbent increased, what could influence metals sequestration in consecutive cycles. BAI et al. (2003) investigated Cr in multiple adsorption/desorption cycles. The reduction of metal capacity was proved. According to the investigators, the efficiency of the beads decreased during the 2nd and 3rd cycle due to incomplete desorption of ions attached to the biomass.

Own research have shown that proportion of adsorbed metals was nearly identical as proportion in adsorbate for activated sludge immobilized in 2% alginate. However, for activated sludge immobilized in 1.5% alginate + 0.5% PVA and for 2% chitin + 0.5% PVA, the percentage of adsorbed Cd was greater than Zn. For 1:1 (50:50%) Cd:Zn ratio in adsorbate the proportions were 59:41% and 66:34%, respectively whereas for 1:2 (33.3:66.7%) Cd:Zn ratio in adsorbate were 37:63% and 46:54%. Analogical proportions were obtained in the eluate.

So far, comparative studies of simultaneous biosorption onto FCAN2 prepared from *Ascophyllum nodosum* seawed biomass crosslinking with formaldehyde were carried out (CHONG, VOLESKY 1995). Equilibrium batch sorption studies were performed using two metal systems containing either Cu-Zn, Cu-Cd or Zn-Cd. A definite preference of the FCAN2 biosorbent for Cd sorbed over Zn could be observed over the equilibrium concentration of examined range.

BENGUELLA, BENAISSA (2002) investigated the effect of copper and zinc co-ions on cadmium biosorption onto chitin. Zinc ions had any appreciate effect on the cadmium uptake capacity of chitin under examined conditions. However, the presence of copper ions alters the affinity of chitin for cadmium. HAMMAINI et al. (2003) demonstrated a competitive uptake of three metal ions: Cu, Cd and Zn by activated sludge with preferential adsorption of Cu followed by Cd and Zn. In Cd-Zn system, the biosorbent exhibited a net of preferences for Cd ion over Zn.

Conclusions

The experiments carried out for multiple adsorption/desorption mixture of cadmium and zinc in adsorbate by immobilized activated sludge have revealed that binding capacity for tested biosorbents was depended on matrix type. It was concerned both the cycles number, adsorption capacity, desorption ability and selectivity in metals binding and their recovery from biosorbents.

For alginate biosorbents, their damage was observed in the 5^{th} or 7^{th} cycle for 2% alginate and 1.5% alginate + 0.5% PVA, respectively. 2% chitin + 0.5% PVA appeared more resistant carrier with the highest number of cycles (17). Another limiting factor was reloading efficiency, which strongly diminished in subsequent cycles. The most significant decrease of reloading efficiency was stated for 2% chitin + 0.5% PVA, which occurred in the 2^{nd}d cycle and was maintained in the further cycles.

Metals in alginate carriers were bound in a reversible way, what confirms the results of complete or nearly complete desorption. However, in chitin carrier the part of metals (about 38%) were bound in an irreversible way.

The carriers were characterized by different selectivity in metals binding. For 2% alginate, Cd and Zn was adsorbed in identical partition in comparison of metals percentage in adsorbate. Cd was preferably uptaken by activated sludge immobilized in 1.5% alginate + 0.5% PVA and to 2% chitin + 0.5% PVA.

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EFFECTIVENESS OF THE POLYMICTIC LAKE GŁĘBOCZEK IN TUCHOLA RESTORATION BY THE PHOSPHORUS INACTIVATION METHOD

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K e y w o r d s: polymictic lakes, phosphorus inactivation, polyaluminium chloride PAX, internal loading, phosphorus.

A b s t r a c t

For decades Lake Głęboczek in Tuchola, Poland (19.1 ha) was a receiving water for domestic waste water as well as organic and nutritive substances from the agricultural drainage basin which resulted in a heavy degradation of the lake. Studied were the determinants and the effectiveness of the restoration of this polymictic lake with the method of phosphorus inactivation using the new generation aluminium coagulant (PAX 18).

Application of the PAX 18 coagulant was the reason for a complete removal of phosphates from the lake water and simultaneously for a considerable reduction of total phosphorus (by 50-60%). The sorptive capacity of the bottom deposits improved which was indicated by the approximately two-fold decrease of phosphorus concentration in the interstitial waters (to 2-3 $mg \cdot dm^{-3}$ P), the increase of mineral phosphorus forms content in the bottom sediments, predominantly in the form of P-Al fraction, and the simultaneous decrease of the amounts of labile phosphorus. Limited phosphorus availability resulted in a significant improvement of the water quality which was displayed by: decreased super-saturation with oxygen of the surface water layers during the vegetation period, reduced organic matter quantity, diminished phytoplankton biomass (particularly blue-green algae), and increased water transparency. The positive changes in the aquatic environment were observed at least two years after the termination of the lake restoration.

EFEKTYWNOŚĆ REKULTYWACJI POLIMIKTYCZNEGO JEZIORA GŁĘBOCZEK W TUCHOLI METODĄ INAKTYWACJI FOSFORU

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S ł o w a k l u c z o w e: jeziora polimiktyczne, inaktywacja fosforu, koagulant glinowy PAX, zasilanie wewnętrzne, fosfor.

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Abstrakt

Jezioro Głęboczek w Tucholi (19,1 ha) przez dziesięciolecia było odbiornikiem ścieków bytowych oraz substancji organicznych i biogennych ze zlewni użytkowanej rolniczo, co doprowadziło do jego silnej degradacji. Badano uwarunkowania i efektywność odnowy tego polimiktycznego jeziora metodą inaktywacji fosforu z użyciem koagulantu glinowego nowej generacji (PAX 18).

Wprowadzenie koagulantu PAX 18 spowodowało całkowite wyeliminowanie fosforanów z wody jeziora, a jednocześnie znaczną redukcję ilości fosforu ogólnego (o 50-60%). Około 2-krotny spadek koncentracji fosforu w wodach interstycjalnych (do 2-3 mg · dm^{-3} P), wzrost zawartości mineralnych form tego pierwiastka w osadach dennych, przede wszystkim w postaci frakcji P-Al, i jednoczesne zmniejszenie ilości fosforu labilnego, świadczyły o poprawie zdolności sorpcyjnych osadów dennych. Ograniczenie dostępności fosforu spowodowało znaczną poprawę jakości wód. Stwierdzono spadek przetlenienia powierzchniowych warstw wód w sezonie wegetacyjnym, redukcję ilości materii organicznej, ograniczenie biomasy fitoplanktonu (zwłaszcza sinic) oraz wzrost przezroczystości wody. Pozytywne zmiany warunków środowiskowych utrzymywały się co najmniej 2 lata po zakończeniu rekultywacji.

Introduction

The ongoing process of degradation in lakes requires application of complicated protection and restoration techniques. Preparation of effective but inexpensive methods providing a chance for factual restoration of the water reservoirs is one of the most important challenges for the modern limnology.

Many authors share the opinion (CARLSON 1995, COOKE et al. 1993, GAWROŃSKA et al. 2002, KLAPPER 2003, WIŚNIEWSKI 1999) that phosphorus inactivation can be regarded as one of such methods. The idea behind is to precipitate, using a coagulant, excessive mineral phosphorus – considered the main eutrophication factor – from the water and immobilize it in the bottom sediments. Recently, the "new generation" coagulants have been applied, such like PAX (polyaluminium chlorides). Compared with the traditional coagulants, PAX has higher efficacy with regard to precipitation of mineral and organic substances from the water and additionally, it is more effective at lower doses (RATNAWEERA et al. 1992, SMOCZYŃSKI et al. 1991).

First time in Poland the PAX coagulant was successfully applied in spring 2001 for the renovation of a dimictic Lake Długie in Olsztyn (GAWROŃSKA et al. 2002). However, it seems necessary to investigate upon the effectiveness of this method in lakes with different morphometric properties and trophic conditions. Investigated in more detail should also be the issue of the conditions determination for the renovation activities, especially regarding optimal coagulant doses, treatment time in the year and technical aspects of the coagulant's application.

The aim of this work was to find out the determinants and the effectiveness of the restoration of the shallow polymictic Lake Głęboczek in Tuchola using the phosphorus inactivation method with the new generation PAX 18 coagulant.

Material and Methods

Study object

Lake Głęboczek is situated in the South Pomeranian Lake District in the administrative borders of the Tuchola town (N 53°35.44', E 17°52.26'). The lake is small (19.1 ha surface area) and shallow, polymictic (max. depth 5.6 m), with relatively small agricultural and urban drainage basin. It plays the roles of the municipal bathing beach and the fishing ground for anglers.

Alike many other urban lakes, it had been heavily eutrophied and yet in the 1990s subjected to the restoration activities. The applied methods, especially the multi-year artificial aeration and the "biostructure" system, did not help to improve the water quality (GOSZCZYŃSKI 2000).

Methods

Restoration of the lake was conducted in two phases, i.e., in autumn 2001 and in spring 2003. To inactivate phosphorus, the total of thirty-five tonnes of an aqueous solution of polyaluminium chloride (PAX 18) was used. The coagulant was introduced under the water surface from boats equipped with systems of containers, transport pipes and perforated pipes with controllable depth of immersion which enabled even distribution of the polyaluminium chloride in the lake water.

The studies were conducted in annual cycles, i.e., February 2001 through November 2003 with the average water sampling frequency of once a month. The vegetation period 2001 was treated as the pre-experimental period.

Water and bottom sediments were sampled from two posts (Figure 1). Temperature and oxygen were measured on each sampling post at every meter of the depth and the profiles done. Water for the physico-chemical laboratory analyses was sampled with a Ruttner apparatus from the surface and the near-bottom water layers. Water transparency was measured with the Secchi disc (0.3 m diameter). The bottom deposits (layers 0-5 cm and 6-10 cm) and the near-bottom waters (10 cm) were sampled with a Kajak's sampling tube (52 mm diameter).

All laboratory analyses of the lake water and the interstitial water were done in accordance with the methods binding in hydrochemical (HERMANOWICZ 1999) and hydrobiological (STARMACH 1989) studies. Chemical composition of the bottom sediments was determined using the methods recommended by JANUSZKIEWICZ (1978) and GOLACHOWSKA (1977a,b,c).

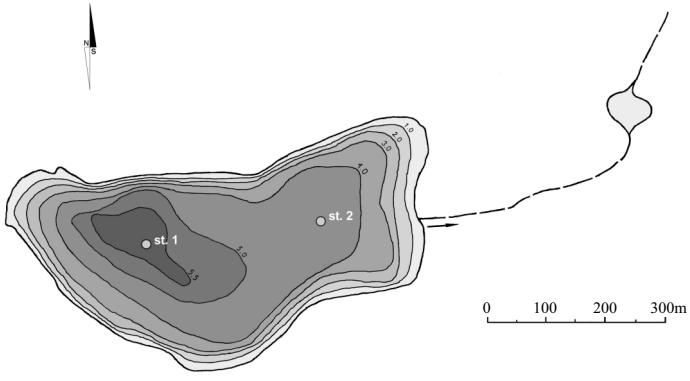

Fig. 1. Bathometric map of Lake Głęboczek in Tuchola with sampling stations

The statistical analysis included comparative statistics, testing the significance of differences between the selected physico-chemical parameters of water and bottom sediments in the matched pairs system. The time space comprised the period before and after the introduction of the factor causing environmental changes in the ecosystem, i.e., the inactivation activities. Normality of continuous variables was checked with the Saphiro-Wilk test followed in case of the positive result with a *t*-test for the dependable variables and with the Wilcoxon Matched-Pairs Signed-Ranks test in case of the negative result.

Statistical work with the data was done with the help of the Statistica 6.0 software.

Results

Phosphorus compounds

Before the restoration, the lake waters were characterised by the high content of total phosphorus, i.e., 0.274 ± 0.064 mg $\cdot$ dm^{-3} on average in the surface water layer and 0.422 ± 0.225 mg $\cdot$ dm^{-3} in the near-bottom layer. Mineral phosphorus was detected in the amounts of 0.042 ± 0.040 and 0.171 ± 0.156 mg $\cdot$ dm^{-3}, respectively, which constituted 15.3% in the surface layer and 40.5% P_{tot} in the near-bottom layer. Already after the first phase of the inactivation it was observed that the reduction of total phosphorus was statistically significant both in the surface waters (to 0.145 ± 0.05 mg $\cdot$ dm^{-3}, $t = 5.51$, $p<0.001$, $df = 9$) and in the near-bottom waters (to $0.163 \pm$

± 0.101 mg · dm^{-3}, $t = 5.72$ $p < 0.001$ $df = 9$). Phosphates were detected only in the near-bottom water layer, in the amount of 0.018 ± 0.047 mg · dm^{-3} on average which comprised 11.0% P_{tot}. After introduction of the second dose of the coagulant, until the end of the experiment, mineral phosphorus was not detected in the water column (Figure 2a).

A lot higher phosphorus concentrations were characteristic for the interstitial waters (Figure 2b). In 2001, the mean content of total phosphorus in the top 5-cm layer of the interstitial waters equalled 3.383 ± 1.411 mg · dm^{-3}. Application of the first dose of PAX 18 significantly ($t = 4.00$, $p = 0.003$ $df = 9$) lowered this value (to 2.123 ± 0.631 mg · dm^{-3}). The second dose application further increased the reduction, to the level of 1.439 ± 0.406 mg · dm^{-3} ($t = 4.734$, $p = 0.001$ $df = 9$). These changes were determined primarily by the reduction of the mineral phosphorus content (Figure 2b) which in both phases of the restoration was statistically significant ($t = 4.260$, $p = 0.002$, $df = 9$ and $t = 5.79$, $p < 0.001$, $df = 9$, respectively).

Introduction of two doses of PAX 18 to the lake waters caused apparent quantitative changes regarding the components responsible for the sorptive properties of the bottom sediments. The aluminium content increased (from 3.19 ± 0.54 to 3.94 ± 0.51% d.w., $t = -2.63$ $p = 0.034$) as well as the content of the phosphorus fraction bound with this element in the top layer (0-5 cm). Simultaneously, the labile phosphorus content diminished (Figure 2c). The total amount of phosphorus in the bottom sediments – compared to the pre-experimental period – increased significantly (2.064 ± 0.167 mg · g^{-1} d.w.), reaching in 2003 the mean value of 2.425 ± 0.255 mg · g^{-1} d.w. ($t = -5.92$, $p < 0.001$).

Other indicators of the trophic condition

In the period preceding the restoration, the high abundance of the lake in nutritive substances resulted in a high primary production (Table 1). It was displayed by the high BOD_5 values, exceeding 8 mg · dm^{-3} O_2, considerable content of chlorophyll "a" (to 100 mg · m^{-3}) and the very high, reaching 55 mg · dm^{-3}, biomass of algae. Intensive "algal blooms" observed practically throughout the vegetation period caused a considerable reduction of the mineral forms of nitrogen and phosphorus, an increase of the water pH in the surface layers (pH > 9.3), total deficiency of free carbon dioxide and the consequent advancement of the biological deliming. In the spring and summer, considerable super-saturation with oxygen was noted in the surface water layers (saturation > 175%) and simultaneously notable oxygen deficits in the deeper water layers or even total de-oxygenation of the near-bottom waters.

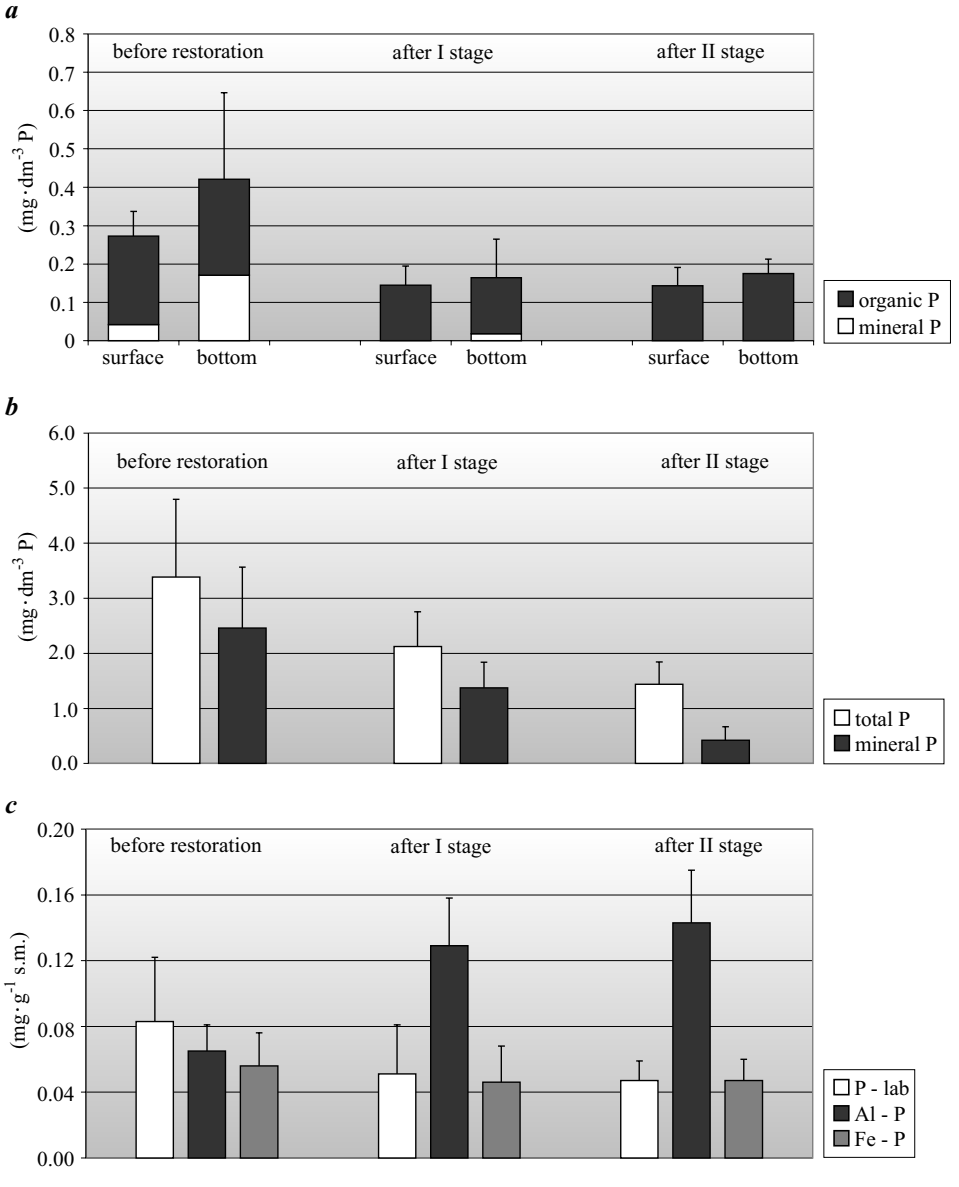

Fig. 2. Changes of phosphorus content in Lake Głęboczek. Medium and SD for comparable periods of the lake year (V-X 2001, V-X 2002, V-X 2003), $n = 10$; *a* – content of P in lake water, *b* – content of P in interstitial water (0–5 cm layer), *c* – labile, Al- and Fe-bounded P in surface (0–5 cm) layer of sediments

After application of PAX 18, the aquatic conditions in the lake improved which was displayed by, among others, an increased Secchi disc visibility in the summer (Figure 3), reduced chlorophyll "a" and organic matter content, and reduced water pH in the surface layers (Table 1).

Table 1

Variation in concentrations of the main trophic condition indicators in Lake Głęboczek

Parameter	Unit	Medium*** ± SD			Significance of the differences****	
		before restoration	after I stage	after II stage	after I stage	after II stage
Visibility*	m	0.45±0.12	0.70±0.19	0.79±0.22	t=-6.27 p<0.001	t=-3.26 p=0.010
Chlorophyll "a"*	μ/l	77.78±27.82	77.97±57.92	37.31±22.69	t=-0.01 p=0.990	T=7.00 p=0.037
Reaction*	pH	8.77±0.49	8.49±0.26	8.38±0.14	t=1.70 p=0.122	t=2.80 p=0.021
Reaction*	pH	7.94±0.41	7.89±0.37	7.84±0.54	t=0.44 p=0.668	t=0.51 p=0.624
BOD_5*	$Mg \cdot dm^{-3}$ O_2	6.73±1.13	7.27±3.09	5.71±2.09	T=25.00 p=0.799	t=2.41 p=0.039
BOD_5**	$Mg \cdot dm^{-3}$ O_2	4.53±0.89	4.39±1.36	6.35±2.34	T=14.00 p=0.169	t=-2.25 p=0.051
$COD_{(KMnO_4)}$*	$Mg \cdot dm^{-3}$ O_2	27.35±4.68	22.26±5.90	18.23±1.63	t=3.77 p=0.004	t=5.22 p<0.001
$COD_{(KMnO_4)}$**	$Mg \cdot dm^{-3}$ O_2	22.79±2.93	18.00±0.75	17.83±1.54	t=4.75 p=0.001	t=4.52 p=0.001
Total phosphorus*	$Mg \cdot dm^{-3}$ P	0.274±0.064	0.145±0.050	0.143±0.048	t=5.51 p<0.001	t=3.71 p=0.005
Total phosphorus**	$Mg \cdot dm^{-3}$ P	0.422±0.225	0.163±0.101	0.175±0.038	t=5.72 p<0.001	T=0.00 p=0.005
Total nitrogen*	$Mg \cdot dm^{-3}$ N	2.79±0.55	2.37±0.60	2.16±0.26	t=1.98 p=0.079	T=8.00 p=0.047
Total nitrogen**	$Mg \cdot dm^{-3}$ N	3.31±1.48	2.36±0.72	2.69±0.49	t=3.10 p=0.013	T=14.00 p=0.169
N/P*	–	10.54±2.88	18.23±8.33	16.37±4.52	T=3.00 p=0.013	t=-2.87 p=0.018
N/P**	–	8.19±1.63	17.00±7.79	16.02±4.27	T=0.00 p=0.005	t=-5.12 p<0.001
Total algal biomass*	$Mg \cdot dm^{-3}$	42.11±14.66	34.82±29.25	11.60±5.41	t=0.71 p=0.524	t=4.98 p=0.016
Cyanophyta biomass*	$Mg \cdot dm^{-3}$	28.90±11.52	2.50±1.67	5.95±11.53	t=4.81 p=0.017	t=4.07 p=0.027

* surface
** bottom
*** for comparable periods of the lake year (V-X 2001, V-X 2002, V-X 2003), n = 10
**** t statistic (t) or Wilcoxon test (T)

In 2002 and 2003 also the modification in the phytoplankton structure was observed (Figure 3). After termination of the restoration, the algal biomass was lowered to 11.60± 5.40 mg · dm^{-3} on average. Simultaneously, the dominance of the *Cyanophyta* was radically reduced and their biomass fell from the values reaching 28.90 mg · dm^{-3} on the average to the maximal level of approximately 2.50 mg · dm^{-3} in 2002 and 5.95 mg · dm^{-3} d.w. in 2003.

After the application of the subsequent doses of the coagulant, a small but statistically significant reduction was observed regarding the content of nitrogen compounds (Table 1).

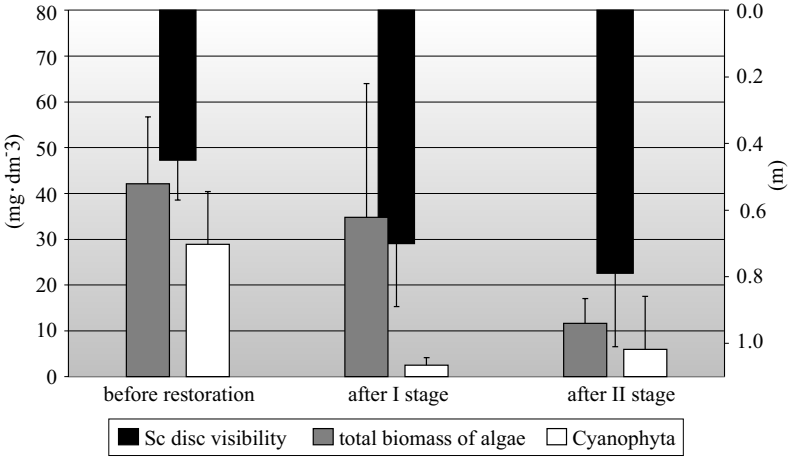

Fig. 3. Changes in Secci disc visibility and algal biomass in Lake Głęboczek. Medium and SD for comparable periods of the lake year (V-X 2001, V-X 2002, V-X 2003), $n = 10$

Discussion

The natural consequence of the intensively occurring processes of wind mixing in polymictic lakes (according to PASCHALSKI 1964 in "pleomictic" lakes) is the contact of the whole surface of the bottom deposits with the water layers in which the primary production processes occur. Lake deposits comprise a practically inexhaustible storage of nutritive substances and can become the secondary source of nutritive matter in an ecosystem. Polymictic lakes, as evidenced by many authors (FORSBERG 1998, GRANÉLI 1999, SØNDEGAARD et al. 2003), are therefore subjected to the fastest degradation and at the same time the symptoms of excessive eutrophication can be fought back with the most difficulty.

The research done in the reference year revealed that the main reason for the lasting low water quality in Lake Głęboczek, despite cutting off most of the external pollution sources (GOSZCZYŃSKI 2000), is the lack of effective mechanisms binding phosphorus in the bottom deposits. The deposits in the lake before the restoration were characterised by low sorptive capacity which was exhibited by the low contents of iron and aluminium (usually below 3-4% d.w.) and the relatively low amount of phosphorus, irrespective of the unfavourable trophic conditions. An effect of that were the exceptionally low amounts of phosphorus bound with iron and aluminium and the relatively high amounts of the labile fraction. The very high – compared to other lakes (ZDANOWSKI 1983) – contents of phosphorus in the near-bottom and interstitial waters and the high phosphorus content in the water column (> 0.2 mg $\cdot$ dm^{-3}), typical for heavily eutrophied reservoirs (ZDANOWSKI 1982), have confirmed the vital role of the internal loading processes.

For that reason, the main goal of the restoration was reduction of the mobility of the major nutritive element in the ecosystem – phosphorus, through precipitation with the PAX 18 aluminium coagulant of the excessive amounts of the mineral form from the water column and inhibition of its release from the bottom deposits.

Selection of the coagulant was done on the ground on the conditions in which it would react after introduction to the lake. Despite the small depth and good oxygenation of the lake waters, the trophic condition of Lake Głęboczek determined the total de-oxygenation of the bottom deposits starting at the depth of a few millimetres. In accordance with the classical concept of Einsele/Mortimer (GOLTERMAN 2001), it causes phosphorus release from the complexes with iron if the redox potential is lower than the boundary value of 0.2 V. Aluminium, unlike iron, is insensitive to the changes of redox potential which means that the influence of anoxic conditions on its sorptive properties in relation to phosphorus is minor (FORSBERG 1989).

The applied technique of the coagulant introduction enabled both the precipitation of phosphorus from the water and its durable immobilization in the deposits. Already the first dose of the coagulant nearly completely eliminated phosphates from the lake water although their periodical occurrence in the near-bottom layers during the water stagnation indicated the insufficient inhibition of phosphorus in the deposits. After application of the second PAX dose the mineral phosphorus was not detected in the water column, even in the deoxygenated near-bottom layers during the summer. These changes, and the simultaneous increase of aluminium and the phosphorus fraction bound with this element in the top layer of the lake deposits, the decrease of the labile phosphorus content, and finally the decrease of phosphorus amounts in the interstitial waters, were the sign of enhanced sorptive capacity of the bottom deposits in Lake Głęboczek.

Small reduction of nitrogen contained in the lake waters and the simultaneous considerable decrease of the phosphorus amounts caused a significant growth of the N/P ratio. The mean value of the ratio in the two-year restoration period oscillated around 17 which was nearly two times more than the values observed in the reference year. As emphasised by many authors (HILLBRICHT-ILKOWSKA 1994, MARSDEN 1989), increase of this parameter's value indicates that phosphorus becomes the factor limiting the reservoir's productivity and values higher than 14-18 give the start to ecologically favourable reconstruction of the phytoplankton structure, displayed by the decay of the blue-green algae "blooms" with the simultaneous improvement of the water quality in the reservoir.

The above hypothesis was confirmed in the surveys of the aquatic conditions in Lake Głęboczek conducted during the restoration. Limitation of the phosphorus abundance in the water caused dramatic changes in the taxonomic and quantitative structure of the planktonic algae. Introduction of the subsequent doses of the coagulant reduced dramatically the phytoplankton biomass – in 2003 the reduction equalled more than 70% of the values observed in the pre-experimental year. The dominance of the blue-green algae in the phytoplankton collapsed and their quantity was lowered more than five times.

The most obvious notable effect of the primary production reduction was the improved water transparency in the vegetation periods, as well as the decrease of the chlorophyll "a" content, especially after the second phase of the restoration, and the decrease of the water pH in the surface layers. Simultaneously a significant reduction of the water's super-saturation with oxygen during the vegetation period was observed, i.e., from over 170% in the pre-experimental period to 95-116% in the surface water layers in 2003. Continuous improvement of the trophic conditions was indicated also by the significant reduction of the organic matter content, particularly of the fraction resistant to degradation.

As reported by WELCH and COOKE (1999), the durability of phosphorus immobilization in shallow lakes can be estimated for a few up to between ten and twenty years. The studies continued in 2004 and 2005 have revealed some positive changes in the aquatic environment for at least two years since the termination of the second phase of the restoration. The still observable too high primary production in the lake points however at the need to increase the dose of the coagulant which has been confirmed in other successful restoration attempts with the use of many times higher amounts of aluminium (SMELZER 1990, CARLSON 1995). In the case of Lake Głęboczek, suffering from the strong activity of the anglers, the durability of the positive effects of the restoration will depend on the proper fish culture. Indispensable seems a diminishment of the benthophagous population destroying the coagulant barrier contained in the surface layer of the bottom deposits.

Conclusions

1. Improvement of the water quality in Lake Głęboczek and the favourable reconstruction of the phytoplankton structure observed as a result of the applied restoration point out that phosphorus inactivation with the use of the new generation aluminium coagulants can be an effective and rather inexpensive restoration technique for eutrophied polymictic lakes.

2. Introduction of the coagulant in the surface water layers not only enabled removal of the excessive phosphorus from the water but most of all, effectively prevented its release from the bottom deposits, even in anaerobic conditions. This indicates that the PAX coagulant can be used also in the heavily degraded lakes.

3. The applied method had no direct impact on the quantity and transformations of the nitrogen compounds. The slow reduction of their content in the water observed during the restoration had a secondary nature, related to the limitation of the intensity of the primary production processes.

4. The prerequisite to acquire radical improvement of the water quality in Lake Głęboczek is the reduction of the total amount of phosphorus compounds to the level controlling the phytoplankton growth. This is an evidence for the needed follow up, i.e., the third phase of the restoration, as previously assumed.

5. The example of Lake Głęboczek illustrates the fact that the desirable trophic condition in a shallow water reservoir restored by phosphorus inactivation can be maintained if supported with the proper fish culture. Mostly it regards effective control of the population of fish praying on benthos and responsible for the re-suspension of the bottom sediments.

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QSAR ANALYSIS IN STUDIES ON THE ACUTE TOXICITY OF INSECTICIDES TO AQUATIC ORGANISMS

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Key words: QSAR, insecticides, toxicity, fish, daphnia, algae.

Abstract

The paper presents the results of QSAR (Quantitative Structure-Activity Relationships) analysis of the acute toxicity of insecticides to aquatic organisms as dependent on their physicochemical parameters (n-octanol-water partition coefficient K_{OW}, water solubility S_{H_2O} and molecular weight M). Four models of correlation and regression were applied to analyze organophosphorus insecticides, carbamates and pyrethroids. Among the chemical classes examined in the study, pyrethroids and organochlorine insecticides were found to be the most toxic to the rainbow trout (*Salmo gairdneri* R.) and daphnia. The analysis of correlation and regression with two variables showed a high ($r > 0.95$) correlation between the water solubility of organophosphorus insecticides and their toxicity to the rainbow trout, daphnia and algae. A similar relationship (IC_{50} determined for algae / solubility) was observed in the case of pyrethroids ($r = 0.98$). Multiple variable models (linear and logarithmic) provided the best prediction of insecticide toxicity. The highest values of coefficient R in linear and logarithmic models with multiple variables (0.952 to 0.965 and 0.955 to 0.992 respectively) were obtained for organophosphorus insecticides.

ANALIZA QSAR W BADANIACH TOKSYCZNOŚCI OSTREJ INSEKTYCYDÓW WOBEC ORGANIZMÓW WODNYCH

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Abstrakt

Przedstawiono wyniki analizy QSAR (*Quantitative Structure-Activity Relationships*) między toksycznością ostrą insektycydów dla organizmów wodnych a ich podstawowymi właściwościami fizykochemicznymi (współczynnikiem podziału *n*-oktanol/woda K_{OW}, rozpuszczalnością w wodzie S_{H_2O} i masą molową *M*). W tym celu wykorzystano analizę korelacji i regresji czterech modeli dla insektycydów fosforoorganicznych, karbaminianów i pyretroidów. Spośród ocenianych grup chemicznych najbardziej toksyczne dla pstrąga tęczowego (*Salmo gairdneri* R.) i dafnii okazały się pyretroidy i węglowodory chlorowane. Analiza korelacji i regresji dwóch zmiennych wykazała wysoką ($r > 0{,}95$) korelację między rozpuszczalnością insektycydów fosforoorganicznych w wodzie a ich toksycznością wobec pstrąga tęczowego, rozwielitki i glonów wodnych. Podobną zależność (IC_{50} dla glonów/rozpuszczalność) odnotowano w przypadku pyretroidów ($r = 0{,}98$). Najefektywniejszymi modelami regresji w predykcji toksyczności insektycydów okazały się modele wielu zmiennych (liniowy i logarytmiczny). Związki fosforoorganiczne były grupą chemiczną insektycydów, w przypadku której uzyskano najwyższe współczynniki *R* dla modelu liniowego wielu zmiennych (od 0,9525 do 0,9650) i logarytmicznego wielu zmiennych (od 0,9550 do 0,9921).

Introduction

QSAR (*Quantitative Structure-Activity Relationships*) analysis determines relationships between the structure of chemical compounds, their physicochemical properties and biological activity. This advanced statistical tool is more and more often applied in pharmacological chemistry, toxicology, ecotoxicology and phytopharmacy (SMIDLI 1997, TAO et al. 2002, CRETTAZ 2003, RONCAGLIONI et al. 2003). Based on the physicochemical parameters of known biologically active substances (drugs, pesticides, etc.) it is possible to predict the biological activity (including toxicity) of newly synthesized substances of a given chemical class. This enables to limit the range of preliminary tests carried out on living organisms, which is of great ethical and economic significance.

Studies on relationships between structure and biological activity are conducted employing both standard statistical methods (SMIDLI 1997) and advanced procedures, making use of artificial neural networks (CLARK et al. 1995, ZAAKARIA et al. 1998, DEVILLERS 2000). Computer program designed especially for this purpose, such as EcoSAR (CASH, NABOLZ 2000) or ToxSys (CRETTAZ 2003), are also applied more and more frequently.

One of the key physicochemical properties of substances analyzed by QSAR procedures is their lipophilic character, reflected by the n-octanol-water partition coefficient K_{OW} (GESTEL et al. 1993, SMITH et al. 1994, YUAN et al. 1997, XU et al. 2000, WANG et al. 2002). Other characteristics commonly examined in QSAR analysis are the soil sorption coefficient K_{OC} (GESTEL et al. 1993), the electrolytic dissociation constant K_a, (SMITH et al. 1994), solvation energy (TANG et al. 1992, XU et al. 2000), energy of the lowest unoccupied

molecular orbital E_{LUMO} (NENDZA 1991, YUAN et al. 1997, WANG et al. 2002) as well as the presence of certain functional groups in a molecule (TAO et al. 2002). The biological activity of chemical compounds is also considerably affected by their chirality, which is taken into account in QSAR modeling (TOMBO, BLASER 1999).

The structure-activity relationship determined in mammals provides the basis for evaluating the effects of both medicines and toxins on humans (NENDZA 1991, ZAAKARIA et al. 1998). Ecotoxicology is another discipline in which QSAR analysis of xenobiotics is employed, using aquatic organisms (SMITH et al. 1994, DIJKMAN et al. 1997, PASSINO-READER et al. 1997) and soil organisms (GESTEL et al. 1993, SVERDRUP et al. 2001) as models.

Insecticides showing neurotoxic properties are a class of pesticides used most often for crop protection as well as for sanitary and veterinary hygiene purposes (FAO 2004). These agents are relatively inexpensive, which contributes to their popularity. The rapid development of the phytopharmaceutical industry was followed by the removal of insecticides considered extremely toxic to humans from the market, and their replacement by new, less toxic agents. Unfortunately, this process is not always accompanied by reducing the toxicity of these substances to aquatic organisms, birds and bees. Still the majority (about 80%) of crop protection chemicals and almost all neurotoxic insecticides are harmful or toxic to water organisms (WARMIŃSKI et al. 2000). Wood preservatives also constitute a serious threat to water biocenoses. These preparations contain not only fungicides, but also insecticides which are particularly harmful to aquatic animals (ŁEBKOWSKA et al. 2003).

The aim of the present study was to determine relationships between the physicochemical parameters of some chemical classes of neurotoxic insecticides and their toxicity to aquatic organisms.

Methods

Toxicological and physicochemical data on insecticides, included in the 12th edition of *The Pesticide Manual* (BCPC 2000), were used for model tests. This reference provides information on pesticides used in ecotoxicological QSAR analysis by many authors (e.g. TAO et al. 2002, TREMOLADA et al. 2004). In this study the indicators of acute toxicity of neurotoxic insecticides to representatives of three links of the food chain in the aquatic environment (i.e. fish, zooplankton and phytoplankton) were determined:

– LC_{50} (median lethal concentration, 96 h) in the rainbow trout (*Salmo gairdneri* R.) ($mg \cdot L^{-1}$);

– EC_{50} (median effective concentration, 48 h) in *Daphnia magna* ($mg \cdot L^{-1}$);

– IC_{50} (median inhibitory concentration, 72 h) in green algae (*Chlorophyta*) ($mg \cdot L^{-1}$).

The following physicochemical parameters were analyzed:

– *n*-octanol-water partition coefficient K_{OW} (a parameter used most often in QSAR analysis),

– water solubility S_{H_2O} (inseparable from the aquatic environment),

– molecular weight M.

The above data were collected for 139 insecticides (organophosphorus insecticides, carbamates, pyrethroids, neonicotinoids and organochlorine insecticides). Arithmetic means, medians and concentrations ranges of LC_{50}, EC_{50} and IC_{50} determined for aquatic organisms are given in Table 1. Neonicotinoids and organochlorine insecticides were not subjected to QSAR analysis since the number of these compounds was too small (N=6). Several models of correlation and regression were used in the study:

a) linear Pearson's model with two variables

$$y = b_0 + b_1 \cdot x_i,$$

b) non-linear model with two variables

$$\log(y^{-1}) = b_0 + b_1 \cdot \log x_i,$$

c) linear model with many variables (multiple)

$$y = b_0 + b_1 \cdot M + b_2 \cdot K_{OW} + b_3 \cdot S_{H_2O},$$

d) non-linear model with many variables (multiple)

$$\log(y^{-1}) = b_0 + b_1 \cdot \log M + b_2 \cdot \log K_{OW} + b_3 \cdot \log S_{H_2O}$$

where:

y – indicator of toxicity to aquatic organisms (LC_{50}, EC_{50}, IC_{50} – dependent variables); x_i, M, K_{OW}, S_{H_2O} – physicochemical properties (independent variables); $x_i = M$, K_{OW} or S_{H_2O}; b_0, b_1, b_2, b_3 – regression coefficients.

Non-linear estimation for multiple variables was based on the least squares loss function $(OBS\text{-}PRED)^2$ and quasi-Newton estimation procedure. The calculations were performed using Statistica 6.1 PL software (StatSoft Inc. 2003).

Table 1

Acute toxicity of neurotoxic insecticides

Organisms	Parameters	Statistics	PHOS	CARB	PYRET	NEONIC	HC
Fish	LC_{50} ($mg \cdot L^{-1}$)	mean	112.5	55.8	1.5	390.5	0.04
		med	2.1	4.0	$2.6 \cdot 10^{-3}$	224.0	0.04
		min	$6.0 \cdot 10^{-3}$	0.03	$6.8 \cdot 10^{-6}$	100.0	0.02
		max	2400.0	960.0	20.5	1000.0	0.08
Daphnia	EC_{50} ($mg \cdot L^{-1}$)	mean	1.8	1.3	0.4	2277.0	0.6
		med	$3.0 \cdot 10^{-3}$	0.2	$1.0 \cdot 10^{-3}$	200.0	0.4
		min	$7.8 \cdot 10^{-5}$	$1.5 \cdot 10^{-3}$	$1.3 \cdot 10^{-5}$	85.0	$7.8 \cdot 10^{-4}$
		max	67.2	10.0	10.0	10 000.0	2.1
Algae	IC_{50} ($mg \cdot L^{-1}$)	mean	68.0	36.9	8.9	76.1	0.7
		med	7.0	20.0	1.0	99.2	0.7
		min	0.4	1.1	$2.0 \cdot 10^{-4}$	6.2	0.6
		max	980.0	140.0	56.2	100.0	0.8
Number of compounds			65	26	36	6	6

med – median,
min – minimum,
max – maximum;
classes of insecticides: PHOS – organophosphorus insecticides, CARB – carbamates, PYRET – pyrethroids, NEONIC – neonicotinoids, HC – organochlorine insecticides.

Results and Discussion

Toxicity of insecticides to aquatic organisms

Insecticides evoked a very strong response in aquatic organisms, especially animals (Table 1). Among the chemical classes examined in the study, pyrethroids (LC_{50} = 1.5 and EC_{50} = 0.4 $mg \cdot L^{-1}$) and organochlorine insecticides (0.04 and 0.6 $mg \cdot L^{-1}$) were found to be the most toxic to fish and daphnia. The range of application of the latter has been considerably limited recently due to their high environmental persistence in the environment, but the former are still widely used today, both for crop protection as well as for sanitary and veterinary hygiene purposes (FAO 2004). Neonicotinoids, a relatively new chemical class (BCPC 2000), were found to be the safest to aquatic organisms of all neurotoxic insecticides (Table 1).

QSAR analysis – models with two variables

Analysis of correlation and simple regression, employed to determine the toxic effect of organophosphorus insecticides on aquatic organisms, demonstrated that the toxicity of these compounds was related primarily to water

solubility ($r = 0.95 \div 0.96$, $p < 0.001$) – Table 2. In the case of pyrethroids a similar relationship was observed only between IC_{50} determined for algae and water solubility. The positive value of the correlation coefficient (r) indicates that the higher the water solubility of these substances the lower their toxicity to aquatic organisms.

Table 2
Correlation matrix of acute toxicity of insecticides and their physicochemical parameters (linear model, two variables $y = f(x_i)$)

Dependent variable (y) – acute toxicity	Independent variables (x_i)		
	M	K_{OW}	S_{H_2O}
PHOS			
LC_{50} – rainbow trout	-0.04	-0.05	0.96**
EC_{50} – *Daphnia magna*	-0.36	-0.06	0.95**
IC_{50} – algae	-0.54	-0.09	0.95**
CARB			
LC_{50} – rainbow trout	-0.15	-0.20	0.11
EC_{50} – *Daphnia magna*	0.54*	0.16	-0.13
IC_{50} – algae	-0.31	-0.35	-0.36
PYRET			
LC_{50} – rainbow trout	-0.13	-0.15	-0.02
EC_{50} – *Daphnia magna*	0.16	-0.13	-0.07
IC_{50} – algae	-0.23	-0.27	0.98**

Regression model: $y = b_0 + b_1 \cdot x_i$
M – molecular weight;
K_{OW} – n-octanol-water partition coefficient;
S_{H_2O} – water solubility;
b_0 – the intercept coefficient;
b_1 – regression coefficient;
*, ** – correlation coefficients significant at $p = 0.01$ and $p = 0.001$ respectively;
Sample size $N = 8 \div 65$;
For details, see Table 1.

Non-linear models, especially when applied to logarithmically transformed data, are more reliable and more frequently used QSAR models (SMIDLI 1997, TREMOLADA et al. 2004). The analysis of the data collected in this study with a logarithmic model revealed additional significant relationships, particularly for organophosphorus insecticides. It should be noted that the absolute values of correlation coefficients were lower than those obtained using a linear model ($|r| = 0.44 \div 0.75$) – Table 3. Apart from water solubility, also molecular weight and the n-octanol-water partition coefficient K_{OW} were found to have an influence on the toxicity of organophosphorus insecticides to daphnia and algae. The toxicity of carbamates to fish (rainbow trout) was affected by water solubility. In this model the positive value of the correlation coefficient (r)

indicates that the higher the value of an independent variable (e.g. K_{OW}) the higher the toxicity of a given insecticide (lower effective concentrations of EC_{50} and lower inhibitory concentrations of IC_{50}). Numerous authors (SMITH et al. 1994, PASSINO-READER et al. 1997, XU et al. 2000) observed a significant correlation between the toxicity of xenobiotics to aquatic organisms and the n-octanol-water partition coefficient K_{OW}. TREMOLADA et al. (2004) reported that in 1996 the European Commission issued a document specifying how to predict the toxicity of new chemicals based on this physicochemical parameter. Higher toxicity of strongly lipophilic xenobiotics is associated with the degree of their bioaccumulation (HERMES 1995) and easier penetration through biological membranes (HANKE, PIOTROWSKI 1984).

Table 3
Correlation matrix of acute toxicity of insecticides and their physicochemical parameters (non-linear model, two variables $y = f(x_i)$)

Dependent variable (y) – acute toxicity	Independent variables (x_i)		
	M	K_{OW}	S_{H_2O}
PHOS			
LC_{50} – rainbow trout	0.29	0.35	-0.33
EC_{50} – *Daphnia magna*	0.52**	0.44*	-0.48*
IC_{50} – algae	0.73**	0.75**	-0.71**
CARB			
LC_{50} – rainbow trout	0.42	0.45	-0.63*
EC_{50} – *Daphnia magna*	-0.02	-0.10	-0.22
IC_{50} – algae	0.37	0.03	-0.14
PYRET			
LC_{50} – rainbow trout	0.14	0.40	-0.14
EC_{50} – *Daphnia magna*	0.17	0.47	-0.12
IC_{50} – algae	-0.21	0.48	-0.57

Regression model: $\log (y^{-1}) = b_0 + b_1 \cdot \log x_i$;
For details, see Table 2.

QSAR analysis – models with multiple variables

Analysis of multiple linear regression, with all three physicochemical parameters (M, K_{OW}, S_{H_2O}) as independent variables, showed a significant (at $p < 0.01$) correlation between the values of these parameters and the toxic effect of organophosphorus insecticides on the rainbow trout, daphnia and algae ($R_{\text{multiple}} = 0.952 \div 0.965$), carbamates on daphnia ($R_{\text{multiple}} = 0.835$) and pyrethroids on algae ($R_{\text{multiple}} = 0.983$) – Table 4.

It was found, similarly as in the case of simple correlation analysis, that the toxicity of organophosphorus insecticides and pyrethroids was determined

Table 4
Results of a multiple linear regression analysis of the acute toxicity of insecticides to aquatic organisms and their physicochemical parameters ($y = f(M, K_{OW}, S_{H_2O})$)

Specifications	PHOS		CARB		PYRET	
	b_i	p	b_i	p	b_i	p
LC_{50} – rainbow trout						
M	0.407	0.012	0.262	0.441	-0.009	0.376
K_{OW}	$-1.0 \cdot 10^{-5}$	0.907	$-1.6 \cdot 10^{-3}$	0.339	$-1.8 \cdot 10^{-7}$	0.527
S_{H_2O}	$1.3 \cdot 10^{-3}$	$6.2 \cdot 10^{-20}$	$-2.8 \cdot 10^{-5}$	0.444	-0.022	0.645
b_o	-126.061	0.009	-40.266	0.574	5.426	0.248
R, p (for model)	$R = 0.965$	$p < 0.001$	$R = 0.370$	$p = 0.741$	$R = 0.255$	$p = 0.727$
EC_{50} – *Daphnia magna*						
M	0.028	0.039	0.049	<0.001	$3.0 \cdot 10^{-4}$	0.345
K_{OW}	$-1.9 \cdot 10^{-6}$	0.768	$-2.1 \cdot 10^{-4}$	0.008	$-6.7 \cdot 10^{-9}$	0.525
S_{H_2O}	$8.4 \cdot 10^{-5}$	$2.2 \cdot 10^{-18}$	$-3.1 \cdot 10^{-6}$	0.227	$-1.7 \cdot 10^{-5}$	0.987
b_o	-8.864	0.033	-9.582	0.001	-0.084	0.550
R, p (for model)	$R = 0.956$	$p < 0.001$	$R = 0.835$	$p = 0.001$	$R = 0.255$	$p = 0.726$
IC_{50} – algae						
M	-0.145	0.678	0.394	0.647	$1.4 \cdot 10^{-3}$	0.958
K_{OW}	$5.7 \cdot 10^{-5}$	0.681	$-8.8 \cdot 10^{-3}$	0.341	$-2.8 \cdot 10^{-7}$	0.483
S_{H_2O}	$1.1 \cdot 10^{-3}$	$3.5 \cdot 10^{-10}$	$-8.3 \cdot 10^{-5}$	0.259	0.569	$2.3 \cdot 10^{-5}$
b_o	51.527	0.605	-19.890	0.910	2.603	0.839
R, p (for model)	$R = 0.952$	$p < 0.001$	$R = 0.628$	$p = 0.527$	$R = 0.983$	$p < 0.001$

Regression model: $y = b_0 + b_1 \cdot M + b_2 \cdot K_{OW} + b_3 \cdot S_{H_2O}$;
y – dependent variable (LC_{50}, EC_{50} or IC_{50});
p – statistical significance (p-level); R – multiple correlation coefficient;
For details, see Table 2.

primarily by their water solubility, which was confirmed by low significance levels (p). It was also demonstrated that molecular weight M and the n-octanol--water partition coefficient K_{OW} (independent variables) made the highest relative contribution to the prediction of the toxic effect of carbamates (dependent variable) on daphnia ($p < 0.001$ and $p = 0.008$).

The highest values of multiple correlation coefficients R were noted in QSAR analysis using a non-linear (logarithmic) model with three independent variables (M, K_{OW}, S_{H_2O}). The coefficients R for organophosphorus insecticides ranged between 0.955 and 0.992 (Table 5). Slightly lower values of R were obtained for pyrethroids (0.948÷0.998). As for carbamates, it was not possible to match models of their toxicity to the rainbow trout and algae ($R < 0,5$).

Xu et al. (2000) also reported that QSAR models with multiple variables, as compared to models with two variables, provided higher accuracy of prediction of the toxicity of xenobiotics to marine luminescent bacteria. In their study the values of coefficient R were 0.772 to 0.828, 0.880 and 0.923 for models with two, three and four variables respectively.

Table 5
Results of non-linear estimation of acute toxicity of insecticides to aquatic organisms and their physicochemical parameters ($y = f(M, K_{OW}, S_{H_2O})$)

Dependent variable (y) – acute toxicity	Regression coefficient				R
	b_0	b_1	b_2	b_3	
PHOS					
LC_{50} – rainbow trout	9.64	2.80	-0.03	-3.22	0.992
EC_{50} – *Daphnia magna*	32.57	29.78	-9.97	-18.76	0.989
IC_{50} – algae	-2.90	6.24	-1.25	-2.60	0.955
CARB					
LC_{50} – rainbow trout	1.14	0.17	-0.48	-0.55	0.401
EC_{50} – *Daphnia magna*	13.49	-14.33	3.93	7.18	0.939
IC_{50} – algae	-1.10	0.63	-0.40	-0.40	0.495
PYRET					
LC_{50} – rainbow trout	-913.20	411.09	-23.09	0.43	0.948
EC_{50} – *Daphnia magna*	142.67	-50.49	-0.83	-0.15	0.974
IC_{50} – algae	2.72	-1.71	0.16	-0.38	0.998

Regression model: $\log(y^{-1}) = b_0 + b_1 \cdot \log M + b_2 \cdot \log K_{OW} + b_3 \cdot \log S_{H_2O}$;
R – multiple correlation coefficient;
For details, see Table 2.

Conclusions

1. It was found, based on analysis of reference data, that among the chemical classes examined in the study pyrethroids and organochlorine insecticides had the strongest toxic effect on aquatic animals, whereas neonicotinoids were the least toxic.

2. The toxicity of insecticides to aquatic organisms was determined primarily by the n-octanol-water partition coefficient K_{OW} and water solubility, which most probably resulted from the mechanisms of penetration of these xenobiotics through biological membranes.

3. The strongest correlation between the physicochemical properties of pyrethroids and organochlorine insecticides and their toxicity to the rainbow trout, daphnia and algae was obtained when non-linear QSAR analysis with many variables was performed (R = 0.948 to 0.998).

4. The toxicity indicator EC_{50} determined for daphnia showed the highest correlation with the physicochemical properties of carbamates.

5. QSAR analysis may be used to predict the ecotoxicity of newly synthesized insecticides to aquatic organisms.

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BACTERIA ACTIVE IN SULFUR CYCLE IN THE UNDERGROUND WATERS OF OMULEWSKI AQUIFER IN THE MAZURIAN LAKE DISTRICT

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K e y w o r d s: *Thiobacillus* spp., sulphate reducing bacteria, *Beggiatoa*, *Thiothrix*, *Thioploca*, underground waters.

A b s t r a c t

The manuscript reports on the result of studies into selected physicochemical parameters as well as the number and/or frequency of occurrence of bacteria oxidizing sulfur and its compounds and those reducing sulfates in underground waters at the area of a hydrogeological unit, i.e. the Omulewski Aquifer. Investigations involved waters originating from: a well in an afforested area (Narty), farm land cultivated by individual farmers (Jedwabno, Kot), a farm of furred animals (Janowo, Sedańsk), and commercial cattle farms (Wesołowo, Przeździęk, Baranowo, Wyżegi) and pigs (Dzierzki, Wielbark), as well as waters from piezometric boreholes at the area of a cattle farm in Wesołówek. Waters of those wells and piezometric boreholes were characterized by a high variability of the physicochemical and microbiological parameters, both between the same wells in the experimental period of 1991-1994 and between wells from different villages. Only the waters from a well located at the area of forester's lodge in Narty corresponded, in terms of chemical composition, to the values reported for a natural hydrochemical background of waters in sands of the Mazuria--Kurpiowski Sandr. Usually, waters of other wells contained more NH_4-N, NO_2-N, NO_3-N, SO_4, Fe; their chemical oxygen demand – measured by $KMnO_4$ consumption – was higher as well. Of the bacteria active in the sulfur cycle, higher counts were reported only for *Thiobacillus thioparus*. They were detected in 80-100% of the samples, in numbers not exceeding 1400 MPN $\cdot$ 100 cm^{-3}. Other bacteria, including *Thiobacillus thiooxidans*, *Thiobacillus denitrificans* and sulfates reducing bacteria of the genus *Desulfovibrio*, occurred in 33-66%, 19-73% and 50-80% of the water samples examined, respectively, in the numbers not exceeding 700, 240 and 460 MPN $\cdot$ 100 cm^{-3}, respectively. Filamentous hydrogen sulfide bacteria were represented by species of the genera *Beggiatoa*, *Thiothrix* and *Thioploca*; and were detected in 16-100% of the water samples analyzed.

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BAKTERIE CZYNNE W OBIEGU SIARKI W WODACH PODZIEMNYCH ZBIORNIKA OMULEWSKIEGO NA POJEZIERZU MAZURSKIM

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S ł o w a k l u c z o w e: *Thiobacillus* spp., bakterie redukujące siarczany, *Beggiatoa*, *Thiothrix*, *Thioploca*, wody podziemne.

A b s t r a k t

Badano niektóre wskaźniki fizyczno-chemiczne oraz liczebność i/lub częstotliwość występowania bakterii utleniających siarkę i jej związki, redukujących siarczany w wodach podziemnych na obszarze jednostki hydrogeologicznej Zbiornika Omulewskiego. W badaniach ujęto wody studni na terenach: zalesionych (Narty), zagospodarowanych przez rolników indywidualnych (Jedwabno, Kot), ferm zwierząt futerkowych (Janowo, Sedańsk), wielkotowarowych ferm bydła (Wesołowo, Przeździęk, Baranowo, Wyżegi) i trzody chlewnej (Dzierzki, Wielbark) oraz wody odwiertów piezometrycznych na terenie fermy bydła w Wesołówku. W wodach tych studni i odwiertów piezometrycznych stwierdzono dużą zmienność parametrów fizykochemicznych i mikrobiologicznych, zarówno w obrębie tych samych studni w okresie badawczym 1991-1994, jak i studni z różnych miejscowości. Jedynie wody studni na terenie leśniczówki w Nartach odpowiadały pod względem chemicznym wartościom podawanym dla naturalnego tła hydrochemicznego wód w piaskach sandru mazursko-kurpiowskiego. Wody innych studni zawierały z reguły więcej NH_4-N, NO_2-N, NO_3-N, SO_4, Fe; również utlenialność mierzona zużyciem $KMnO_4$ była wyższa. Spośród bakterii czynnych w obiegu siarki, liczniej występowały tylko *Thiobacillus thioparus*. Stwierdzano je w 80-100% prób, w ilościach nie przekraczających 1400 MPN · 100 cm^{-3}. Inne bakterie, jak *Thiobacillus thiooxidans*, *Thiobacillus denitrificans* i bakterie redukujące siarczany z rodzaju *Desulfovibrio,* były obecne odpowiednio w 33-66%, 19-73% i 50-80% badanych prób wody, w ilościach nie przekraczających odpowiednio 700, 240 i 460 MPN · 100 cm^{-3}. Nitkowate bakterie siarkowodorowe były reprezentowane przez gatunki z rodzajów *Beggiatoa*, *Thiothrix* i *Thioploca*; znajdywano je w 16-100% badanych prób wody.

Introduction

Underground waters constitute a major source of drinking water and water for household purposes in general, and in rural areas in particular. Hence, the protection of that type of water resources, their quality as well as contamination hazards have been the subject of great concern worldwide. According to NIEDZIELSKI, 1915, cit. after KOCHAŃSKA (1991), in Poland at the beginning of the last century, pollution with nitrogen compounds and hydrogen sulfide has been reported to occur only sporadically. Currently, waters of shallow wells do not comply with hygienic and sanitary standards. Well waters from villages located at the area of the Wigry National Park may serve as an example (NIEWOLAK 1998). The quality of waters drawn from deeper intakes, unprotec-

ted with an impermeable layer of geological formation, is subject to ever-increasing deterioration (SZCZEPKOWSKI 1983). The deterioration of the chemical and bacteriological quality of waters is likely to result from the penetration of sewage pollutants, mineral fertilizers and industrial contaminants from the ground surface (NIEWOLAK 1994a). A number of these pollutants are subject to microbiological transformation in soil and underground waters. In the course of those transformations, a significant function is served by a variety of bacteria, including bacteria oxidizing sulfur and its compounds and sulfate reducing bacteria. In some water-bearing formations, they can constitute a predominating group of bacteria (GROSSMAN, DESROCHER 2001). They are likely to exert a considerable effect on the quality of underground waters. The oxidation of sulfides by thionic bacteria may result in water acidification due to the production of sulfuric acid. The reduction of sulfates by bacteria of the genus *Desulfovibrio* and some other bacteria (CASTRO et al. 2000, SASS, CYPIONKA 2004, SASS et al. 2002) may lead to a decrease in the concentration of toxic metals as a consequence of precipitation of insoluble sulfides (BRIERLEY, BRIERLEY 1996). At a lack of metals forming insoluble sulfides, the accumulation of detrimental levels of hydrogen sulfide is likely to occur (BERNER 1987). That gas may pose problems once its concentration in water reaches a perceptible level, i.e. 0.03 $mg \cdot dm^{-3}$ (HAO et al. 1996). The presence of hydrogen sulfide in water may lead to the corrosion of appliances and a water-pipe network (IVERSON 1972, POSTGATE 1984), and to the deterioration of water odor. In addition, the sulfate reducing bacteria can contribute to biological colmatation of gravel ridging surrounding a well's filter or the water-bearing layer adhering to a borehole, and to incrust in the water-pipe network. Part of incrustation's mass may detach and transfer to the transmitted water, which is likely to occur at rapid changes of pressure in the network (OLAŃCZUK-NEYMAN 2001). As a consequence, the turbidity of water increases and its color and taste deteriorate. A mass increase in the number of filamentous hydrogen sulfide bacteria, in turn, causes clogging of free spaces in well and water-pipe network filters as a result of the production of "sulfur slime" (WILLIAMS, UNZ 1985a, 1985b). No data exists on the occurrence of active bacteria of the sulfur cycle in underground waters of the north-eastern areas of Poland.

Bearing in mind the significance of these bacteria in these types of waters, the objective of this study was to analyze the occurrence of bacteria oxidizing sulfur and its compounds (*Thiobacillus thioparus*, *Thiobacillus thiooxidans*, *Thiobacillus denitrificans*) and sulfate reducing bacteria (*Desulfovibrio* sp.) in the waters of wells and piezometric boreholes covering underground waters, not isolated with an impermeable layer from the ground's surface, of a hydrological unit referred to as Omulewski Aquifer.

In the area of the aquifer, several large commercial farms of cattle and pigs have been functioning since the 1970s. Their impact on the contamination of underground waters of this area has been reflected in an altered, unfavorable composition: both chemical (KOCHAŃSKA 1991) and bacteriological (NIEWOLAK 1994b), of water drawn from the deepest intakes.

Material and Methods

Omulewski Aquifer. The Omulewski Aquifer is located in the western part of the Large Mazuria-Kurpiowski Sandr, in the catchment area of Omulew and Narew and partly of the Orzyc River (Figure 1). In the Mazurian Lake District, it is the largest hydrogeological unit with renewable, shallowly deposited water resources not posing any exploitation difficulties. SZCZEPKOWSKI (1983), who included it into a group of aquifers of underground waters without isolation from the surface with impermeable formation, emphasizes a potential risk of the penetration of chemical and bacteriological contaminations from the ground's surface. It is of key importance due to the location of large commercial farms of cattle and pigs at this area. The surface area of the aquifer is 820 km^2. The surface level of the underground water of the aquifer is at a depth of from 1 to several meters and is subject to vertical variation, ± 1.5 m per year on average. The highest thickness of the aquifer accounts for 120 m and occurs in its central part, gradually becoming shallow towards the south-east and the Narew River. The water flow is presumably two-directional: the upper layers run towards the Narew river, whereas the bottom ones either run in the opposite direction following the bottom's inclination or stagnate (SZCZEPKOWSKI 1983). The underground waters may directly contact the surface waters of this area, especially those of the Omulew and Orzyc Rivers and of Lake Omulew, Świętajno-Narty, Brajnickie and Warchały. Lands by the Omulewski Aquifer are included (according to agricultural maps) in the Szczycieński Region. Over half of their total area is covered with forests, 24% are constituted by arable lands, 12% by grasslands, and others by waters of rivers and lakes as well as farm buildings. Periodically or constantly dry soils predominate in the entire area, whereas periodically excessively damp or even waterlogged soils also occur at lower situated areas. Most of them require constant mineral and organic fertilization and even liming. Considerable afforestation and grasslands constitute a natural protection of underground waters against penetration of pollutants. The greatest hazard is posed by non-utilized components of mineral fertilizers, slurry and pesticides applied on grasslands and arable lands. Risks may also result from spot sources of contamination, including industrial waste waters (Jedwabno, Kot), domestic sewage (Janowo,

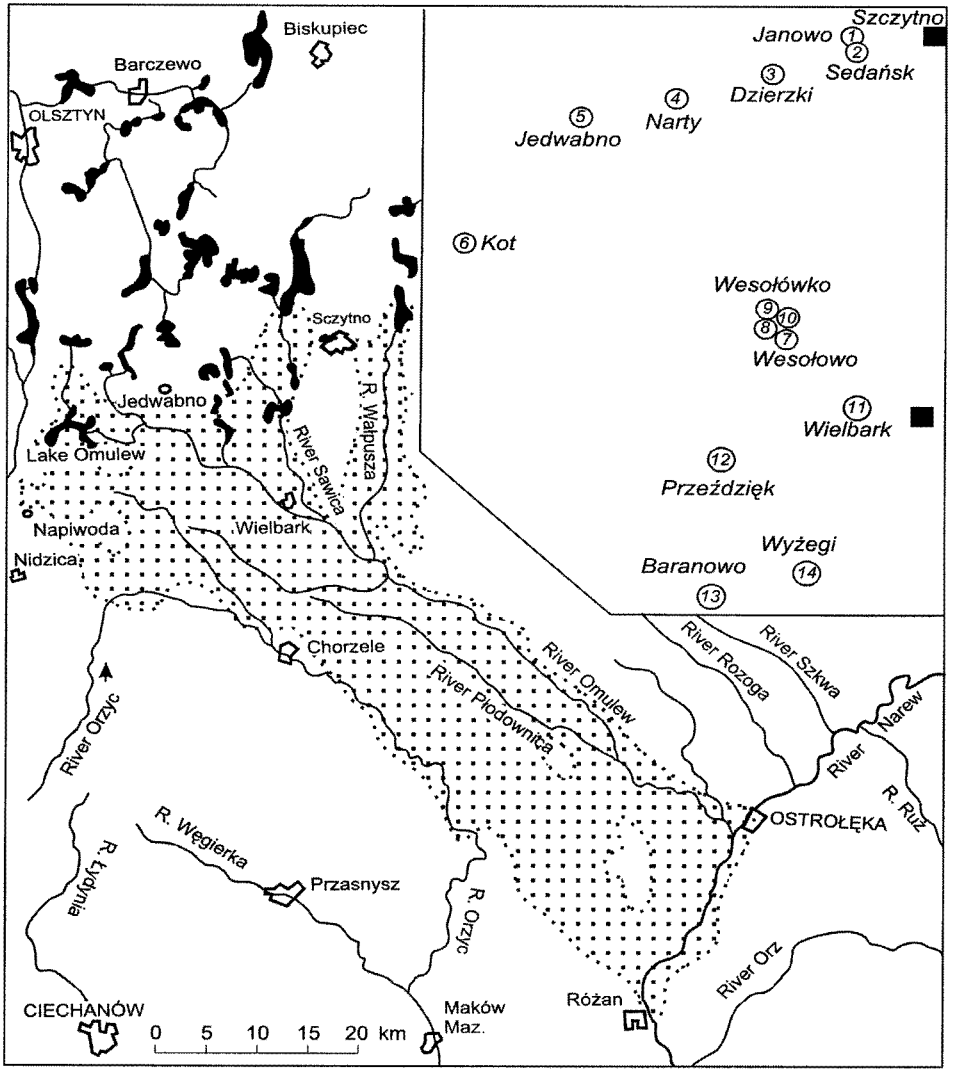

Fig. 1. Situational draft of the Omulewski Reservoir Aquifer according to SZCZEPKOWSKI (1983)

Sedańsk), and especially those originating from animal farming (Dzierzki, Wesołowo, Wielbark, Przeździęk, Baranowo, Wyżegi) including farming run with the non-litter system. Until 1992, several large farms of cattle and pigs had been located herein, before some of them were closed.

Wells and piezometric boreholes. Eleven wells and three piezometric boreholes located in the northern and central part of the Omulewski Aquifer were selected for the study (Figure 1).

They were located in:

1) a completely afforested area (Sedańsk, Narty);

2) the area of fox rearing farm in the vicinity of a trout farm (Janowo);

3) farm lands managed by individual farmers (Jedwabno, Kot);

4) an area with the prevalence of large commercial farms of cattle and pigs (Dzierzki, Wielbark, Wesołowo, Przeździęk, Baranowo, Wyżegi, Wesołowo).

Piezometric boreholes were situated in an area belonging to a cattle farm in Wesołowo-Wesołówek:

1) at an afforested area close to farm buildings (places where mineral fertilizers used to be stored);

2) in a quarter formerly used to keep straw;

3) in a quarter used as a cattle-run and for additional feeding of young cattle in the open air, close to a cow shed and adjacent containers for slurry.

Waters of wells in Janowo, Sedańsk, Dzierzki, Narty and Wielbark were of the carbonate-calcium-magnesium type, those of wells located in Kot, Wesołowo and Przeździęk villages – of the carbonate-calcium type, those of the well in Baranowo – of the carbonate-chloride-calcium type; and those of the well in Wyżegi – of the carbonate-calcium-sulfate type (KOCHAŃSKA 1991). Some technical data referring to these wells is provided in Table 1.

Water samples to be analyzed were collected from wells and piezometric boreholes once every three months, from March 1989 till September 1994. In Janowo, Sedańsk, Dzierzki, Narty, Przeździęk, Baranowo and Wyżegi villages, water samples were collected directly from valves of water collecting pipes of the hydrophore, whereas in Jedwabno, Kot, Wesołowo (Wesołówko) and Wielbark villages – after the hydrophore from a user's tap, due to the lack of technical possibility of direct collection of well waters. Water sample collection for bacteriological examinations, from 11 wells, were preceded by disinfection of valves and taps and 10-min pumping. In the case of water samples from the piezometric boreholes, samples were collected each time after pumping out ca. 100 dm^3 of stagnating water using a manual water pump. The water samples collected were transported to a laboratory in containers providing a temperature of 4°C.

Chemical analyses of the water samples were carried out in the years 1989-1992, following the methodology described by ALLEN, STEWART (1975), HERMANOWICZ et al. (1976) and other data provided in Polish Standards. They included, among others, such parameters as: water reaction (pH), content of dissolved oxygen, oxidability (measured as $KMnO_4$), NH_4^+, NO_2^-, NO_3^-, Cl^-, SO_4^-, Fe, total hardness, alkalinity and color. Results obtained at this stage of the study were referred to the values of parameters from a control well in Narty village that corresponded to the natural hydrochemical background of the Mazuria-Kurpiowski Sandr (MACIOSZCZYK 1986).

Table 1

Characteristics of wells and piezometric bore-holes (basic geological-technical data) on the area of Omulewski Aquifer

Localization	Year of construction	Depth (m)		Type of filter	Length of working part of filter	Gravel wall of filter P – sand	Yield ($m^3 \cdot 24\ h^{-1}$)		Age of geological layer	Type of water	Utilizer	High above sea level (m)
		total	afiltration				exploiting	maximal				
Janowo	1965	27.0	20.0-24.0	A	4.2	$P< = 2$ mm	55.0	55.0	IV	CO_3/Ca/Mg**	fox farm (2 000)*	158.0
Sedańsk	1978	40.0	19.5-36.5	B	17.0	$P< = 2$ mm	49.0	66.1	IV	CO_3/Ca/Mg	fox farm	140.0
Dzierzki	1972	25.0	11.0-17.0	B	6.0	–	29.0	33.8	IV	CO_3/Ca/Mg	swine farm (2 500)	134.0
Narty	–	40.0	16.5-30.0	C	–	–	–	–	–	CO_3/Ca/Mg	forester's lodge	–
Jedwabno	1972	49.5	37.0-47.0	D	–	$P< = 2$ mm	60.0	50.1	IV	–	dairy	138.1
Kot	–	20.0	–	D	–	–	–	–	–	CO_3/Ca***	polythene processing plant	–
Wesołowo	1976	41.0	28.1-38.0	D	9.9	$P< = 2$ mm	54.0	57.0	IV (plei)	CO_3/Ca	cattle farm (700)	127.8
Wesołówko (1)	1976	8.0	6.5-7.5	D	1.0	–	–	5.0	IV	–	cattle farm	127.4
Wesołówko (2)	1976	29.0	27.5-28.0	D	1.0	–	–	5.0	IV (plei)	–	cattle farm	128.0
Wesołówko (3)	1976	29.0	27.5-28.0	D	1.0	–	–	5.0	IV (plei)	–	cattle farm	127.5
Wielbark	–	60.0	1.3-44.0	D	–	–	–	–	–	CO_3/Ca/Mg	swine farm (10 000)	126.0
Przeździęk	1978	34.0	23.2-32.3	B	9.1	$P< = 2$ mm	52.0	60.0	IV (plei)	CO_3/Ca	cattle farm (500)	131.4
Baranowo	1979	84.0	49.1-62.4	B	13.3	$P< = 2$ mm	20.0	25.1	IV (plei)	CO_3/Cl/Ca****	cattle farm (500)	127.0
Wyżegi	1975	25.0	14.9-23.0	B	8.1	–	–	60.0	IV	CO_3/Ca/SO_4*****	cattle farm (1 000)	126.0

(1, 2, 3) – piezometric bore-holes; A – gravel, steel; B – steel, stilon net; C – asbestos sealing; D – steel, unknown net;
(–) no data;
IV – guaternary (pleistocene);
* – number of animals in brackets; ** – carbonate-calcite-magnezite, *** – carbonate-calcite, **** – carbonate-chloride-calcite, ***** – carbonate-calcite-sulphate.

Microbiological analyses were carried out in the years 1990-1994 and involved determinations of the number and/or frequency of occurrence of bacteria oxidizing sulfur and its compounds (*Thiobacillus thioparus*, *Thiobacillus thiooxidans*, *Thiobacillus denitrificans*), sulfate reducing bacteria (*Desulfovibrio* spp. and others) later on referred to as SRB, and filamentous hydrogen sulfide bacteria. The number of bacteria oxidizing sulfur and its compounds and that of SRB were determined on selective media under culture conditions described by SPANDOWSKA et al. (1979) and provided in Table 2. The occurrence of hydrogen sulfide bacteria was determined with a microscopic technique of glass incrusting described in a paper on the occurrence of bacteria of the family *Siderocapsaceae* (NIEWOLAK, GOŁAŚ 2003). The microscopic picture of filamentous hydrogen sulfide bacteria was compared with data reported by: HUETTEL et al. (1996), BRIGMON et al. (1994a, 1994b), *Standard methods...* (1992), EIKELBOOM, VAN BUIJSEN (1992), JENKINS et al. (1986), WILLIAMS, UNZ (1985a, 1985b), BLAND, STANLEY (1978), and STROHL, LARKIN (1978). In total, 224-280 water samples were examined.

Table 2

Growth media and incubation regime in the determination of bacteria active in oxidation of sulphur and sulphur compounds and reduction of sulphate in underground waters of Omulewski Aquifer (Mazurian Lake District)

Bacteria	Nutrient medium	Condition of grown	Incubation	
			temperature (°C)	time (days)
Thiobacillus thioparus	* Starkey (pH 7.8)	aerobic	25	28
Thiobacillus thiooxidans	* Waksman (pH 4.8)	aerobic	25	28
Thiobacillus denitrificans	** Lieske with Durham's tube	anaerobic	25	28
Sulphate-reducing bacteria	*** Tauson in Szturm's modification (pH 7.5)	anaerobic	25	14

* after A.P.H.A. (1992);
** after SPANDOWSKA et al. (1979);
*** after RODINA (1968).

Results

The values of intervals of some physicochemical parameters were presented in Table 3. It indicates that waters of the wells analyzed were characterized by a reaction close to neutral or slightly basic. The content of oxygen was the lowest in water of wells from Wielbark and Przeździęk as well as from piezometric borehole located at the cattle farm in Wesołowo (Wesołówek), whereas the highest was in well water from Narty village (constant in the entire experimental period). In water of the other wells, it was subject to

Table 3

The values of intervals of some physico-chemical parameters in the waters of wells and piezometric bore-holes in the Omulewski Aquifer in 1990-1992 years

Localization	pH (units)	Oxygen ($mg \cdot dm^{-3}$ O_2)	Oxidability with $KMnO_4$ ($mg \cdot dm^{-3}$ O_2)	Ammonium ($mg \cdot dm^{-3}$ NH_4-N)	Nitrites ($mg \cdot dm^{-3}$ NO_2-N)	Nitrates ($mg \cdot dm^{-3}$ NO_3-N)	Chlorides ($mg \cdot dm^{-3}$ Cl)	Sulphates ($mg \cdot dm^{-3}$ SO_4)	Fe-total ($mg \cdot dm^{-3}$ Fe)	Total hardnes ($mval \cdot dm^{-3}$)	Alcalinity ($mval \cdot dm^{-3}$)	Colour as ^{o}Pt dm^{-3}
Wells:												
Janowo	6.68-7.85	1.1-4.6	1.9-8.8	0.20-0.76	0.000-0.014	0.00-2.79	7.0-11.7	9.8-22.5	0.01-1.50	4.30-5.70	2.1-4.8	20
Sedańsk	6.90-7.90	1.0-6.4	1.9-7.5	0.00-0.27	0.001-0.014	0.00-0.11	5.4-9.0	9.0-43.6	1.17-1.70	4.60-5.90	2.3-4.6	20
Narty	7.30-8.04	8.0-8.4	0.7-1.4	0.00-0.17	0.000-0.006	0.04-0.27	2.7-15.3	21.0-32.5	0.01-0.35	3.50-4.30	1.7-3.6	–
Jedwabno	7.00-7.95	5.8-7.8	1.0-3.3	0.00-0.60	0.000-0.047	0.62-12.3	16.2-36.0	19.8-73.2	0.01-0.15	5.40-7.50	2.8-6.2	20
Kot	7.05-7.91	1.0-5.9	2.4-4.2	0.37-1.52	0.030-0.190	1.52-29.0	16.2-40.0	23.4-46.0	0.08-0.46	4.60-6.60	2.1-3.9	–
Dzierzki	6.98-7.90	1.3-13.6	2.8-7.0	0.00-0.94	0.000-0.028	0.01-2.0	3.0-9.1	5.8-43.0	0.01-1.90	0.34-0.48	1.9-4.0	10
Wielbark	7.08-8.15	0.6-1.8	3.0-6.2	0.11-0.56	0.000-0.016	0.00-0.22	2.0-36.0	2.5-16.9	0.20-1.89	0.45-0.93	2.8-5.3	60
Przeździęk	7.11-7.80	0.3-2.4	5.9-13.4	0.25-1.33	0.000-0.020	0.05-0.85	12.7-50.4	17.7-69.1	0.42-7.48	0.37-0.60	2.8-4.2	70
Baranowo	7.00-7.78	2.0-7.0	6.5-8.2	0.38-3.56	0.009-0.030	0.05-1.00	16.0-108.5	37.2-60.0	1.08-7.00	0.43-0.69	2.0-3.9	70
Wyżegi	6.70-7.75	1.0-4.4	4.6-16.0	0.67-7.70	0.003-0.065	0.30-5.70	17.0-52.8	22.0-204.9	0.60-2.69	0.39-0.67	2.6-3.5	15
Wesołowo	6.89-7.39	0.5-8.2	3.6-6.0	0.00-4.12	0.000-0.047	0.00-0.20	6.3-22.5	21.4-62.9	0.00-4.58	0.41-0.52	3.4-4.8	20
Bore-holes:												
No.1	7.05-8.45	1.0-1.6	2.8-11.4	0.00-4.03	0.000-0.021	0.00-0.22	8.0-15.2	1.25-30.0	0.00-0.28	0.24-0.52	2.3-3.0	–
No.2	7.17-8.15	1.0-2.0	2.0-3.5	0.00-0.69	0.000-0.010	0.10-0.16	7.6-17.1	6.00-20.0	0.00-0.78	0.25-0.43	2.3-3.0	–
No.3	7.18-7.58	0.0-1.8	10.0-21.5	0.16-3.99	0.000-0.030	0.09-0.18	16.2-37.1	71.80-171.60	0.00-7.30	0.41-0.84	3.5-4.2	–

(–) no tested

smaller (Janowo, Sedańsk, Jedwabno, Kot, Wyżegi) or greater (Dzierzki, Wesołowo, Baranowo) fluctuations. Oxidability (measured as $KMNO_4$ consumption) in water of the well in Narty was within the range reported for a natural hydrochemical background of sands of the Mazuria-Kurpiowski Sandr. In water from wells located in Przeździęk, Baranowo and Wyżegi villages and in that from piezometric boreholes No. 1 and 3 at the cattle farm in Wesołowo (in May of 1990 and March of 1989 and 1991), it occasionally exceeded the maximal permissible values for drinking water (5 mg $O_2 \cdot dm^{-3}$). The waters of these wells also contained higher concentrations of NH_4-N as compared to those reported for the hydrochemical background. The higher concentrations of NH_4-N also occurred in the waters of wells located in Dzierzki, Wesołowo (Wesołówko), Jedwabno, Kot and Wielbark villages; in the latter three – at lower oxidability values. Nitrites only in well water from Janowo, Sedańsk, Narty and in water from piezometric boreholes in Wesołowo-Wesołówek, nitrates only in well water from Sedańsk, Narty and Wielbark villages and in water of piezometric borehole No. 1 in Wesołowo (Wesołówek), and chlorides only in well water from Sedańsk and Dzierzki villages were within the range reported for the natural hydrochemical background of sands of the Mazuria-Kurpiowski Sandr. The content of sulfates exceeded 100 mg $SO_4 \cdot dm^{-3}$ only in well water from Wyżegi and water of piezometric borehole No. 1 (at the storage area of mineral fertilizers); whereas the values of total Fe were within the maximal permissible range for drinking water only in well water from Narty, Jedwabno and Kot villages and water of piezometric borehole No. 1. Most of the wells supplied water with hardness established for a natural hydrochemical background of sands of the Mazuria--Kurpiowski Sandr. The color of water sampled from a well in Przeździęk reached the maximal values permissible for drinking water, whereas in water collected from wells in Baranowo and Wyżegi – it exceeded these values. More detailed data on these and other physicochemical parameters of the analyzed samples of underground waters from the Omulewski Aquifer were reported in separate papers (KOCHAŃSKA, NIEWOLAK 1995, 1997).

The bacteria oxidizing sulfur and its compounds. Of this group of bacteria, in waters of the examined wells and piezometric boreholes, *Thiobacillus thioparus* occurred the most frequently (in 80-100%). Their number ranged from < 3 to 1400 MPN $\cdot$ 100 cm^{-3}. In the experimental period, their lower numbers were usually reported from December of 1990 till September of 1992, whereas higher ones – in different seasons of 1993 and 1994 (Table 4).

Thiobacillus thiooxidans occurred in 33-66% of the water samples analyzed, less frequently in the water of wells from Sedańsk, Narty, Kot, Dzierzki and Wielbark villages and in water of the piezometric borehole No. 1 in Wesołowo, and more frequently in well water from Janowo, Jedwabno,

Table 4

Number (MPN · 100 cm^{-3}) of *Thiobacillus thioparus* in underground waters of the Omulewski Aquifer (Mazurian Lake District) in 1990-1994 years

Date (month, year) of sampling	Janowo	Sedańsk	Narty	Jedwabno	Kot	Dzierzki	Wielbark	Przeździęk	Baranowo	Wyżegi	Wesołowo	Wesołówko		
												bore-holes		
	wells											no. 1	no. 2	no. 3
12.1990	11	–*	–	3	3	11	930	28	1400	1400	4	–	–	–
03.1991	450	–	15	43	23	1400	93	–	240	1400	240	240	1400	240
06.1991	1400	–	150	23	1400	23	–	<3	<3	<3	–	23	23	23
09.1991	21	21	9	4	15	21	28	–	15	<3	15	15	28	15
12.1991	<3	–	<3	<3	<3	4	4	4	<3	<3	–	<3	4	<3
03.1992	3	–	4	<3	28	<3	9	3	9	7	–	–	–	–
06.1992	6	5	6	5	62	62	5	23	5	6	6	6	5	6
09.1992	5	6	5	5	5	62	23	6	6	5	23	6	5	13
12.1992	460	1400	15	21	460	15	1400	39	39	46	33	1400	1400	1400
03.1993	1400	7	43	14	93	1400	–	–	35	–	–	1400	–	–
06.1993	1400	1100	210	1400	3	93	210	20	210	240	–	1400	1400	460
10.1993	240	–	1100	460	460	1100	460	240	1400	460	1100	1100	1400	1100
12.1993	150	–	210	43	20	20	14	75	43	9	15	460	43	120
03.1994	210	–	93	<3	<3	240	240	240	43	93	21	21	28	240
06.1994	1100	–	75	240	1400	1100	1400	1400	1100	1400	460	1400	1400	1400
09.1994	150	–	210	43	20	20	14	75	43	9	15	460	43	120
(%)	93	100	93	81	87	100	100	92	87	80	100	93	100	92

* – no tested

% – percent positive samples

Przeździęk, Baranowo and Wesołowo. Their number fluctuated from < 3 to 700 MPN · 100 cm^{-3}. They occurred in higher numbers in June of 1992, and less frequently in the remaining experimental period (Table 5).

Thiobacillus denitrificans was detected in 19-73% of the well water samples and in 30-50% of water samples from piezometric boreholes. It occurred less frequently in water of wells located in Narty, Dzierzki and Wielbark an in the water of piezometric borehole No. 3 from Wesołowo, and most frequently in the well water from Sedańsk and Wyżegi. Their number ranged from < 3 to 240 MPN · 100 cm^{-3} in well water from Janowo and to 300 MPN · 100 cm^{-3} in the well water from Wyżegi. In the water of the other wells it did not exceed 93 MPN · 100 cm^{-3}, whereas in water of piezometric boreholes it was not higher than 24 MPN · 100 cm^{-3}. In waters of different wells and piezometric boreholes, their slightly higher numbers were reported in different experimental periods (Table 6).

The sulfate-reducing bacteria (SRB) were present in 50-80% of the analyzed water samples from wells located in different villages and in 58-75% of water samples from piezometric boreholes in Wesołowo. They were observed the least frequently in well water from Sedańsk and Kot villages, and the most frequently in well water from Wyżegi. They number ranged from < 3 to 460 MPN · 100 cm^{-3}. In the water of the wells examined, their higher numbers occurred in December of 1992, whereas in the other months they appeared less frequently. In the water of piezometric boreholes their number fluctuated from < 3 to 93 MPN · 100 cm^{-3}. Their higher numbers were found in December of 1992 (piezometric borehole No. 2) and in September of 1994 (piezometric boreholes No. 1 and 3) – Table 7.

The filamentous hydrogen sulfide oxidizing bacteria. These bacteria occurred in 5-35% of the analyzed samples of well water and in 10% of those of piezometric boreholes. Their lowest frequency was observed for water samples collected in Kot and the highest were in water samples from Narty and Baranowo. Bacteria of the genus *Thiotrix* and *Thioploca* were identified more frequently, compared to those of the genus *Beggiatoa* (Table 8).

Discussion

The predominating contribution of *Thiobacillus thioparus* in water of the wells and piezometric boreholes examined is explained by the nature of geological formations of the Omulewski Aquifer. The sandy formations are also highly permeable for oxygen, whereas their redox potential usually exceeds E_h 300 mV (GROSSMAN, DESROCHER 2001). The reaction close to neutral can be a factor discriminating *Thiobacillus thioparus* from the other species of thionic

Table 5

Number (MPN · 100 cm^{-3}) of *Thiobacillus thiooxidans* in underground waters of the Omulewski Aquifer (Mazurian Lake District) in 1990-1994 years

Date (month, year) of sampling	Janowo	Sedańsk	Narty	Jedwabno	Kot	Dzierzki	Wielbark	Przeździęk	Baranowo	Wyżegi	Wesołowo	Wesołówko		
												bore-holes		
	wells											no. 1	no. 2	no. 3
12.1990	3	–*	–	9	<3	<3	<3	<3	15	<3	<3	–	–	–
03.1991	<3	–	<3	<3	<3	<3	<3	–	<3	<3	23	<3	<3	<3
06.1991	<3	–	<3	60	130	<3	<3	<3	<3	<3	–	<3	<3	<3
09.1991	21	9	9	<3	9	<3	4	–	4	4	9	9	9	4
12.1991	15	–	9	9	9	9	20	9	20	9	–	15	20	20
03.1992	15	–	<3	<3	<3	<3	<3	<3	<3	<3	–	–	–	–
06.1992	23	23	700	240	240	23	23	62	240	240	240	240	23	23
09.1992	240	12	23	23	23	21	23	6	23	23	23	23	23	23
12.1992	28	<3	<3	3	<3	3	3	7	7	7	3	3	3	28
03.1993	<3	<3	–	<3	<3	<3	<3	<3	–	<3	–	<3	<3	<3
06.1993	28	<3	<3	3	<3	3	<3	7	7	7	3	3	3	28
10.1993	<3	–	4	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3
12.1993	3	–	<3	3	<3	3	3	3	3	3	3	<3	3	3
03.1994	<3	–	<3	<3	<3	<3	<3	3	3	<3	<3	<3	<3	<3
06.1994	<3	–	23	21	<3	<3	<3	<3	<3	3	<3	<3	<3	<3
09.1994	7	–	210	–	–	20	11	75	43	9	15	460	43	120
(%)	62	50	50	60	33	44	44	57	66	56	66	50	57	57

* – no tested

% – percent positive samples

Table 6

Number (MPN · 100 cm^{-3}) of *Thiobacillus denitrificans* in underground waters of the Omulewski Aquifer (Mazurian Lake District) in 1990-1994 years

Date (month, year) of sampling	Janowo	Sedańsk	Narty	Jedwabno	Kot	Dzierzki	Wielbark	Przeździęk	Baranowo	Wyżegi	Wesołowo	Wesołówko		
												bore-holes		
	wells											no. 1	no. 2	no. 3
12.1990	<3	–*	–	<3	<3	<3	<3	<3	9	4	<3	–	–	–
03.1991	<3	–	<3	<3	4	<3	<3	–	<3	23	23	<3	<3	4
06.1991	<3	–	<3	<3	<3	<3	<3	<3	4	<3	–	<3	<3	24
09.1991	<3	<3	<3	<3	<3	<3	<3	–	<3	4	<3	<3	<3	9
12.1991	<3	–	<3	3	<3	<3	<3	6	<3	6	–	<3	<3	<3
03.1992	4	–	9	9	23	<3	<3	9	23	300	–	–	–	–
06.1992	23	23	–	14	15	23	28	9	21	28	9	9	9	<3
09.1992	5	5	5	5	6	5	5	5	5	6	6	5	5	5
12.1992	<3	<3	4	3	4	<3	<3	3	<3	9	<3	<3	4	<3
03.1993	<3	4	<3	23	<3	<3	<3	–	4	–	–	9	<3	–
06.1993	240	28	14	28	4	11	<3	3	<3	9	<3	<3	4	<3
10.1993	<3	–	3	11	<3	<3	<3	<3	<3	3	4	<3	<3	<3
12.1993	7	–	<3	<3	<3	4	<3	<3	20	<3	<3	3	7	<3
03.1994	3	–	4	23	93	4	4	<3	21	<3	4	<3	7	<3
06.1994	<3	–	<3	<3	<3	<3	<3	9	21	110	<3	<3	<3	<3
09.1994	7	–	<3	–	–	4	<3	<3	20	<3	<3	3	7	<3
(%)	44	66	36	60	47	37	19	54	62	73	42	35	50	30

* – no tested

% – percent positive samples

Table 7

Number (MPN · 100 cm^3) of sulphate-reducing bacteria in underground waters of the Omulewski Aquifer (Mazurian Lake District) in 1990-1994 years

Date (month, year) of sampling	Janowo	Sedańsk	Narty	Jedwabno	Kot	Dzierzki	Wielbark	Przeździęk	Baranowo	Wyżegi	Wesołowo	Wesołówko		
												bore-holes		
	wells											no. 1	no. 2	no. 3
12.1990	<3	–	–	–	<3	<3	23	23	23	23	<3	–	–	–
03.1991	–*	–	<3	–	–	–	–	–	–	–	–	–	–	–
06.1991	<3	–	<3	<3	<3	<3	4	–	<3	15	<3	<3	4	23
09.1991	7	7	<3	28	<3	7	<3	3	–	15	–	3	3	7
12.1991	<3	–	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3
03.1992	<3	–	<3	4	3	4	<3	<3	<3	<3	–	–	–	–
06.1992	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3
09.1992	6	6	23	6	23	<3	6	5	6	6	6	23	6	6
12.1992	40	120	150	110	150	120	28	140	140	210	3	11	28	11
03.1993	23	<3	4	9	<3	23	–	–	23	23	7	23	–	–
06.1993	23	<3	3	460	7	150	<3	23	39	15	–	<3	<3	<3
10.1993	4	–	4	<3	4	<3	11	<3	4	23	23	4	<3	9
12.1993	110	–	11	210	460	240	11	150	28	28	11	4	11	28
03.1994	75	–	4	4	23	460	15	9	43	23	9	23	20	15
06.1994	23	–	15	23	64	7	<3	21	9	9	43	13	9	7
09.1994	23	–	3	<3	<3	4	4	23	150	210	210	93	<3	93
(%)	66	50	60	64	53	60	57	69	71	80	66	69	58	75

* – no tested

% – percent positive samples

Table 8

The occurrence of filamentous hydrogen sulphide oxidizing bacteria (*Beggiatoa*, *Thioploca*, *Thiothrix*) in underground waters of the Omulewski Aquifer (Mazurian Lake District) in 1990-1995 years

Date (month, year) of sampling	Janowo	Sedańsk	Narty	Jedwabno	Kot	Dzierzki	Wielbark	Przeździęk	Baranowo	Wyżegi	Wesołowo	Wesołówko		
												bore-holes		
	wells											no. 1	no. 2	no. 3
12.1990	–*	?	?	–	–	–	–	Th	–	–	–	–	–	–
03.1991	–	?	–	–	–	–	–	?	–	–	–	–	–	–
06.1991	–	?	–	–	–	–	–	B	–	–	–	–	–	–
09.1991	–	–	Tp	–	–	–	B	–	–	–	–	–	–	–
12.1991	–	?	–	B	–	–	–	–	B	Tp	–	B	–	B
03.1992	–	?	Tp	B	–	B	B	–	–	Tp	–	–	–	–
06.1992	B	–	B	–	–	–	–	B	B	–	B	–	–	Tp
09.1992	–	–	–	–	–	–	B	–	B	–	–	–	B	–
12.1992	–	B	–	–	–	Th	–	B	–	–	–	–	–	–
03.1993	B	–	?	Tp	–	B	Tp, B	–	Th	–	–	–	–	–
06.1993	–	–	B	–	–	–	–	–	–	–	–	–	–	–
10.1993	?	–	B	–	–	–	–	–	Th	B	B	–	–	–
12.1993	?	–	–	–	–	–	–	–	–	–	–	–	–	–
03.1994	?	–	–	B	–	–	–	–	–	–	–	–	–	–
06.1994	?	–	–	–	–	–	–	–	–	–	–	–	–	–
09.1994	?	–	?	–	–	–	–	–	–	–	–	–	–	–
12.1994	–	B	–	–	–	–	–	B	–	–	B	–	–	–
03.1995	–	B	B	–	B	–	B	–	–	–	B	B	B	–
06.1995	–	–	–	B	–	–	–	–	B	–	–	–	–	–
09.1995	–	–	–	–	–	–	–	–	–	Tp	–	–	–	–
(%)	13	20	35	25	5	15	25	26	30	22	22	10	10	10

* – no stated;
? – no tested;
B – *Beggiatoa*, Tp – *Thioploca*, Th – *Thiothrix*;
% – percent positive samples.

bacteria requiring rather lower pH values (FJERDINGSTAD et al. 1976). In waters of the wells and piezometric boreholes, these bacteria usually occurred in lower numbers when the content of dissolved oxygen was lower. For example, in well water from Wielbark (water of the carbonate-calcium-magnesium type) a lower number of those bacteria (< 3-62 MPN · 100 cm^{-3}) were reported from March of 1991 till September of 1992 when the content of dissolved oxygen ranged from 0.6 to 1.8 mg · dm^{-3} O_2; in the same period, the sulfate content of water fluctuated from 7 to 31 mg · dm^{-3} SO_4. In well water from Wyżegi (waters of the carbonate-calcium-sulfate type), in an analogous period, the number of *Thiobacillus thioparus* ranged from < 3 to 46 MPN · 100 cm^{-3}, the content of dissolved oxygen – from 1.2 to 2.0 mg · dm^{-3} O_2, whereas that of sulfates reached 96-129 mg · dm^{-3} SO_4. Waters of that well were also reported to contain from 0.60 to 2.69 mg · dm^{-3} Fe (KOCHAŃSKA 1991, KOCHAŃSKA, NIEWOLAK 1997). Fluctuations in the number of *Thiobacillus thioparus* in the waters of the wells and piezometric boreholes investigated at the area of the hydrological unit of Omulewski Aquifer were more or less 10-times lower than those reported by OLAŃCZUK-NEYMAN, OLESZKIEWICZ-GOŹDZIELEWSKA (1992) for underground waters of the Cretaceous horizon in the Regional Water-bearing System of Gdańsk.

Water reaction in the wells and piezometric boreholes analyzed was one of the significant parameters limiting the count of *Thiobacillus thiooxidans*. These bacteria prefer environments with pH lower than 4.0 (GROSSMAN, DESROCHES 2001). Niches with such a reaction could occur in terrestrial subsurface environments, from where those bacteria could have been washed out to underground waters. The latter can serve as an explanation of the higher numbers of these bacteria in the waters of wells and piezometric boreholes in June of 1992 and September of 1994 when rainfalls were more intensive. SOUTHAM, BEVERIDGE (1992) detected low numbers of those bacteria in such micro-environments with a neutral reaction in which acidic micro-niches are likely to occur. A factor that reduced the number of *Thiobacillus thiooxidans* in the underground waters of the hydrogeological unit of the Omulewski Aquifer could have also been their temperature. These bacteria are typical mesophiles with an optimal growth temperature of 30-35°C (NORRIS 1990), whereas the water temperature of the hydrogeological unit did not exceed 10-12°C (KOCHAŃSKA 1991).

The maximal numbers of *Thiobacillus denitrificans* in waters of the wells and piezometric boreholes investigated were 10-times lower as compared to those determined for underground waters of northern areas of Germany, containing up to 472 mg · dm^{-3} SO_4 and up to 114 mg · dm^3 Fe (KÖLLE et al. 1983). In turn, ALFÖLDI (1988) reported that in underground waters of Vituki in Finland, the numbers of these bacteria were so low that they were of no significance to the reduction of nitrates.

Fluctuations in the numbers of SRB in waters of the wells and piezometric boreholes of the hydrogeological unit of the Omulewski Aquifer were at least 10-times higher that those reported for five intakes of underground waters of the Cretaceous horizon in the Regional Water-bearing System of Gdańsk and of the Quaternary-Tertiary horizon of the Kaszubska Marginal Stream Valley (WARGIN 2002). A factor reducing the SRB count in the water of the wells and piezometric boreholes examined at the area of the hydrogeological unit of the Omulewski Aquifer could have been the oxygen conditions. In December of 1992, the maximal numbers of SRB in water of wells from Janowo, Kot, Dzierzki, Wielbark, Przeździęk and Wyżegi villages were linked with oxygen contents of 1.2-2.2 mg $\cdot$ dm^{-3} O_2. In water originating from wells located in Sedańsk, Narty, Jedwabno and Baranowo villages, similar numbers of these bacteria were observed at an oxygen content of 6.4-8.0 mg $O_2 \cdot dm^{-3}$. JOHNSON, WOOD (1992, 1993) detected these bacteria in water collected from boreholes in the London Basin, containing 1.1-3.3 mg $O_2 \cdot dm^{-3}$. In natural, well oxygenated habitats, however, there are likely to occur niches with an appropriately low redox potential (E_h -150 to -200 mV), where SRB may survive and use oxygen in respiratory processes (SASS et al. 1997, ESCHEMANN et al. 1999). Reference data provide a number of examples for the occurrence of SRB in apparently aerobic environments. In the bottom sediments of the Kattegat Bay, JØRGENSEN, BAK (1991) detected those bacteria in similar or higher numbers in oxic surface layers compared to the anoxic ones. FUKUJI, TAKII (1990) postulated that SRB could survive in the surface layer of bottom sediments of lakes in particles larger than 10 μm organic matter and that sulfates were more abundant in the surface sediment than in the deeper sediment. According to SASS et al. (1997), SRB strains isolated from the oxic layers of sediments demonstrate a higher tolerance to oxygen and capacity for oxic respiration, compared to the strains isolated from the anoxic layers. Though SRB can occur in such micro-habitats, still they cannot reduce sulfates (JØRGENSEN 1977, FUKUJI, TAKII 1990). GROSSMAN, DROSCHER (2001) reported a lack of that process in waters containing more than 1.0 mg $O_2 \cdot dm^{-3}$ as a result of an increase in redox potential. It is assumed that oxygen inactivates or inhibits enzymes or proteins active in the reduction process of sulfates (CANFIELD, DES MARAIS 1991). The letter may serve as an explanation of a lack of perceptible presence of hydrogen sulfide in water samples from the wells and piezometric boreholes at the area of the hydrogeological unit of the Omulewski Aquifer. In addition, DOCKINS et al. (1980) determined up to 24 000 MPN $\cdot$ 100 cm^{-3} of SRB in water of 25 wells located in the south-eastern Montana (Fort Union Formation), however, the aroma of hydrogen sulfide was perceptible in water of only 1 well. In turn, OLAŃCZUK-NEYMAN, OLESZKIEWICZ-GOŹDZIELEWSKA (1992) and WARGIN (2002) detected the presence of hydrogen sulfide in the underground waters of the Cretaceous horizon in the Regional Water-bearing System of Gdańsk as well as in under-

ground waters of one out of five intakes of the Quaternary-Tertiary horizon of the Kaszubska Marginal Stream Valley, exploiting the Pleistocene water-bearing level, in which the number of SRB did not exceed 18 NLP · 100 cm^{-3}. A factor modifying both the number and activity of SRB in underground waters could be the differences in the content and type of organic matter as well as the possibility of the occurrence of anaerobic niches. According to WIDDEL (1988), if waters contain sufficient quantities of organic matter, the SRB can be active in respective micro-niches despite a lack of oxygen in water. According to ASS et al. (2002), the SRB that oxidize the organic matter only to acetates (*Desulfovibrio*, *Desulfomicrobium*, *Desulfolubus*) prefer simple products of fermentation (hydrogen, lactates, ethanol) that are formed in larger amounts at the oxic-anoxic interface of sediments or even in the oxic layers; whereas the SRB that completely oxidize the organic matter to CO_2 (*Desulfonema*) are likely to occur in the oxic zones of incrustations. Most known SRB (CASTRO et al. 2000) may survive temporary contact with oxygen and even possess mechanisms of oxygen reduction (BRUNE et al. 2000, CYPIONKA 2000, LEMOS 2001). What is more, they can transfer and form clusters (aggregates) of cells (KREKELER et al. 1997) facilitating their survival. Fluctuations in the numbers of SRB in the examined samples of well water may result from the intensity of its intake in a given village and consequent desorption of those bacteria from the deposit.

Sporadic occurrence of filamentous hydrogen sulfide bacteria in water collected from wells and piezometric boreholes at the area of the hydrogeological unit of the Omulewski Aquifer is likely to be linked with leakage of contaminations from the ground's surface. It especially refers to wells in Dzierzki, Wielbark (pig farms), Przeździęk, Baranowo and Wyżegi (cattle farms), drilled at the area of household yards, where spot sources of contamination (putrefying and slurry containers, leaky containers with silage, cow sheds) occurred within the range of a depression sink. A typical trait of those wells was a depth not exceeding 64 m and the possibility of penetration of selected pollutants to underground waters (KOCHAŃSKA 1991, NIEWOLAK 1994b, NIEWOLAK, GOŁAŚ 2000).

Conclusions

1. Of the bacteria active in the sulfur cycle in water of the wells and piezometric boreholes examined, filamentous hydrogen sulfide bacteria and *Thiobacillus denitrificans* were detected the least frequently (in 5-35% and 19-73% of samples, respectively), whereas *Thiobacillus thiooxidans* and *Desulfovibrio* spp. more frequently (33-66% and 50-80% of the samples, respectively), and *Thiobacillus thioparus* the most frequently (80-100% of the samples).

2. Thiobacillus thioparus were detected more often in water from wells located in Janowo, Dzierzki, Kot and Wielbark villages; *Thiobacillus thiooxidans* in well water from Janowo, Kot and Wesołowo; *Thiobacillus denitrificans* and *Desulfovibrio* spp. in well water from Wyżegi; whereas filamentous hydrogen sulfide bacteria in well waters from Narty, Wielbark, Przeździęk and Baranowo.

3. In the experimental period, when the oxygen content of waters from the wells examined was lower, lower numbers of *Thiobacillus thioparus* and higher numbers of *Desulfovibrio* spp. were often reported. The higher counts of *Thiobacillus thioparus* (up to 1400 MPN/100 cm^3) were observed periodically in well water from Wyżegi and in water from the piezometric borehole in Wesołowo when the content of sulfates reached the highest values (93 and 98 $mg \cdot dm^{-3}$ SO_4, respectively).

4. Not frequent and not numerous occurrence of sulfate reducing bacteria as well as a lack of perceptible aroma of hydrogen sulfide in the water of the wells and piezometric boreholes examined enable assuming that they do not provide the SRB conditions suitable for their biochemical activity. A factor that reduces their activity could be aerobic conditions rather facilitating the oxidation of sulfides by non-acidophilic thionic bacteria (*Thiobacillus thioparus*).

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CARBON DIOXIDE EMISSION FROM FLY ASH ENRICHED IN SEWAGE SLUDGE

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K e y w o r d s: fly ash, carbon, sewage sludge, carbon dioxide, emission.

A b s t r a c t

Fly ash obtained from the Municipal Heating Plant in Olsztyn and sewage sludge obtained from the Municipal Wastewater Treatment Plant in Olsztyn were used in the study. Different quantities of fly ash and sewage sludge were mixed, and the treatments were additionally fertilized with NPK. Organic carbon content was determined in the material collected from particular treatments, and carbon dioxide emission was measured at 20°C and at moisture content of 40, 60 and 80% of total available water capacity. Samples were incubated in a controlled environment chamber for 1, 2 and 3 days. It was found that carbon dioxide emission was significantly affected by sewage sludge rates and moisture content. The highest CO_2 emission levels were recorded in substratum with a moisture content of 80%, containing 50% of sludge.

EMISJA DWUTLENKU WĘGLA Z POPIOŁÓW LOTNYCH WZBOGACONYCH OSADEM ŚCIEKOWYM

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S ł o w a k l u c z o w e: popiół lotny, węgiel, osady ściekowe, dwutlenek węgla, emisja.

A b s t r a k t

W doświadczeniu zastosowano popioły lotne z Miejskiego Przedsiębiorstwa Energetyki Cieplnej w Olsztynie oraz osad ściekowy z komunalnej oczyszczalni ścieków. Osad z popiołem połączono w różnych dawkach, wybrane obiekty dodatkowo nawożono NPK. W materiale z obiektów

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doświadczalnych określono zawartość węgla organicznego oraz oznaczono emisję dwutlenku węgla w temp. 20°C oraz wilgotności 40, 60 i 80% maksymalnej pojemności wodnej. Próby inkubowano w komorze klimatyzacyjnej przez 1, 2 i 3 doby. Stwierdzono, że na wydzielanie dwutlenku węgla istotny wpływ miała dawka osadu ściekowego i wilgotność. Najintensywniejsze wydzielanie CO_2 stwierdzono w podłożach o wilgotności 80% zawierających 50% osadu ściekowego.

Introduction

Combustion wastes (mixtures of ash and slag) are produced during the combustion of solid fuels, such as hard coal, brown coal and other. Due to their specific properties, combustion wastes may have an adverse impact on the natural environment. Ash disposal sites are a source of dust pollution, thus posing a serious threat to surface and underground waters as well as to soils, mostly due to high concentrations of heavy metals. Moreover, they may cause changes in the landscape and surface features. About 50% of these wastes are stored at disposal sites.

The development of arable soils is determined by organic matter content. The rate of organic matter mineralization is dependent on carbon dioxide concentration, which enables to evaluate soil respiratory activity (BARAN et al. 1999; SĄDEJ, MAZUR 2000). According to ROGALSKI et al. (2004), this is of particular importance for reclamation and amendment of soils degraded by industrial activities. The supply of organic substance in the form of sewage sludge is followed by an increase in soil respiratory activity (GOSTKOWSKA et al. 1989).

Soil carbon dioxide emissions may vary widely depending on the type of soil, its moisture content, temperature, reaction, management system, application of mineral and organic fertilizers or pesticides, as well as on other factors. WŁODARCZYK et al. (2001), AZAM et al. (2002) and WANG et al. (2003) reported that CO_2 emission from sandy, loamy, brown and steppe black soils ranged from 4.4 to 239.86 mg $CO_2 \cdot kg^{-1} \cdot d^{-1}$. According to GOSTKOWSKA et al. (1989), KOPEĆ & NOWOROLNIK (1999) and ROGALSKI et al. (2005), soils fertilizers with mineral and organic fertilizers (sewage sludge, wastewater, animal slurry, manure) emitted between 11 and 651 mg $CO_2 \cdot kg^{-1} \cdot d^{-1}$. CO_2 emission from sandy forest soils and grassland was respectively 2.2 to 24.6 mg $CO_2 \cdot kg^{-1} \cdot d^{-1}$ (PINZARI et al. 1999) and 47.06 mg $CO_2 \cdot kg^{-1} \cdot d^{-1}$ (ONDRÁŠEK, GÁBORČÍK 1998). In the study conducted by DZIEJOWSKI et al. (2001) soils treated with pesticides emitted on average 65 mg $CO_2 \cdot kg^{-1} \cdot d^{-1}$, and in the experiment performed by ONDRAŠEK et al. (1997) soil with different Cd concentrations emitted 275.8 to 612.8 mg $CO_2 \cdot kg^{-1} \cdot d^{-1}$.

The aim of the present study was to determine carbon dioxide emission from mixtures of fly ash and sewage sludge from municipal wastes, since

literature on the subject provides no such data. Relationships between the carbon content of mixtures of fly ash and sewage sludge and carbon dioxide emission rates were also analyzed.

Materials and Methods

A pot experiment was performed in a greenhouse. Fly ash was enriched in sewage sludge, and the treatments were additionally fertilized with NPK (N – 1 g, P – 0.2 g, K – 1.25 g, i.e. 2.2 g of urea + 1.1 g of triple superphosphate + 2.5 g of potash salt per pot). Each pot contained 10 kg of a mixture of fly ash and sewage sludge (5 or 7.5 kg fly ash and 5 or 2.5 kg of sludge, depending on the ratio between them). The proportions were determined for fresh sewage sludge. The sludge used in the experiment had a sticky-greasy consistency and was obtained from sludge drying beds of the Municipal Wastewater Treatment Plant in Olsztyn. It was abundant in nutrients: N – 5, P – 3.3, K – 2.6 $g \cdot kg^{-1}$ d.m. and in organic carbon – 74.8 $g \cdot kg^{-1}$ d.m.; pH measured in H_2O was 7.9. The dry matter content of fresh sludge was 54.32%. Fly ash produced during hard coal combustion was obtained from the Municipal Heating Plant (MPEC) in Olsztyn. Ignition losses at 800°C were 29.53%. In this paper mixtures of fly ash and sewage sludge as well as individual components are referred to as substratum, after KALEMBASA & WYSOKIŃSKI (1999), CZYŻ et al. (2001) and ROGALSKI et al. (2001).

A grass mixture composed of red fescue (*Festuca rubra* L.) – 40%, perennial ryegrass (*Lolium perenne* L.) – 30% and meadow bluegrass (*Poa pratensis* L.) – 30% was sown in pots with substratum of a specified composition.

Carbon dioxide emission was studied at a laboratory, using the material collected in particular treatments prior to the growing season. Samples of particular substratum were brought to a moisture content of 40, 60 and 80% of total available water capacity determined in accordance with the Polish Standard PN-ISO 11269-1. Carbon dioxide emission levels were measured in 1 l sealed jars, as described by ISERMEYER (1952). The jars containing samples of substratum (50 g) and a NaOH solution at a concentration of 0.05 $mol \cdot dm^{-3}$ (25 ml) were placed in a controlled environment chamber (Microclima 1000, Snijders Scientific B.V.). Incubation was carried out for 3 days at 20°C + 0.3. Carbon dioxide emission was measured every 24 h. The jars were aired out each time. After a specified period of time vessels with sodium hydroxide were taken out of the jars. Titration with hydrochloric acid at a concentration of 0.05 $mol \cdot dm^{-3}$ was carried out in the presence of an excess of barium chloride and phenolphthalein. Control samples (empty) were incubated at the same time.

The amount of CO_2 emitted during the experiment was calculated using the following formula:

$$CO_2 = \frac{(V_0 - V) \cdot 1.1}{m} \text{ (mg)},$$

where:
V_0 – amount of HCl used for control sample titration (ml),
V – amount of HCl used for titration of samples taken from particular substratum (ml),
m – sample weight (g d.m.),
1.1 – conversion factor (1 ml 0.05 mol · dm^{-3} NaOH equals 1.1 mg CO_2).

The dry matter content of particular substratum was determined in accordance with the Polish Standard PN 88/R-04013. Organic carbon content was determined by the Tiurin method.

Results were verified statistically by analysis of variance (F test). The statistical significance of differences was estimated by the Duncan's test at a significance level of $p = 0.01$. The results of post-hoc tests are presented as homogenous groups. Relationships between the carbon content of mixtures of fly ash and sewage sludge and carbon dioxide emission rates were determined by analysis of Pearson linear correlation between two variables.

Results and Discussion

The analysis of variance showed that in the treatments analyzed in the experiment carbon content was significantly affected by the type of substratum and NPK fertilization as well as the interaction between these two factors (Table 1). In our study the carbon content of fly ash was on average 14.01% (Table 2), while reference values vary greatly. According to BOGACZ (1995), it ranges between 3.47 and 4.52%, whereas in the studies conducted by ROGALSKI et al. (1998) GAIND et al. (2003), BALTRUS et al. (2002), STYSZKO-GROCHOWIAK et al. (2004), and MCCARTY et al. (1997) the carbon content of fly ash was 1.81%, 0.29%, 16%, 20% and 24.9% respectively. Such high values as those reported by BALTRUS et al. (2002) and STYSZKO-GROCHOWIAK et al. (2004) result from the presence of the residues of uncombusted coal. In our experiment the addition of sewage sludge to fly ash caused a significant decrease in the carbon content of substratum. In the 75% + 25% mixture of fly ash and sewage sludge the mean C concentration was 8.33%, as compared with 10.04% in the mixture containing 50% of sewage sludge. In treatments with mineral

fertilization carbon content was significantly higher. Mineral fertilization had a positive effect on the increase in the concentration of this element, in comparison with unfertilized treatments. This was most probably related to a faster growth of plant mass, including underground parts.

Table 1

Analysis of variance (F test) of organic carbon content

Factors	Empirical F values	Significance level
S	71.033*	0.000004
F	48.836*	0.000114
SxF	6.021*	0.018963

S – substratum, F – fertilization, SxF – interaction between experimental factors, *Femp. > Ftab. – relationship significant at $p = 0.01$

Table 2

Organic carbon content in particular treatments (% d.m.)

Treatment	Fertilized	Unfertilized	Mean
Sewage sludge	6.39^{ab}	4.68^{a}	5.54^{a}
Fly ash	16.83^{e}	11.19^{bc}	14.01^{d}
Fly ash + sewage sludge (75% + 25%)	8.82^{bcd}	7.83^{b}	8.33^{b}
Fly ash + sewage sludge (50% + 50%)	11.74^{d}	8.33^{bc}	10.04^{c}
Mean	10.95^{y}	8.01^{x}	

Values followed by different letters differ statistically at $p = 0.01$; values followed by (a, b) – comparison of substratum and interactions, values followed by (x, y) – comparison of fertilized and unfertilized treatments; these values belong to different homogenous groups (based on post-hoc tests).

The analysis of variance of carbon dioxide emission from mixtures of fly ash and sewage sludge indicated that after one day CO_2 emission was significantly affected by the type of substratum, its moisture content, NPK fertilization as well as by interactions between moisture content and the type of substratum, and the type of substratum and fertilization levels (Table 3). The measurement performed after two days revealed that the effect of fertilization and the moisture content x fertilization interaction as well as interactions among all experimental factors were statistically non-significant. After three days carbon dioxide emission was found to be significantly affected by all factors and interactions among them, except for the moisture content x fertilization interaction.

Table 3

Analysis of variance (*F* test) of carbon dioxide emission after 1, 2 and 3 days

Factors	1 d		2 d		3 d	
	Emp *F*	*p*	Emp *F*	*p*	Emp *F*	*p*
E	39.89*	< 0.000001	79.52*	< 0.000001	42.375*	< 0.000001
S	1082.64*	< 0.000001	998.55*	< 0.000001	360.429*	< 0.000001
F	23.07*	0.000068	0.07	0.790128	9.782*	0.004574
ExS	9.39*	0.000024	33.16*	< 0.000001	3.777*	0.008643
ExF	2.96	0.70760	2.60	0.094929	0.556	0.580895
SxF	69.86*	< 0.000001	17.17*	0.000004	56.437*	< 0.000001
ExSxF	2.11	0.090184	1.00	0.446533	4.932*	0.002044

E – carbon dioxide emission, *S* – substratum, *F* – fertilization, *SxF*, *ExF*, *ExS*, *ExSxF* – interaction between experimental factors;
**F*emp. > *F*tab. – relationship significant at $p = 0.01$, *E*mp *F* – empirical *F* values, *p* – significance level.

The measurement of mean levels of CO_2 emission in all experimental variants after one day showed that they were the highest in treatments with a moisture content of 60 and 80%, and the lowest in those where moisture content was 40% (Table 4). The 25% addition of sewage sludge to fly ash resulted in higher carbon dioxide emissions, as compared with the 50% addition. NPK fertilization reduced CO_2 emission.

Table 4

Carbon dioxide emission (mg $CO_2 \cdot kg^{-1}$ d.m. of substratum/day) in particular treatments after 1 day

Treatment		Moisture content (%)			Mean
		40	60	80	
Sewage sludge		270.69^{d}	325.75^{e}	354.39^{f}	$\mathbf{316.94}^{D}$
Fly ash		64.46^{a}	67.85^{a}	76.41^{a}	$\mathbf{69.57}^{A}$
Fly ash + sewage sludge (75% + 25%)		248.16^{d}	266.60^{d}	263.57^{d}	$\mathbf{259.44}^{C}$
Fly ash + sewage sludge (50% + 50%)		170.86^{b}	192.29^{bc}	197.48^{c}	$\mathbf{186.88}^{B}$
Mean	fertilized	$133.55^{a'}$	$154.78^{a'}$	$151.25^{a'}$	$\mathbf{146.53}^{A'}$
	unfertilized	$155.73^{b'}$	$175.52^{b'}$	$175.70^{b'}$	$\mathbf{168.98}^{B'}$
Mean		$\mathbf{144.64}^{x}$	$\mathbf{165.15}^{y}$	$\mathbf{163.48}^{y}$	

Values followed by different letters differ statistically at $p = 0.01$; values followed by (*a, b,...*) – comparison of substratum and moisture content, values followed by (*A, B,...*) – comparison of substratum; values followed by (*A'*, *B'*) – effect of fertilization; values followed by (*a'*, *b'*) – comparison of interactions between fertilization and moisture content; values followed by (*x, y, z*) – effect of moisture content carbon dioxide emission; these values belong to different homogenous groups (based on post-hoc tests).

Similar tendencies were observed after two days of measurements. The highest carbon dioxide emission rates were recorded in treatments with a moisture content of 80% (Table 5). Just like after one day, carbon

dioxide emission was at a lower level in treatments where the amount of sewage sludge was 50%.

Table 5

Carbon dioxide emission (mg $CO_2 \cdot kg^{-1}$ d.m. of substratum/day) in particular treatments after 2 days

Treatment		Moisture content (%)			Mean
		40	60	80	
Sewage sludge		146.93^{d}	165.85^{e}	230.10^{f}	**180.96**^{D}
Fly ash		34.05^{a}	34.03^{a}	28.38^{a}	**32.14**^{A}
Fly ash + sewage sludge (75% + 25%)		139.76^{d}	171.81^{e}	168.27^{e}	**159.95**^{C}
Fly ash + sewage sludge (50% + 50%)		86.89^{b}	108.04^{c}	111.02^{c}	**101.98**^{B}
Mean	fertilized	$77.03^{a'}$	$84.04^{a'}$	$90.75^{a'}$	**83.94**$^{A'}$
	unfertilized	$75.70^{a'}$	$92.44^{a'}$	$101.98^{a'}$	**90.04**$^{A'}$
Mean		**76.36**^{x}	**88.24**^{y}	**96.36**^{z}	

Explanations as in Table 4

After three days the highest carbon dioxide emission was observed in treatments with a moisture content of 80%. CO_2 emission in treatments containing 25 and 50% of sewage sludge was at a statistically comparable level (Table 6). Significantly higher CO_2 emission rates were recorded in treatments fertilized with NPK (an average 64 mg $CO_2 \cdot kg^{-1}$ d.m. of soil per day).

The analysis of carbon dioxide emissions at 24-hour intervals indicates that the most CO_2 was emitted after the first day. Then the emission levels decreased, and after three days were almost threefold lower than on the first day.

Table 6

Carbon dioxide emission (mg $CO_2 \cdot kg^{-1}$ d.m. of substratum/day) in particular treatments after 3 days

Treatment		Moisture content (%)			Mean
		40	60	80	
Sewage sludge		88.20^{b}	96.78^{bc}	122.03^{de}	**102.34**^{B}
Fly ash		17.96^{a}	15.72^{a}	21.95^{a}	**18.54**^{A}
Fly ash + sewage sludge (75% + 25%)		91.44^{b}	111.62^{cd}	130.63^{e}	**111.23**^{BC}
Fly ash + sewage sludge (50% + 50%)		100.86^{bc}	110.83^{cd}	130.44^{e}	**114.04**^{C}
Mean	fertilized	$56.14^{b'}$	$61.38^{b'}$	$74.48^{b'}$	**64.00**$^{B'}$
	unfertilized	$52.87^{a'}$	$62.24^{a'}$	$70.82^{a'}$	**61.98**$^{A'}$
Mean		**54.51**^{x}	**61.81**^{y}	**72.65**^{z}	

Explanations as in Table 4

The analysis of Pearson simple correlation between two variables revealed a significant negative correlation ($R = -0.81$) between carbon dioxide emission from a mixture of fly ash and sewage sludge and carbon concentrations in treatments. Figure 1 illustrates these relationships and a regression equation, taking into account total carbon dioxide emissions from treatments in which moisture content was 40, 60 and 80%. The coefficients of correlation determined for individual treatments with various moisture content were also significant and negative. Positive correlations were observed between the moisture content of a substratum and carbon dioxide emission (Figure 2).

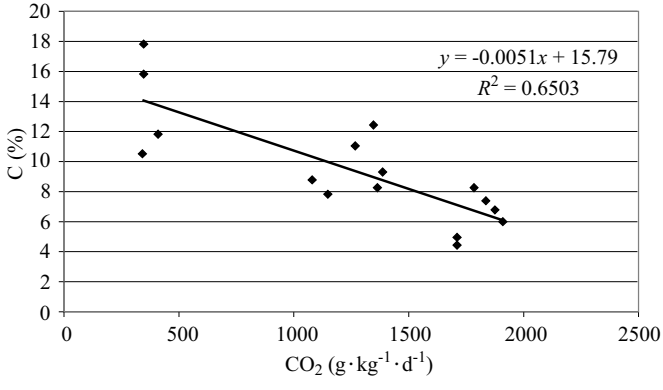

Fig. 1. Relationship between carbon dioxide and CO_2 emission from fly ash enriched in sewage sludge

Carbon dioxide emission in treatments with fly ash was relatively low despite high carbon concentrations in substratum (the mean carbon content of all treatments with fly ash was 10.79%). This was related to the presence of the residues of uncombusted coal in fly ash. FAKOUSSA and HOFRICHTER (1999) demonstrated that the rate of microbiological degradation of organic substance contained in coal is very slow due to, among others, the high degree of its hydrophobicity as well as the presence of persistent ingredients. In addition, the pH of treatments with fly ash was above 7. According to MCCARTY et al. (1997), part of carbon dioxide contained in soil whose pH is higher than 6.5 is converted into calcium carbonate. As a result, carbon dioxide emission from soils with high pH levels is lower, compared to soils with pH below 6. Most probably this was the case with the tested substratum. Thus, the authors decided to carry out further research in order to describe in more detail the effect of the pH of a substratum on CO_2 emission.

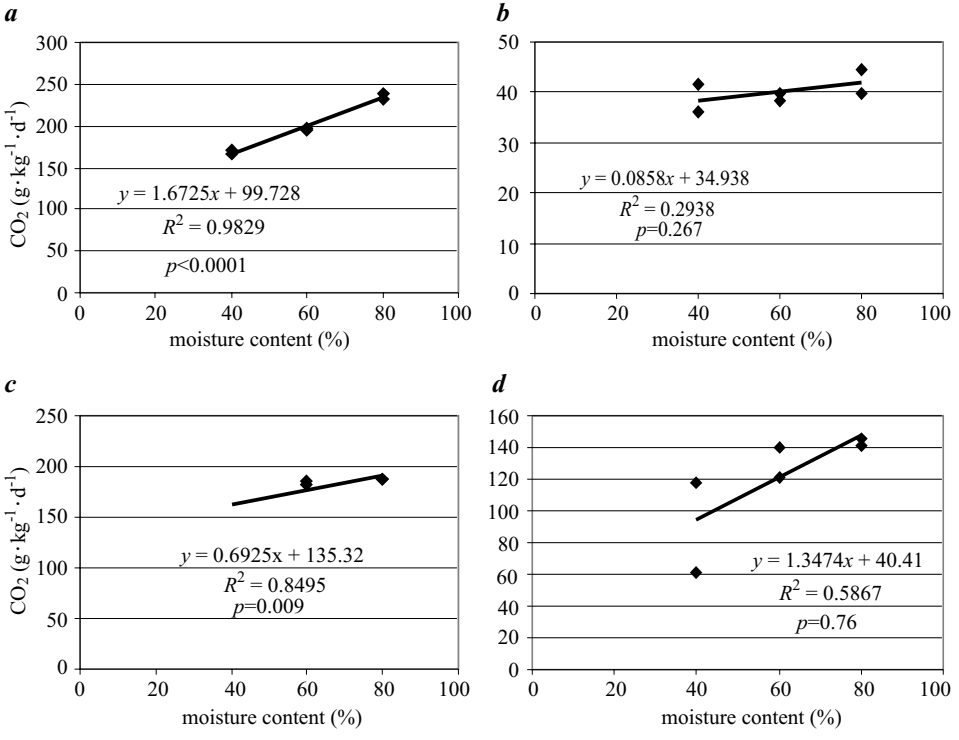

Fig. 2. Relationship between the moisture content of the substratum and CO_2 emission: *a* – sewage sludge, *b* – fly ash, *c* – fly ash + sewage sludge (25% + 75%), *d* – fly ash + sewage sludge (50% + 50%)

Conclusions

1. There was a positive correlation between carbon dioxide emission from mixtures of fly ash with sewage sludge and an increase in the moisture content of substratum, to 80% of total available water capacity. The increase in moisture content from 40 to 80% increased CO_2 emission, on average by 24%.

2. In all treatments the highest carbon dioxide emission was recorded after the first day of the experiment (on average 158 mg CO_2 per kg d.m.). After three days it decreased to 63 mg CO_2 per kg d.m. Higher levels of carbon dioxide emission were observed in treatments fertilized with NPK.

3. A negative correlation was found between CO_2 emission from mixtures of fly ash and sewage sludge and carbon concentrations in substratum, which was related to the presence of uncombusted coal particles in fly ash.

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BACTERIOLOGICAL ANALYSIS OF THE WATERS OF SMALL MUNICIPAL LAKE

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K e y w o r d s: bacteriological analysis, sanitary state bacteria, lake.

A b s t r a c t

Studies on water samples collected from small municipal Rusalka lake were performed on the monthly basis from January to December in 2001. We present data on pollution degree indicator bacteria i.e. TVC 37°C and TVC 20°C, sanitary state bacteria, i.e. total coliform bacteria (TC), fecal coliform bacteria (FC) and fecal streptococcus bacteria (FS) as well as bacteria of selected physiological groups – denitrifying bacteria, ammonifying bacteria, sulphate- and sulphite-reducing bacteria, and also one physicochemical indicator – a temperature from two sites. The number of TVC 37°C, TVC 20°C and sanitary state bacteria shows significant pollution of the examined lake. The samples of polluted water were collected particularly at the inflow site of the lake. The recorded results were higher than the data of similar studies on this lake obtained in the previous years and also higher than the date obtained in analogous studies carried out on others municipal lakes in Poland.

The obtained results confirm that it is of great importance to conduct monitoring of this reservoir.

ANALIZA BAKTERIOLOGICZNA WÓD MAŁEGO JEZIORA ŚRÓDMIEJSKIEGO

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S ł o w a k l u c z o w e: analiza bakteriologiczna, bakterie wskaźnikowe stanu sanitarnego, jezioro.

A b s t r a k t

Celem pracy była analiza mikrobiologiczna wód jeziora Rusałka. Próby wody pobierano z dwóch punktów, w miesięcznych odstępach, w okresie od stycznia do grudnia 2001 r. Badania oparto na modelu własnym, określając następujące wskaźniki bakteriologiczne: bakterie wskaźnikowe

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stopnia zanieczyszczenia, tj. TVC 37°C i TVC 20°C, bakterie stanu sanitarnego, tj. bakterie grupy coli (TC), bakterie grupy coli typu kałowego (FC) i paciorkowce kałowe (FS), oraz bakterie wybranych grup fizjologicznych, tj. bakterie denitryfikacyjne, amonifikacyjne, bakterie redukujące siarczyny, redukujące siarczany, a także jeden wskaźnik fizykochemiczny – temperaturę. Wykazano znaczne zanieczyszczenie wód jeziora Rusałka, szczególnie bakteriami wskaźnikowymi stopnia zanieczyszczenia i stanu sanitarnego, w punkcie dopływu cieku wodnego do zbiornika. Uzyskane wartości były wyższe od analogicznych wartości stwierdzonych w innych zbiornikach śródmiejskich. Badania potwierdzają konieczność monitoringu wód tego zbiornika.

Introduction

Small municipal lakes playing an esthetic and recreational function are very important part of cities parks and forests. Very often wastewater produced by the city which can consist of industrial waste, human waste (or sewage), or runoff from rainwater flow into those basins posing a threat to public health, safety and environment. Unfortunately in studies on small lakes of the area less then 50 ha are not carried out according to the National Environmental Monitoring scheme. According to the National Environmental Monitoring studies on lakes of the area above 100 ha are conducted (*National Environmental*...) In addition assessments are performed on lakes which are characterized as being valuable or unique from various standpoints including economics and ecology (10 representative lakes are selected by Provincial Environmental Protetcion Inspectorate in coordination with Chief Environmental Protection Inspectorate). Lakes examination and classification is based on "Guidelines for lake primary monitoring" that considers assessing water quality by using 24 physiochemical indicators and only one microbiological index – fecal coliform count. The most recent applied water classification system revised the number of water classes (*Ordinance of*.). The new classification schema has 5 class categories unlike to the previous one which divided waters into 3 classes. In addition according to the new schema 2 microbiological indicators are considered to be determined – coliform bacterial count and fecal coliform bacterial count. It should be stressed however that the mentioned above new schema was in force till the end of 2004 only. According to the information given on the website of Ministry of Environmental Protection the projects of the new schema – has been under constuction (http://www.mos.gov.pl/1prace-legislacyjne/aktualny-stan/2005.05.shtml), projects of new regulations referring to the surface water monitoring – "Draft regulation of the Minister of Environment on the assessment of surface and underground waters conditions" and "Draft regulation of the Minister of Environment on the method of conducting the surface and underground waters monitoring" – have been under construction.

Rusalka lake is a small flow through reservoir located in the one of Szczecin's parks. The water body supports recreational values and esthetic values of the park. Unfortunately cities wastewater flowing from the vicinity into the lake contributed to overall poor water quality and may contain bacteria and viruses that can be harmful for public health (GOŁAŚ et al. 2002, NIEWOLAK, MINDROW 1998, BOSCH 1998, DEPTUŁA et al. 1995, KOCWA-HALUCH 2001, ŚWIĄTECKI 1997).

The studies of Rusalka Lake were based on the own model developed by the Department of Microbiology and Immunology that considers determination of a number microbiological and only few physicochemical parameters (DEPTUŁA et al. 1998). The assessment based on the microbiological indicators allows to evaluate contamination of the reservoir and also enable to trace down the self-purification process in water as it is microbial component of aquatic ecosystems that provides the self-purification capacity of natural waters. The data on microbiological studies on municipal lakes available in the literature provide information on reservoirs bigger (NIEWOLAK 1972, 1989) and significantly bigger then the one examined by us (GODLEWSKA--LIPOWA et al. 1974, NIEWOLAK 1978b, SZULKOWSKA-WOJACZEK 1972, ZMYSŁOWSKA, SOBIERAJSKA 1977, ZMYSŁOWSKA, SOBIERAJSKA 1980). Only few studies present date on lakes of the area below 10 ha (NAHURSKA et al. 1999, 2003, NAHURSKA, DEPTUŁA 2003, 2004, NIEWOLAK et al. 1997). Furthermore most of the work on microbiological component of municipal lakes has focused only on sanitary parameters i.e. e.coli, fecal e.coli and fecal streptococci (GODLEWSKA-LIPOWA et al. 1974, NAHURSKA et al. 1999, 2003, NAHURSKA, DEPTUŁA 2003, 2004, NIEWOLAK 1972, 1978a,b, 1989, NIEWOLAK et al. 1997, STAPF, DEPTUŁA 1994, SZULKOWSKA-WOJACZEK 1972, ZMYSŁOWSKA, SOBIERAJSKA 1977, 1980), only several previous studies demonstrated data on physiological bacteria (GODLEWSKA-LIPOWA et al. 1974, NAHURSKA, DEPTUŁA 2003, ZMYSŁOWSKA, SOBIERAJSKA 1977, 1980).

Aim

The study aimed at microbiological evaluation of water samples originating from Rusałka lake. The examined variables included presence of water pollution index bacteria (TVC 37°C, TVC 20°C) and of sanitary status index bacteria: coli group bacteria (TC), foecal type coli group bacteria (FC), foecal streptococci (FC), bacteria which decompose organic substances (of selected physiological groups), i.e., denitrification bacteria, amonification bacteria, sulphite-reducing bacteria and sulphate-reducing bacteria.

Material and Methods

The goal of the study was microbiological assessment of the water samples derived from Rusalka Lake. It was studied a pollution degree indicator bacteria i.e. TVC 37°C and TVC 20°C, sanitary state bacteria, i.e. coliform indicator bacteria (TC), fecal coliform bacteria (FC) and fecal streptococcus bacteria (FS) as well as bacteria of selected physiological groups – denitrifying bacteria, ammonifying bacteria, sulphate- and sulphite-reducing bacteria, and also one physicochemical indicator – a temperature from two sites were examined.

Water samples were derived from two sites (A and B) from Rusalka Lake located in one of Szczecin's parks (Figure 1). The reservoir is of elongated shape of 3.7 ha, about 40 m wide and about 2 m deep, since the lake is shallow thermal stratification and wave motion do not occur. The lake bed is flat and

Fig. 1. Location of sampling site A and B in Rusałka Lake

heavily muddy and polluted. As a result of physicochemical studies conducted in the spring and summer 2001 on water of the container (POLESZCZUK, WAWRZYNIAK 2002), Rusałka lake was classified to the IIIrd water purity class, according to the criteria of the Appraisal System of Water Quality. Rusałka is a flow through reservoir that is supplied by Osowka stream which flows into

the basin, water flows out from the lake through underground pipeline through Niecka Niebuszewska and Szczecin Shipyard C.O. into the Western Odra River. The sampling sites were selected on the basis of their topography and ecological survey. The water inflow, outflow and sewage inflow point were taken into consideration. Sampling site A is located at the water inflow point in the western part of the reservoir while sampling site B is situated at the outflow water from the lake to the underground pipeline in its eastern part Water samples, not less than 200 ml, were collected according to Polish Standard (Polish Standard PN-74/C-04620/02, Polish Standard PN-75/C--04615/00) directly to sterile glass bottles from the depth of approximately 15-20 cm below the water surface and then transported to the laboratory in an isothermic container at 4°C. The time from sample collection to analysis did not exceed 4 hours. The examination was carried out on the monthly basis from January to December 2001.

Microbiological examinations of the water from the Lake Rusałka were carried out basing on the research model developed in the Department of Microbiology and Immunology of the Faculty of Natural Sciences of the University of Szczecin (DEPTUŁA et al. 1998). This model is diametrically opposed to the state monitoring schema, which considers determination of only 2 microbiological parameters – coliform bacterial count and fecal coliform bacterial count (*Ordinance of...*).

The own model (DEPTUŁA et al. 1998) includes determination of the following microbiological parameters:

Pollution degree indicator bacteria:

1) Total bacterial count in 1 ml water on nutritive agar:
   a) after 24 h of culturing at. 37°C (TVC 37°C);
   b) after 72 h of culturing at 20°C (TVC 20°C) (Polish Standard PN-75/C--04615/03);

Sanitary state bacteria:

2) MPN (most probable number) in 100 ml water and coliform bacteria titre (TC), determined with fermentation test-tube method (Polish Standard PN-75/C-04615/05,);
3) MPN in 100 ml water and fecal coliform bacteria titre (FC), determined with fermentation test-tube method (Polish Standard PN-77/C-04615/07);
4) MPN of fecal streptococcus bacteria in 100 ml water (FS), determined with membrane filter method (Polish Standard PN-82/C-04615/25);

Bacteria of selected physiological groups:

5) MPN in 100 ml water and denitrifying bacteria titre, determined with test-tube method (Polish Standard PN-75/C-04615/19);
6) MPN in 100 ml water and ammonifying bacteria titre, determined with test-tube method (Polish Standard PN-75/C-04615/16);

7) MPN of anaerobic, spore-forming, sulphite-reducing bacteria *(Clostridium)* in 100 ml water, determined with liquid medium culturing method (Polish Standard PN-79/C-04615/12);
8) Presence of thermophilous, spore-forming, sulphate-reducing bacteria *(Desulfotomaculum nigrificans)*, determined with liquid medium culturing method (Polish Standard PN-82/C-04615/29).

Moreover, basing on the obtained results the ratio of fecal coliform bacteria (FC) to fecal streptococcus bacteria (FS) was calculated (GELDREICH 1976).

Statistical analysis

Results of the studies were subjected to statistical analysis using the Statistica 6.0 software. The employed statistical tests included W test of Shapiro-Wilk, employed to detect other than normal distribution of studied parameter values which was linked to application of non-parametric statistics for calculation of R. Spearman's correlation coefficients. Differences and correlations were assumed to be significant at $p < 0.05$.

Results

Results of the studies are presented in Tables 1, 2, 3 and in Figures 2-8. Tables 4 and 5 present results of the statistical analysis. Analysis of the obtained results permitted to note that in the sampling point A the lowest levels of water pollution index bacteria, both of TVC 37°C and TVC 20°C, were observed in January while in the sampling point B the lowest levels of TVC 37°C were seen in April while the lowest levels of TVC 20°C were detected in July. The highest levels of the bacteria were detected in both sampling points in August (Table 2, Figure 2). In the cases of TC and FC bacteria, their lowest level in point A was detected in July and August, respectively, while in point B in the spring, i.e. in March and in March to May, respectively. In turn the highest levels of TC and FC were noted mainly in autumn, i.e. in October, except of FC in point B in which the highest values were detected in June (Table 1, Figs. 3, 5). In the case of FS no such bacteria could be detected in point A in October and in point B in March, April, July, October, November and December. The highest values of FS were detected in November in point A and in February in point B (Table 1, Figure 4).

In the range of physiological group bacteria the lowest levels of denitrifying bacteria were detected in both sampling sites in March, the highest levels were seen in both sampling sites in March while their peak levels were observed in

Table 1

The amount of pollution degree indicator bacteria (TVC 37°C, TVC 20°C) and sanitary state bacteria (total coliform bacteria, fecal coliform bacteria, fecal streptococcus bacteria) in water samples from the Lake Rusałka – sites A and B

Sampling site		Temp of water (°C)	Temp. of air (°C)	TVC 37°C	TVC 20°C	MPN · 100 ml^{-1} total coliforms	Coli titre	MPN · 100 ml^{-1} fecal coliforms	Fecal coliforms titre	MPN · 100 ml^{-1} fecal streptococci
A	range	0.0-17.7	-2.0-22.0	128-300 000	50-138 000	240-240 000	0.4-0.0004	60-130 000	1.7-0.0008	0-2900
	average	**8.8**	**9.0**	**27 572**	**115 432**	**33 370**	**0.043**	**16 932**	**0.3636**	**750**
B	range	0.0-21.0	-2.0-22.0	74-91 000	320-242 000	62-6200	2.0-0.02	23-620	4.0-0.2	0-700
	average	**7.8**	**9.2**	**7 879**	**24 569**	**845**	**0.460**	**236**	**1.3082**	**158**

Table 2

The amount of organic matter decomposing bacteria (of selected physiological groups) – denitrifying bacteria, ammonifying bacteria and sulphate- and sulphite bacteria in water samples from the Lake Rusałka – sites A and B

Sampling site		MPN · 100 ml^{-1} of denitrifying bacteria	Titre of denitrifying bacteria	MPN · 100 ml^{-1} of amonifying bacteria	Titre of amonifying bacteria	MPN · 100 ml^{-1} of sulphite reducing bacteria	Presence of sulphate reducing bacteria
A	range	24 000-2 400 000	0.004-0.00004	93-240 000 000	1.0-0.0000004	23-2400	present
	average	**403 333**	**0.001**	**21 284 341**	**0.084**	**369**	–
B	range	4300-4 300 000	0.02-0.00002	2300-240 000 000	0.04-0.0000004	9-240	present
	average	**538 133**	**0.005**	**44 026 633**	**0.0045**	**107**	–

Table 3
Percentage distribution of values of ratio FC:FS in the water of Rusałka Lake

FC:FS	Sampling sites	
	A (%)	B (%)
> 4	92	59
0.7-4	0	33
< 0.7	8	8

Table 4
R. Spearman's correlation coefficients of pollution degree indicator bacteria and sanitary state bacteria in water samples originating from Rusałka lake

Specification	Temp. of water	TVC 20°C	TVC 37°C	TC	FC	FS
Temp. of water	–	–	0.44	–	–	–
TVC 20°C	–	–	0.67	–	–	0.59
TVC 37°C	0.44	0.67	–	–	0.44	0.55
TC	–	–	–	–	0.61	–
FC	–	–	0.44	0.61	–	–
FS	–	0.59	0.55	–	–	–

(–) not significant

Table 5
R. Spearman's correlation coefficients of bacteria of selected physiological groups in water samples originating from Rusałka lake

Specification	Temp. of water	TVC 20°C	TVC 37°C	Denitrifying bacteria	Amonifying bacteria	Sulphite reducing bacteria
Temp. of water	–	–	0.43	–	–	-0.76
TVC 20°C	–	–	0.67	0.43	0.45	–
TVC 20°C	0.44	0.67	–	–	–	–
Denitrifying bacteria	–	0.43	–	–	0.49	–
Amonifying bacteria	–	0.45	–	0.49	–	–
Sulphite reducing bacteria	-0.76	–	–	–	–	–

(–) not significant

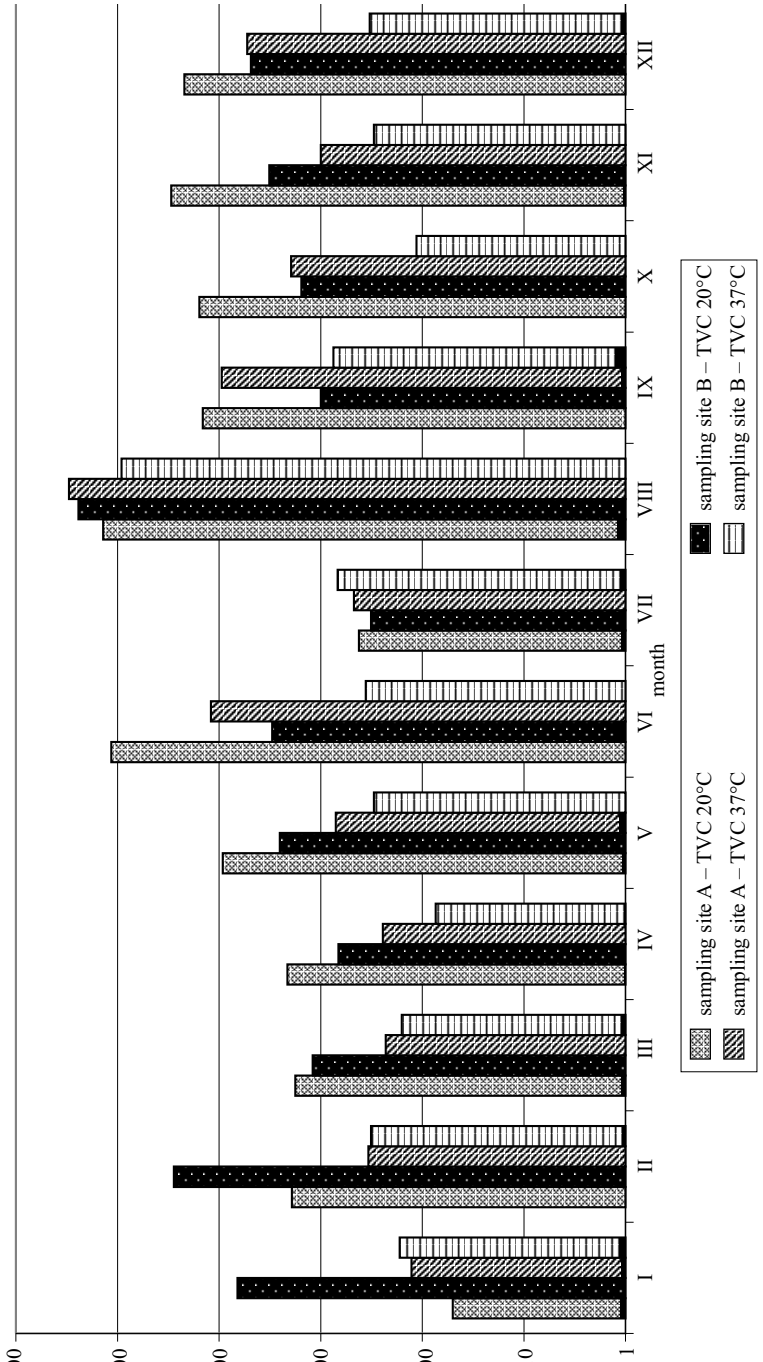

Fig. 2. Total viable count at 37°C (TVC 37°C) and at 20°C (TVC 20°C) in water samples originating from Rusałka Lake

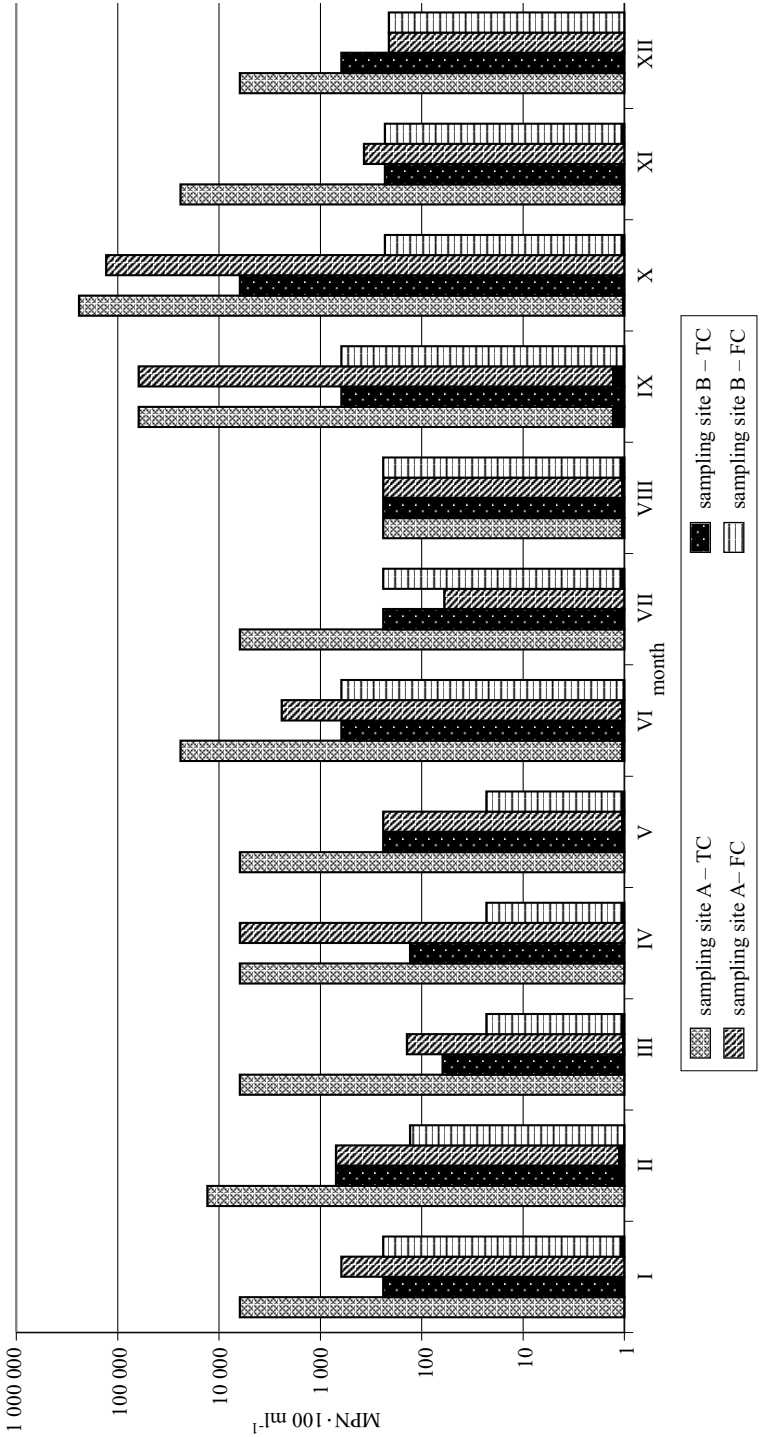

Fig. 3. Most probable number (MPN · 100 ml^{-1}) of total coliforms (TC) and fecal coliforms (FC) in water samples originating from Rusałka Lake

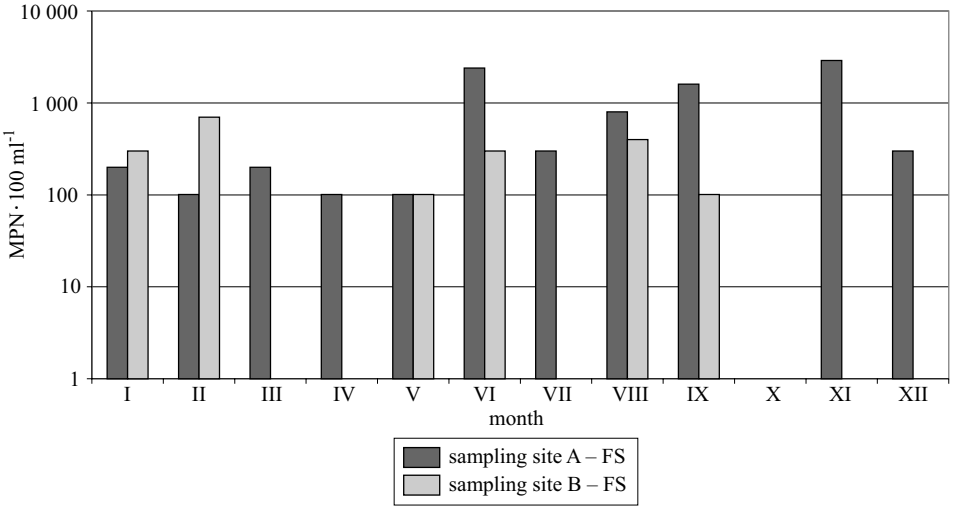

Fig. 4. Most probable number (MPN · 100 ml^{-1}) of fecal streptococci (FS) in water samples originating from Rusałka Lake

June in point A and in December in point B (Table 2, Figures 6, 7). The lowest numbers of amonifying bacteria were detected in March in point A, in July in point B while their peak levels were noted in June in point A and in February and June in point B (Table 2, Figures 6, 7). In turn, the lowest levels of sulphite-reducing bacteria were observed in July and August in points A and B, respectively, while their peak levels were detected in November in point A and in January – March plus in December in point B (Table 2, Figure 8).

When analysing mean annual values, it should be stated that in case of pollution degree indicator bacteria and sanitary state bacteria the values recorded at the sampling site A were at least four times higher than those at the sampling site B (Table 1). On the other hand, considerably higher abundance of physiological bacteria was observed at the sampling site B (except sulphite-reducing bacteria, which were three times less than as numerous at the sampling site A and titre of ammonifying bacteria) – Table 2.

Table 3 presents percentage distribution of FC/FS ratio, i.e. the proportion of fecal coliform bacteria to fecal streptococcus bacteria in the water samples from the sampling sites A and B. This ratio points to the source of origin of fecal pollutants. At the water sampling site A, the determined FC/FS ratio exceeding 4 was calculated for 92% of water samples, while in the remaining ones, i.e. in 8%, this ratio was below 0.7. At the sampling site B, the FC/FS ratio exceeding 4 was determined in more than half of water samples; water samples, for which FC/FS ratio was within 0.7 – 4, were also found (33%), as well as those with the ratio being below 0.7 (8%).

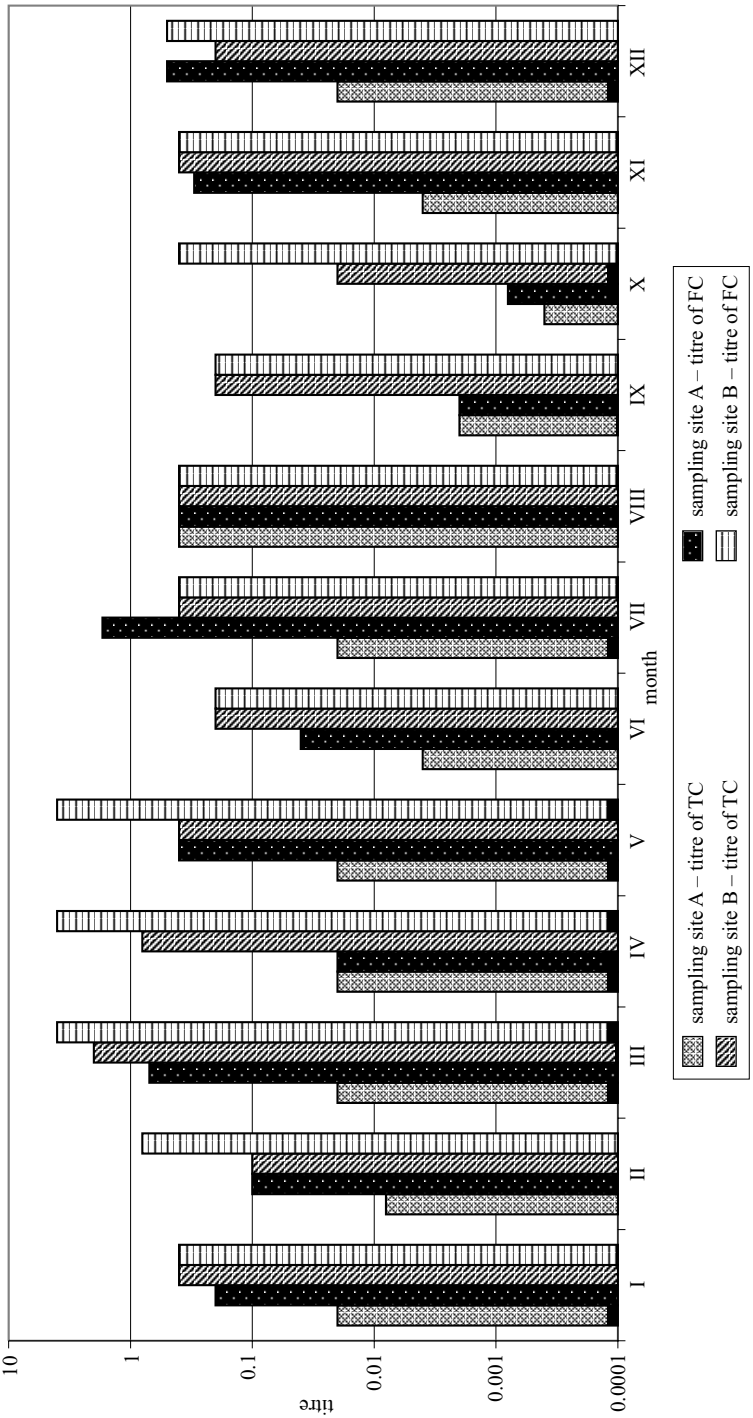

Fig. 5. Titre of total coliforms (TC) and fecal coliforms (FC) in water samples originating from Rusałka Lake

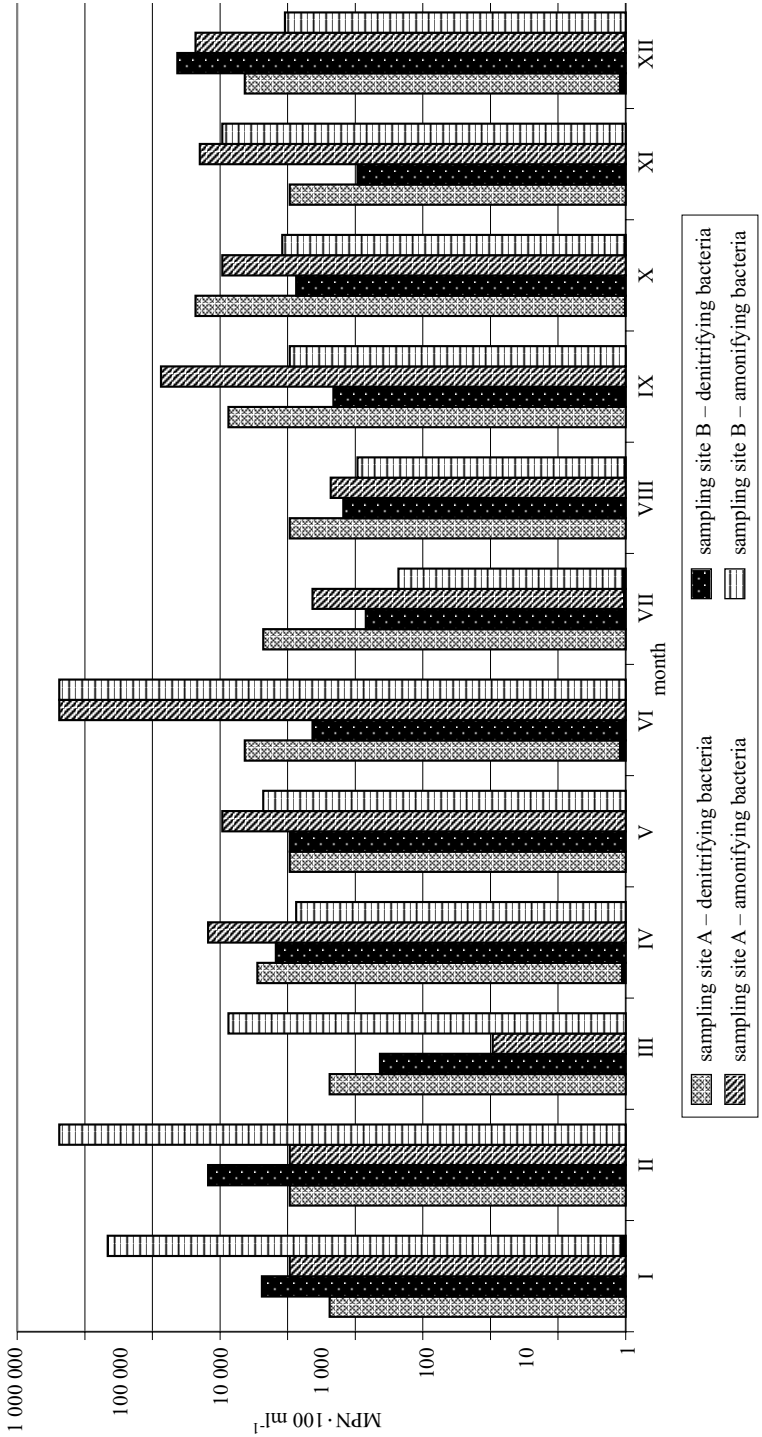

Fig. 6. Most probable number (MPN · 100 ml^{-1}) of denitrifying and amonifying bacteria in water samples originating from Rusałka Lake

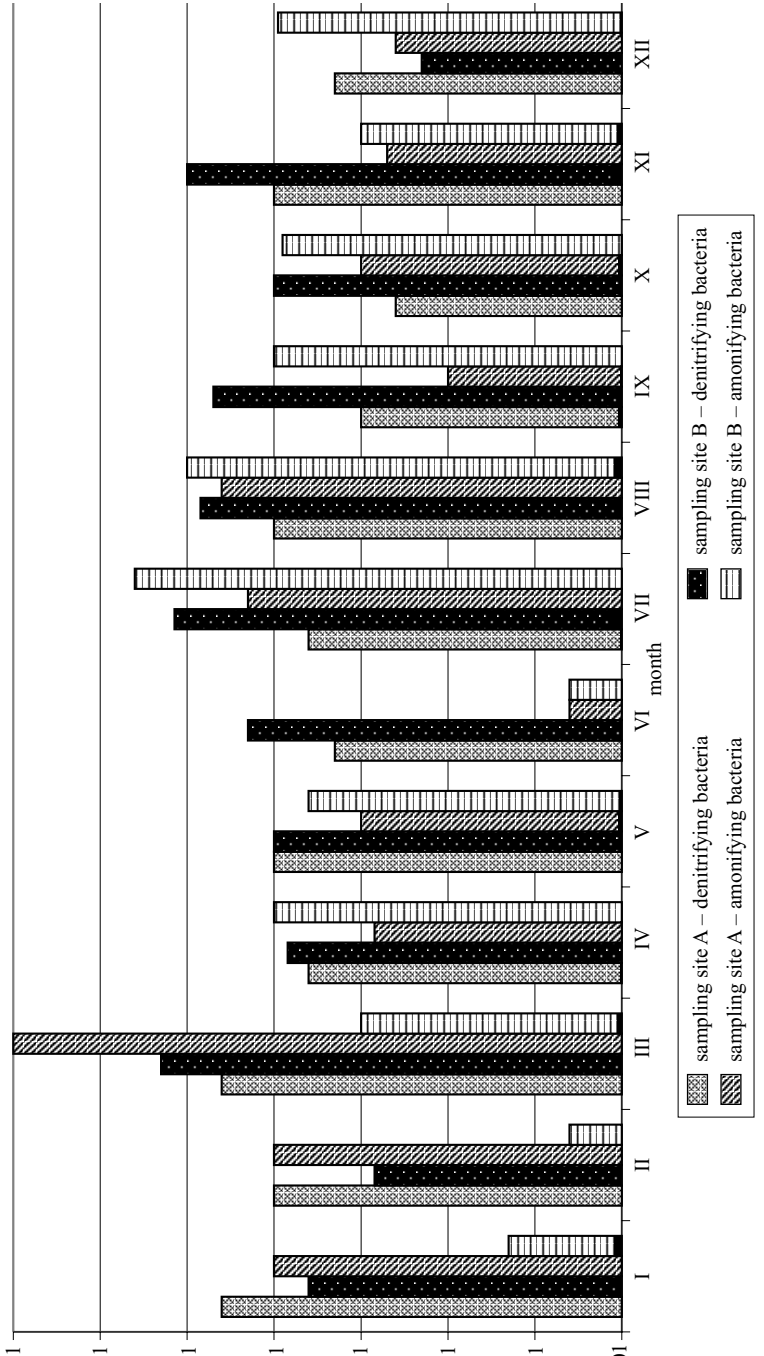

Fig. 7. Titre of denitrifying and amonifying bacteria in water samples originating from Rusałka Lake

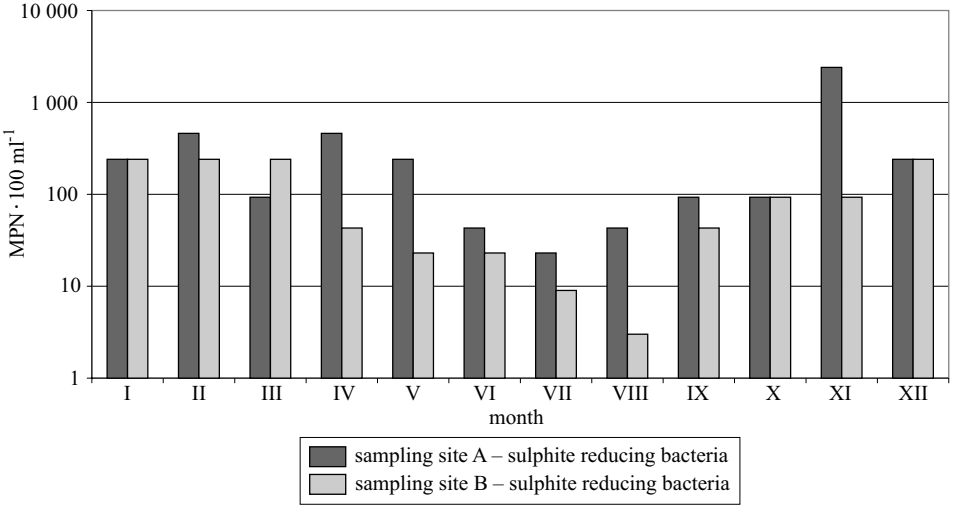

Fig. 8. Most probable number (MPN · 100 ml^{-1}) of sulphite reducing bacteria in water samples originating from Rusałka Lake

Discussion

Comparison of our own results with those of other authors remains difficult due to the absence of respective results of analogous studies on small water containers, including small municipal lakes. Studies carried on by other authors have been usually limited to determination of the number of pollution degree indicator bacteria, i.e. TVC 20°C, TVC 37°C (GODLEWSKA-LIPOWA et al. 1974, NAHURSKA et al. 1999, 2003, NIEWOLAK 1972, 1978a,b, 1989, STAPF, DEPTUŁA 1994, SZULKOWSKA-WOJACZEK 1972, ZMYSŁOWSKA, SOBIERAJSKA 1977, 1980) and water sanitary analysis – rather frequently was determined coliform bacteria count (GODLEWSKA-LIPOWA et al. 1974, NAHURSKA et al. 1999, 2003, NIEWOLAK 1972, STAPF, DEPTUŁA 1994, SZULKOWSKA-WOJACZEK 1972, ZMYSŁOWSKA, SOBIERAJSKA 1977, 1980), while more seldom fecal coliform bacteria count (NAHURSKA et al. 1999, 2003, NIEWOLAK 1978a,b, NIEWOLAK et al. 1997, STAPF, DEPTUŁA 1994) and fecal streptococcus bacteria count (NAHURSKA et al. 1999, 2003, NIEWOLAK 1978a,b, NIEWOLAK et al. 1997, STAPF, DEPTUŁA 1994). Few papers referred to identification of bacteria decomposing organic matter, such as for example ammonifying bacteria (GODLEWSKA--LIPOWA et al. 1974, NAHURSKA, DEPTUŁA 2004, ZMYSŁOWSKA, SOBIERAJSKA 1977, 1980). Furthermore, the data on microbiological studies of municipal lakes concern mostly much bigger lakes than the one discussed in the present paper. From among the lakes examined by other authors, alike Lake Rusałka

in respect of size (3.7 ha) are the following lakes: the Jeziorak Mały lake (26 ha) (NIEWOLAK 1972), the Starodworskie lake (6.7 ha) (NIEWOLAK et al.1997), the Bęskie lake (56 ha) (NIEWOLAK 1989), and the Syrenie Stawy lakes (5 ha) (NAHURSKA et al. 1999, NAHURSKA et al. 2003, NAHURSKA, DEPTUŁA 2004). The others examined municipal lakes, i.e. the Długie and the Miejskie lakes in w Szczytno (GODLEWSKA-LIPOWA et al. 1974), the Jeziorak lake, the Miejskie lake, the Ełckie lake (NIEWOLAK 1972), the Kortowskie lake (NIEWOLAK 1972, NIEWOLAK 1978a, ZMYSŁOWSKA, SOBIERAJSKA 1977), the Ukiel lake (NIEWOLAK 1978b), the Charzykowo lake (SZULKOWSKA-WOJACZEK 1972), the Długie lake in Olsztyn (ZMYSLOWSKA, SOBIERAJSKA 1980), are much larger.

When comparing the results presented currently with those obtained in previous years from the same water basin (NAHURSKA et al. 1999, NAHURSKA, DEPTUŁA 2004), it should be stated that TVC 20°C and TVC 37°C values are higher, while sanitary conditions indicator bacteria values are similar. The comparison of the values of the mentioned above parameters with the results obtained by the other authors examining alike water bodies allows for stating that the values obtained in the own study were higher than those obtained by them (NIEWOLAK 1972, 1989, NIEWOLAK et al. 1997) or similar, as is the case of the Syrenie Stawy lakes (NAHURSKA et al. 2003, NAHURSKA, DEPTUŁA 2003). Only in few papers are presented analyses concerning selected bacteria of physiological groups (GODLEWSKA-LIPOWA et al. 1974, NAHURSKA, DEPTUŁA 2003, ZMYSŁOWSKA, SOBIERAJSKA 1977, 1980). The values obtained in the own study for ammonifying bacteria are higher than those given by other authors (GODLEWSKA-LIPOWA et al. 1974, ZMYSŁOWSKA, SOBIERAJSKA 1977, 1980). Similar number of ammonifying bacteria and sulphite-reducing bacteria as in the own study was found in the Syrenie Stawy lakes only, whereas that of denitrifying bacteria was higher (NAHURSKA, DEPTUŁA 2003). The present study showed higher number of sanitary state indicator bacteria at the sampling site situated at the inflow of stream than at the sampling site located at the outflow water from the lake. High numbers of ammonifying and denitrifying bacteria, bacteria decomposing organic matter, were found at the sampling site situated at the outflow of water from the lake.

The annual fluctuations in the number of pollution degree indicator bacteria observed in the own study as well as the lack of regularity in seasonal development of these microorganisms result most likely from the pollution of this lake by municipal wastes, which is linked to the abundance of easily available food materials, the presence of which is probably the main factor that controls their growth (DONDERSKI 1983, ŚWIĄTECKI 1997). Similar situation was observed by other authors who examined municipal lakes of the Masurian Lakeland, being receiving bodies for municipal wastes (NIEWOLAK 1972). Also algal blooms and phytoplacton decomposition which cause the enrichment of

the water body in organic matters that are easily available for bacteria may be important in this process, and increase the bacteria growth rate (DONDERSKI, KALWASIŃSKA 2003). Seasonal changes in bacteria numbers may be influenced also by grazing – consumption of bacteria by protozoa (KOTON-CZARNECKA, CHRÓST 2001). Another factor that may shape the number of bacteria in annual cycle is temperature, though according to Świątecki (ŚWIĄTECKI 1997) the temperature is a factor, which limits bacteria growth rate solely at the level below 10°C, no correlation between this factor and the number of bacteria has been observed at higher temperatures, especially in summer.

The considerably higher number of fecal coliform bacteria than that of fecal streptococcus bacteria observed in the own study, in particular at the sampling site A, shows the inflow of fecal pollutants of human origin, which are brought in to the water body together with the waters of stream supplying it. The FC/FS ratio at the sampling site B points to a contribution of fecal pollutants of mixed origin, both human and animal, which may be linked to the fact that this water body is inhabited by water-fowl bringing in fecal pollutants as well.

In analysis of specific correlation coefficients the detected correlation between TVC 37°C and water temperature is consistent with thermal properties of the group of microbes. The correlation between TVC 20°C and TVC 37°C probably reflects the fact that TVC 37°C bacteria enter the water container with sewages, which in parallel introduce significant amounts of organic matter. Supplementation of the water in the container with nutrients may also induce increase in number of TVC 20°C bacteria. The relationship between number of TVC 37°C bacteria and NPL of fecal coliform bacteria and number of fecal streptococci may reflect the fact that FC and FS bacteria represent allochtonic flora of water environment and develop well at the temperature of 37°C. In turn, the interrelationship between index bacteria of sanitary status, i.e. TC and FC can be explained by coexistence of the microbial groups in faeces of humans and homothermal animals, with which the bacteria are directly or indirectly drained to water reservoirs.

The interrelationship between amonifying and denitrifying bacteria stems from the fact that both groups of the bacteria are involved in metabolism of nitrogen compounds and their development is linked to levels of the compounds in their environment.

Conclusions

1. The values of determined parameters vary considerably during the whole year, due to the regular inflow of pollutants into Rusalka Lake.

2. Higher values for sanitary state indicator bacteria were observed at the

sampling site A, i.e. at the water inflow point to the lake, than at the sampling site B situated at the water outlet point to the underground pipeline. Furthermore, high number of bacteria of physiological groups tells about the great abundance of organic matter in the waters of the Rusalka lake, and shows that there is remineralisation process going on in the reservoir.

3. The results obtained in the own study show in most cases higher values from the analogous results recorded in the same water body in previous years as well as from those given by the others authors for municipal lakes in Poland.

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ECOLOGICAL ASPECTS OF NITROGEN TRANSFORMATION IN A MESOTROPHIC LAKE (LAKE DŁUGIE WIGIERSKIE) IN THE PRESENCE OF BLACK CORMORANTS (*PHALOCROCORAX CARBO*)

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K e y w o r d s: nitrogen cycle bacteria, mesotrophic lake.

A b s t r a c t

The paper comprises results of assays of ammonium, nitrite, nitrate, and organic nitrogen, mineral and total phosphorus, chlorophyll *a*, temperature, oxygen, pH, Secchi's disc visibility and count of ammonifying, the 1st phase nitrifying (*Nitrosomonas*) and the 2nd phase nitrifying (*Nitrobacter*), reducing NO_3^- to NO_2^-, denitrifying (reducing NO_3^- to N_2O/N_2) and atmospheric nitrogen fixing bacteria (aerobic and anaerobic) in the waters of Długie Wigierskie Lake. The study was completed during three successive vegetative seasons in 1999-2001, at sites located in the deepest parts of the lake (sites 3 and 7) as well as its inflow and outflow (sites 5 and 8); in addition, microbiological assays were carried out at 10 sites located in some characteristic points (especially near habitats of black cormorants). Water samples were collected from the surface layer and near the bottom; at sites 3 and 7 additional samples were taken from the depths of 7 and 5 m, respectively. Water was sampled at 1-month intervals. The amounts of nitrate nitrogen, mineral and total phosphorus and chlorophyll a corresponded to the values characterizing water classified as purity class I. Sporadically (in bottom water during the summer stagnation period) the content of ammonium nitrogen reached the values which corresponded to class II of water purity, while the amounts of nitrite nitrogen and oxygen indicated water purity classes II and III. The counts of nitrogen cycle bacteria suggested relatively low eutrophication of the lake. Although on many occasions more bacteria were detected in water samples collected near black cormorant habitats (sites 9, 10, 11 and 12), statistical differences between those and the other sites were either non-significant or very small.

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EKOLOGICZNE ASPEKTY PRZEMIAN AZOTU W WODZIE JEZIORA DŁUGIEGO WIGIERSKIEGO W WARUNKACH BYTOWANIA KORMORANA CZARNEGO (*PHALOCROCORAX CARBO*)

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Słowa kluczowe: bakterie cyklu azotowego, jezioro mezotroficzne.

Abstrakt

Praca obejmuje wyniki badań zawartości azotu amonowego, azotynowego, azotanowego, organicznego, fosforu mineralnego i całkowitego, chlorofilu *a*, sestonu, temperatury, tlenu, pH, widzialności krążka Secchiego oraz liczebności bakterii amonifikacyjnych, nitryfikacyjnych I i II fazy, redukujących NO_3^- do NO_2^-, denitryfikacyjnych (redukujących NO_3^- do N_2O/N_2) oraz wiążących azot atmosferyczny w wodzie Jeziora Długiego Wigierskiego. Badania przeprowadzono w latach 1999--2001, w 3 kolejnych okresach wegetacyjnych, na stanowiskach usytuowanych w najgłębszych miejscach jeziora, w jego dopływie i odpływie; dodatkowo badania mikrobiologiczne prowadzono na 10 stanowiskach usytuowanych w charakterystycznych miejscach (ze szczególnym uwzględnieniem siedlisk kormorana czarnego). Próby wody do badań pobierano z warstwy powierzchniowej i przydennej, na stanowiskach 3 i 7, dodatkowo z głębokości odpowiednio 7 i 5 m, w odstępach 1-miesięcznych. Ilości azotu azotanowego, fosforu mineralnego i całkowitego oraz chlorofilu *a* odpowiadały wartościom przyjętym dla wód I klasy czystości. Sporadycznie (w wodzie przydennej podczas letniej stagnacji) ilości azotu amonowego osiągały wartości przyjęte dla wód II klasy, azotu azotynowego i tlenu zaś dla II i III klasy czystości. Liczebność bakterii czynnych w obiegu azotu świadczy o względnie niskim stopniu troficznym wody jeziora. Chociaż niejednokrotnie więcej tych bakterii stwierdzano w próbach wody pobieranych na stanowiskach usytuowanych w pobliżu siedlisk kormorana czarnego, to statystycznie różnice te były nieistotne lub mało istotne.

Introduction

Processes of atmospheric nitrogen fixing and mineralization of organic nitrogen compounds in surface waters are of utmost importance in nature. Microorganisms involved in those processes are widely distributed in all kinds of water reservoirs. Their count in water can vary depending on the type of a water body, type of the basin (arable, forested, pasture), season of the year, the weather. In Wigry National Park birds and wild animals may also affect the number of bacteria in water. The fact that waterfowl (ducks, geese, swans and seagulls) contribute to bacteriological contamination of surface waters is well documented (ALDERISIO and DELUCA 1999, BUCK 1990, LÉVESQUE et al. 2000, KUHN et al. 2002). The influence of birds on the number of bacteria actively participating in nitrogen compound conversion in lake waters seems to be indirect, i.e. via excreta. As the literature seems to lack the relevant data on this question, it was interesting to undertake the present study. The tests were conducted on Długie Wigierskie Lake, which stands out among 43 other lakes

in Wigry National Park as a habitat holding a colony of ca 1000 black cormorants (*Phalocrocorax carbo*) (MELLIN and MIROWSKA-IBRON 1994). Excrements of those birds, their droppings and expectorated fish can be washed into the lake during rain, thus enriching its water with nitrogen compounds. The paper presents some physicochemical parameters of the water of Długie Wigierskie Lake and spatial distribution as well as seasonal fluctuations of the counts of some bacteria which are involved in nitrogen cycling. The study was carried out in spring, summer and autumn of 1999-2001, on samples of surface and bottom water collected at sites located in some characteristic parts of the lake, especially near habitats of black cormorants. The purpose of this research was to find out if and to what extent black cormorants affected the presence and number of microorganisms actively participating in nitrogen cycle.

Materials and Methods

Study area. Długie Wigierskie Lake is situated in Wigry National Park. The surface area of the lake is 0.8 km^2. The maximum depth reaches 14.8 m, while the average depth is 6.4 m. The lake divides into two sections separated with a rather deep narrowing: the north-western and the south-eastern parts (Figure 1). The lake is connected with two other lakes via quite wide canals: to the north it is joined with Muliczne Lake, and to the west it is linked with Okrągłe Lake. Since 1985 all those lakes together with some forested land and surrounding swamps (of the total area of 2.94 km^2) have been under strict nature conservation protection. There are three small islets (Ostrówek, Szaniec and Grądzik) on Długie Wigierskie Lake, which together cover an area of 0.16 km^2. Habitats of black cormorants can be found on Ostrówek Isle and at two other places near the lake shore, in the south-eastern part of the lake. It is a non-nesting colony, comprising ca 1000 birds, which come to the lake to feed. One characteristic feature of the lake basin is a poorly developed river system – apart from the canals there are no streams or rivers connecting the lakes. The immediate lake basin consists of meadows and pastures (20%), arable land (23%) and forested land (57%), which includes the northern part of Augustów Forest, lying around Wigry Lake (BAJKIEWICZ-GRABOWSKA et al. 1992). Within the lake basin there are a few farmsteads, several summer cottages and some buildings which belong to the State Forests.

Water sampling. Water samples for the assays were collected on Długie Wigierskie Lake from April to November (at 1-month intervals) at 14 sites in the years 1999-2001. All the sampling sites were situated at some characteristic parts of the lake, with special attention given to the areas settled by cormorants (Figure 1). The north-western part of the lake held sites 1-4, while

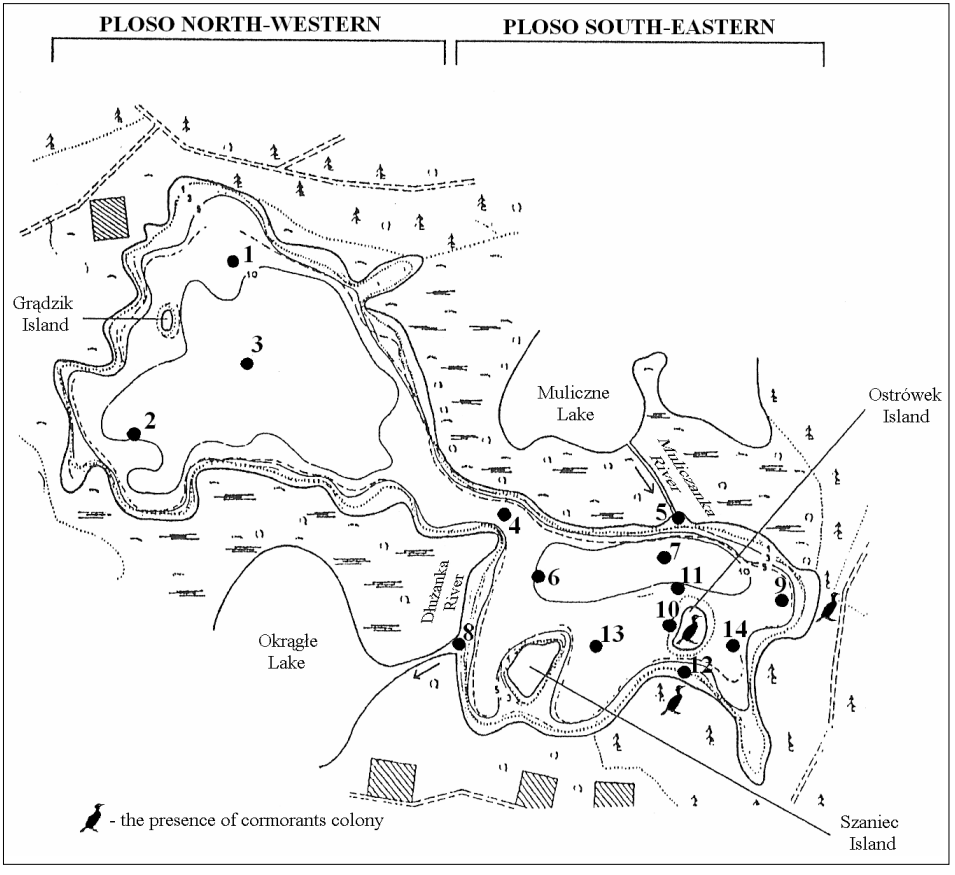

Fig. 1. Location sketch of the Długie Wigierskie Lake. 1, 2 ..., 14 – water sampling sites

the south-eastern area contained sites 5-14. Sites 3 and 7 were located in the deepest parts of the lake (14.8 and 10.0 m, respectively), while sites 9, 10 and 12 were situated near black cormorant habitats. Site 5 was situated near the water inflow from Muliczne Lake, whereas site 8 was near the water outflow to Okrągłe Lake. Samples of water for laboratory analyses were taken from the surface layer (0.3 m) and near the lake bottom (0.2 m above the bottom). In addition, at sites 3 and 7 (the deepest ones) water was also sampled at 7 and 5 m depth, respectively. Water samples from the surface layer were collected directly to sterile 300 dm^3 bottles; water samples from deeper layers or near the bottom were poured into identical bottles using a 5 dm^3 capacity Ruttner sampler. The water samples were cooled to 4-6°C and transported to the laboratory in thermobags. The elapsed time from water sampling to analyses

was less than 12 hours. In total, 500 water samples were collected and analyzed. Parallel to water sampling, the following measurements were made: water temperature, chlorophill *a*, Secchi disc visibility, seston and water pH. Additionally, water for chemical analyses (contents of: oxygen, $N\text{-}NO_3$, $N\text{-}NO_2$, $N\text{-}NH_4$, N_{org}, P_{total} and $P\text{-}PO_4$) was collected. Meteorological observations included air temperature on water sampling days, total atmospheric precipitation in 7 and 30 days before water sampling as well as wind power and direction in 2 days before water sampling. The detailed chemical, physical and meteorological data can be obtained from the authors.

Microbiological analyses. Microbiological analyses included the number of atmospheric nitrogen-fixing bacteria under aerobic (*Azotobacter* and others) and anaerobic (*Clostridium pasteurianum*) conditions, ammonifying bacteria, the 1st phase nitrifying (*Nitrosomonas*) and the 2nd phase nitrifying (*Nitrobacter*) bacteria, reducing NO_3^- to NO_2^-, denitrifying (reducing NO_3^- to N_2O/N_2) bacteria on media and under conditions specified in Table 1. The measurements were carried out in 3 parallel repetitions of the same sample. When measuring the number of atmospheric nitrogen fixing bacteria under aerobic conditions, only typical colonies were counted, whereas when measuring the number of ammonifying bacteria all the grown colonies were counted. Positive results of the presence of 1st phase and the 2nd phase nitrifying bacteria, bacteria reducing NO_3^- to NO_2^-, denitrifying bacteria (reducing NO_3^- to N_2O/N_2) and atmospheric nitrogen-fixing bacteria under aerobic conditions were con-

Table 1

Nutrient medium, temperature and time of incubation of bacteria active in nitrogen cycle in water of Długie Wigierskie Lake, its inflow and outflow

Microorganisms	Media used to enumerate bacteria	Incubation	
		temperature (°C)	time (days)
Ammonifying bacteria	broth-agar + 3% bacto pepton (Difco)	25	3
NH_4 -oxidizers (*Nitrosomonas*)	mineral medium with $(NH_4)SO_4$ acc. Winogradsky	28	14
NO_2 -oxidizers (*Nitrobacter*)	mineral medium with $NaNO_2$ acc. Winogradsky	28	21
NO_3 to NO_2 reducers	Giltay's medium with Durham's tubes	25	7
Denitrifying bacteria (NO_3 to $N_2/NO/N_2O$ reducers)	Giltay's medium with Durham's tubes	25	7
Aerobic nitrogen fixers	Fiodorow's medium with mannit (2%)	25	7
Anaerobic nitrogen fixers	Winogradsky's medium	25	7

firmed according to the methodology described by ALEF and NANNIPIERI (1995) and RODINA (1968). The most probable number (MPN) of those bacteria was read from McGrady's tables (MEYNELL and MEYNELL 1970).

Statistical evaluation. In order to estimate significance of differences in counts of microorganisms in the lake waters during the 3-year time of the study, a single factor analysis of variance (ANOVA) was conducted, verifying the hypothesis of the equality of means (H_0:$x_1 = x_2 = ... = x_5$) at the level of significance $\alpha = 0.05$, assuming that the variance for the numerousness of the bacteria groups under study are uniform. The uniformity of variance was tested with Levene's test. If the test proved significant, the hypothesis was rejected. Next, the Kruskal-Wallis test was applied, which is a non-parametric equivalent of the analysis of variance (STANISZ 2006). Estimation by Spearman; correlation between numbers of studied microorganisms and physicochemical and meteorological data were used in this study too.

Results

This paper presents only the general numbers of bacteria fluctuations; the detailed results can be obtained from the authors. Highly significant statistical differences in the counts of all groups of studied bacteria were noticed between water samples collected in summer versus those obtained in spring and autumn (Figure 2); no such differences in the number of studied microorganisms were detected between water samples taken from different sites and depths of the lake. According statistic estimation by Spearman' correlation all nitrogen cycle bacteria analyzed was most weakly correlated with the concentration of N-NH_4 and the content of oxygen in water whereas the strongest correlation was revealed with the concentration of P-PO_4 and pH of water (Table 2). Counts of most studied bacteria were directly proportional (negative or positive correlation) to the 7-days before water sampling and one-month precipitation.

Atmospheric nitrogen fixing bacteria (Figure 2). The number of bacteria binding atmospheric nitrogen under aerobic conditions (*Azotobacter* and others) did not exceed 400 cfu · cm^{-3}. Frequently, no such bacteria were

→

Fig. 2. Averages numbers (+ standard deviation and + random mean square-RMS) of: *a* – aerobic nitrogen fixing bacteria (*Azotobacter*) (cfu · cm^{-3}), *b* – anaerobic nitrogen fixing bacteria (*Clostridium pasteurianum*) (MPN · 100 cm^{-3}), *c* – ammonifying bacteria (cfu · cm^{-3}), *d* – the 1st phase nitrifying bacteria (MPN · 100 cm^{-3}), *e* – the 2nd phase nitrifying bacteria (MPN · 100 cm^{-3}) and *f* – denitrifying bacteria (MPN · 100 cm^{-3}) in the water of Długie Wigierskie Lake during whole 3-years period of study. Independent variable (assembling): time. ANOVA test of Kruskal-Wallis ranges

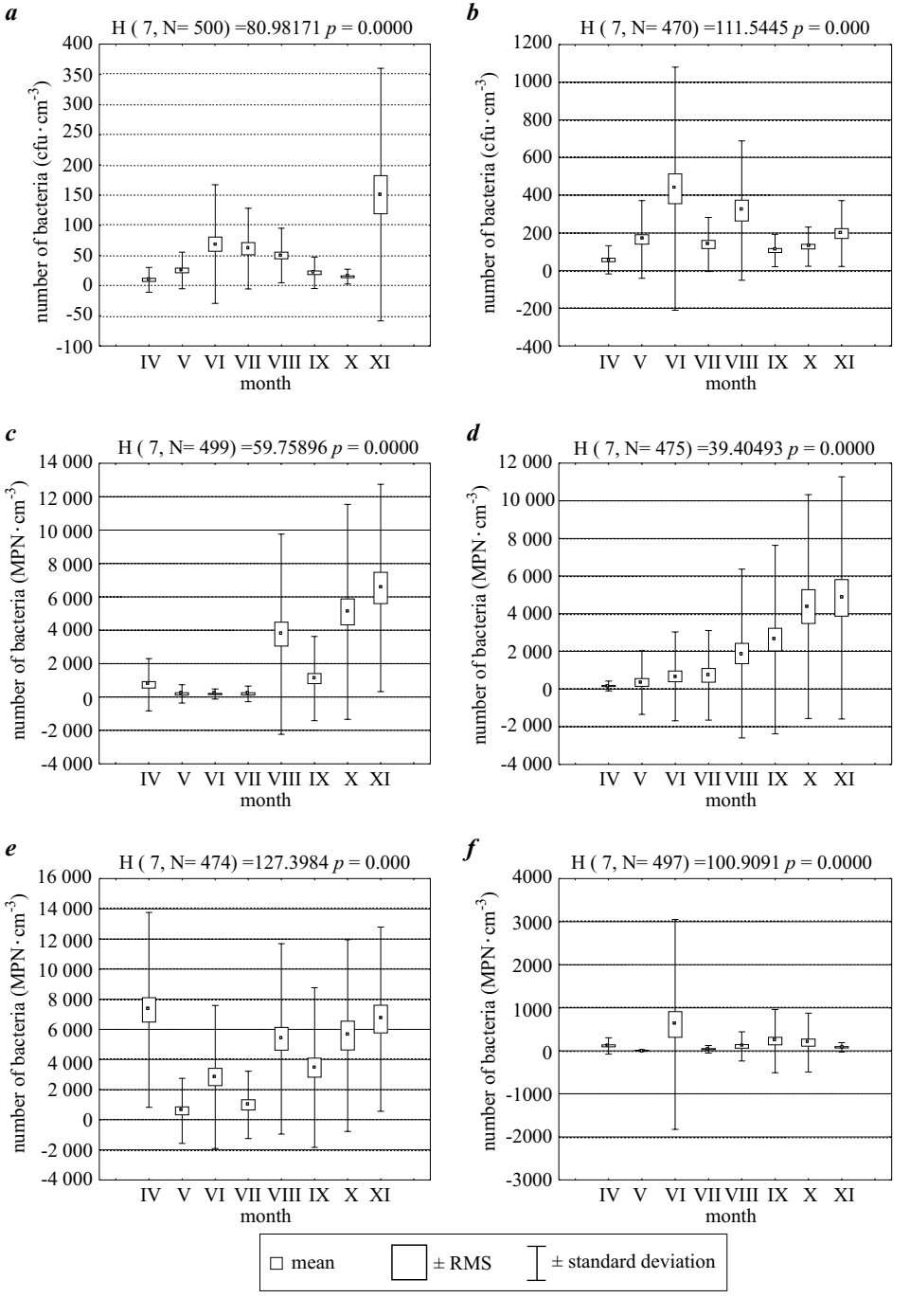
a
H (7, N= 500) =80.98171 p = 0.0000
number of bacteria (cfu · cm-3)
400
350
300
250
200
150
100
50
0
-50
-100
IV V VI VII VIII IX X XI
month
b
H (7, N= 470) =111.5445 p = 0.000
number of bacteria (cfu · cm-3)
1200
1000
800
600
400
200
0
-200
-400
IV V VI VII VIII IX X XI
month
c
H (7, N= 499) =59.75896 p = 0.0000
number of bacteria (MPN · cm-3)
14 000
12 000
10 000
8 000
6 000
4 000
2 000
0
-2 000
-4 000
IV V VI VII VIII IX X XI
month
d
H (7, N= 475) =39.40493 p = 0.0000
number of bacteria (MPN · cm-3)
12 000
10 000
8 000
6 000
4 000
2 000
0
-2 000
-4 000
IV V VI VII VIII IX X XI
month
e
H (7, N= 474) =127.3984 p = 0.000
number of bacteria (MPN · cm-3)
16 000
14 000
12 000
10 000
8 000
6 000
4 000
2 000
0
-2 000
-4 000
IV V VI VII VIII IX X XI
month
f
H (7, N= 497) =100.9091 p = 0.0000
number of bacteria (MPN · cm-3)
4000
3000
2000
1000
0
-1000
-2000
-3000
IV V VI VII VIII IX X XI
month
mean
± RMS
± standard deviation

Table 2

Statistic estimation by Spearman; correlation between numbers (cfu · cm^{-3}/MPN · 100 cm^{-3}) of studied nitrogen cycle microorganisms recovered from the water of Długie Wigierskie Lake during whole time of study and some chemical compounds (mg · dm^{-3}) in water. BD eliminated in couple. Important correlations ($p < 0.05000$) marked

Specification	*Azotobacter*	*Clostridium pasteurianum*	Ammonifying bacteria	The 1st phase nitrifying bacteria	The 2nd phase nitrifying bacteria	NO_3 to NO_2 reducers	Denitrifying bacteria
Azotobacter	1.000	-0.064	0.260	0.074	0.119	0.339	0.017
Clostridium pasteurianum	-0.064	1.000	-0.103	0.468	0.282	0.115	-0.050
Ammonifying bacteria	0.260	-0.103	1.000	-0.171	-0.199	0.097	-0.125
The 1st phase nitrifying bacteria	0.074	0.468	-0.171	1.000	0.299	0.331	0.072
The 2nd phase nitrifying bacteria	0.119	0.282	-0.199	0.299	1.000	0.213	0.292
NO_3 to NO_2 reducers	0.339	0.115	0.097	0.331	0.213	1.000	-0.034
Denitrifying bacteria	0.017	-0.050	-0.125	0.072	0.292	-0.034	1.000
N-NO_3	-0.246	-0.162	-0.106	0.020	0.023	-0.133	-0.046
N-NO_2	0.065	0.143	0.255	0.065	-0.011	0.176	-0.109
N-NH_4	0.032	0.150	-0.011	-0.011	0.059	0.054	0.028
N_{org}	0.010	-0.215	0.066	-0.064	-0.189	0.110	0.056
P_{total}	-0.148	0.362	0.015	0.102	0.027	-0.137	0.004
P-PO_4	-0.227	0.343	-0.223	0.352	0.057	-0.147	0.068
Temperature of water (°C)	0.105	0.139	0.143	0.071	-0.280	0.038	-0.144
Oxygen	-0.052	-0.104	-0.131	-0.003	-0.077	-0.114	-0.089
pH	0.476	0.159	-0.573	0.244	0.560	-0.193	0.137
Chlorophill a (μg · dm^{-3})	-0.176	0.151	-0.499	0.113	0.393	-0.127	0.101
Seston	-0.041	-0.067	0.385	0.067	-0.245	0.119	-0.111
Secchi disc visibility (m)	-0.056	0.105	0.016	0.084	0.052	-0.185	0.202
Temperature during the day of sampling (°C)	0.241	0.053	0.049	0.092	-0.026	0.022	-0.094
Month average temperature (°C)	0.211	-0.071	-0.057	-0.051	-0.118	0.209	-0.061
7-days rainfall (mm)	-0.131	-0.099	0.064	-0.205	0.006	-0.191	0.210
1-month rainfall (mm)	0.069	0.131	0.076	0.067	0.155	-0.089	0.355
Wind (m · s^{-1})	-0.265	-0.174	-0.122	-0.333	0.020	-0.338	0.119

determined in the analysed volume of water. The highest count of those bacteria was recorded in November during whole time of study; such high numbers were only sporadically noticed in other time periods (June, July). There were little differences in the number of aerobically N-fixing bacteria in water sampled at different depths and sites. The count of bacteria fixing atmospheric nitrogen under anaerobic conditions (*Clostridium pasteurianum*) varied from < 3 MPN $\cdot$ 100 cm^{-3} to $1.4 \cdot 10^3$ MPN $\cdot$ 100 cm^{-3}. The mean numbers of the tested bacteria were the lowest in April and the highest in June and August.

Bacteria actively involved in nitrogen compound conversions (Figure 2). Among bacteria which actively participate in conversions of nitrogen compounds, the waters of Długie Wigierskie Lake contained the highest number of ammonifying bacteria. Seasonal fluctuations of ammonifying bacteria were smaller in May, June and July and the largest in autumn (October and November). Differences in the counts of ammonifying bacteria found between the sampling sites were ambiguous. Sometimes the highest numbers were determined in the north-western part of the lake, at sites 2 and 3, and sometimes there were more ammonifying bacteria in the south-eastern area of the lake, at sites 9, 10, 11 and 12. This group of bacteria occurred in higher numbers in bottom water. The number of the 1st phase (*Nitrosomonas*) and the 2nd phase (*Nitrobacter*) nitrifying bacteria did not exceed $1.4 \cdot 10^4$ MPN $\cdot$ $\cdot$ 100 cm^{-3}. On many occasions no nitrifying bacteria were found. The 1st phase (*Nitrosomonas*) nitrifying bacteria were found in smaller numbers during spring months, the highest during autumnal months. The 2nd phase (*Nitrobacter*) nitrifying bacteria appeared more abundantly in April and from August to November (less often in September). The number of denitrifying bacteria (reducing NO_3^- to N_2O/N_2) ranged from < 3 MPN $\cdot$ 100 cm^{-3} in most of the water samples analysed to $1.4 \cdot 10^4$ MPN $\cdot$ 100 cm^{-3}. The highest numbers of this group of bacteria were found in June, in other time of study there were at the similar level. Several-fold more such bacteria were detected in bottom water.

Discussion

Processes of mineralization of organic matter produced in situ or introduced to surface waters from catchment are conditioned by the biochemical activity of various physiological groups of bacteria. Nitrogen fixing, ammonifying, nitrifying and denitrifying bacteria are particularly important in those processes. In the waters of Długie Wigierskie Lake bacteria binding atmospheric nitrogen constituted rather a small group of microorganisms. The mean number of bacteria fixing nitrogen under aerobic conditions (28-50 cfu $\cdot$ cm^{-3})

was 2- to 3-fold higher, while those binding nitrogen under anaerobic condition ($1.8\text{-}2.8 \cdot 10^3$ MPN $\cdot$ 100 cm^{-3}) appeared at 5- to 10-fold higher counts than the same group of bacteria determined in waters in dystrophic lakes in Wigry National Park (KUCZYŃSKA and NIEWOLAK 2004). Higher numbers of those microorganisms were assessed in eutrophic lakes near Olsztyn (NIEWOLAK 1983, EL-SHENAWY 1989, NIEWOLAK et al. 2005). Very small differences in the counts of those bacteria in water samples collected from different sites at Długie Wigierskie Lake could be attributed to the lake's small size (0.8 km^2). The development of those bacteria is affected by a number of factors, including aerobic conditions, presence of easily available resources of carbon and energy, produced *in situ* via photosynthesis processes carried out by phytoplankton and higher water plants, as well as the supplied from the lake's basin during spring thaws and summer rains. Maximum counts of aerobic nitrogen fixing bacteria (*Azotobacter* and others) in the water of Długie Wigierskie Lake could be associated with increased concentrations of chlorophyll *a* at the same time. Similarly high number of anaerobic nitrogen fixing bacteria (*Clostridium pasteurianum*) at the same site was determined when concentrations of chlorophyll *a* were higher. Higher numbers of anaerobic nitrogen fixing bacteria (*Clostridium pasteurianum*) in June may have been caused by the circulation of water masses and transmission of microorganisms from the surface layer of bottom sediments. It is known from the literature (NIEWOLAK 1970) that bottom sediments in lakes contain much more nitrogen fixing bacteria than the water above the bottom. Among the bacteria which are actively involved in conversion of some forms of nitrogen into other forms of this element, the most numerous ones in the waters of Długie Wigierskie Lake were ammonifying and the 2nd phase nitrifying bacteria (*Nitrobacter*). The count of ammonifying bacteria, however, did not diverge from the analogous numbers determined in dystrophic lakes lying in Wigry National Park (KUCZYŃSKA and NIEWOLAK 2004) or in the waters of oligotrophic Hańcza Lake (GOTKOWSKA-PŁACHTA et al. 2005); that number was typically 10 to 100-fold lower than analogous counts determined earlier in lakes of Masurian Lake Distric (NIEWOLAK et al. 1978, NIEWOLAK and POTOCKA 1988, NIEWOLAK et al. 2005). Higher numbers of such bacteria in Długie Wigierskie Lake detected from August to November are mirrored by increased concentrations of $N\text{-}NH_4$ and $N\text{-}NO_2$ (especially in bottom water). This may suggest increased intensity of mineralization of organic nitrogen compounds, for example in the surface layer of the bottom. Differences in the amount of $N\text{-}NH_4$ in bottom and surface water could have been related to the processes of $N\text{-}NH_4$ nitrification and/or the uptake of that compound by phytoplankton. Increased concentration of $N\text{-}NH_4$ in bottom water was not infrequently accompanied by elevated amounts of $N\text{-}NO_3$ in the same layer of water. $N\text{-}NO_3$ could have been derived

from oxygenation of ammonium nitrogen by the 1st phase nitrifying bacteria (*Nitrosomonas*) and/or heterotrophic bacteria, as well as from the reduction of nitrates to nitrites by those microorganisms (depending on the environmental conditions). Some previous reports (GÜDE and OVERBECK 1972) claim that during the maximum development ($1 \cdot 10^5$ cells per 1 cm^3) of heterotrophic bacteria which oxygenate ammonium to nitrites (heterotrophic nitrification) in the waters of Plusse Lake in Holstein can accumulate 1 or more mg dm^{-3} $N\text{-}NO_2$. This calculation is based on the fact that 1 cell of heterotrophic bacteria can oxygenate $4 \cdot 10^{-7}$ – $1 \cdot 10^{-8}$ μg $N\text{-}NH_4$ an hour. At the same time, $N\text{-}NO_3$ did not accumulate in large amounts in the lake water despite an increase in the number of the 2nd phase nitrifying bacteria (*Nitrobacter*), which may have been connected with the uptake of $N\text{-}NO_3$ by phytoplankton. This event was accompanied by a decrease in the concentration of $P\text{-}PO_4$, which occurred in higher amounts only in autumn, when phytoplankton began to die out. The presence of higher concentrations of $N\text{-}NH_4$ and $N\text{-}NO_2$ in bottom water of Długie Wigierskie Lake in summer may have been associated with an inhibition of the 2nd phase nitrification process (oxygenation of NO_2^- to NO_3^-) under conditions of limited amount or complete lack of water dissolved oxygen and presence of sulphur hydrogen. $N\text{-}NO_2$ found at that time in the bottom water of Długie Wigierskie Lake could also have been derived from the reduction of nitrates to nitrites by heterotrophic bacteria, which were determined to occur in higher numbers denitrifying bacteria, which reduced NO_3^- to N_2/N_2O.

The counts of bacteria involved in nitrogen cycle determined in the waters of Długie Wigierskie Lake may suggest a low level of pollution of the lake and a small content of organic mater (*Rozporządzenie...* 2004). They are not highly varied between the 14 sampling sites or between layers of water. Seasonal fluctuations in the number of nitrogen cycle bacteria usually reached peak values in summer, exceptionally in spring or autumn. Lack of statistically significant differences in the count of those bacteria between the sites located close or far from habitats of black cormorants may imply that those birds either had no effect on the contamination of the lake, or else that the influence was marginally small.

Conclusions

1. The counts of nitrogen – fixing bacteria under aerobic and anaerobic conditions and actively participating in nitrogen cycle (ammonifying, the 1st and the 2nd phase nitrifying, reducing nitrates to nitrites, denitrifying – reducing nitrates to elemental nitrogen and/or nitrogen oxides) suggest a low level of the contamination of the lake and its relatively low trophy.

2. The counts of the nitrogen cycle bacteria determined in the course of the study did not show any significant spatial or vertical diversity. Seasonal changes occurred in the number of ammonifying bacteria, which peaked in the summer.

3. No large differences in the counts of the nitrogen cycle bacteria assessed in the study at particular sampling sites, or else only slightly higher microbial counts (ammonifying and NO_3^- reducing bacteria at shore sampling sites near habitats of black cormorants) suggest that those birds do not play any important role in the pollution and eutrophization of Długie Wigierskie Lake.

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RESEARCH FOR RESISTANCE TO ANTIBIOTICS DOMINANT BACTERIA ISOLATED FROM WATER AND CARP (*CYPRINUS CARPIO* L.) DURING FISH WINTERING IN POST-COOLING WATER

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Key words: water, fish, bacteria, antibiotic, resistance.

Abstract

Studies were carried out to identify and determine the resistance to antibiotics exhibited by potentially pathogenic bacteria isolated from the water, mucous, skin surface and alimentary tract of fish. The isolates were studied for resistance to 12 antibiotics commonly used in the prevention and therapy people and animals. Twenty bacterial strains were isolated and identified based on the phenotype traits: *Aeromonas hydrophila* (13), *Shewanella putrefaciens* (4), *Aeromonas salmonicida* (2) *Aeromonas sobria* (1) and *Pseudomonas putida* (1). About 67% of the strains isolated from the alimentary tract and 78% of the strains isolated from the mucous exhibited resistance to more than one antibiotic. All strains isolated from water showed resistance to 4 to 8 antibiotics. The largest number of different bacteria was resistant to oxacilin (18), fewer strains were resistant to ampicilin (15), vancomycin (15), trimethoprim (5) and individual strains were resistant to neomycin, streptomycin, oxytetracyclin, kanamycin, erythromycin, gentamycin and novobiocin. All of the examined strains were sensitive to nalidixic acid.

BADANIA ANTYBIOTYKOOPORNOŚCI DOMINUJĄCYCH BAKTERII WYIZOLOWANYCH Z WODY I KARPIA (*CYPRINUS CARPIO* L.) W CZASIE ZIMOWANIA RYB W WODZIE POCHŁODNICZEJ

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Słowa kluczowe: woda, ryby, bakterie, antybiotyki, oporność.

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Abstrakt

W pracy badano antybiotykooporność szczepów bakteryjnych wyizolowanych z intensywnej hodowli ryb w czasie ich zimowania. Wyizolowano 25 szczepów bakteryjnych (6 z wody, 8 z przewodu pokarmowego i 11 ze śluzu z powierzchni skóry ryb), z których 20 zidentyfikowano do gatunku na podstawie cech morfologicznych i testów biochemicznych API 20 NE firmy „bioMerieux". Większość taksonów stanowiły *Aeromonas hydrophila* (13 szczepów), a ponadto oznaczono: *Shewanella putrefaciens* (4 szczepy), *Aeromonas salmonicida* (2 szczepy), *Aeromonas sobria* (1 szczep) i *Pseudomonas putida* (1 szczep). Oznaczano wrażliwość zidentyfikowanych szczepów na 12 rodzajów antybiotyków, stosowanych często w profilaktyce i leczeniu ludzi oraz zwierząt. Około 67% szczepów wyizolowanych z przewodu pokarmowego ryb i ok. 78% szczepów wyizolowanych ze śluzu z powierzchni skóry było opornych na więcej niż 1 rodzaj antybiotyku. Wszystkie szczepy bakteryjne wyizolowane z wody wykazywały oporność na 4 do 8 antybiotyków. Najwięcej bakterii wykazywało oporność na oksacylinę (18 szczepów), a mniej na: ampicylinę (15 szczepów), wankomycynę (15 szczepów), trimetoprim (5 szczepów). Pojedyncze szczepy wykazywały oporność na pozostałe antybiotyki, takie jak: neomycyna, streptomycyna, oksytetracyklina, kanamycyna, erytromycyna, nowobiocyna. Wszystkie badane szczepy bakterii były wrażliwe na kwas nalidyksowy.

Introduction

During recent years, an increased frequency of cases of infections caused by bacteria resistant to many antibiotics has been observed worldwide. This is a consequence of the common use of antibiotics, not only for therapeutic purposes but also in preventative medicine and in animal rearing. The phenomenon of acquired resistance to medical drugs and contact of people and animals with bacteria resistant to antibiotics is important not only in case of pathogens, but also for microorganisms not showing pathogenic characteristics (McPhearson et al. 1991, Zmysłowska et al. 2004b). Saprophytes developed a number of antibiotic resistance coding genes. As a result, various disturbances in biocenoses and vast changes in the natural habitats occur, which have serious implications for the environment (Miranda, Zamelman 2001a, 2001b).

Extensive rearing of fish in large numbers per spatial unit using antibiotics results in a continual increase in resistance of pathogenic bacteria and relative pathogens to them (Krause, Zmysłowska 2004, Zmysłowska et al. 2004b). That is why determination of resistance to antibiotics is one of many important aspects in microbiological diagnostics that should also be considered in the case of extensive fish production, particularly during an invasion of pathogenic bacteria (Szczerba, Dwornik 2004).

Among the bacteria isolated from fish and aquatic environments, particularly polluted ones, genus *Aeromonas* bacteria have a large percentage share (Lewandowska et al. 2001, Zmysłowska et al. 2002, Harnisz, Zmysłowska 2004). It should be stressed that these microorganisms represent an increasing threat for human and animal health and their characteristics pose major problems in identification and prevention of the wide spectrum of diseases they

cause. Among the many reasons for the increased significance of *Aeromonas* bacteria in animal and human diseases, an increase of environment pollution, particularly in aquatic environments with various types of organic substances that support the growth and spread of these pathogens should be mentioned (RHODES, KATOR 1994, HARNISZ, ZMYSŁOWSKA 2004, ZMYSŁOWSKA et al. 2004a).

This study aimed at identifying and determining the resistance to antibiotics of dominant Gram-negative bacteria isolated from the water, skin mucus and alimentary tract content of carp (*Cyprinus carpio* L.) during the wintering period.

Materials and Methods

Water and fish for the study were collected during wintering of carp in fish bins positioned in a canal containing post-cooling water (flow rate 12 $m \cdot s^{-1}$) from a CHP plant. The material for further studies consisted of Gram-negative bacteria isolated from the water, alimentary tract contents and mucus of fish skin of *Cyprinus carpio* (L.), on TSA medium (triptose-soy agar). The isolated bacterial strains were identified on the basis of the phenotype characteristics determining the morphological colony and cell features and their biochemical properties on the basis of API 20 NE tests by bioMmrieux.

The resistance to antibiotics of the isolates was determined by the diffusion method in a Mueller-Hinton medium (BAUER et al. 1996). For the tests, discs containing the following antibiotics were used: neomycin N (30 μg), oxacylin OX (1 μg), streptomycin S (10 μg), vancomycin VA (30 μg), ampicilin AMP (10 μg), oxytetracyclin OT (30 μg), trimethoprim W (5 μg), kanamycin K (30 μg), erythromycin E (15 μg), nalidixic acid NA (30 μg), gentamycin CN (10 μg) and novobiocin NV (30 μg).

The results of the antibiotic-resistance of the bacterial strains under study were analyzed based on the size of the zones of growth inhibition, according to NCCLS standards (1998).

Results

Among the isolated bacteria, a total of 20 strains were identified, including 5 strains from water, 9 strains from fish skin mucose and 6 strains from alimentary tract contents.

The majority of the bacterial strains showed multiple resistances to the applied antibiotics. 100% of strains isolated from water were resistant to between 4 to 8 antibiotics. Around 78% of strains isolated from the mucose and

67% of strains isolated from the alimentary tract of the fish showed resistance to more than one antibiotic. Among the tested strains isolated from water 100% were resistant to: oxacylin OX, vancomycin VA and ampicilin AM, while 80 and 60% of strains were resistant to: trimethoprim W, novobiocin NV, respectively. 77% of bacteria originated from mucus of skin were not susceptible to oxacylin OX and ampicilin AM. Whereas they were resistant to: trimethoprim W, novobiocin NV and vancomycin VA in 11%, 22% and 55%, respectively. The tested strains isolated from alimentary tract content were not susceptible to vancomycin VA (83%), oxacylin OX (67%) and ampicilin AM (55%). Whereas none of bacterial strains originated from this environment not demonstrated to resistance for trimethoprim W and novobiocin NV (Figure 1).

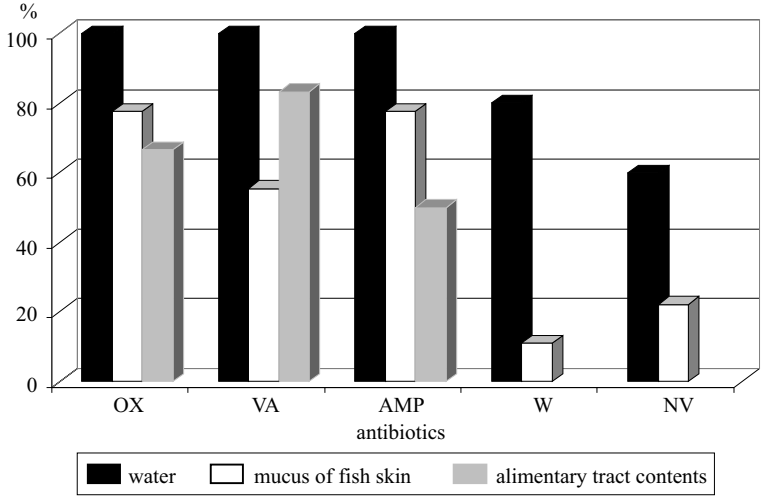

Fig. 1. Frequency of resistance (%) to selected antibiotics among strains isolated from water and fish (*Cyprinus carpio* L.) during wintering: OX – oxacylin, VA – vancomycin, AMP – ampicilin, W – trimethoprim, NV – novobiocin

The antibiotic resistance of gram-negative rods isolated from water and fish *Cyprinus carpio* L. during wintering was provided in Table 1.

It was established that the tested strains showed the highest resistance to oxacylin OX (18 strains), ampicilin AMP (15 strains) and vancomycin VA (15 strains). Five strains were resistant to trimethoprim W and novobiocin NV while one of the strains was resistant to one of the other antibiotics (N, S, OT, K, E, CN). All tested strains, on the other hand, were sensitive to nalidixic acid NA.

Table 1

The antibiotic resistance of gram-negative rods isolated from water and fish – *Cyprinus carpio* L. during wintering

Environment	Number of strain	Genus	Antibiotics											
			N	OX	S	VA	W	AMP	OT	K	E	NA	CN	NV
Water	1	*A. hydrophila*		+		+		+						+
	2	*A. hydrophila*		+		+	+	+						
	3	*A. hydrophila*		++		+	+	+						
	4	*A. hydrophila*		++		+	+				+		+	
	5	*Sh. putrefaciens*	+	+	+	+	+	+		+			+	
Mucus of fish skin	6	*A. hydrophila*		+		+		+						
	7	*A. hydrophila*		+		+		+						
	8	*A. hydrophila*		+				+						
	9	*A. hydrophila*		+				+						
	10	*A. hydrophila*		+				+						
	11	*A. salmonicida*		+				+						
	12	*Sh. putrefaciens*				+								+
	13	*Sh. putrefaciens*				+								
	14	*Ps. putida*		+		+	+	+						+
Alimentary tract contents	15	A. hydrophila		+		+		+						
	16	*A. hydrophila*		+		+		+						
	17	*A. hydrophila*		+		+		+						
	18	*A. salmonicida*				+			+					
	19	*A. sobria*		+										
	20	*Sh. putrefaciens*				+								

Explanation: + resistance, type of antibiotic: N – neomycin, OX – oxacylin, S – streptomycin, VA – vancomycin, W – trimetoprim, AMP – ampicilin, OT – oxytetracyclin, K – kanamycin, E – erythromycin, NA – nalidixic acid, CN – gentamycin, NV – novobiocin

The largest number of strains resistant to multiple antibiotics was isolated from water. For example the strain *Shewanella putrefaciens* showed resistance to 8 antibiotics while resistance of *Aeromonas hydrophila* strains differed: one strain was resistant to 6 antibiotics, one to 5 and two to 4 antibiotics. Among the bacteria isolated from the mucose, the strain *Pseudomonas putida* was resistant to 5 antibiotics and two strains of *Aeromonas hydrophila* to 3 antibiotics, three strains of *Aeromonas hydrophila* to 2 antibiotics and two strains of *Shewanella putrefaciens* to 1 antibiotic.

Strains of *Aeromonas hydrophila* isolated from fish alimentary tract content were resistant to 3 antibiotics (OX, VA, AMP). Strain *Aeromonas salmonicida* showed resistance to 2 antibiotics (VA, OT), *Aeromonas sobria* and *Shewanella putrefaciens* to 1 antibiotic each (OT and VA respectively) – Table 1.

Discussion

Intensive fish breeding may pose a threat to aquatic ecosystems as it favours the growth of various organisms contaminating water reservoirs and breeding ponds and, consequently, the water bodies where the post-production waters are run off (ZMYSŁOWSKA et al. 2001). This refers to products of fish metabolism, saprophytic and potentially pathogenic microorganisms and the pathogens associated with intensive of fish breeding. Among the microorganisms which should be given particular attention are the ubiquitous bacteria of the *Aeromonas* genus (LEWANDOWSKA et al. 2001, ZMYSŁOWSKA et al. 2004a), which the authors of this study have most frequently found both in fish and in water. In addition, the problem of the increasing resistance of microorganisms to antibiotics applied in the therapy and prophylaxis of fish diseases has been observed in recent years (ZMYSŁOWSKA et al. 2004b). This was confirmed in our study, in which most of the bacterial strains were found to be resistant to three antibiotics: penicillin, vancomycin, ampicilin. The high frequency with which the resistance of these bacteria, particularly those isolated from water during the experiment (60-100%) to antibiotics occurs indicates that fish in intensive breeding can act as reservoirs of bacteria with various degrees of resistance to antibiotics and can be a source of bacteria transfer to other animals and to humans. The bacteria can be transferred directly through water or directly through fish, both raw and processed for consumption.

Conclusions

1. Domination of *Aeromonas* sp. bacteria among the microflora of water and fish highlights the need to constantly monitor facilities of intensive fish breeding for the quantity of the bacteria and their resistance to antibiotics.

2. The high frequency with which the resistance to antibiotics occurs (60-100%) among the bacteria isolated from water and slightly lower (11-78%) found in the bacteria isolated from carp, indicates the threat which may be posed by fish in intensive breeding to the natural environment, humans and animals.

3. Among bacteria isolated from water and fish (*Cyprinus carpio* L.) most of the strains were found to be resistant to: oxacylin, vancomycin and ampicilin, whereas they were susceptible to: nalidixic acid, neomycin, streptomycin, oxytetracyclin, kanamycyne, erythromycin and gentamicin.

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CHIRONOMUS USENICUS LOGINOVA, BELYANINA, 1994 (DIPTERA, CHIRONOMIDAE) A SPECIES CYTOTAXOINOMICALLY IDENTIFIED FROM KORTOWSKIE LAKE, POLAND

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K e y w o r d s: *Chironomus usenicus,* larva, imago, polytene chromosomes, inversions.

A b s t r a c t

The cytotaxonomic characterization (external morphology of larvae and adults, and karyotype) of *Chironomus usenicus* from Poland is presented. The karyotype of this species is characterized by chromosome set $2n=8$. Cytogenetically it belongs to "thummi" complex with chromosome arm combinations: AB CD EF G and its larva belongs to *plumosus* type. The population examined is distinguished from other European populations of *C. usenicus* by its monomorphism as well as by fixed homozygous inversions in arms A and D. The reasons for the monomorphous characters of *C. usenicus* karyotype are discussed.

CYTOTAKSONOMICZNA IDENTYFIKACJA GATUNKU *CHIRONOMUS USENICUS* LOGINOVA, BELYANINA, 1994 (DIPTERA, CHIRONOMIDAE) Z JEZIORA KORTOWSKIEGO, POLSKA

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S ł o w a k l u c z o w e: *Chironomus usenicus*, larwa, imago, chromosomy politeniczne, inwersje.

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Abstrakt

Przedstawiono cytotaksonomiczną charakterystykę *Chironomus usenicus* (kariotyp oraz morfologię larwy i postaci dorosłe). Takson ten ($2n=8$) cytogenetycznie należy do kompleksu „thummi" z układem ramion chromosomów AB CD EF G, natomiast larwa jest zaliczana do typu *plumosus*. Analizowana populacja różni się od innych populacji europejskich monomorfizmem i stałą inwersją w ramionach A i D. W pracy dyskutowano przyczyny monomorficznego charakteru kariotypu *Chironomus usenicus*.

Introduction

Many species in larva stage from the genus *Chironomus* are used for limnological and ecological studies. However, due to great variation in the external morphology of larvae and adults, the taxonomy of this genus is still confusing (WÜLKER 1997). Some larvae of this genus can be divided into *plumosus*, *reductus, semireductus* or *plumosus-reductus* types depending on the length of the larval tubules (CHERNOVSKII 1949). However, nowadays the species identification within this genus is based mainly on the karyotype characteristics of salivary gland chromosomes (KEYL 1962, WÜLKER and BUTLER 1983, MICHAILOVA 1989, KIKNADZE et al. 1991, 1998). On the basis of chromosome arm combinations of these chromosomes the species of the genus are involved in different cytocomplexes (KEYL 1962).

In the present paper the salivary gland chromosomes of *Chironomus usenicus* Loginova, Belyanina from Poland, a species identified cytotaxonomically for the first time in Poland. In addition, we compared the band sequences of the chromosomes with those from other populations: Russian and Bulgarian (LOGINOVA and BELYANINA 1994, MICHAILOVA 2004). Also, the larval and adult morphology are considered on the basis of the reared material.

Material and Methods

The material analyzed in the study was collected from Kortowskie Lake, which lies in the Mazurian Lake District, in the northeastern part of Poland. The surface area of the lake is about 90 ha, its maximum depth is 17.2 m and the average depth is 5.9 m (MIENTKI and DUNALASKA 2001). The lake bowl has two overdeepenings: in the northern part with the maximum depth 15.7 m and in the southern part with the maximum depth 17.2 m. The samples of the larvae were collected in the latter part of the lake, at 5-7 m of depth. The lake water supply consists of several small tributaries into which communal savage is loaded directly. In addition, they are infiltrated by subsoil run-off with pollution from housing estates built in the direct vicinity of the lake. The

inflow of biogenic compounds from external sources is still large. The lake's yearly average total phosphorus load during the years 1990-1999 amounted to 920 kg (ca 1 $g \cdot m^{-2}$), while that of nitrogen was 9242 kg – ca 10 $g \cdot m^{-2}$ (WIŚNIEWSKI and MIENTKI 2002). Oxygen is deficient in the hypolimnion waters and on the surface of bottom deposits. Consequently, the circulation of substances, especially phosphorus, in the lake is substantially accelerated.

For cytogenetic analysis larvae of IVth instar stage were collected. Part of the material was fixed in ethanol (96%) and glacial acetic acid (3:1), whereas the remainder was reared in order to obtain pupae and adults. Forty-five larvae were analyzed cytogenetically (25 females and 20 males). External morphological analysis was done on 45 specimens.

Isolated salivary glands were used for preparing chromosome preparations. They were done by routine aceto-orcein method (MICHAILOVA 1989). Permanent slides have been prepared according to MICHAILOVA (2004). The rest of the larva's body and head capsule were mounted on slides for morphological analysis.

Standardization of banding pattern in arms E, F was carried out according to KEYL (1962), to LOGINOVA and BELYANINA (1994), and to MICHAILOVA (2004). Indication of banding patterns of arms C, D followed the description of DEVAI et al. (1989). Arms A, B and G were identified in comparison with banding pattern sequences of *C. plumosus* done by BUTLER et al. (1999).

Results and Discussion

Karyotype

The species has chromosome set $2n=8$, with chromosome arm combinations: AB, CD, EF and G. The species belongs to *thummi* cytocomplex. Chromosomes AB, CD are metacentric, EF – submetacentric and G – acrocentric. Centromeric heterochromatin is well expressed in all chromosomes. Arms B and G have BRs: BR1 and BR2, BR3 respectively.

Arm A has the following band sequences: 1-2-10ac-13ab-4-2-9-5-4d-2hk-3--12-19 (Figure 1a). This was the only sequence found in the population studied. It differed from the Bulgarian (MICHAILOVA 2004) and Russian (LOGINOVA and BELYANINA 1994) populations by fixed homozygous inversion. The band sequences corresponded to *C. plumosus* A2.2 as described by BUTLER et al. (1999).

Arm B is similar to *C. plumosus* B1.1 for the Palearctic region, described by BUTLER et al. (1999). It has the following band sequences: 20gc-22-23a--20ab-19-15-16-17-6ad-5-4d-6ef-7-8ba-19a-18-8ec-9-10-11-13-14-23-28

– Figure 1a. However, a heterozygous inversion was found in arm B (B1.2) (Figure 1e), although it occurred at a low frequency.

Arm C has a band sequence same as those described by MICHAILOVA (2004) from Bulgaria (Figure 1b). The band sequences are: 1-2ac-6cf-7-16-17-6hg--11dh-12-4-5-6ab-11ca-10-9-8-15-14-13-3-2-17b-18-22.

Arm D is the same as in *C. usenicus* described for the Russian populations (LOGINOVA and BELYANINA 1994). It has the following band sequences: 1-3-11--12-13a-9-18a-8-7-4-10ab-13-14-15-17-18c-19-20-21-22-24 (Figure 1b). These band sequences were distinguished from the Bulgarian population (MICHAILOVA 2004) by two steps of homozygous inversions.

Arm E is identical with *C. usenicus* from Bulgaria (MICHAILOVA 2004) (Figure 1c). It is worth underling that this arm has basic sequences established in most species of genus Chironomus. The band sequences are: 1-3e-5-10b-4-3f-10-13.

Arm F is like band sequences of *C. usenicus* from the Bulgarian and Russian populations (MICHAILOVA 2004, LOGINOVA and BELYANINA 1994) – Figure 1c. The sequences are observed in all the populations studied. The band sequences are: 1de-6-1e-7-10-18-11-17-19-23.

Arm G has the bands sequences: 1-2-3-4-5-6-7-8, with two Balbiani rings and one Nucleolar organizer (Figure 1d). The same sequences were observed in the Bulgarian population (MICHAILOVA 2004). However, in the Russian population there are several sequences distinguished from each other by homozygous inversion (LOGINOVA and BELYANINA 1994).

External morphology

Larva. Dark red body, length up to 25 mm, plumosus type (2 pairs of tubules longer than the posterior parapod tubule or at least equal to the posterior parapods). Head: brown, gula with dark area (like in the Russian population) (LOGINOVA and BELYANINA 1994).

Mandible: with 4th dark teeth, dorsal tooth rounded and dark at the top (Figure 2). Subdental seta slims, about 20 μm. Internal setae – 1st and 2nd simple, 3rd and 4th branch (like in the Bulgarian population) (MICHAILOVA 2004).

SI about 75 μm, resembling the population from Bulgaria (MICHAILOVA 2004) – long, with hairs on one side, apical hairs on both sides. SII simple, thin, about 120 μm.

Premandible dark, with 2 teeth (Figure 3). The ventral tooth is thinner and slightly longer than the dorsal tooth, as in the Bulgarian population (MICHAILOVA 2004). Pectin epipharings with 16 teeth usually (Figure 4).

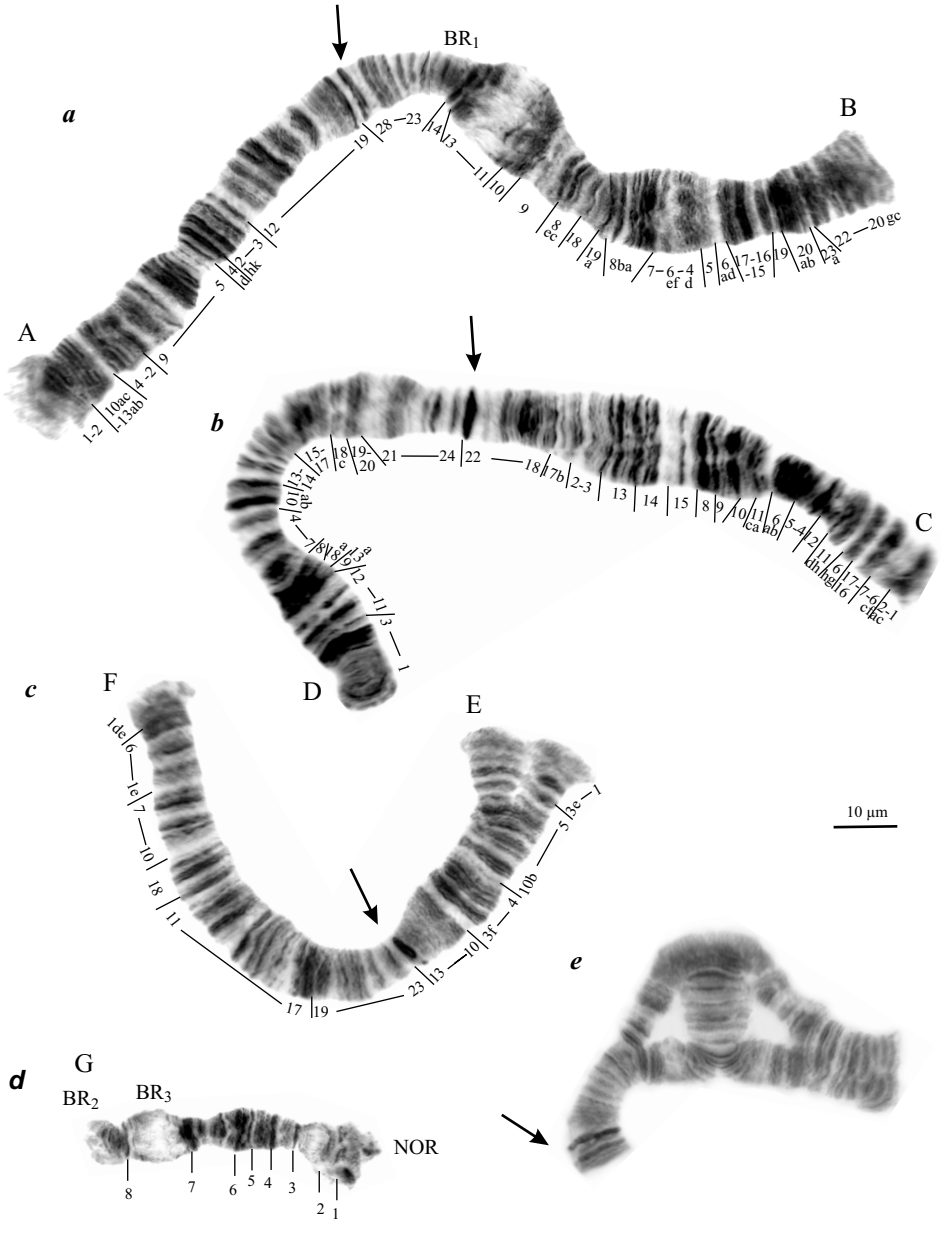

Fig. 1. Chromosome map of *C. usenicus*: *a* – chromosome AB, *b* – chromosome CD, *c* – chromosome EF, *d* – chromosome G, *e* – heterozygous inversion in arm B, BR – Balbiani ring, NOR – Nucleolar organizer, arrow – centromere region

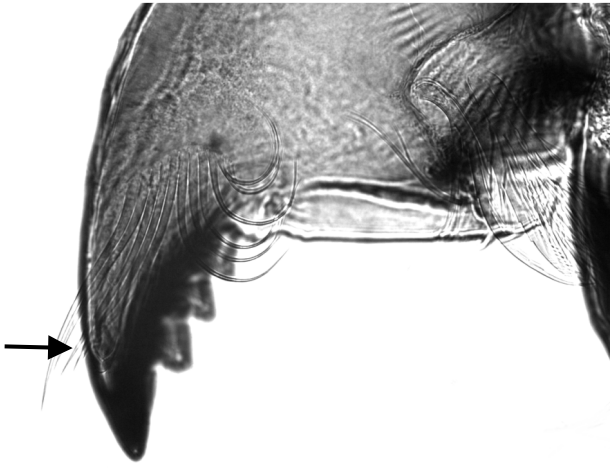

Fig. 2. Mandible

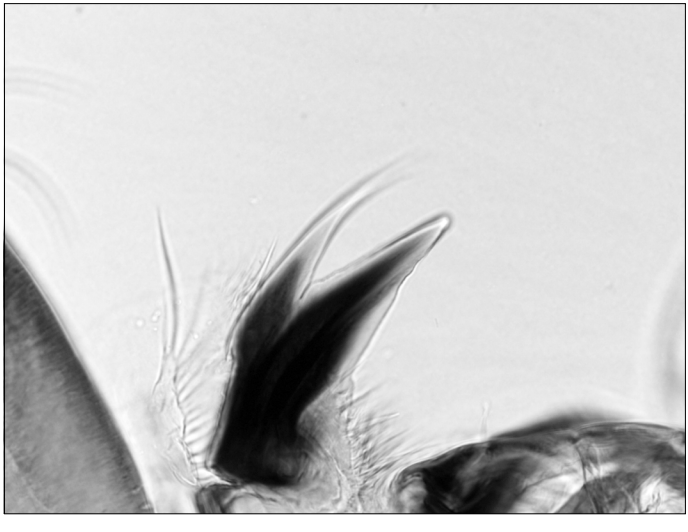

Fig. 3. Premandibles

The antennae are about 250 μm long. The antennal blade reaches to 5th segment. RO located in the proximal part of the first segment, as in the Bulgarian population (Figure 5). Proportions of the lengths of the segments: 75:12:4:6:3. Mentum (Figure 6) is dark, median tooth trifid, rounded at the top, lower in comparison with the near lateral tooth. The lateral teeth are decreasing from 6 to 1 tooth. The inner surface of ventromental plates is serrated.

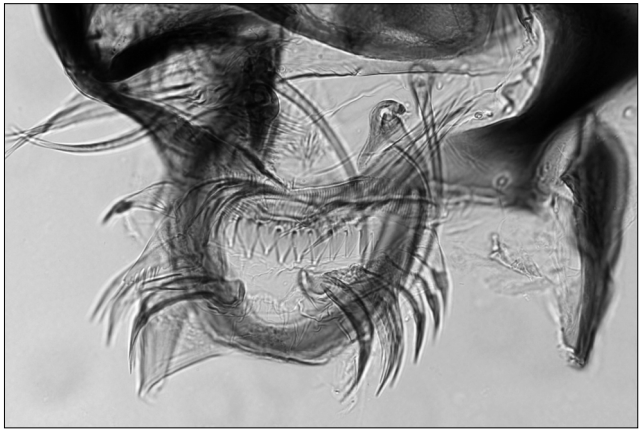

Fig. 4. Pecten epipharings

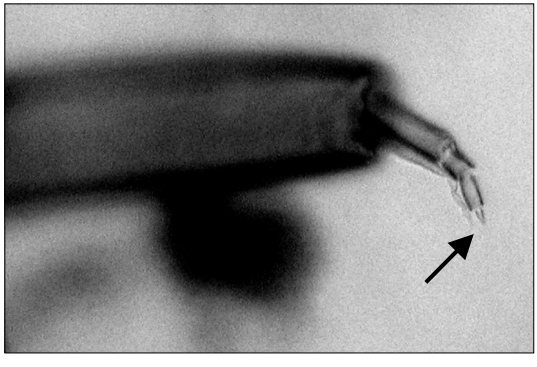

Fig. 5. Antenna

According to the length of ventral tubules of *C.usenicus* larvae from Polish population, the larvae can be considered as *plumosus* type. However, the larvae of the same species from Russian and Bulgarian populations belong to *semireductus* type. It might be possible that the differences of the length of tubules can be affected by the larval habitat as has been discovered by (WÜLKER and BUTLER 1983). For instance, less oxygen produced large tubule ventralis (Haas and STRENZKE 1957). So, the deficit of oxygen established in the lake could be the reason of the enlarged larval tubules of the studied species.

Pupa. The pupa has been sent to Dr Langton, who confirmed the taxonomical status of *C. usenicus*.

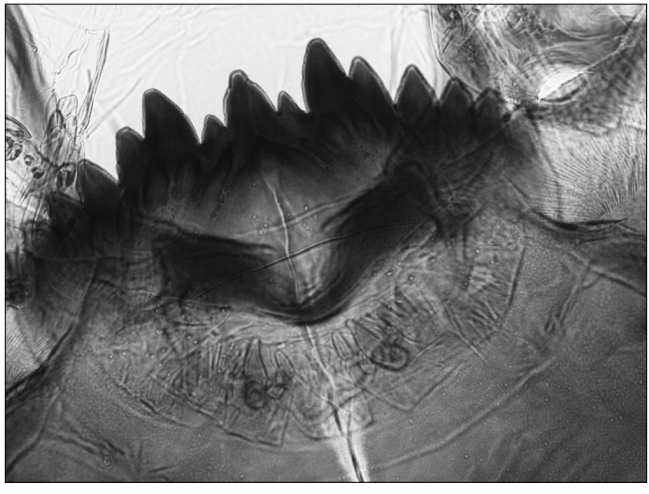

Fig. 6. Mentum

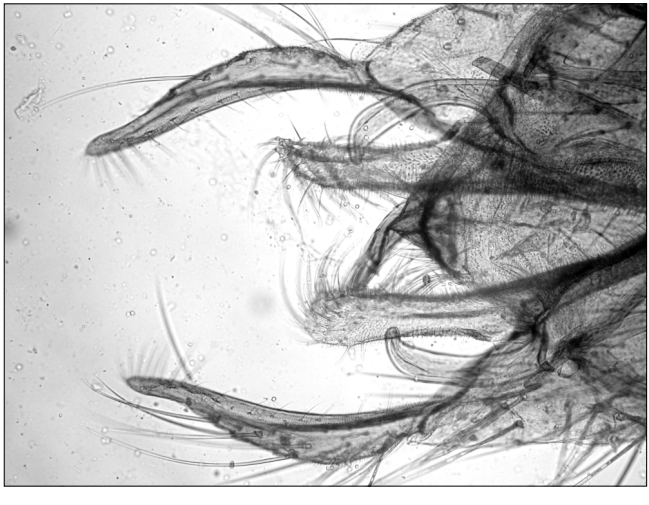

Fig. 7. Hypopygium

Imago. The studies on the hypopygium morphology (Figure 7) in male specimens from Poland proved that it had the same morphology as the other populations (LOGINOVA and BELYANINA 1994, MICHAILOVA 2004).

Chromosome polymorphism

Chironomus usenicus from different Palearctic populations showed different levels of chromosome polymorphism. It is necessary to notice that the Polish population was practically monomorphous. It differed from the other two populations by fixed homozygous inversions in arms A and B. In arm B there was only one heterozygous inversion occurring at a very low frequency (one individual). Arms C, D, E, F, G are monomorphous. In the other two Palearctic populations (Bulgaria and Russia), beside a high level of chromosome polymorphism expressed by heterozygous inversions, the genome polymorphism was detected. The "B" chromosome appearing at a high frequency was recorded for both populations: Russian and Bulgarian (LOGINOVA and BELYANINA 1994, MICHAILOVA 2004). It is quite possible that certain ecological factors such as temperature, oxygen content, pollution, etc. might have an influence on the polymorphism of these populations. It cannot be ruled out that the homozygous sequences of arm A and monomorphous karyotype of *C. usenicus* from the population analyzed had to become adapted to specific niches during the larval stage spent in Kortowskie Lake. The lake has been resorted since 1956. The outflow of near-bottom waters was introduced as a restoration method by Olszewski (OLSZEWSKI 1959). Long-term measurements showed that the maximal water temperature in water layer 1 m under the surface varied between 19.5 and 27.6°C. The mean value calculated for the maximal surface temperatures for the period of 49 years equaled 22.7°C, while in near the bottom layers, at the deepest site in the lake, the average temperature equaled 10.6°C. The water temperature at 1 m depth over the bottom at the beginning of summer stagnation ranged from 3.8 to 11.3°C (WIŚNIEWSKI and MIENTKI 2003). The mean time of ice cover occurrence was 94 days (MIENTKI and WIŚNIEWSKI 2003).

It is unknown what abiotic factors this monomorphous karyotype is adapted to. The process of adaptation of specific karyotypes to specific ecological factors has also been found in *C. staegeri* (MARTIN and WÜLKER 1971). The clearly expressed dimorphism of inversion sequences has been discovered in *C. anthracinus* by KIKNADZE et al. (2005). These authors reported a high level of chromosome polymorphism in populations of this species from shallow water where environmental conditions are very changeable. *C. anthracinus* from deep water bodies has a low level of polymorphism. *C. usenicus* from Poland has been found in a deep lake which freezes in winter time on the surface but remain above freezing at the bottom and showed stability in environmental conditions. The homozygous sequences in all arms of *C. usenicus* in this population might better adapt the larvae to these conditions. However, in the future should be done more precise analysis of the

ecological factors in Kortowskie Lake which might be affected the monomorphous characters of *C. usenicus* karyotype.

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INITIAL REARING OF VIMBA LARVAE (*VIMBA VIMBA* L.) UNDER CONTROLLED CONDITIONSON NATURAL FOOD AND COMMERCIAL FODDER

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K e y w o r d s: *Vimba vimba,* larvae, controlled rearing, natural food, formulated diets.

A b s t r a c t

Vimba (*Vimba vimba* L.) larvae were initially reared at 25.0 ± 0.5°C for the period of 20 days. The stock in 25-liter tanks was 1000 larvae per tank. The first natural food (naupliuses of artemia) was substituted by feeds by Aller Aqua intended for commercial rearing of carp (Aller Uni Starter) and trout (SGP 493 Krystal). Application of commercial starter feeds by Aller Aqua, after 10 days of feeding the larvae on natural food proved useful and justified for rearing and practical reasons. Better rearing results expressed by indicators (survival, ITL, RGR, RBR) were achieved substituting the natural food with formulated starter carp diet by Aller Aqua.

PODCHÓW LARW CERTY (*VIMBA VIMBA* L.) W WARUNKACH KONTROLOWANYCH NA POKARMIE NATURALNYM I PASZY KOMERCYJNEJ

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S ł o w a k l u c z o w e: *Vimba vimba,* larwa, chów kontrolowany, pokarm naturalny, pasze komercyjne.

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Abstrakt

Larwy certy (*Vimba vimba* L.) podchowywano w temp. 25,0 ± 0,5°C przez 20 dni. Obsada 25-litrowych zbiorników wynosiła 1000 osobników. Pierwszy pokarm naturalny (naupliusy solowca) zastępowano paszami firmy Aller Aqua, przeznaczonymi do komercyjnych podchowów larw karpia (Aller Uni Starter) i pstrąga (SGP 493 Krystal). Zastosowanie komercyjnych pasz startowych firmy Aller Aqua, po 10 dniach karmienia larw pokarmem naturalnym, ze względów hodowlanych i praktycznych okazało się celowe i uzasadnione. Lepsze wyniki hodowlane, wyrażone wskaźnikami (przeżywalność, ITL, RGR, RBR), osiągnięto, zastępując pokarm naturalny komercyjną paszą dla karpi typu starter, firmy Aller Aqua.

Introduction

Deteriorating natural environment conditions and regulation of riverbeds have resulted in many irreversible changes in aquatic ecosystems. Construction of barriers caused destruction of natural spawning sites and initial growth sites for young reophile fish in many places and made traveling of double habitat fish up the rivers for spawning (BACKIEL 1985, BACKIEL and BONTEMPS 1995). Sedimentation of organic substances caused silting up of riverbeds, which in turn caused deterioration in quality and reduction in area of the spawning sites. In so strongly transformed aquatic environment the fish rarely engage in spawning and the spawn laid under such conditions frequently dies out within a short time.

Cumulating effects of numerous unfavorable factors has lead to a significant decrease in the number and reach of many species of reophile fish (MARSZAŁ and PRZYBYLSKI 1996, WITKOWSKI 1996a,b, BŁACHUTA 1998), and, as a consequence, classifying some of them as vanishing species or species threatened by extinction (WITKOWSKI 1992, WITKOWSKI et al. 1999). Among reophile cyprinid fish, vimba (*Vimba vimba* (L.) is the species particularly sensitive to deterioration of water quality (JAKUBOWSKI et al. 1988, PENCZAK et al. 1995, 1996, WITKOWSKI and HEESE 1996).

Vimba is the only anadromic traveling cyprinid fish found in the inland waters of Poland (PLISZKA 1953, BONTEMPS 1960). It favors mainly cool flowing waters with high contents of oxygen dissolved in the water. It belongs to the litophile reproduction group (BALON 1975, 1981, MANN 1996).

Maintaining the numbers of fish at the level securing survival of the local requires both protection of the natural spawning grounds and the necessity of substituting natural spawning with artificial one, incubation of spawn under controlled conditions and next initial rearing of the material obtained (WOJDA 1997).

Controlled initial rearing of the larvae is a very important stage in the material production. During that rearing the larvae can be fed on

live food (naupliuses of *Artemia* sp.) and/or formulated diets (SCHUMPBERGER et al. 1976, KAMLER et al. 1987, OPUSZYŃSKI et al. 1989, FORESTI 2000). So far, however, no formulated starter diet has been developed for the larvae of reophile cyprinid fish. On industrial scale attempts are taken at feeding the larvae with starters generally available in the market composed for trout or carp larvae. There is little information available, however, on the possibilities of effective substituting of natural food with commercially available formulated feeds. Absence of detailed studies on those issues was the inspiration to take up studies aiming at assessment of possibilities of vimba initial rearing using commercially available starters fed after initial feeding on natural food and defining the minimum necessary period of feeding vimba larvae on natural food.

Material and Methods

Material – Fish

The study used larvae of vimba (*Vimba vimba* Linnaeus, 1758). The ready to spawn specimens of vimba originated from Ostrowieckie lake (HLIWA et al. 2002) through which the Płociczna River flows. After catching them from the natural environment and transporting to the hatching-initial rearing hall, they were placed in 1000 dm^3 tanks. The larvae of vimba were obtained from semi-artificial reproduction (the fish were injected with hormones and released into the tank, where the substrate for placing spawn was prepared). Hormonal stimulation of reproduction was done using Ovopel (HORVATH et al. 1997). Following the stimulating injection, the tank water temperature was increased from 20 to 22°C. The fish started spawning after 24 hours. After spawning the fish were caught.

Mass hatching of the larvae occurred one day since observing the first hatched individuals. They stayed in the tank until the end of gull bladder resorption period. The spawn incubation conditions and vimba larvae characteristics are presented in Table 1.

Methods

The initial rearing of the larvae using the natural food and starter type feed was done in tanks supplied with water from closed circuit designed by KUJAWA et al. (2000). Fish larvae, on the second day after starting free floating and filling the air bladders with air, were placed in 14 flow-through tanks with the

Table 1

Conditions of incubation and characteristics of vimba larvae (*Vimba vimba* L.)

Parameter	
Water temperature during incubation (°C)	20-22
Time of incubation (days)	4
Larvae length (longitudo totalis) after hatching (mm)	5.0 ± 0.1
Water temperature during yolk sac resorption (°C)	20 ± 0.5
Yolk sac resorption time (days)	6
Larvae length (*longitudo totalis*) at the start of feeding (mm)	8.1 ± 0.2

Mean ± standard deviation

working capacity of 40 dm^3. The total water replacement time in individual tanks was ca 20 minutes. Water temperature during initial raring was 25.0 ± 0.5°C. The initial density of larvae in tanks was 25 larvae per 1 dm^3. The content of air dissolved in water ranged from 6.1 to 7.9 mg $O_2 \cdot dm^{-3}$, and the pH from 7.3 to 7.5. During the experiment no presence of ammonia in water was detected. During the entire raring period the day light cycle was constant at 12 hours of light and 12 hours of darkness.

The first food (naupliuses of *Artemia* sp.) was fed to the larvae after 24 hours from placing them in the raring tanks (VANHAECKE et al. 1990). Fish were fed *ad libitum*. To assure their continuous access to food they were fed six times a day, every 2 hours. The natural food was substituted with formulated diet after 5 and 10 days of initial feeding on naupliuses of artemia. Initially the daily dose of the formulated diet for vimba larvae was 120% of the biomass. Every four days it was decreased by 20% as compared to the initial value. Finally, at the end of raring, the daily dose was 20% of the larvae biomass. The experiment was conducted in 7 experimental groups, each in two repetitions. Three control groups were identified. One consisted of larvae that were fed with naupliuses of artemia only during the entire time of initial raring (20 days) (KA20), the second one was fed on the carp formulated diet only (KK20) and the third one on the trout formulated diet only (KP20).

The natural food was substituted by Aller Aqua formulated diets for commercial initial raring of carp larvae (Aller Uni Starter) or trout larvae (SGP 493 Krystal). The compositions of the individual formulated diets used in the experiment is presented in table 2. In planning the setup of the experiments the earlier works on development of reophile fish larvae were considered (WOLNICKI 1996, KUJAWA et al. 1998a-d, KUJAWA 1998, 2004).

Table 2
Chemical composition of *Artemia nauplii* and starter diets produced by Aller Aqua (% dry weight)

Food	Component (%)				
	protein	fat	carbohydrate	ash	phosphorus
Artemia sp.	47.0	21.5	10.6	9.5	–
Aller Uni Starter	55.0	7.0	21.0	9.0	1.4
SGP 493 Krystal	53.0	14.0	14.0	10.0	1.4

Measurements and data analysis

In case of initial raring of larvae on different diets the control sample (2 times 30 larvae) was collected on the day of filling the tanks, before commencement of feeding. The consecutive samples (of 30 larvae each) were collected every four days. Collection of samples was done immediately after switching on the lights and before commencement of feeding. Before measurements the fish caught were subjected to short anesthesia in the solution of 2-phenoxy-ethanol at the concentration of 0.1-0.3 $cm3 \cdot dm^{-3}$. The larvae were weighted with the accuracy of up to 1 mg and their total length (*longitudo totalis* – l. t.) was measured with the accuracy of up to 0.1 mm. After measurements the fish were returned to the appropriate tanks. On the basis of the data obtained the length increase over a time unit *ITL* ($mm \cdot d^{-1}$) was calculated according to the formula (PEŇÁZ et al. 1989):

$$ITL = \frac{TL(n_2) - TL(n_1)}{\Delta t},$$

where:
TL – average total length (*longitudo totalis*),
n_1 – beginning of raring,
n_2 – end of raring,
Δt – period of raring (days – *d*).

The relative weight increase rate SGR and the relative biomass increase rate (SBR) from the time of commencement of feeding until the completion of the experiment were calculated according to the formulas (BROWN 1957):

$$SGR = 100 \cdot \frac{\ln W_2 - \ln W_1}{\Delta t} \text{ and}$$

$$SBR = 100 \cdot \frac{\ln (n_2 \cdot W_2 - \ln (n_1 \cdot W_1)}{\Delta t},$$

where:
W_1 – average initial bodyweight of individual (mg) during raring,
W_2 – average end bodyweight of individual (mg) during raring,
N_1 – number of individuals at the beginning of raring,
N_2 – number of individuals at the end of raring,
Δt – duration of raring (days).

Next the relative mass increase ratio for the individual RGR and the relative biomass increase rate since commencement of feeding until the end of the experiment were calculated according to the formulas (MYSZKOWSKI 1997):

$$RGR = 100 \left(e^{\frac{SGR}{100}} - 1 \right) \text{ and}$$

$$RBR = 100 \left(e^{\frac{SBR}{100}} - 1 \right).$$

The rates of increases (SGR and RGR) for the length were calculated in the same way.

The biomass of fish in the tanks was calculated as the product of the average individual weight and the number of live individuals. The obtained value was divided by the tank volume. The obtained biomass was expressed in $g \cdot dm^{-3}$.

The number of dead fish recorded daily served preparation of cumulated mortality curves for individual raring variants. The statistical differences in mortality of larvae between population groups were analyzed using the χ^2 test (GREŃ 1984, ŁOMNICKI 2003). The χ^2 test was used to verify the zero hypothesis (H0) that the mortality in the compared groups was the same.

Statistical differences between experimental groups were established using the DUNCAN test (1955) at the significance level $\alpha = 0.05$. Statistical processing of the results was done using the Excel 9.0 and Statistica 6.0 for Windows software.

Results

The larvae of vimba were willingly consuming both the natural food and the starter diet. Mass and length increases of vimba larvae achieved

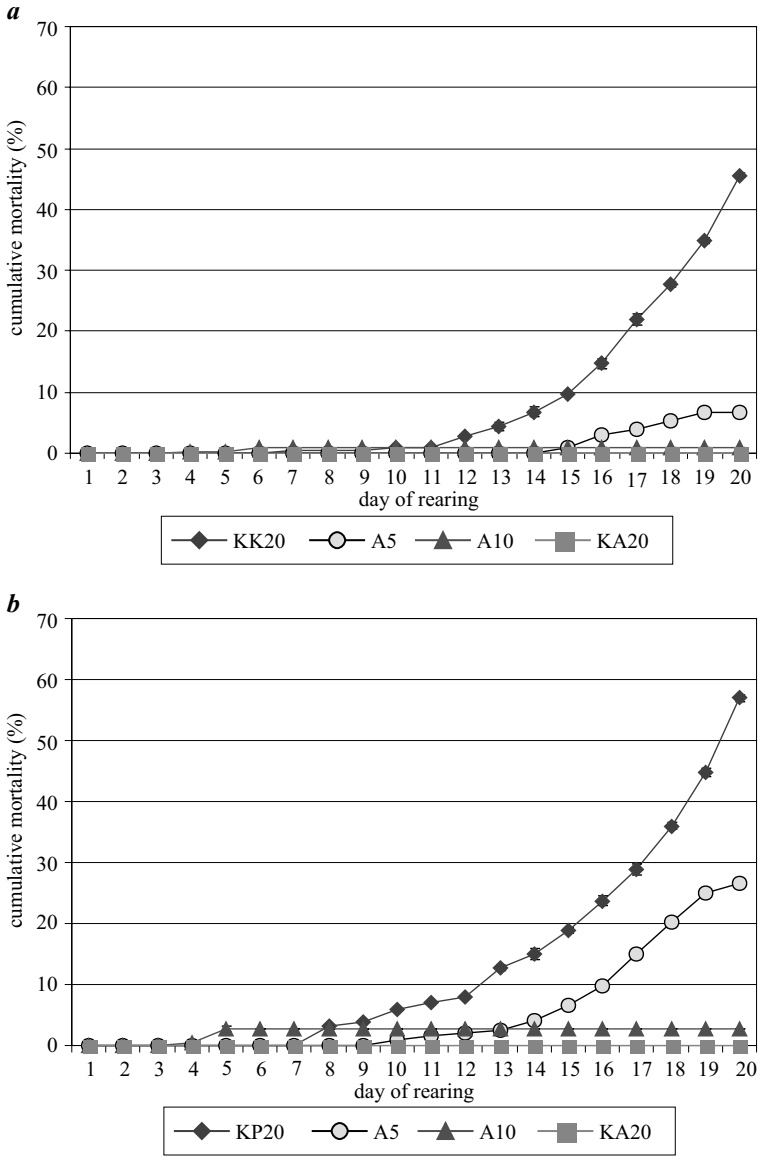

Fig. 1. Cumulative mortality of vimba larvae (*Vimba vimba* L.) reared on natural (*Artemia* sp. – A) and artificial foods: carp (*a*) or trout (*b*) starters. Transfer to artificial food after 5, 10 days

at the end of raring on the natural food were the largest. Similar results were also obtained when the natural food was substituted after 10 days with carp starter. During raring of vimba larvae using different types of feed a larger mass and length of fish were obtained when feeding

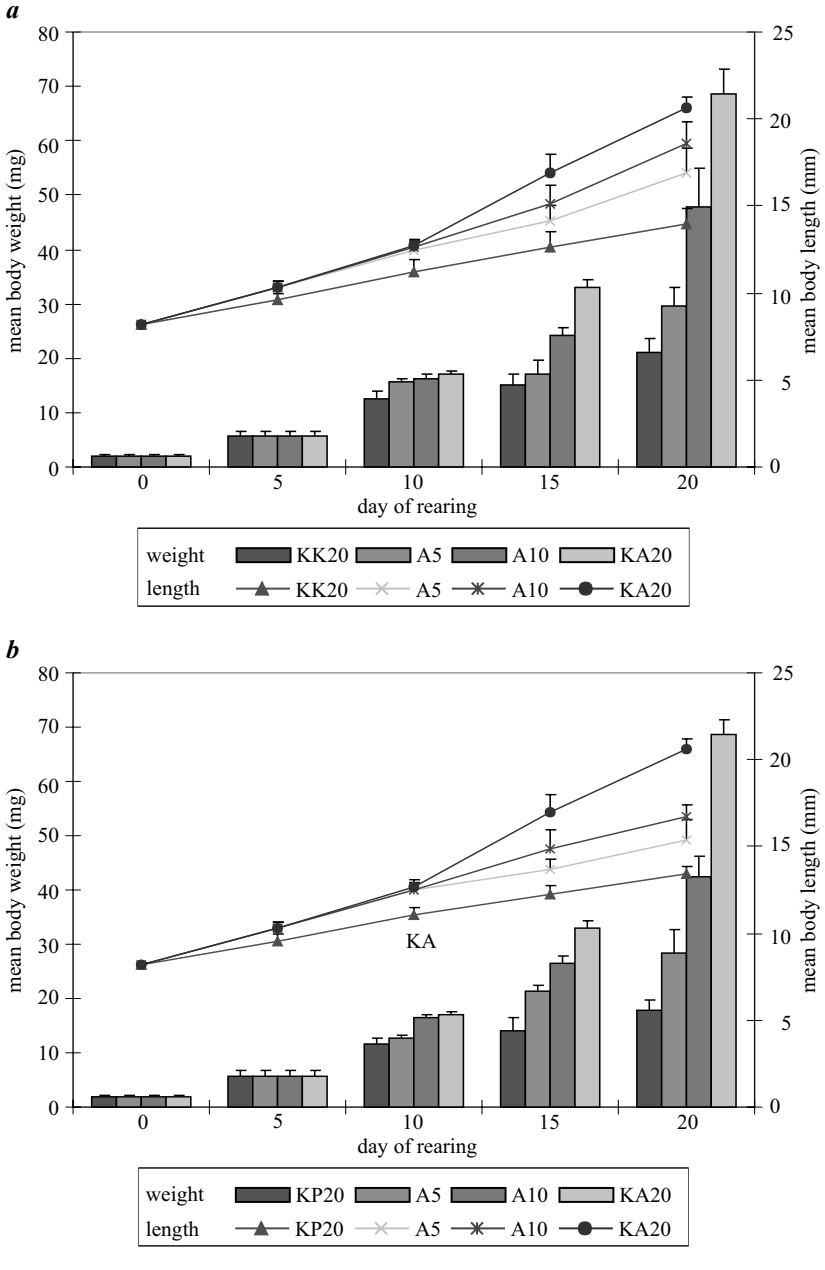

Fig. 2. Growth of vimba larvae (*Vimba vimba* L) reared on natural (*Artemia* sp. – A) and artificial foods: carp (*a*) or trout (*b*) starters. Transfer to artificial food after 5, 10 days

them with carp starter (Table 3.). The differences in the average mass and length of larvae in individual feed groups appeared after 15 days of raring only (Figure 1 a,b). The average weight of fish fed during the entire period with the naupliuses of artemia was the highest and reached 68.7 mg at the end of raring while the average length reached 20.6 mm. The larvae fed on carp starter from the first day reached the average weight of 21.0 mg and the average length of 14 mm while those receiving trout starter 17.9 mg only and the average length of 13.4 mm.

The vimba larvae fed from day 6 or 11 with carp starter reached the average weight of 29.5 and 47.9 mg and average length of 16.9 and 18.6 mm respectively. Much poorer results were obtained feeding the vimba larvae with trout starter as of day 6 or 11. Clear differences in mortality of larvae appeared in the groups where the larvae were fed on starters as of day 1 (Figure 2a, b). It was 57% in the group receiving trout starter as of day 1 and 45.6% in the group receiving carp starter as of day 1. The survival of vimba larvae in the other groups was high and ranged from 97.3 to 99.9%. The length increase rate per time unit was between 0.26 and 0.62 mm $\cdot$ d^{-1}. The biomass of fish in both feeding variants differed significantly and ranged from 0.83 to 1.10 g $\cdot$ dm^{-3} in groups fed on formulated diets as of day 6. Fish fed with naupliuses of artemia during the entire experiment reached 2.75 g $\cdot$ dm^{-3}. In groups where as of day 11 the natural food was replaced by carp or trout starter s of RGR and RBR calculated for them were also closest to the values calculated for the control group (Table 3).

Discussion

Securing appropriate feed quality and volume is one of the major issues during raring of fish larvae under controlled conditions.

Fresh water zooplankton or naupliuses of artemia *Artemia* sp. are the most frequently applied live feeds characterized by high availability and acceptance by the majority of fish larvae (BRYANT and MATTY 1980). That can be the only feed during the initial life period of he larvae, but the starter formulated diets can be used as supplements (WIGGINS et al. 1986, FERMIN and RECOMETA 1988, ABI-AYAD, KESTEMONT 1994). The larvae of cyprinid fish should initially be fed on the natural food because only after some time they can effectively assimilate nutrients contained in starter feeds (GRUDNIEWSKI et al. 1979, DABROWSKI 1984a, DĄBROWSKI and POCZYCZYŃSKI 1988).

In case of reophile cyprinid fish the group of digestive enzymes during the initial period of life is poor and hence feeding them from the start on formulated diets is not recommended (DABROWSKI 1984b, STANNY 1984).

Table 3

Results of rearing vimba (*Vimba vimba* L.) larvae fed with Artemia and transferred to carp starter or trout starter after 5 or 10 days

Parameter	Carp starter			Trout starter			Natural food
	feeding period with starter (days)						
	10(A10)	15(A5)	20(KK20)	10(A10)	15(A5)	20(KP20)	20 (KA20)
Initial mean body weight (mg)	2.0 ± 0.2^{a}	2.0 ± 0.2^{a}	2.0 ± 0.2^{a}	2.0 ± 0.2^{a}	2.0 ± 0.2^{a}	2.0 ± 0.2^{a}	2.0 ± 0.2^{a}
Final mean body weight (mg)	47.9 ± 7.1^{d}	29.5 ± 3.6^{c}	21.0 ± 4.3^{b}	42.4 ± 3.7^{d}	28.3 ± 2.1^{c}	17.9 ± 2.4^{a}	$68.7 \pm 2.6e$
Initial mean body length (mm)	8.2 ± 0.1^{a}	8.2 ± 0.1^{a}	8.2 ± 0.1^{a}	8.2 ± 0.1^{a}	8.2 ± 0.1^{a}	8.2 ± 0.1^{a}	8.1 ± 0.1^{a}
Final mean body length (mm)	18.6 ± 1.3^{c}	16.9 ± 1.4^{bc}	14.0 ± 0.9^{b}	16.7 ± 0.7^{bc}	15.4 ± 0.9^{b}	13.4 ± 0.4^{a}	20.6 ± 0.6^{d}
Initial stock (indiv.)	1000	1000	1000	1000	1000	1000	1000
Final stock (indiv.)	991 ± 3.5^{f}	934 ± 6.4^{d}	544 ± 6.4^{b}	972 ± 2.1^{e}	733 ± 9.9^{c}	430 ± 14.1^{a}	999 ± 1.4^{f}
Survival (%)	99.1 ± 0.3^{f}	93.4 ± 0.6^{d}	54.4 ± 0.6^{b}	97.2 ± 0.2^{e}	73.3 ± 0.9^{c}	43.0 ± 1.3^{a}	99.9 ± 0.1^{f}
Increase in total length (ITL) ($mm \cdot d^{-1}$)	0.52 ± 0.0^{e}	0.44 ± 0.0^{d}	0.29 ± 0.0^{b}	0.43 ± 0.0^{d}	0.36 ± 0.0^{c}	0.26 ± 0.0^{a}	0.62 ± 0.0^{f}
Relative growth rate (RGR) of weight ($\% \cdot d^{-1}$)	15.88 ± 0.4^{d}	13.46 ± 1.0^{c}	11.76 ± 1.0^{ab}	15.27 ± 0.5^{d}	13.25 ± 0.4^{bc}	10.96 ± 1.0^{a}	17.68 ± 0.4^{e}
Relative growth rate (RGR) of length ($\% \cdot d^{-1}$)	4.10 ± 0.1^{d}	3.62 ± 0.3^{b}	2.66 ± 0.3^{a}	3.56 ± 0.1^{d}	3.15 ± 0.1^{c}	2.46 ± 0.3^{a}	4.61 ± 0.2^{e}
Relative growth rate (RBR) of biomass ($\% \cdot d^{-1}$)	15.83 ± 0.4^{d}	13.11 ± 1.0^{c}	8.71 ± 1.0^{b}	15.13 ± 0.5^{d}	11.70 ± 0.4^{c}	6.74 ± 1.0^{a}	17.68 ± 0.4^{e}
Biomass ($g \cdot dm^{-3}$)	1.90 ± 0.1^{e}	1.10 ± 0.2^{c}	0.46 ± 0.2^{b}	1.65 ± 0.1^{d}	0.83 ± 0.1^{c}	0.31 ± 0.1^{a}	2.75 ± 0.2^{f}

Mean value ± S.D. Results in rows with the same letter index are not statistically significantly different ($p \leq 0.05$)

The larvae of those species of fish compensate the shortage of their own digestive enzymes partly with the enzymes of consumed zooplankton (POCZYCZYŃSKI 1996). Only after some time, different in different species, they can digest ingested granulated feed.

Defining the optimum period during which the larvae should be fed on natural food (to be able to substitute it with formulated diets) is very important during controlled raring. Attempts at feeding the larvae of cyprinid reophile fish (excluding the larvae of barbell and nase) from the very beginning on formulated starters usually end at stopping the growth and decreasing the resistance to diseases, which, as a consequence leads to mass deaths (WOLNICKI 1997, KUJAWA 2004).

Some authors believe that feeding cyprinid fish larvae with formulated diet can start when they reach the unit mass of 5-15 mg (BRYANT and MATTY 1981) or 10-12 mg (STANNY 1984),and that during the initial period of life they should be fed on live food. That allows fast bodyweight increase and achievement of consecutive stages of development. That would explain to a certain extent the fact that the larvae of barbell can practically from the start be fed with starter feeds (KUJAWA et al. 1998d, FIALA and SPURNY 2001).

Feeding the larvae of other cyprinids with starter type feeds gives much worse results (lower bodyweight increase and low survival rate) as compared to feeding with zooplankton (STANNY 1984). That is also confirmed by the studies on raring nase larvae with natural feed that after a defined time was substituted by starter feeds (KUJAWA et al. 1998a). That is also confirmed by studies on raring cyprinid fish using starter formulated diets (WOLNICKI 1996, WOLNICKI and GÓRNY 1995ab, WOLNICKI and MYSZKOWSKI 1999ab). They indicate that species of fish such as barbell and nase can be fed from the start on starter formulated diets while the other need several days of raring on natural food.

After a certain period of feeding on natural food, the larvae of cyprinid fish can be fed with quite satisfactory results on formulated diets. Application of naupliuses of artemia substituted during raring with commercially available starter feeds allowed in own studies on defining the time for which the larvae must receive natural food to be able later to assimilate nutrients contained in feeds. The results obtained confirm earlier observations (KUJAWA 1998a-d) that during the first period of life – different for different cyprinid fish – the larvae must receive natural food. It was shown that after 10 days on natural food substitution with starter feeds does not influence survival of the larvae significantly. The growth rate of larvae receiving naupliuses of artemia and feed was lower than that of fish fed all the time on naupliuses of artemia only. Similar results were obtained by LITTAK et al. (1979) for raring of carp larvae when the natural food after 10 days was formulated by formulated diet.

The above data indicate that using feeds only during raring does not allow full use of growth potential of the larvae, which was also confirmed by studies by WOLNICKI and KORWIN-KOSSAKOWSKI (1993), concerning tench (*Tinca tinca* L.) larvae raring.

The studies presented in this paper confirm the earlier observations and results of studies that vimba larvae can be fed on starter type fodders only after a period of feeding on natural food (WOLNICKI 1996, 2000, WOLNICKI et al. 2000, KUJAWA 2004).

Knowledge of the minimum period of feeding on natural food and automation of feeding operation by applying feeder (CHARON and BERGOT 1984, 1986) can highly increase vimba stocking material production potential under controlled conditions.

Conclusion

1. Early substitution of natural food with tested starter type feeds offered significantly worse results (lower bodyweight increase and low survival rate) than feeding the larvae on naupliuses of artemia.

2. The longer the period of natural food availability, the better the raring effects.

3. Application of commercial starter formulated diets by Aller Aqua, after 10 days of earlier feeding on natural food is useful and justified for raring and practical reasons.

4. Better raring results are achieved when natural food is substituted by carp starter formulated diet by Aller Aqua than by trout starter formulated diet by the same manufacturer.

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AROMA COMPOUNDS IN GOUDA CHEESE PRODUCED WITH ADDITION OF PROBIOTIC STRAINS

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K e y w o r d s: Gouda cheese, probiotic strains, aroma compounds, sensory quality.

A b s t r a c t

Gouda type cheeses were produced in a dairy plant with mesophilic streptococcus lactis cultures and without (control) and with addition of probiotic lactobacillus strains i.e. *Lactobacillus casei, Lactobacillus acidophilus* and *Lactobacillus rhamnosus.*

Contents of volatile components of flavour in control and experimental cheeses confirmed modification of sensory features resulted of probiotic lactobacilli presence. The highest sensory quality was found in cheeses produced with *L. acidophilus* addition and it contained the highest amount of butyric acid, high of propionic acid, and much less of acetone, ethanol and also of acetoin and diacetyl determined by solid phase microextraction method.

Reference of volatile aroma compounds level to punctual sensory evaluation does not allow to identification of those components that determine taste and aroma of cheese, because sensoric quality is determined by the proportion of main aroma components and also, various composition of paracasein degradation products.

KOMPONENTY AROMATU W SERACH GOUDA WYPRODUKOWANYCH Z DODATKIEM SZCZEPÓW PROBIOTYCZNYCH

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S ł o w a k l u c z o w e: sery gouda, szczepy probiotyczne, komponenty aromatu, jakość sensoryczna.

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Abstrakt

Sery gouda produkowano w warunkach przemysłowych z zastosowaniem mezofilnych paciorkowców mlekowych bez dodatku (wyroby kontrolne) lub z dodatkiem szczepów probiotycznych: *Lactobacillus casei, Lactobacillus acidophilus* i *Lactobacillus rhamnosus*.

Zawartość lotnych komponentów aromatu w kontrolnych i doświadczalnych serach potwierdza modyfikację cech sensorycznych pod wpływem probiotycznych pałeczek mlekowych. Najwyższą jakość sensoryczną stwierdzono w przypadku serów wyprodukowanych z *L. acidophilus*. Strefy te zawierały najwięcej kwasu masłowego, kwasu propionowego, znacznie mniej acetonu, etanolu, a także acetoiny i diacetylu, oznaczanych metodą mikroekstrakcji do fazy stałej.

Odniesienie zawartości lotnych komponentów aromatu do punktowej oceny sensorycznej nie pozwala na identyfikację komponentów determinujących smak i zapach serów, ponieważ jakość sensoryczna jest zależna od proporcji poszczególnych komponentów aromatu, a także stopnia degradacji parakazeiny.

Introduction

One of the serious problems in quantitative analysis of aroma producing substances in cheese is prevention of their losses. VANDEWEGHE and REINECCIUS (1990) identified volatile taste and aroma producing components of cheddar cheese by three different analytical methods: distillation, dialysis and extraction. The smallest amount of taste and aroma isolate was obtained by extraction method. Application of dialysis led to the most concentrated isolate, though many active components of cheese taste and aroma were below the detection limit of gas chromatograph.

One of the recent techniques is solid phase microextraction (SPME) that makes possible both qualitative and quantitative analysis of volatile taste and aroma compounds, also in ripening cheeses (PAWLISZYN 1997). The principle of the method is binding of volatile compounds by accordant fiber placed in syringe. Transfer of analyte from fiber on chromatographic column does net need expensive desorber and the fiber can be used several times. Another advantage of SPME is the possibility of miniaturization – volatile compounds from very small samples can be analysed (ZHANG and PAWLISZYN 1993a, b).

Chin and others (1996) found the same volatile compounds in Swiss, cheddar and Italian cheeses by SPME. Sensory profiles of the cheeses were different. Propionic acid and dodecanoic acid and acetic acids dominated among the taste and aroma substances complex of Swiss cheese and total amount of dodecanoic acid and acetic acids was lower than that of propionic acid. In cheddar cheese dodecanoic acid and acetic acids dominated. Lipolysis products of fat were dominant in Italian cheese.

Because of the technological differences, especially of higher water content and shorter ripening time, sensory profiles of Dutch type cheeses can be different. Products of paracasein hydrolysis (CICHOSZ et al. 2003b) and volatile aroma components that, after reaction with free aminoacids or in their

presence, generate the full range of heterogeneous aromas (GRIFFITH and HAMMOND 1989), dominate in the taste and aroma substances complex.

Paracasein degradation is more influenced by non-starter lactic acid bacteria than by starter cultures (CICHOSZ et al. 2003a). Dutch type cheeses produced with addition of various strains of probiotic were characterised by shorter ripening time (CICHOSZ et al. 2006).

The aim of the study was to compare of sensory quality of Gouda cheeses produced with different probiotic Lactobacillus strains.

Materials and Methods

Gouda type cheeses were produced in a dairy plant (Mlekpol, Grajewo, Poland, cheese-dairy Kolno). Control cheeses were produced with mesophilic streptococcus lactis cultures only. For experimental cheeses production addition of selected probiotic strains i.e. *Lactobacillus casei, Lactobacillus acidophilus* and *Lactobacillus rhamnosus* was applied beside mesophilic streptococcus lactis cultures.

Changes of acidity were measured during cheese ripening. Sensory evaluation of cheese was completed after 4 and 6 weeks of ripening and sensory profiles of control and experimental cheeses were defined. Volatile aroma components were determined by SPME method.

Evaluation of sensory quality of cheese

Graduation method (according to Polish Norm PN – ISO 6658, PN – ISO 11035) consists in classification of product in dependence on selected features evaluation. In the study the following quality descriptors: colour, eyeing, consistency, aroma and taste were chosen for evaluation.

For every descriptors importance coefficients were set (colour – 0.15; eyeing – 0.15 consistency – 0.20 aroma – 0.25 and taste – 0.25) and numerical values of serial scale degrees were defined. Six-point scale was applied for sensory evaluation. Characteristics of the quality descriptors were worked up along quality requirements for ripening rennet cheeses of Gouda type defined by Polish norm PN – 68/A – 86230 and IDF Standard 99C:1997.

Evaluator's duty was to classify the cheese quality descriptors by the quality marks scale and marks filled in the mark chart. Every time 7 persons participated in the evaluation and 3 cheeses from the lot were tested.

Sample preparation

Cheese samples for SPME – GC analysis were prepared according to the method described by Chin and others (1996). Samples were homogenized using a laboratory homogenizer MPW – 120 (Med-Instrument, Warsaw, Poland). 20 g of sample was placed in glass jar and tightly closed by teflon caps using a hand crimper and heated 40 min at 60°C in water bath. Volatile compounds from cheese were extracted using a solid phase microextraction device (SPME, Supleco, Bellefonte, PA). Fiber holder was inserted into jar with sample through the semptum. SPME fiber (75 um Carboxen/Polydimethylsiloxane) was exposed to the headspace for 20 min in the same temperature.

Gas chromatography

SPME fiber was immediately inserted into GC injector (2 min splitless mode) for 10 min for thermal desorption of volatiles. GC analysis was performed using Hewlett – Packard 6890 series chromatograph with flame ionization detector. Separation was carried out on HP-Innowax capillary column (60 m x 0.25 mm id. x 0.5 um film thickness). The column temperature was programmed from 40°C (2 min hold) then increased to 220°C (at $5^{\circ}C \cdot min^{-1}$). Detector was heated to 250°C, injector – 225°C. Helium ($18\ cm \cdot s^{-1}$) was used as a carrier gas. Compounds were identified by comparing retention time with standards (Aldrich, Milwaukee, USA).

Proportional share of components of the Gouda cheese aromas in relation to total peak area of the sample was counted to compare the sensory profiles. Arithmetic average of triplicates was calculated.

Results

Typical for Gouda cheese changes of acidity were determined during cheese ripening. No significant changes were found between control cheeses and those produced with addition of probiotic lactobacilli (Table 1).

Sensory quality

The consequence of various microbiological compositions was different sensory features of the cheeses. All the cheeses were of intensive cream-yellow colour, uniform in the whole mass. Colour of all cheeses was very highly judged

Table 1

Changes of acidity (pH) during Gouda cheese ripening

Cheese		After pressing	After salting	After 4 weeks of ripening	After 4 weeks of ripening
Control	A	5.22	5.25	5.36	5.49
	B	5.18	5.28	5.40	5.45
	C	5.24	5.36	5.44	5.47
L. casei	D	5.15	5.30	5.42	5.50
	E	5.43	5.43	5.48	5.51
	F	5.51	5.42	5.45	5.58
L. acidophilus	G	5.38	5.44	5.49	5.56
	H	5.38	5.42	5.43	5.45
	I	5.43	5.40	5.47	5.49
L. rhamnosus	J	5.44	5.39	5.46	5.52
	K	5.47	5.45	5.49	5.56
	L	5.53	5.33	5.38	5.42

both after 4 and 6 weeks of ripening. In 8 cheeses favourable changes, i.e. higher marks, were found after 6 weeks of ripening and the highest marks for colour were attributed to experimental cheeses produces with addition of *L. acidophilus* and *L. rhamnosus* cultures (Figures 1, 2).

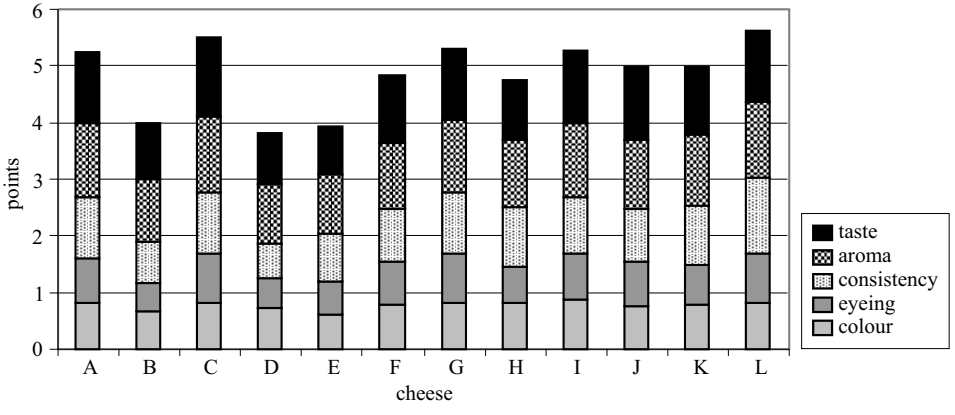

Fig. 1. Mean sensory quality of cheeses after 4 weeks of ripening

Eyeing of the cheeses was more differentiated then colour. After 4 weeks of ripening 7 (of 12) cheeses were characterized by very good eyeing (over 5.0 pt) and only 3 were judged as good (4.0 or less). Comparing parallel control and experimental cheeses we can conclude that cheeses produced with addition of *L. acidophilus* and *L. rhamnosus* showed better eyeing, though the average

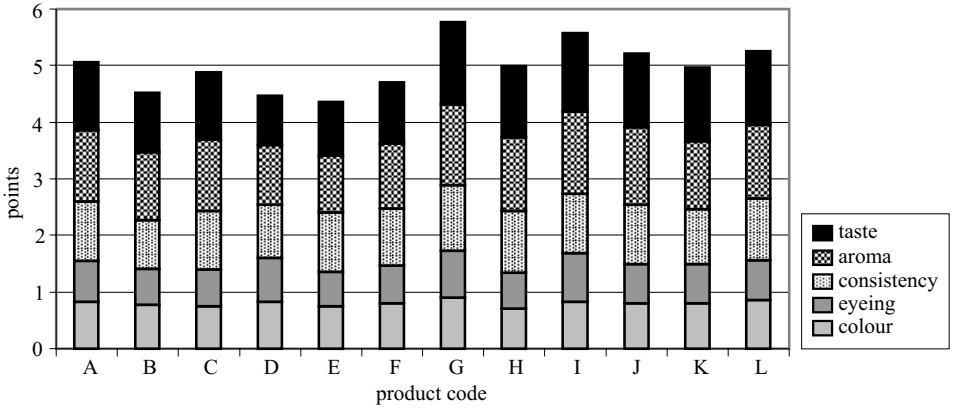

Fig. 2. Mean sensory quality of cheeses after 6 weeks of ripening

punctual mark was lower after 6 then after 4 weeks of ripening. Eyeing of control and with *L. casei* addition cheeses was similar (Figures 1, 2) Consistency of the cheeses was different. In most cases i.e. in 9 ripened cheeses consistency was correct and only in 3 products the mark was below 5.0. Consistency of most cheeses was better after 6 then after 4 weeks. The highest value of punctual evaluation of experimental cheeses was for cheese produced with addition of *L. acidophilus* and slightly lower for that produced with *L. rhamnosus.*

Similarly to the previously described descriptors of the sensory quality, average punctual mark for aroma was higher for cheeses produced with addition of *L. acidophilus* and *L. rhamnosus*. The cheese with *L. casei* addition was judged considerably lower than the control one. *L. casei* cultures intensified cheese aroma powerfully but undesirable; the aroma reminded overriped or soft cheese aroma.

After 4 weeks of ripening most of the cheeses possessed typical, sweet, delicate aroma and received very high marks. Only two cheeses (D,E) received marks below 4.0. Also, after 6 weeks many cheeses were judged at 5.0 or more (G-L) but not in every case there were the same products as after 4 weeks of ripening (Figures 1, 2).

Also, taste of the cheeses produced with addition of *L. acidophilus* and *L. rhamnosus* received the highest marks at the punctual evaluation. Taste of cheese with *L. casei* was judged considerably lower than taste of control and the rest of experimental cheeses (Figures 1, 2).

Results of general evaluation, taking importance coefficients into consideration, confirm good sensory quality of cheeses, both control and experimental ones. Most of them was judged at 5.0 and over, both after 4 weeks of ripening (A, C, G, I, J-L,) and after 6 weeks (A, G-L). General sensory quality of

experimental cheeses was higher (except F) after 6 than after 4 weeks of ripening; the phenomenon was not observed with control cheeses (Figures 1, 2).

Characteristics of sensory profile

Sensory profiles of cheeses produces without and with *Lactobacillus* strains addition were similar but not the same (Figures 3-6). Acetic acid, acetoin, butyric acid and diacetyl were dominant components of cheese aroma. Share of the compounds in cheese taste and aroma generation was different and dependent on ripening time.

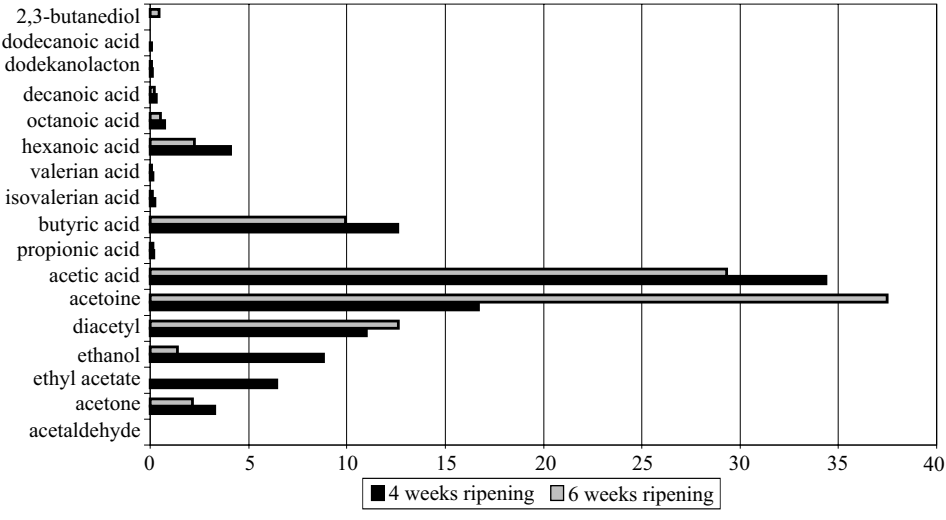

Fig. 3. Volatile components of flavour in control cheese

After 4 weeks of ripening sensory profiles of cheeses produced with *L. casei* and *L. rhamnosus* were more alike control ones then of cheeses with *L. acidophilus*. Amount of some volatile components of aroma decreased. In both control and experimental cheeses, acetaldehyde and acetate were not determined.

Sensory profile of control cheeses changed less than that of experimental ones. Relation and amount of the most important components did not changed considerably after 4 and 6 weeks of control cheeses ripening. Acetic acid, then acetoin, butyric acid and diacetyl remained dominant. Nevertheless, during the last ripening stage acetoin and butyric acid content increased at 37% and 12%, respectively, and diacetyl content decreased at 29%.

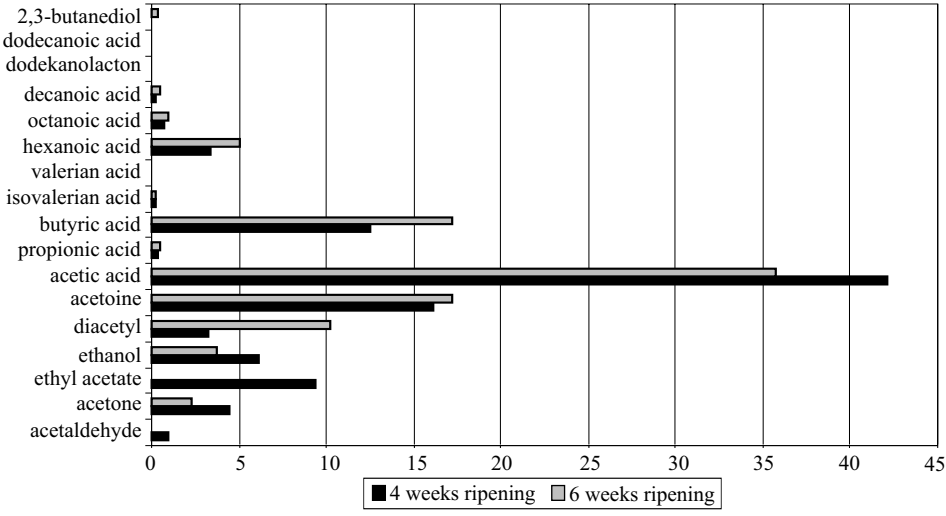

Fig. 4. Volatile components of flavour in cheese produced with addition of *L. casei*

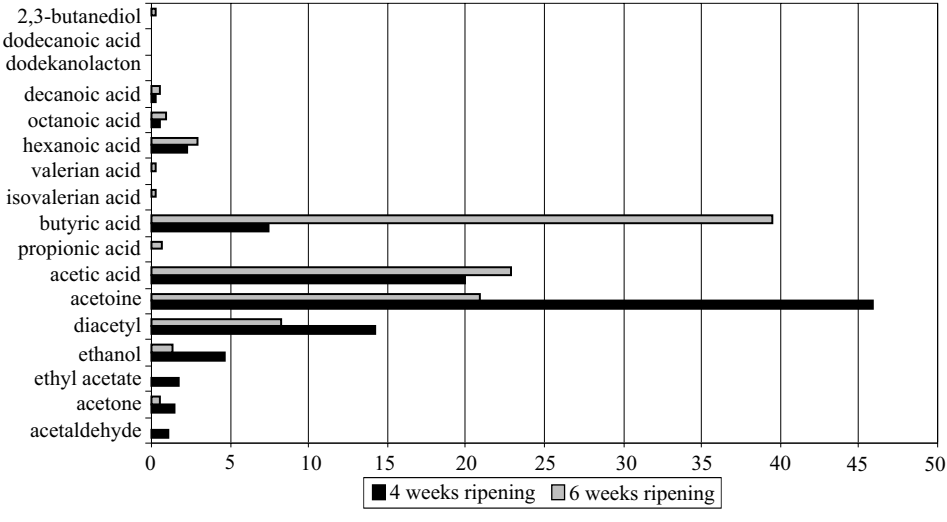

Fig. 5. Volatile components of flavour in cheese produced with addition of *L .acidophilus*

Sensory profile of cheese produced with *L. casei* addition changed not much (Figure 4). After 4 and 6 weeks of ripening acetic acid, acetoin and butyric acid and diacetyl were dominant volatile aroma compounds. During the last two weeks of ripening content of butyric acid decreased and of the rest components

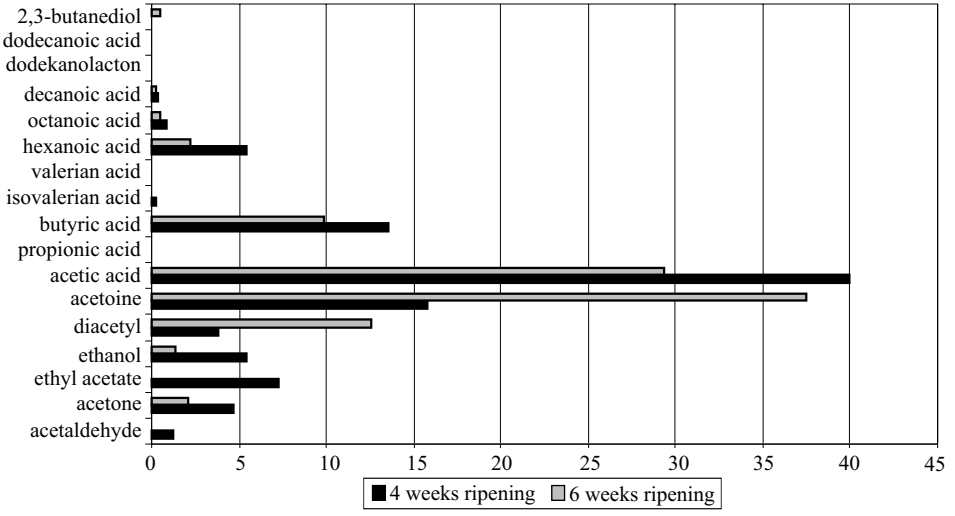

Fig. 6. Volatile components of flavour in cheese produced with addition of *L. rhamnosus*

increased. Significant increase of diacetyl (220%) and hexanoic acid (66%) were found. Ethanol and acetone content decreased (61 and 50%, respectively).

Sensory profile of cheese produced with *L. acidophilus* changed much at the last stage of ripening (Figure 5). Relation and amount of the most important components changed considerably after 6 weeks in comparison to 4 weeks of ripening. After 6 weeks butyric acid was the dominant component and its content increased 5.3-times. Acetic acid content increased much less but acetoin and diacetyl content decreased two-times. Also ethanol and acetone content decreased considerably: 3.5 and 3.0- times, respectively.

Unlike cheese with *L. acidophilus,* in cheese with *L. rhamnosus* (Figure 6) after 6 weeks of ripening the dominant compound was acetoin (2.4-times increase). Also, diacetyl content increased from level of 4% after 4 weeks to 13% after 6 weeks of ripening (over 3-times), however, content of acetic acid, which dominated in 4-weeks-cheese, decreased of 36%. Content of acetone, ethanol and hexanoic acid also decreased (54%, almost 300% and about 100%).

Cheese with *L. rhamnosus* contained more diacetyl and acetoin in comparison to the rest of the cheeses. Content of acetic acid was lower after 6 than after 4 weeks, also butyric acid content decreased. Because of its ability to reduct acetate addition of *L. rhamnosus* strain is used in dairy industry to control butyric fermentation (MÄYRÄ-MÄKINEN, SUOMALAINEN 1996).

The SPME technique appeared to be very useful in analysis of volatile cheese aroma composition. The results were precise and repeatable. However, comparison of our results with data reported by other authors is impossible. Sensory

quality of ripening cheeses depends on many various parameters, e.g. heat treatment of milk (MUIR et al. 1997), starter cultures (MUIR et al. 1996), fat content (CHIN and ROSENBERG 1997, DIMOS et al. 1996), ripening time (ENGELS et al. 1997), temperature and method of sample preparation (MUIR et al. 1995).

Composition of volatile aroma components in cheddar cheese (DACREMONT and VICKERS 1994, ARORA et al. 1995) and in Swiss one (JOU and HARPER 1998, YANG and MIN 1994) is similar. Anyway, different proportion of the most important aroma components, and also different composition of paracasein degradation products, results in completely different sensory features (ENGELS and VISSER 1994).

Conclusions

Application of probiotic *Lactobacillus* strains in technology of Gouda cheese makes possible to product cheeses of different sensory quality. Cheeses produced with addition of *L. acidophilus* and *L. rhamnosus* were characterized by better taste and aroma, then the control ones. However, unfavorable sensory quality changes were observed in cheese with *L. casei*; aroma, and in lesser extent taste, were characteristic for overriped cheese.

Relation of sensory profile to punctual sensory evaluation of cheese showed that diacetyl and acetoin were not the most important components of cheese taste and aroma: sensory quality of cheese produced with *L. rhamnosus,* though the highest content of diacetyl and acetoin, was lower then the quality of cheese with *L. acidophilus* containing low levels of the compounds. Aroma of the cheeses was better after 4 then after 6 weeks of ripening. The best sensory quality was established for cheese produced with addition of *L. acidophilus* which contained much more of butyric acid and much less of acetone and ethanol in comparison to the rest of the cheeses. They contained also the more of propionic acid.

Translated by KRYSTYNA SKIBNIEWSKA

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DESIGN OF FOOD PROTEIN PROTEOLYSIS WITH A VIEW TO OBTAINING BIOACTIVE PEPTIDES

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K e y w o r d s: food proteins, peptide and protein database, bioactive peptides, proteolysis simulation.

A b s t r a c t

The work was undertaken to select proteolytic enzymes useful for release biologically active peptides from their protein precursors. To achieve this aim 23 out of 44 activities present in BIOPEP database (www.uwm.edu.pl/biochemia) were chosen according to the frequency of occurrence of fragments exhibiting given activity in protein chains (*A* parameter). 149 amino acid sequences of proteins and 24 enzyme activites were analysed.

For each biological activity, families of the proteins containing fragments with a given activity were determined. The richest families were formed by proteins being precursors of antihypertensive, dipeptidyl-peptidase IV inhibitors, ubiquitin-mediated proteolysis stimulating and opioid peptides. The poorest families contained precursors of chemotactic, red blood cell creation stimulating, smooth muscle contracting and haemolytic peptides. The richest ones were divided into subfamilies according to the *A* value.

We assumed that increase of the *A* value enhances the probability of release of bioactive peptides by proteases. Proteolysis was simulated only for the richest subfamilies defined according to differences in *A* values) in rich families. For few-member families, all proteins were analysed. For each activity, proteases or pairs of them, liberating active protein fragments, were determined.

Based on the results, peptides introducible to food, exhibiting e.g. antihypertensive, immunomodulating, antibacterial and antioxidative activity, should usually not be hydrolysed by gastrointestinal proteinases.

The simulation results, confirmed experimentally for tryptic hydrolysis of bovine κ-casein, indicated the high possibility of bioactive peptide obtainment.

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PROJEKTOWANIE PROTEOLIZY BIAŁEK ŻYWNOŚCI W KONTEKŚCIE OTRZYMYWANIA BIOAKTYWNYCH PEPTYDÓW

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S ł o w a k l u c z e: białka żywności, baza danych białek i peptydów, bioaktywne peptydy, symulacja proteolizy.

A b s t r a k t

Celem pracy była selekcja enzymów proteolitycznych uwalniających bioaktywne peptydy z białkowych prekursorów. Na podstawie częstości występowania fragmentów o danej aktywności (parametr *A*) wybrano 23 spośród 44 rodzajów aktywności opisanych w bazie danych BIOPEP (http://www.uwm.edu.pl/biochemia). Analizie poddano 149 sekwencji aminokwasowych białek i 24 enzymy proteolityczne. Zdefiniowano rodziny białek zawierających fragmenty o danej aktywności. Najbogatsze rodziny tworzyły białka będące potencjalnymi prekursorami peptydów przeciwnadciśnieniowych, inhibitorów dipeptydylopeptydazy IV, peptydów stymulujących proteolizę ubikwitynowanych białek oraz peptydów opioidowych. Najuboższe rodziny zawierały prekursory peptydów chemotaktycznych, hemolitycznych, stymulujących wytwarzanie czerwonych ciałek krwi oraz stymulujących skurcz mięśni gładkich. Najbogatsze rodziny dzielono na podrodziny na podstawie wartości parametru *A*.

Założono, że wzrost wartości *A* zwiększa prawdopodobieństwo uwolnienia bioaktywnych peptydów przez enzymy proteolityczne. Symulację proteolizy przeprowadzono tylko dla najbogatszych podrodzin (zdefiniowanych na podstawie parametru *A*) w bogatych rodzinach. W rodzinach liczących po kilka białek analizowano wszystkie sekwencje aminokwasowe. W przypadku rodzaju aktywności określono enzymy lub pary enzymów uwalniające aktywne peptydy.

Stwierdzono, że peptydy o aktywności przeciwnadciśnieniowej, immunomodulacyjnej, antybakteryjnej i przeciwutleniającej mogące być składnikami żywności są zwykle odporne na działanie endopeptydaz przewodu pokarmowego.

Rezultaty symulacji, potwierdzone eksperymentalnie dla trypsynowych hydrolizatów krowiej kazeiny-κ, wskazują na znaczne możliwości otrzymywania bioaktywnych peptydów.

Introduction

Thanks to a wide range of action of bioactive peptides, they are recommended as components of so called functional food i.e. food designed in order to obtain the desired functional and biological functions (ARAI 1996, KORHONEN et al. 1998, MEISEL 2001). The interest in such food is growing worldwide (DE FELICE 1995). Studies are under way concerning the inclusion of bioactive peptides as physiological food components or nutraceutics obtained industrially for the production of so-called phormones. Particular attention is being paid to antihypertensive peptides in connection with phosphopeptides and immunomodulative peptides because of their health benefits (GOBBETTI et al. 2002).

The emergence of new peptides from various sources has resulted in the need to sort out the information as well as to develop new criteria for evaluating peptides as precursors of bioactive peptides. The BIOPEP database (www.uwm.edu.pl/biochemia), developed by our group (DZIUBA et al. 2003 b), allows for determining the profiles of potential biological activity of proteins (type and position of the active fragment in the protein chain sequence) and to determine the protein value based on the newly developed quantitative criteria, i.e. frequency of occurrence of bioactive fragments in the protein chain sequence (*A*) and the potential activity of protein fragments (*B*) (DZIUBA et al. 2003 a, b, IWANIAK et al. 2005). The study had to be extended by the work conducted *in silico* and devoted to the possibility of releasing bioactive fragments from a precursor sequence and of verifying the results with analytic methods.

Numerous research groups use computer aided evaluation techniques to achieve modelling the physical and chemical properties of proteins (LACKNER et al. 1999), predicting their secondary and tertiary structure (FISCHER 2006) and seeking homology between proteins and determining their functions based on this (BRAY et al. 2000, KRIVENTSEVA et al. 2001) requires analyses based on protein sequences or sequence motifs databases (JELIC et al. 2003, WU et al. 2006, ZAMYATNIN et al. 2006, FÄLTH et al. 2006). The research is complemented by the strategy of studying proteins and bioactive peptides with a view to obtaining them from certain groups of proteins. Therefore, the study aimed at: developing an application for the BIOPEP database for proteolysis design; dividing proteins according to a specific criterion into families with similar profiles of biological activity; designing processes of proteolysis of proteins from the richest families-peptide precursors with the following types of activities: antiamnestic, antithrombotic, antihypertensive, immunomodulating, chemotactic, smooth muscle contracting, coeliac-toxic, embryotoxic, antioxidant, dipeptidyl-peptidase IV inhibitor, opioid and opioid antagonist, stimulating red blood cells creation, haemolytic, metal ion binding and transporting, ligands of bacterial permease, anorectic, activating ubiquitin-mediated proteolysis, regulating ion flow, neuropeptide inhibitor, regulating stomach mucosal membrane activity, antibacterial and antiviral, regulating phosphoinositole mechanism as well as analytic verification, using several examples, of the results of computer aided design of proteolysis aimed at releasing bioactive peptides.

Materials and Methods

The research into the release of bioactive peptides *in silico* was based on the BIOPEP database of protein and bioactive peptides. The following types of

activity: antiamnestic, antithrombotic, antihypertensive, immunomodulating, chemotactic, smooth muscle contracting, coeliac-toxic, embryotoxic, antioxidative, dipeptidyl-peptidase IV inhibitor, opioid and opioid antagonist, stimulating red blood cells creation, haemolytic, metal ion binding and transporting, ligands of bacterial permease, anorectic, activating ubiquitin-mediated proteolysis, regulating ion flow, neuropeptide inhibitor, regulating of stomach mucosal membrane activity, antibacterial and antiviral, regulating phosphoinositole mechanism, were analysed (23 among 44 types of activity present in the BIOPEP database). The choice was affected by the frequency of occurrence of particular types of peptides or their interesting health or technological properties. 149 amino acid sequences of proteins, gathered in the BIOPEP database, were used in the study.

Evaluation of proteins as a precursors of bioactive peptides via the BIOPEP program

The "Operations on records" window in the BIOPEP program containing the following functions: create a list of proteins or bioactive peptides with a given activity by selecting the option "List of proteins" or "List of peptides with given activity"; determine the type, number and position of the active fragments in a protein chain – determine its peptide profile ("Profiles of proteins biological activity"); calculate the parameters, which characterize the value of protein as a source of bioactive peptides – "*A, B, Y* Calculation", was used.

The value of proteins as precursors of bioactive peptides was assessed with the use of the frequency of occurrence of bioactive fragments in a protein chain (*A*) defined as:

$$A = a/N \tag{1}$$

where:
a – the number of fragments with a given type of activity in a protein chain,
N – the number of amino acid residues.

Division of proteins, precursors of peptides with similar activity, into subfamilies

Each family of proteins – precursors of peptides of a given activity – was divided into subfamilies with reference to the frequency of occurrence of active

fragments in a protein sequence, expressed as the value of the *A* parameter. The division was performed with the use of iteration procedure, which enabled the appropriate number of proteins to be assigned to a given subfamily (ranges). Such ranges are described as class ranges, and the differences between the upper and the lower limit of a class range number *i* the span of a range hi.

$$h_i = A_{1i} - A_{0i} \tag{2}$$

where:
A_{0i}, i A_{1i} (i = 1, ..., k) are limits of the range number i.

The procedure of classification of proteins being potential precursors of peptides with given activity according to *A* values included the following steps. The *A* values of individual proteins were divided into groups using equal ranges according to the rules of stem-and-leaf procedure (LANE 2003) although without use stem-and-leaf plot notation. The span intervals were defined individually for particular protein families. One protein could form separate subfamily only if its *A* value was the lowest or the highest in the data set. In other case the single protein was inserted into the nearest subfamily. Then three possibilities were considered:

1. Maximum difference between *A* values within subfamily is smaller than differences between the extreme values in this subfamily and the extreme values of neighboring subfamilies. ⇒ Subfamily should be left without changes.

2. Maximum difference between *A* values within subfamily is bigger than one of differences between the extreme values in this subfamily and the extreme values of neighboring subfamilies. ⇒ Subfamily should be connected with the nearer of two neighboring subfamilies.

3. Maximum difference between *A* values within subfamily is bigger than both differences between the extreme values in this subfamily and the extreme values of neighboring subfamilies. ⇒ Subfamily should be divided into two new ones. The first of them should be connected with the preceding, the second with the following subfamily. The maximum difference within subfamily becomes thus the difference between extreme A values of two new subfamilies.

The procedure was stopped when all subfamilies fulfilled conditions described as case 1.

Proteolysis design in the BIOPEP database

Simulation of proteolysis was conducted using the following options: "Enzymes action" → "hydrolysis with a single enzyme" (24 enzymes) or "hydrolysis with a combination of two enzymes" (276 combinations). The data of the following 24 proteolytic enzymes have been gathered in the BIOPEP database: chymotrypsin A, trypsin, pepsin, proteinase K, pancreatic elastase, prolyl oligopeptidase, V-8 protease (glutamyl endopeptidase), thermolysin, plasmin, cathepsin G, clostripain, chymase, papain, ficin, leucocyte elastase, chymotrypsin C, metridin, thrombin bromelain, pancreatic elastase II, glutamyl endopeptidase II, oligopeptidase B, calpain and glycyl endopeptidase. For each enzyme, the following information has been gathered: ID number; classification number – EC; recognition sequence – a sequence of amino acids recognised by a given enzyme; cleavage site – an amino acid residue, characteristic of a given enzyme, which forms the bond hydrolysed by the enzyme.

Following the selection of a protein for analysis and a single enzyme (or a double combination thereof) and running the "View the report with the results" option, the program automatically displays a form with sequences and positions of all the fragments released by the enzymes.

Verifying the results with the use of mass spectrometry

The results of *in silico* predictions have been verified on the basis of results of tryptic hydrolysis of bovine κ-casein (Access. No in the UniProtKB database: P02668), purchased from Sigma. The protein was purified using solid phase extraction with Visiprep™ apparatus on Superclean™ LC-18 3 ml columns (Supelco) as follows: 2.5 mg of the sample was dissolved in 1 ml of 0.05 M phosphate buffer (pH 6.6), then 0.5 ml of this solution was mixed with 0.5 ml of buffer A (acetonitrile : deionized water : trifluoroacetic acid; 100 : 900 : 1 v/v/v). Acetonitrile and trifluoroacetic acid were from Baker. The resulting solution (1 ml volume) was loaded onto the column. Before use the column was washed with 2 ml of deionized water (Milli-Q; Millipore). After sample loading column was washed with 2 ml of buffer A diluted twice with deionized water. Finally purified proteins adsorbed on the column were eluted with 1 ml of buffer B (acetonitrile : deionized water : trifluoroacetic acid; 900 : 100 : 0.7 v/v/v). The protein purity was checked using reversed-phase high-performance liquid chromatography after reduction conducted directly according to MINKIEWICZ et al. (2005). The separated fractions were identified on the basis on third derivatives of UV spectra (MINKIEWICZ et al. 2003, 2006) as well as MALDI-ToF mass spectrometry of intact protein using Ettan MALDI-ToF Pro (Amersham

Biosciences) mass spectrometer (FLENSBURG et al. 2004). Protein was hydrolysed in a solution of ammonium bicarbonate, pH 8.5, with trypsin for proteomic studies (Sigma) and the enzyme: substrate ratio of 1 : 50 (w/w). The hydrolysis was carried out for 24 hours at 37°C. The samples were subsequently subjected to the MS analysis performed using Ettan MALDI-ToF Pro (Amersham Biosciences) mass spectrometer in a reflectron mode. The positive ions analysis and accelerating voltage of 20 kV were applied. The samples were prepared by the dried-droplet method. To this end, 2 μl of a sample was mixed with 2 μl of the matrix solution containing 1 mg/ml α-cyano-4-hydroxycinnamic acid (Sigma) in a solution of 50% acetonitrile and 0.1% trifluoroacetic acid. 0.3 μl of the prepared mixture was applied onto a slide. The apparatus was externally or internally calibrated with the following as the standards: a fragment 1-7 of bradykinin $(M + H)^+$ – 757.399 Da and a fragment 18-39 of ACTH $(M + H)^+$ – 2465.199 Da (Sigma). The ProFound software (ZHANG, CHAIT 2000) connected to NCBI (National Centre for Biotechnology Information) protein database was used for peptide identification via peptide mass fingerprinting (PMF) mode. The sequences of peptides identified via ProFound program were processed via the BIOPEP program using option: "Record operations" → "Search your sequence in bioactive peptide database".

Results and Discussion

The characteristics of selected precursor proteins – creating families and subfamilies of examined proteins with similar activity profiles; depending on the value of parameter *A*.

All the protein set gathered in the database have been divided into families. The adopted criterion of including into a family was the presence of motifs of a given activity in the protein sequence. This gave rise to 23 families of proteins – precursors of peptides with the following types of activity: antihypertensive – 148 proteins; antithrombotic – 82 proteins; antiamnestic – 83 proteins; immunomodulating – 89 proteins; antioxidative – 79 proteins; coeliac-toxic – 13 proteins; smooth muscle contracting – 2 proteins; chemotactic – 6 proteins; embryotoxic – 3 proteins; opioid – 111 proteins; regulating ion flow – 41 proteins; regulating the stomach mucosal membrane activity – 79 proteins; dipeptidyl-peptidase IV inhibitor – 148 proteins; activating ubiquitin-mediated proteolysis – 124 proteins); regulating phosphoinositole mechanism – 28 proteins); neuropeptide inhibitor – 71 proteins; ligands of bacterial permease – 80 proteins; stimulating red blood cells creation – 2 proteins; haemolytic – 1 protein; anorectic – 12 proteins; opioid antagonist – 6 proteins; metal ion

binding and transporting – 3 proteins; as well as antibacterial and antiviral – 10 proteins. The choice of these activities was a result of their frequent occurrence in a protein sequence and of the possibility of using bioactive peptides with pro-health properties as physiologic food components or nutraceuticals (DZIEZAK 1986, KILARA, PANYAM 2003).

As a quality parameter, the biological activity profile did not allow for a full evaluation of proteins as precursors of biologically active peptides. This is why parameter *A* was employed, which allows for a quantitative presentation of a characteristic in question, i.e. a protein value as a source of biologically active peptides. Example results of division of proteins – precursors of peptides regulating the phosphoinositole action are shown in Table 1. The resulting

Table 1

Subfamilies of proteins – precursors of peptides regulating phosphoinositole action

Subfamily	Proteins		Parameter *A*	Class range of given characteristics
	BIOPEP ID	protein name		
I	1105	β-lactoglobulin, sheep *(Ovis aries)*	0.001387	0.001387--0.003061
	1236	lactoferrin precursor, bovine *(Bos taurus)*	0.001412	
	1121	lactoferrin *(Homo sapiens)*	0.001445	
	1212	lactoferrin bovine *(Bos taurus)*	0.001451	
	1235	cruciferin, 11S globulin, rape *(Brassica napus)*	0.002058	
	1076	chicken connectin (titin), fragment *(Gallus gallus)*	0.002266	
	1123	myosin subfragment-1, chicken *(Gallus gallus)*	0.003061	
II	1222	probable cell division protein ftsW	0.004762	0.004762--0.005464
	1087	α_{S1}-casein gen. var. B *(Bos taurus)*	0.005025	
	1088	α_{S1}-casein gen. var. C *(Bos taurus)*	0.005025	
	1089	α_{S1}-casein gen. var. D *(Bos taurus)*	0.005025	
	1086	α_{S1}-casein gen. var. A *(Bos taurus)*	0.005376	
	1198	retinol binding protein RBP, bovine *(Bos taurus)*	0.005464	
III	1192	PR-10 protein, white lupine *(Lupinus albus)*	0.006329	0.006329--0.007407
	1225	hemoglobin β-A chain, goat *(Capra hircus)*	0.006896	
	1226	hemoglobin β-C chain, goat *(Capra hircus)*	0.007092	
	1084	α-lactalbumin, rat *(Rattus norvegicus)*	0.007143	
	1125	Myoglobin	0.007407	
IV	1077	α-lactalbumin *(Homo sapiens)*	0.008130	0.008130--0.008333
	1078	α-lactalbumin, horse *(Equus caballus)*	0.008130	
	1079	α-lactalbumin, goat *(Capra hircus)*	0.008130	
	1082	α-lactalbumin, sheep *(Ovis aries)*	0.008130	
	1083	α-lactalbumin, guinea pig *(Cavia porcellus)*	0.008130	
	1085	α-lactalbumin, arabian camel *(Camelus dromedarius)*	0.008130	
	1115	α-lactalbumin, bovine *(Bos taurus)*	0.008197	
	1081	α-lactalbumin, red-necked wallaby *(Macropus rufogriseus)*	0.008264	
	1093	lysozyme precursor, silk moth *(Bombyx mori)*	0.008333	
V	1195	major urinary protein 1 precursor, mouse *(Mus musculus)*	0.009259	

subfamilies differ from these obtained using equal ranges of the parameter using for classification as used in classic statistical procedures (GAWĘCKI, WAGNER 1984, RÓSZKIEWICZ 2002) and in simplest stem-and-leaf plot analysis (LANE 2003). The proposed procedure mimics intuition, which tends to use the maximum differences between neighboring values as a borders between subfamilies.

The numbers of proteins in particular subfamilies were highly variable. That was the case in dividing the families with the following types of activity: antithrombotic, antiamnestic, regulating ion flow, regulating the stomach mucosal membrane activity and neuropeptide inhibitors. For the peptide precursors with the following types of activity: coeliac-toxic, smooth muscle contracting, chemotactic, embryotoxic, stimulating blood cell creation, haemolytic, anorectic, opioid antagonist, metal ion binding and transporting as well as antibacterial and antiviral, subfamilies were not created because of a small number of proteins in them.

The resulting classification was used to choose proteins for designing proteolysis aiming at releasing peptides with a specific type of activity. The richest family or two richest subfamilies (for the following types of activity: antithrombotic, antiamnestic, antioxidative, opioid, regulating ion flow, regulating the stomach mucosal membrane activity, dipeptidyl-peptidase IV inhibitor, activating ubiquitin-mediated proteolysis and regulating phosphoinositole mechanism) were choosen. For the ligands of bacterial permease three richest subfamilies of proteins were analysed.

Releasing bioactive peptides from selected precursor proteins by enzymes or their combinations as in the example of multi-functional peptides with antithrombotic, antiamnestic and the stomach mucosal membrane regulating activity.

Based on earlier research (DZIUBA et al. 1999 b, 2002) it has been shown that the more bioactive fragments there are in the protein peptide profile, the higher the probability is of releasing the fragments by proteolytic enzymes. Therefore, those which made up the most numerous families were chosen for the "*in silico*" research of proteolysis design. Small families, for which all the proteins were examined, were exceptional. An assumption was made in the study that the more sequencing motifs corresponding to bioactive peptides is present in a protein, the more frequently such fragments can be potentially released by proteolytic enzymes. The assumption was generally true, but in some cases bioactive peptides are more intensely released from proteins with potentially fewer bioactive fragments. For example, chymotrypsin C and papain release only one active fragment each from blueberry monellin (ID 1170), which is the richest precursor of peptides regulating ion flow

(A = 0.022222), while such enzymes as proteinase K, papain, ficin and bromelain release from one to three active fragments from green pea narbonin (ID 1191), which is a less rich precursor of peptides with the same activity (A = 0.010309).

The assumption is a certain simplification and does not take into account such aspects as the effect of the position of active fragments in the amino acid sequence of a protein molecule, the structure of a protein molecule or the specificity of the applied enzymes. Hence, the bioactive motifs situated in the vicinity of bonds easily hydrolysed by proteolytic enzymes can be released more effectively, while those situated close to proteolysis-resistant bonds remain within longer sequences, regardless of the biological value of the protein precursor. Factor A is also used as the measure of the selection of proteins – precursors of biological activity – and determines the number of bioactive fragments in a protein in relation to the number of amino acids making up the whole protein sequence. Therefore, the proteins with a short sequence, which contain fewer bioactive fragments, can frequently be classified as a better precursor than ones with a relatively long sequence containing fewer bioactive fragments.

One of the best-researched groups of multifunctional peptides is a family of short chains containing frequently occurring residues of glycine (G) and proline (P) (Otaki et al. 2005), e.g.: PG, GP, PGP. They show the following types of activities: antithrombotic, antiamnestic, regulating stomach mucosal membrane activity and dipeptidyl-peptidase IV inhibitor (the GP fragment only). These peptides were frequently present in the sequences of precursor peptides and created the most numerous group among the peptides with the activities: antithrombotic, antamnestic and regulating stomach mucosal membrane activity. In addition, they were the most frequently predicted to be released by proteolytic enzymes. Because of the multi-functionality of the PG and GP peptides – the main representatives of anti-coagulative, antiamnestic and regulating stomach mucosal membrane activity – and their most frequent occurrence in the sequence of the same proteins, the results of proteolysis simulation are presented together, taking into account the differences concerning only one of the activities.

Among the proteins – precursors of peptides with anticoagulative, antiamnestic and regulating stomach mucosal membrane activity, representatives of two richest subfamilies were selected for the proteolysis simulation stage. The subfamilies included the following proteins: collagen α 1(III) chain (*Bos taurus*) (ID 1111); collagen α 1(I) chain (fragment) (*Bos taurus*) (ID 1112); collagen α 1(I) chain precursor, chicken (*Gallus gallus*) (ID 1113). They included in their sequences 295, 208 and 301 peptides with these activities, respectively. Table 2 shows the enzymes which potentially release multifunctional peptides with

anticoagulative, antiamnestic and stomach mucosal membrane activity regulating activity, as well as the number of obtained fragments as a result of their use. The most frequently released fragments were GP and PG peptides. Proteinase K, prolyl oligopeptidase and chymotrypsin C released *in silico* fragments with GP sequence, while the others released PG fragments.

Table 2
Enzymes releasing antithrombotic, antiamnestic and stomach mucosal membrane activity regulating peptides

Enzyme	Number of fragments with antithrombotic, antiamnestic and regulating stomach mucosal membrane activity released from precursor proteins		
	protein ID 1111	protein ID 1112	protein ID 1113
Proteinase K	57	47	64
Pancreatic elastase	48	28	32
Prolyl oligopeptidase	57	34	52
Chymotrypsin C	57	46	70
Papain	55	29	45
Ficin	52	31	46
Bromelain	38	19	30
Glycyl endopeptidase	9	–	–

Table 3 shows the enzymes which release *in silico* bioactive peptides from precursor proteins. Among the analysed protein sequences, animal proteins, and particularly those from milk, showed the highest potential of releasing bioactive peptides. The resulting fragments are peptides with the following types of activity: antihypertensive, opioid, immunomodulating, anti-coagulative, regulating ion flow, antiamnestic and dipeptidyl-peptidase IV inhibitor. Among the enzymes specific to milk proteins, which produced *in silico* the largest amounts of the peptides, were elastase, chymotrypsin and trypsin. Di- and tri-peptides, e.g. RL, GY, AY, VPL, QP, LW, FY, FP, YL, FGK, RF, AP were predicted to be released from all the milk proteins. These short peptides are most easily absorbed from the digestive system to blood (SIEMENSMA et al. 1993, DZIUBA et al. 1999 a, b, VERMEIRSSEN et al. 2004 a).

Table 3
Enzymes releasing bioactive peptides from precursor proteins *in silico*

Activity	Proteolytic enzymes
1	2
Antithrombotic, Antiamnestic, Regulating stomach mucosal membrane activity	Proteinase K (EC.3.4.21.14), Pancreatic elastase (EC 3.4.21.36), Prolyl oligopeptidase (EC 3.4.21.26), Chymotrypsin C (EC 3.4.21.2), Papain (EC 3.4.22.2),

cont. Table 3

1	2
	Ficin (EC 3.4.22.3), Bromelain (EC 3.4.22.4), Glycyl endopeptidase (EC 3.4.22.25) Thermolysin (EC 3.4.24.27)/Chymotrypsin A (EC 3.4.21.1), Thermolysin (EC 3.4.24.27)/Trypsin (EC 3.4.21.4), Thermolysin (EC 3.4.24.27)/Pepsin (EC 3.4.23.1), Thermolysin (EC 3.4.24.27)/V-8 protease (Glutamyl endopeptidase)(EC 3.4.21.19), Thermolysin (EC 3.4.24.27)/Plasmin (EC 3.4.21.7), Thermolysin (EC 3.4.24.27)/Cathepsin G (EC 3.4.21.20), Thermolysin (EC 3.4.24.27)/Clostripain (EC 3.4.22.8), Thermolysin (EC 3.4.24.27)/Chymase (EC 3.4.21.39), Thermolysin (EC 3.4.24.27)/Leukocyte elastase (EC 3.4.21.37), Thermolysin (EC 3.4.24.27)/ Metridin (EC 3.4.21.3), Thermolysin (EC 3.4.24.27)/Trombin (EC 3.4.21.5), Thermolysin (EC 3.4.24.27)/ Pancreatic elastase II (EC 3.4.21.71), Thermolysin (EC 3.4.24.27)/Glutamyl endopeptidase II(EC 3.4.21.82), Thermolysin (EC 3.4.24.27)/ Oligopeptidase B (EC 3.4.21.83), Leukocyte elastase (EC 3.4.21.37)/ Trypsin (EC 3.4.21.4), Leukocyte elastase (EC 3.4.21.37)/Oligopeptidase B (EC 3.4.21.83), Leukocyte elastase (EC 3.4.21.37)/Cathepsin G (EC 3.4.21.20),
Antihypertensive	Chymotrypsin A (EC 3.4.21.1), Trypsin (EC 3.4.21.4), Pepsin (EC 3.4.23.1), Prolyl oligopeptidase (EC 3.4.21.26), Thermolysin (EC 3.4.24.27), Chymotrypsin C (EC 3.4.21.2), Plasmin (EC 3.4.21.7), Cathepsin G (EC 3.4.21.20), Papain (EC 3.4.22.2), Metridin (EC 3.4.21.3), Pancreatic elastase II (EC 3.4.21.71), Bromelain (EC 3.4.22.4), Oligopeptidase B (EC 3.4.21.83), Chymase (EC 3.4.21.39)/Pancreatic elastase (EC 3.4.21.36), Chymase (EC 3.4.21.39)/Clostripain (EC 3.4.22.8), Chymase (EC 3.4.21.39)/ Leukocyte elastase (EC 3.4.21.37)
Antioxidative	Proteinase K (EC.3.4.21.14), Pancreatic elastase (EC 3.4.21.36), Thermolysin (EC 3.4.24.27), Cathepsin G (EC 3.4.21.20), Papain (EC 3.4.22.2), Ficin (EC 3.4.22.3), Chymotrypsin C (EC 3.4.21.2),
Immunomodulating	Pancreatic elastase (EC 3.4.21.36), Glycyl endopeptidase (EC 3.4.22.25), Chymase (EC 3.4.21.39), Thermolysin (EC 3.4.24.27)/V-8 protease (Glutamyl endopeptidase)(EC 3.4.21.19), Chymase (EC 3.4.21.39)/ Glutamyl endopeptidase II(EC 3.4.21.82), Thermolysin (EC 3.4.24.27)/ Trypsin (EC 3.4.21.4)
Coeliac toxic	Termolysin (EC 3.4.24.27)/Clostripain (EC 3.4.22.8),
Smooth muscle contracting	Trypsin (EC 3.4.21.4), Plasmin (EC 3.4.21.7), Oligopeptidase B (EC 3.4.21.83)
Embryotoxic	Thermolysin (EC 3.4.24.27)/Leukocyte elastase (EC 3.4.21.37),
Chemotactic	–

cont. Table 3

1	2
Opioid	Pepsin (EC 3.4.23.1), Pancreatic elastase (EC 3.4.21.36), Prolyl oligopeptidase (EC 3.4.21.26), Leukocyte elastase (EC 3.4.21.37),
Regulating ion flow	Proteinase K (EC.3.4.21.14), Chymotrypsin C (EC 3.4.21.2), Papain (EC 3.4.22.2), Ficin (EC 3.4.22.3), Bromelain (EC 3.4.22.4),
Dipeptydyl-peptidase IV inhibitory	Proteinase K (EC.3.4.21.14), Pancreatic elastase (EC 3.4.21.36), Prolyl oligopeptidase (EC 3.4.21.26), Thermolysin (EC 3.4.24.27), Chymotrypsin C (EC 3.4.21.2), Papain (EC 3.4.22.2), Ficin (EC 3.4.22.3), Leukocyte elastase (EC 3.4.21.37), Bromelain (EC 3.4.22.4),
Activating ubiquitin mediated proteolysis	Pancreatic elastase (EC 3.4.21.36), Papain (EC 3.4.22.2), Ficin (EC 3.4.22.3), Leukocyte elastase (EC 3.4.21.37), Bromelain (EC 3.4.22.4),
Regulating phosphoinositole mechanism	Chymase (EC 3.4.21.39), Calpain (EC 3.4.22.17)/ Prolyl oligopeptidase (EC 3.4.21.26), Calpain (EC 3.4.22.17)/V-8 protease (Glutamyl endopeptidase) (EC 3.4.21.19), Calpain (EC 3.4.22.17)/Plasmin (EC 3.4.21.7), Calpain (EC 3.4.22.17)/Clostripain (EC 3.4.22.8), Calpain (EC 3.4.22.17)/Leukocyte elastase (EC 3.4.21.37), Calpain (EC 3.4.22.17)/Thrombin (EC 3.4.21.5), Calpain (EC 3.4.22.17)/Glutamyl endopeptidase II (EC 3.4.21.82), Calpain (EC 3.4.22.17)/Oligopeptidase B (EC 3.4.21.83)
Neuropeptide inhibitor	Chymotrypsin C (EC 3.4.21.2), Thermolysin (EC 3.4.24.27)/Proteinase K (EC.3.4.21.14), Thermolysin (EC 3.4.24.27)/Prolyl oligopeptidase (EC 3.4.21.26),
Ligand of bacterial permease	Leukocyte elastase (EC 3.4.21.37)
Stimulating red blond cells creation	–
Haemolytic	–
Anorectic	Thermolysin (EC 3.4.24.27)/Chymotrypsin A (EC 3.4.21.1), Thermolysin (EC 3.4.24.27)/Pepsin (EC 3.4.23.1), Thermolysin (EC 3.4.24.27)/Cathepsin G (EC 3.4.21.20), Thermolysin (EC 3.4.24.27)/ Chymase (EC 3.4.21.39), Thermolysin (EC 3.4.24.27)/ /Metridin (EC 3.4.21.3), Thermolysin (EC 3.4.24.27)/Pancreatic elastase II (EC 3.4.21.71), Thermolysin (EC 3.4.24.27)/ V-8 protease (Glutamyl endopeptidase)(EC 3.4.21.19), Thermolysin (EC 3.4.24.27)/Clostripain (EC 3.4.22.8), Thermolysin (EC 3.4.24.27)/Glutamyl endopeptidase II(EC 3.4.21.82), Thermolysin (EC 3.4.24.27)/Oligopeptidase B (EC 3.4.21.83)
Opioid antagonist	Trypsin (EC 3.4.21.4), Plasmin (EC 3.4.21.7), Oligopeptidase B (EC 3.4.21.83)
Metal ion binding	Pancreatic elastase II (EC 3.4.21.71),

cont. Table 3

1	2
Antibacterial and antiviral	Chymotrypsin A (EC 3.4.21.1), Trypsin (EC 3.4.21.4), Thermolysin (EC 3.4.24.27), Plasmin (EC 3.4.21.7), Chymase (EC 3.4.21.39), Metridin (EC 3.4.21.3), Bromelain (EC 3.4.22.4), Oligopeptidase B (EC 3.4.21.83), Glutamyl endopeptidase II(EC 3.4.21.82)/ Pepsin (EC 3.4.23.1), Glutamyl endopeptidase II(EC 3.4.21.82)/ Chymase (EC 3.4.21.39), Glutamyl endopeptidase II(EC 3.4.21.82)/ Metridin (EC 3.4.21.3), Glutamyl endopeptidase II(EC 3.4.21.82)/ Pancreatic elastase II (EC 3.4.21.71), Ficin (EC 3.4.22.3)/ V-8 protease (Glutamyl endopeptidase)(EC 3.4.21.19), Ficin (EC 3.4.22.3)/ Chymotrypsin C (EC 3.4.21.2), Ficin (EC 3.4.22.3)/ Glutamyl endopeptidase II(EC 3.4.21.82), Bromelain (EC 3.4.22.4)/ V-8 protease (Glutamyl endopeptidase)(EC 3.4.21.19), Bromelain (EC 3.4.22.4)/ Chymotrypsin C (EC 3.4.21.2), Bromelain (EC 3.4.22.4)/ Glutamyl endopeptidase II(EC 3.4.21.82)

As is seen from the study, the peptides which can be introduced to food as physiologically active substances, namely antihypertensive, responsible for metal ion transport, immunomodulating, antibacterial and antioxidative ones, were not in general hydrolysed by proteolytic enzymes of the alimentary tract. The BIOPEP database contains information about the major proteolytic enzymes of the alimentary tract. These are: pepsin, trypsin, chymotrypsin, pancreatic elastase and proline oligopeptidase. All these enzymes hydrolyse peptide bonds formed with group carboxyl group of the following amino acids: A, G, V, L, Y, F, M, W, E, Q, K, R and P. Comparing the specificity of action of digestive enzymes with the sequence of physiologically important peptides confirms their general resistance to proteolysis. Among the exceptions there are those peptides which contain proline (P) inside the sequence motif. Such peptides are hydrolysed by prolyl oligopeptidase. Therefore, the design process of an addition to a bioactive peptide should take into account the potential hydrolysis of bonds within peptide sequence by digestive enzymes.

Vegetable proteins are generally a worse source of bioactive polypeptides as compared with these of animal origin. The peptides released from wheat gliadin show anti-hypertensive, opioid, antioxidative and dipeptidyl-peptidase IV inhibitory activity. The largest number of bioactive peptides can be released by proteinase K, proline endopeptidase and elastase. Such enzymes as trypsin, clostripain, collagenase, pronase, papain, plasmin, thermolysin, cathepsin and

bacterial proteinases do not release any active fragments. Their release is favoured by the hydrophilic surrounding of the active sequences in the proteins under study (DZIUBA et al. 2002, 2004, 2005).

In the case of antihypertensive, smooth muscle contracting, ion flow regulating, activating ubiquitin-mediated proteolysis, neuropeptide inhibitor, ligands of bacterial permease, opioid antagonist, metal ion binding and transporting, a coupled action of two enzymes did not improve the proteolysis effectiveness. Applying combinations of two enzymes for the following types of activity: antithrombotic, antiamnestic, regulating of stomach mucosal membrane activity, antioxidative, immunomodulating, opioid, dipeptidyl--peptidase IV inhibitor improved the effectiveness of releasing the relevant fragments. Thanks to the use of two enzymes for hydrolysis for phosphoinositole mechanism regulating as well as antibacterial and antiviral activities, fragments were obtained with the sequences not released by single enzymes. Coeliac-toxic and embryotoxic peptides were released only by two-enzyme combinations.

When analysing the possibility of releasing bioactive peptides by proteolytic enzymes, the relative number of bioactive fragments, the effect of physical and chemical conditions (temperature, pH, enzyme specificity) necessary to conduct effective hydrolysis, physiological aspects which determine the release and absorbing the bioactive peptides in the organism should be taken into account (ADLER-NISSEN 1986, FRIEDMAN 1996, VOROB'EV, GONCHAROVA 1998, VERMEIRSSEN et al. 2004 a). A study of a possibility of releasing bioactive peptides can be conducted experimentally or with the use of computer techniques (*in silico*) (VOROB'EV, GONCHAROVA 1998, VERMEIRSSEN et al. 2004 b, CALLEBAUT et al. 2005, PRIPP 2005, BOLSCHER et al. 2006). Concerning the latter, it must be borne in mind that only in some cases does a possibility exist to program a computer simulation of proteolysis which takes into account the conditions prevalent, e.g. in the alimentary tract. Consequently, the results obtained *in silico* can partly correspond to those of laboratory experiments. Among the examples may be β-lactoglobulin, which, in theory, has many bonds which can be hydrolysed by pepsin, but is, in fact, resistant to the enzyme (CHOBERT et al. 1997). The resistance of β-lactoglobulin is a result of the fact that the protein molecule has a concise, globular structure. The peptide bonds which could be hydrolysed by pepsin are situated inside the molecule and are therefore inaccessible to the enzyme. Heat treatment can unfold the protein chain and reveal the inaccessible bonds (VOROB'EV, GONCHAROVA 1998, DZIUBA, DAREWICZ 2000).

Identifying selected biopeptides released from bovine κ-casein by trypsin

Table 4 shows of bovine κ-casein data set as an example of a profile of potential biological activity of protein. In the profile of potential biological activity of κ-casein the active fragments predicted to be released *in silico* by trypsin are marked bold.

Table 4

The profile of potential biological activity of cow's κ-casein (ID 1117)

ID of protein:	1117	Name:	κ-casein, genetic variant A, bovine (*Bos taurus*)
Protein sequence:	QEQNQEQPIRCEKDERFFSDKIAKYIPIQYVLSRYPSYGLNYYQQKPVALINNQ FLPYPYYAKPAAVRSPAQILQWQVLSDTVPAKSCQAQPTTMARHPHPHLSFMA IPPKKNQDKTEIPTINTIASGEPTSTPTTEAVESTVATLEDSPEVIESPPEINTVQ VTSTAV		

ID[*,**]	name of peptide	activity	number of repetitions	sequence	location
1	2	3	4	5	6
3258	β-lactokinin	antihypertensive	1	IR	[9-10]
3372		antihypertensive	1	PYP	[57-59]
3380	ACE inhibitor	antihypertensive	1	RY	[34-35]
3489	ACE inhibitor from sake lees	antihypertensive	1	RF	[16-17]
3495	**ACE inhibitor from κ-casein**	**antihypertensive**	**1**	**YIPIQYVLSR**	**[25-34]**
3522	ACE inhibitor	antihypertensive	1	IPP	[108-110]
3553	ACE inhibitor	antihypertensive	1	YG	[38-39]
3597		antihypertensive	1	AIP	[107-109]
3666		antihypertensive	2	YP	[35-36], [58-59]
3973	ACE inhibitor	antihypertensive	1	YGL	[38-40]
3291	**bovine κ-casein fr. 113-116**	**antithrombotic**	**1**	**NQDK**	**[113-116]**
3292	bovine κ-casein fr.106-116	antithrombotic	1	MAIPPKKNQ DK	[106-116]
3293	bovine κ-casein fr. 106-111	antithrombotic	1	MAIPPK	[106-111]
3294	bovine κ-casein fr. 106-112	antithrombotic	1	MAIPPKK	[106-112]
3213	**Casoxin from bovine** κ-casein fr. 25-34	immunomodulating	1	YIPIQYVLSR	[25-34]
3770		immunomodulating	1	YG	[38-39]
2882	opioid fragment of α-lactorphin fr. 50-51	opioid	1	YG	[38-39]
3214	Casoxin from bovine κ-casein fr: 35-41	opioid antagonist	1	YPSYGLN	[35-41]

cont. Table 4

1	2	3	4	5	6
3218	Casoxin	opioid antagonist	1	YPYY	[58-61]
3306		antioxidative	1	HPHL	[100-103]
3307		antioxidative	1	PYY	[59-61]
3311		antioxidative	2	HPH	[98-100], [100-102]
3317		antioxidative	1	HL	[102-103]
3215	**casoxin C**	**smooth muscle contracting**	**1**	**YIPIQYVLSR**	**[25-34]**
3813		smooth muscle contracting	1	YVLSR	[30-34]
3751		ligand of bacterial permease	1	KK	[111-112]
2796	platelet inhibitor (fr. 106-116 of bovine κ-casein)	antithrombotic	1	MAIPPKKN QDK	[106-116]
3167	diprotin A	enzyme inhibitor	1	IPI	[26-28]
3170	dipeptidyl peptidase IV inhibitor	enzyme inhibitor	2	PP	[109-110], [156-157]
3172	dipeptidyl-aminopeptidase IV inhibitor	enzyme inhibitor	2	VA	[48-49], [143-144]
3173	dipeptidyl-aminopeptidase IV inhibitor	enzyme inhibitor	2	MA	[95-96], [106-107]
3179	dipeptidyl-aminopeptidase IV inhibitor	enzyme inhibitor	3	PA	[64-65], [70-71], [84-85]
3180	dipeptidyl-aminopeptidase IV inhibitor	enzyme inhibitor	1	LP	[56-57]
3181	dipeptidyl-aminopeptidase IV inhibitor	enzyme inhibitor	1	VP	[83-84]
3203	chymosin inhibitor (fr. 99-105 of bovine κ-casein)	enzyme inhibitor	1	PHPHLSF	[99-105]
3216	**casoxin C**	**opioid antagonist**	**1**	**YIPIQYVLSR**	**[25-34]**
3217	casoxin (fr. 33-38 of bovine κ-casein)	opioid antagonist	1	SRYPSY	[33-38]

* – Bioactive fragments potentially released by trypsin are marked bold

** – The fragment 25-34 is multifunctional and thus inserted into BIOPEP database with more than one accession number. Every number corresponds to separate activity.

The protein was analysed by chromatography. Figure 1 shows an example chromatogram of cow's κ-casein. The UV spectroscopy and MS revealed purity of the protein used.

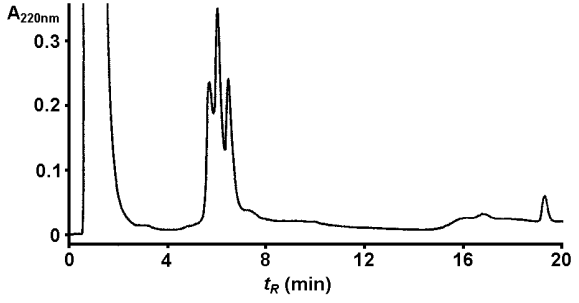

Fig. 1. Chromatogram (RP-HPLC) of bovine κ-casein

Figure 2 shows the mass spectrum of the tryptic hydrolysate of κ-casein. The most intensive peak at 1251 Da corresponded to the fragment with the sequence YIPIQYVLSR (fragment 25 – 34 κ-casein). This was a multifunctional peptide with antihypertensive, opioid antagonist, immunomodulating and smooth muscle contracting activity (CHIBA et al. 1989, TAKAHASHI et al. 1997, MEISEL 1998).

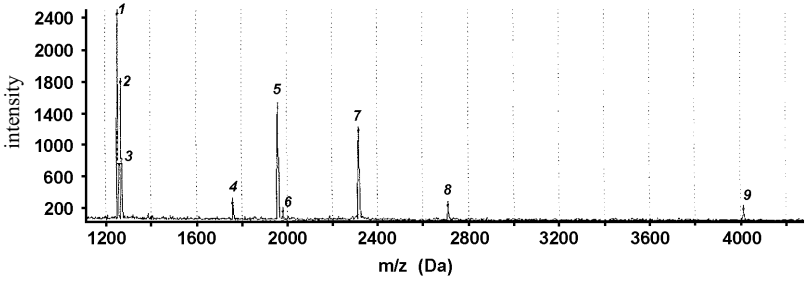

Fig. 2. Mass spectrum of a tryptic hydrolysate of bovine κ-casein. 1 – mass to charge (m/z) ratio 1251.809 Da (peptide: YIPIQYVLSR); 2 – m/z = 1267.826 Da; 3 – m/z = 1269.607 Da; 4 – m/z = = 1760.148 Da; 5 – m/z = 1960.210 Da; 6 – 1980.361 Da; 7 – m/z = 2317.434 Da; 8 – 2708.686 Da; 9 – 4011.239 Da

The results of a computer simulation of proteolysis, confirmed in the laboratory for κ-casein hydrolysed by trypsin indicated a relatively high possibility of obtaining bioactive peptides from this protein. The phrase "relatively high possibility" refers to a comparison of the results of computer

simulation of proteolysis with experimental results and not to the overall number of bioactive motifs in precursor proteins. Bioinformatic methods applied in biotechnology or biochemistry are becoming more and more popular thanks to a reduced time of waiting for results, low cost, possibility to record the results in text files, which makes them repeatable, and to their constant development with progress in information technology.

Earlier (IWANIAK et al. 2005) and current research allowed for the preparation of a strategy of protein and bioactive peptide research. The strategy is being constantly developed by us and can be illustrated by the diagram shown in Figure 3.

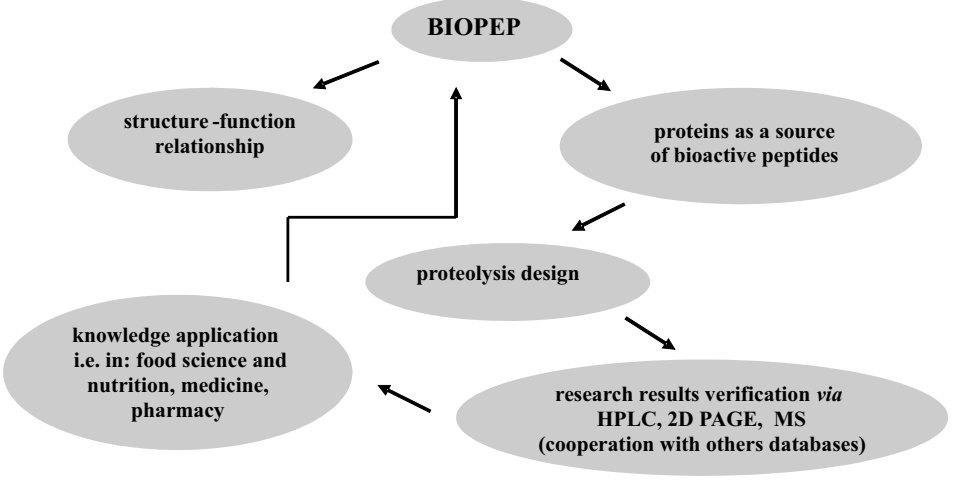

Fig. 3. Strategy of research on proteins and bioactive peptides

Conclusions

1. Based on the frequency of occurrence of bioactive fragments in a protein sequence all proteins can be classified into families – precursors of peptides of specific biological activity, and into subfamilies.

2. The number of proteins in families varies. The largest ones comprise proteins – precursors of antihypertensive peptides and of dipeptidyl-peptidase IV inhibitors, while the smallest ones are precursors of haemolytic peptides.

3. The applied proteolytic enzymes can release peptides from their protein precursors with twenty out of the twenty-three types of activity. None of the

examined enzymes released chemotactic, red blood cells synthesis stimulating or haemolytic peptides from their protein precursors.

4. Depending on a hydrolysed protein, combinations of two enzymes enhanced the effectiveness of releasing bioactive peptides for the following types of activity: antithrombotic, antiamnestic, regulating of stomach mucosal membrane activity, antioxidative, immunomodulating, opioid, dipeptidyl-peptidase IV inhibitory. Thanks to the use of two enzymes there is possible to release phosphoinositole action regulating, antibacterial, antiviral, coeliac-toxic and embryotoxic fragments not released by single enzymes.

5. The results of computer simulation of proteolysis, confirmed in laboratory for κ-casein hydrolysed by trypsin indicate the relatively high possibility of obtaining bioactive peptides from it. The phrase "relatively high possibility" refers to a comparison of the results of computer simulation of proteolysis with experimental results and not to the overall number of bioactive motifs in precursor proteins.

6. The results obtained in the study indicate that many peptides, which can be introduced to food as physiologically active components, such as antihypertensive, metal ion transporting, immunomodulating, antibacterial and antioxidative peptides, are not generally hydrolysed by proteolytic enzymes of the alimentary tract.

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MILLING OF WHEAT GRAIN AS THE WAY TO CONTROL THE PROTEIN CONTENT IN FLOUR*

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K e y w o r d s: wheat grain, variety, milling, flour, protein content, rheology of dough.

A b s t r a c t

In the last decade the number of adult consumers indicated the intolerance of wheat proteins is growing. It means the need to produce cereals or bread of reduced content of gliadins. The first step was to test if the milling of grains and their sizes differentiated the protein content in flour and its quality. The material of experiment were three Polish varieties of wheat of known growing technology. The grains were sieving to obtain the fractions of proper sizes. The milling process was conducted to produce the flour of different content of break and reduction flours. It was found that the content of proteins in flour of I break is lowest compare to others flours (break flours + reduction flours) and depends of variety and size of grains. The rheological quality of dough is statistical significant among investigated flours. The investigation will be continued in proteomic analysis of proteins of I break flour.

PRZEMIAŁ ZIARNA PSZENICY A ZAWARTOŚĆ BIAŁKA W MĄCE

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S ł o w a k l u c z o w e: ziarno pszenicy, odmiana, przemiał, mąka, zawartość białka, reologia ciasta.

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Abstrakt

W ostatnim dziesięcioleciu zwiększyła się liczba osób dorosłych z nietolerancją pokarmową białek zbożowych. Wynika z tego potrzeba produkcji przetworów zbożowych i piekarskich o obniżonej zawartości gliadyn. Pierwszym etapem badań w tym zakresie było sprawdzenie, czy operacje technologiczne w przemiale ziarna pszenicy i jego cechy geometryczne różnicują zawartość białka w mące oraz w jakim stopniu zmieniają się cechy reologiczne ciasta. Materiałem badań było ziarno trzech polskich odmian pszenicy o znanej technologii produkcji. Ziarno przed przemiałem było frakcjonowane wg wielkości, a frakcje stanowiące pozostałość na sitach kierowano oddzielnie do przemiału. W schemacie przemiałowym uwzględniono komponowanie mąki z różnym udziałem mąki śrutowej i wymiałowej. Stwierdzono, że zawartość białka w mące I śrutu jest najniższa, w porównaniu z mąkami komponowanymi (śruty + wymiały), i zależy od odmiany i wielkości ziaren. Cechy reologiczne ciasta różniły się istotnie statystycznie między badanymi mąkami. Kontynuacją badań będą badania proteomiczne białek mąki I śrutu.

Introduction

In the last decade, increasing attention has been paid to the functions and significance of toxic and allergenic proteins of seeds in the increasing incidence of atopic celiac disease (RUJNER, SOCHA 2001). The toxicity of plant proteins that induce food intolerance, especially prolamines of cereal grains and glycine of legume seeds, is linked with an amino acid sequence of terminal groups rich in proline, tyrosine or threonine (CORNELL, WILLS-JOHNSON 2001). Of great significance is also the molecular weight of a peptide, the frequency of proline occurrence and the number of hydrogen bonds. These relationships have been elucidated, to a great extent, by proteomic researches, with special attention paid to the following amino acid sequences: PSQQ, QQQP, QQPY.

The processing of cereal grains and legume seeds causes a number of changes in the biochemical characteristics and content of proteins in cereal products, which are connected with action of: temperature, water or mechanical forces. Particular significance can also be attributed to such characteristics of raw material as: species, variety and size distribution of grains. Among the technological operations, consideration should be given to sorting raw material, variety homogeneity, milling schemes, and modeling flour composition in terms of reduced gliadin content.

The investigations of KUBIAK and FORNAL (1994, 1995) as well as MAJEWSKA et al. (1997, 2000) have indicated that, independence of variety, wheat grains constituting remnants on a screen with a mesh size of > 3.15 mm x x 25 mm and < 2.2 mm x 25 mm are characterized by a different concentration of protein per area unit (mm^2). The proteomic characteristics of the proteins present in wheat grains of these fractions is unknown. Such studies should be preceded by confirming that composing flour during wheat grain milling may be applied in the production of cereals and bakery products with a reduced content of proteins inducing food intolerance.

The following assumptions were adopted in this study: grains of winter wheat varieties with various technological characteristics (Sukces, Tonacja) and grains of spring wheat variety (Nawra), fractionation, milling of isolated fractions and an analysis of the quality of experimentally-composed flour.

Material and Methods

The experimental material were two winter wheat varieties: Sukces (according to Polish Standard quality class A) and Tonacja (quality class B), and one spring variety Nawra (quality class A). The grain originated from 2004 harvest of the Production and Experimental Station Bałcyny Ltd. Elements of wheat cultivation technology are presented in Table 1. The cultivation technology applied involved the complete protection of plants against development of fungal diseases which restrict the synthesis of protective proteins that are considered to be toxic. It was assumed, then, that the grains applied in the study were free of that group of proteins.

Grains after the removal of impurities (fraction F_0) were sorted on screens. Two fractions were obtained: fraction F_1 of grains sifted through a sieve with a mesh size of < 2.2 mm x 25 mm, and a fraction of residues on a sieve with a mesh size of > 2.2 mm x 25 mm which was then divided into the following subfractions:

– 2.2 mm x 25 mm < F_2 < 2.5 mm x 25 mm;
– 2.5 mm x 25 mm < F_3 < 2.8 mm x 25 mm;
– F_4 > 2.8 mm x 25 mm.

Each grain fraction that was sorted from the sieves was characterized by the primary geometric features with the use of a digital image analysis (DIA) with LUCIA G ver. 4.80 software (KOZIROK 2005). The grains were milled in a MLU-202 grinder, assuming the scheme of experimental flour composition presented in Table 2. The MLU-202 laboratory mill consists of three break and three reduction passages. Each passage is coupled with a sifting machine. In technical design, the milling scheme of the laboratory mill simulates the basic operations of grain milling in an industrial scale.

The evaluation of grain and flour included determination of: protein content (PN-75/A-040018), Zeleny's sedimentation index (PN-ISO5529), and falling number (PN-ISO3093). The rheological properties of dough were evaluated by determining viscosity and temperature range of flour suspension gelatinization – with the use of amylographic (PN-ISO7973), farinographic (PN-ISO5530-1) and alveographic tests (PN-ISO5530-4).

Statistical analyses, including determination of average mean, significance of differences between the means (one-way analysis of variance with Duncan's test, $p = 0.05$) and correlations, were all carried out with the use of Statistica 6.0 PL software.

Table 1

The conditions of grain production technology

Variety	Term of treatment	Fertilization	Chemical treatment of plant (name of pesticides)
Sukces Tonacja	14.09.2003	100 kg potasium salt (56% K_2O)	
	18.09.2003	sieving, seed dressing with RAXIL 060 FS, 50 $cm^3 \cdot (100\ kg)^{-1}$ of seeds	
	19.09.2003	Legan 25 g · ha	
	01.04.2004	ammonium nitrate 250 kg · ha (85 kg N · ha^{-1})	
	30.04.2004		Juwell TT 1.2 l · ha^{-1} Ekolist 2 l · ha^{-1} Cycocel 2 l · ha^{-1}
	01.06.2004	ammonium nitrate 100 kg – 34 kg N · ha^{-1}	
	20.06.2004		Swing 1.5 l · ha^{-1}
	07.07.2004		Alphacyper 0.1 l · ha^{-1}
Nawra	01.04.2004	100 kg potasium salt (56% K_2O) 100 kg ammonium phosphate 12% N, 52% P_2O_5	
	03.04.2004	sieving – seed dressing MAXIM 025 FS	
	23.04.2004	RSM 32% 70 kg N · ha^{-1}	
	12.05.2004		Mocarz 200 g · ha^{-1}
	17.05.2004		Bavistin 0,5 l · ha^{-1} Corbel 0,5 l · ha^{-1} Stabilan 750 l · ha^{-1}
	09.06.2004	RSM 32% 35 kg N · ha^{-1}	
	28.06.2004		Alphacyper 0.1 l · ha^{-1} TANGO 1.5 l · ha^{-1}

Table 2

A scheme of milling modeling

Variety	Fraction – size of grain	Type of flour
Sukces Tonacja Nawra	F_0 – random sample	total flour (I-III brake + I-III reduction) I brake flour I reduction flour II brake flour + II reduction flour III brake flour + III reduction flour
Sukces Tonacja Nawra	F_1 < 2.2 mm x 25 mm	total flour (I-III brake + I-III reduction) I brake flour I reduction flour II brake flour + II reduction flour III brake flour + III reduction flour
Sukces Tonacja Nawra	2.2 mm x 25 mm < F_2 < 2.5 mm x 25 mm	total flour (I-III brake + I-III reduction) I brake flour I reduction flour II brake flour + II reduction flour III brake flour + III reduction flour
Sukces Tonacja Nawra	2.5 mm x 25 mm < F_3 < 2.8 mm x 25 mm	total flour (I-III brake + I-III reduction) I brake flour I reduction flour II brake flour + II reduction flour III brake flour + III reduction flour
Sukces Tonacja Nawra	F_4 > 2.8 mm x 25 mm	total flour (I-III brake + I-III reduction) I brake flour I reduction flour II brake flour + II reduction flour III brake flour + III reduction flour

Results and Discussion

Granulometric characteristics of wheat grain

The size distribution was determined based on the fractionation of a mixture of grains on sieves (Table 3). Among the examined varieties, the greatest share was observed for fraction F_4, and the lowest were for fractions F_2 and F_1. The share of grains constituting residues and fines on a sieve with a mesh size of 2.2 mm x 25 mm ranged from ca 5% to 17%, depending on the variety. This fraction of grain may be of significance in further discussion on proving the research thesis. The question then arises as to whether the actual sizes of these fractions are differentiated enough in the automation of the process of their discrimination. Hence, the mean values of geometric features appear to

discriminate the greatest extent fractions F_1 and F_4 (Table 3). This is also presented in the graphical distribution of the occurrence of mean values of the analyzed features (Figures 1, 2). The dependency between the width and length of grains and concentration of their sizes indicates the possibility of combining fractions F_4 and F_3 as well as fractions F_2 and F_1 and their isolation in consideration of directed milling (Figure 3).

Table 3

Size distribution and geometrical features of grain

Variety	Fraction	Content of fraction (%)	D* (mm)	d* (mm)	S* (mm^2)	P* (mm)	W* (–)
Sukces	F_1	6.7	6.46	2.67	12.91	16.08	0.42
	F_2	11.5	6.67	3.14	15.76	17.01	0.40
	F_3	27.1	7.10	3.59	19.19	18.45	0.51
	F_4	54.8	7.22	3.76	20.73	19.12	0.52
Tonacja	F_1	4.1	6.20	2.82	12.88	15.44	0.46
	F_2	6.8	6.71	3.13	15.46	16.73	0.47
	F_3	21.7	7.22	3.43	18.32	18.17	0.48
	F_4	67.4	7.49	3.76	21.27	19.33	0.50
Nawra	F_1	2.1	6.29	2.72	12.76	15.59	0.43
	F_2	2.8	6.48	2.91	13.97	16.41	0.45
	F_3	22.4	7.12	3.29	17.56	17.98	0.46
	F_4	72.8	7.44	3.50	19.91	19.08	0.47

Symbols: D – length, d – width, S – surface, P – perimeter, W – shape ratio determined by formula W = d/D;
* – average of 200 measurements

Chemical characteristics of grain

The chemical characteristics of grain involved determination of such traits as protein content, Zeleny's sedimentation index and falling number (Table 4). The content of protein in grains was relatively low; however, the highest was in grain of the Nawra variety. In contrast, the protein content in grains of the isolated fractions of winter varieties Sukces and Tonacja indicated only their similarity and simultaneous diversity compared to the Nawra variety. Values of the sedimentation index, indirectly indicating the content of high-molecular weight glutenins, were differentiated to the greatest extent as affected by variety and grain size. This refers mainly Sukces and Nawra varieties (quality class A). The falling number showed to a low activity of α-amylase that was statistically different between varieties. A relatively low falling number was reported for grains of Tonacja variety, which may indicate greater synthesis of albumin as a variety-specific properties.

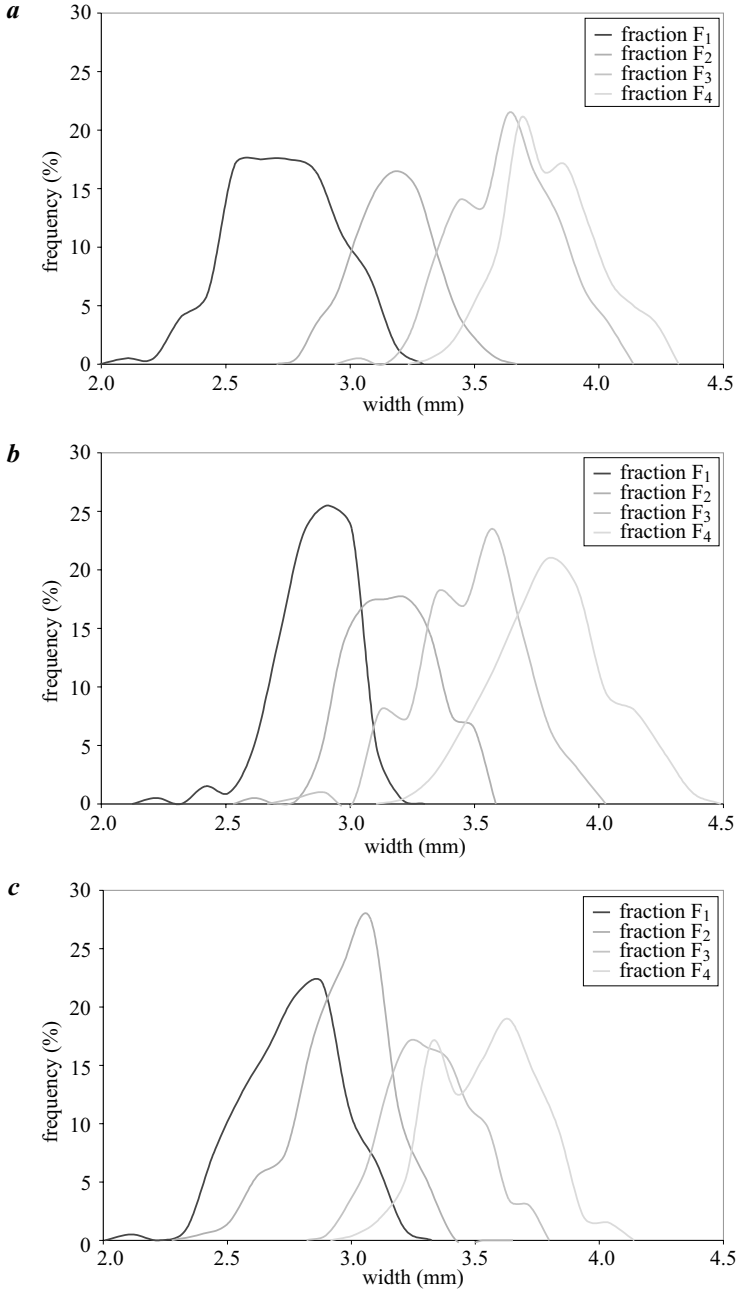

Fig. 1. Distributions of width values of the wheat grain varieties: *a* – Sukces, *b* – Tonacja, *c* – Nawra

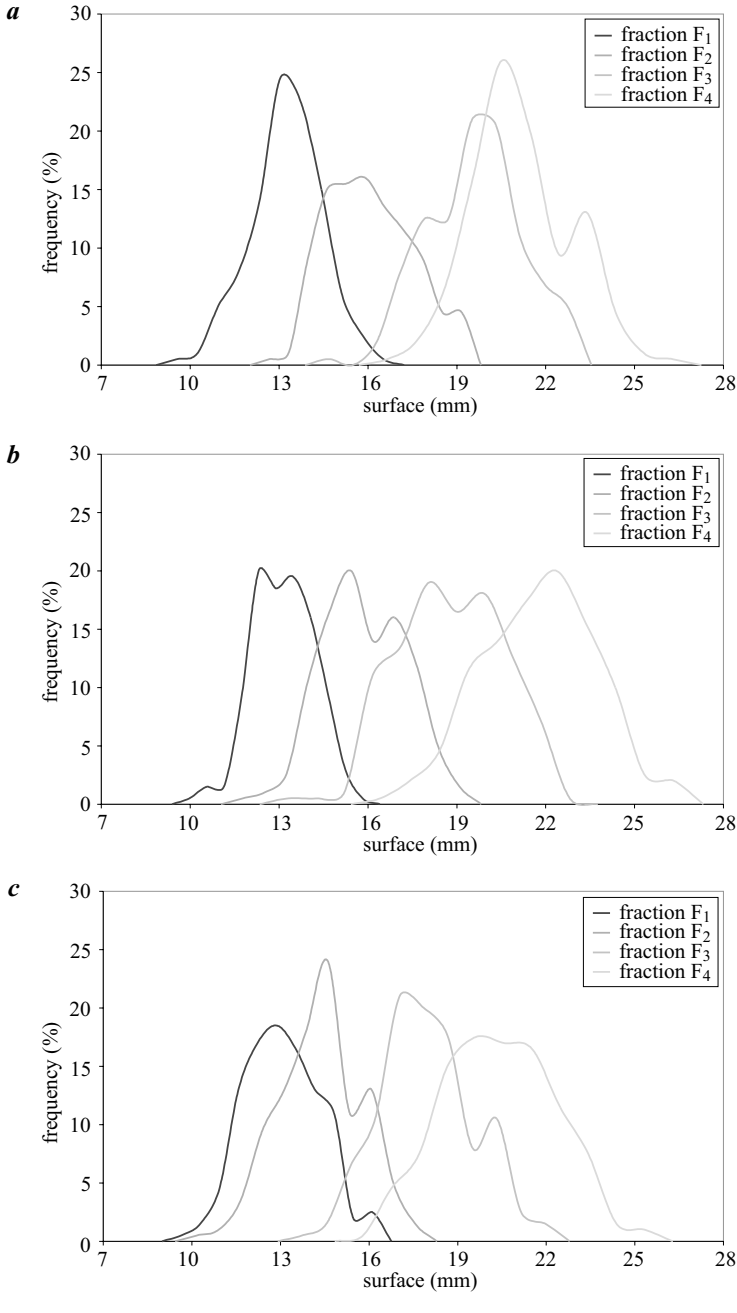

Fig. 2. Distributions of surface values of wheat grain varieties: *a* – Sukces, *b* – Tonacja, *c* – Nawra

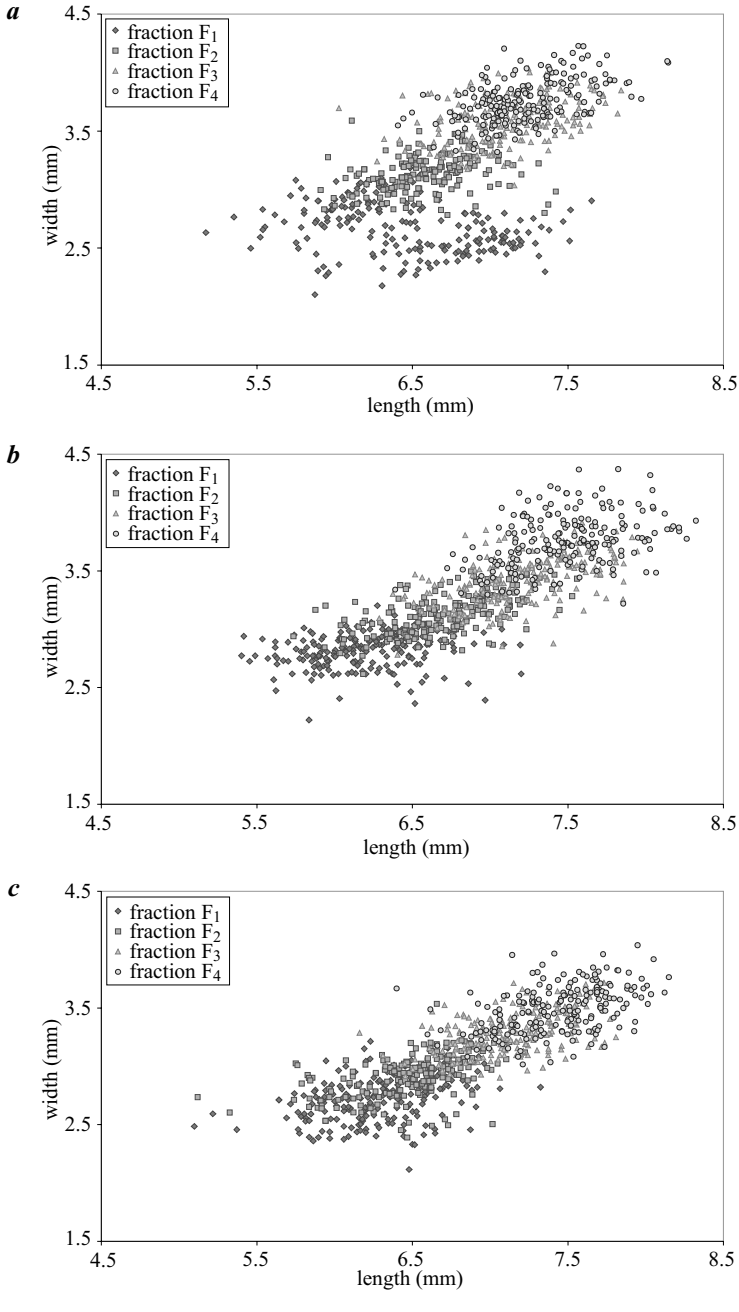

Fig. 3. The relation of length values against width values of wheat grain varieties: *a* – Sukces, *b* – Tonacja, *c* – Nawra

Table 4
Protein content, value of Zeleny's sedimentation test and falling number in grains depending on variety, grain size and milling scheme

Variety	Fraction	Protein content (N · 5.7) (% d.m.)	Zeleny's sedimentation test (cm^3)	Falling number (s)
Sukces	F_0	10.9	31	394
	F_1	10.2	28	383
	F_2	10.5	28	409
	F_3	10.9	32	396
	F_4	10.1	33	378
	$x_{av.}$	10.5^a	30^a	392^a
Tonacja	F_0	11.0	34	284
	F_1	10.4	31	276
	F_2	10.4	33	314
	F_3	10.8	35	322
	F_4	11.2	35	271
	$x_{av.}$	10.8^a	34^{ab}	293^b
Nawra	F_0	12.1	42	355
	F_1	11.6	33	300
	F_2	10.9	32	331
	F_3	11.5	35	374
	F_4	12.4	34	324
	$x_{av.}$.	11.7^b	35^b	337^c

Symbols: a, b, c – mean values in lines marked by the same letter are not significantly different ($p = 0.05$)

Protein content and sedimentation index of flour

The milling of grain according to the designed scheme indicated a significant effect of flour composition on total protein content (Table 5). The highest protein content was reported for III break flour + III reduction flour, however, the highest was always found in flour from grains of fraction F_4. A factor that differentiated this tendency was variety. In turn, the lowest protein content was determined in I break flour from grains of the smallest sizes (Table 5).

The statistical analysis demonstrated that the mean contents of protein in total extracted flour and I break flour from grains of the Sukces variety do not differ significantly (Table 5). Only the protein content in III break flour + III reduction flour was significantly different. In contrast, no statistically significant differences were observed as affected by grain size. Statistically significant differences were also observed in the mean content of protein in total flour and III break flour + III reduction flour in the case of the Nawra variety as well as between the protein content of total flour and that of I break and III break + III reduction from grains of the Tonacja variety.

Values of the sedimentation index were the lowest in I break flour, irrespective of grain size and variety (Table 5). Only III break flour + III reduction flour of fractions F_2 and F_4 of the Nawra variety were also characterized by the lowest values of that index. The statistical analysis demonstrated

Table 5
Protein content and value of Zeleny's sedimentation test in flour depending on variety, grain size and milling scheme

Variety	Fraction	Total flour		I break flour		III break flour + III reduction flour	
		B (% d.m.)	WS (cm^3)	B (% s.m.)	WS (cm^3)	B (% d.m.)	WS (cm^3)
Sukces	F_0	9.7	33	8.8	26	12.7	38
	F_1	9.0	31	7.8	24	10.4	29
	F_2	9.2	32	8.0	25	10.4	29
	F_3	9.5	36	8.6	28	12.1	36
	F_4	9.8	36	8.9	28	13.2	39
	$x_{av.}$	9.4^{aA}	33^{aA}	8.4^{aA}	26^{bA}	11.8^{bA}	34^{aA}
Tonacja	F_0	9.7	35	8.6	29	13.1	41
	F_1	9.2	34	8.0	27	11.0	35
	F_2	9.0	36	7.8	26	10.5	32
	F_3	9.4	37	8.4	28	12.0	40
	F_4	9.7	37	8.7	28	13.2	41
	$x_{av.}$	9.4^{aA}	36^{aA}	8.3^{bA}	28^{bA}	12.0^{cA}	38^{aA}
Nawra	F_0	10.7	40	9.9	32	14.0	31
	F_1	10.2	38	8.9	29	11.9	32
	F_2	9.6	36	8.1	27	11.7	19
	F_3	10.0	39	8.6	30	12.3	30
	F_4	10.9	42	10.1	34	14.4	24
	$x_{av.}$	10.3^{aB}	39^{aB}	9.1^{aA}	30^{bB}	12.9^{bA}	27^{bB}

Symbols: B – protein content (N · 5.7), WS – value of Zeleny's sedimentation test;
a, b, c – mean values in lines marked by the same letter are not significantly different ($p = 0.05$), *A, B* – mean values in columns marked by the same letter are not significantly different ($p = 0.05$)

that the mean values of the sedimentation index of I break flour were differ significantly, irrespective of variety and grains size.

The results described above confirm the possibilities of modeling grain milling so that the flour had low protein content. Its application in the production of flour with a reduced content of proteins inducing food intolerance depends on the biochemical characteristics of proteins of I break flour according to proteomic studies. It will also require prognosing which mass of grains with the smallest sizes may be considered. The data collated in Tables 3 and 5 indicate that a combination of two fractions, F_1 and F_2, may constitute from 5 to 17% of commercial mass.

Evaluation of the rheological properties of dough

The content of protein and its fraction composition, lipid profile and properties of starch determine, to a great extent, viscosity, fermentation capacity and the rheological properties of dough (ROTKIEWICZ et al. 2003).

The activity of α-amylase determined by using the test of falling number (results not shown) was low in all examined flours from grains of the Sukces and Nawra varieties. Only flour from grains of the Tonacja variety was differed in this respect. This is highly in line with the activity of α-amylase in grain. In addition, I break flour of this variety, irrespective of grains size, was characterized by a high activity of α-amylase. These observations were confirmed by the results of amylographic evaluation (Table 6, Figure 4). A high viscosity was observed at the maximum temperature of starch gelatinization – of flour from grains of the Sukces and Nawra varieties. Only flour from grains of the Tonacja variety demonstrated a viscosity lower than 500 AU (Figure 4). Results of the statistical analysis showed that the maximum viscosity and final temperature of starch gelatinization – of I break flour are statistically significantly different than the other composed flours.

In turn, the rheological properties of dough determined with the alveographic and farinographic method pointed to the significance of both variety, grain size and the milling scheme applied (Tables 7, 8). Flour of I break from grains of the smallest fraction had the lowest water absorption, time of dough development and stability of dough (Table 8). These properties of I break flour were common and statistically significant for the investigated varieties.

Table 6
Viscosity and temperature of gelatinization depending on variety, grain size and milling scheme

Variety	Fraction	Total flour			I break flour		
		max L (AU)	Tp (°C)	Tk (°C)	max L (AU)	Tp (°C)	Tk (°C)
Sukces	F_0	750	58.0	89.0	770	58.5	89.5
	F_1	750	57.5	89.5	770	58.5	89.5
	F_4	900	57.0	88.5	930	57.0	87.0
	$x_{av.}$	800^{aA}	57.5^{aA}	89.0^{aA}	823^{aA}	58.0^{aA}	88.7^{aA}
Tonacja	F_0	300	55.5	78.0	190	56.0	71.0
	F_1	310	57.0	78.5	190	57.0	72.0
	F_4	230	57.0	76.5	150	55.5	70.0
	$x_{av.}$	280^{aB}	56.5^{aA}	77.7^{aB}	177^{bB}	56.2^{aB}	71.0^{bB}
Nawra	F_0	870	57.0	86.0	800	58.5	90.0
	F_1	630	57.0	84.0	540	58.5	84.0
	F_4	860	57.0	88.5	750	58.5	88.0
	$x_{av.}$	787^{aA}	57.0^{aA}	86.2^{a}	697^{aA}	58.5^{bA}	87.3^{aA}

Symbols: Max L – maximal viscosity, Tp – initial temperature of gelatinization, Tk – final temperature of gelatinization;
a, b, c – mean values in lines marked by the same letter are not significantly different ($p = 0.05$), A, B – mean values in columns marked by the same letter are not significantly different ($p = 0.05$)

Table 7

Alveographic evaluation of flour depending on variety, grain size and milling scheme

Variety	Fraction	Total flour						I break flour					
		W	P	L	P/L	G	I_e	W	P	L	P/L	G	I_e
Sukces	F_0	229	93	68	1.37	18.4	55	180	79	59	1.35	17.1	56
	F_1	139	74	57	1.28	16.8	41	126	57	68	0.85	18.3	45
	F_4	239	107	58	1.84	17.0	56	190	83	59	1.41	17.1	57
	$x_{av.}$	202^{aA}	91^{aA}	61^{aA}	1.50^{aA}	17.4^{aA}	51^{aA}	165^{aAB}	73^{aA}	62^{aA}	1.20^{aA}	17.5^{aA}	53^{aAB}
Tonacja	F_0	172	68	84	0.81	20.5	46	143	58	79	0.73	19.8	47
	F_1	158	63	90	0.70	21.2	43	125	49	89	0.55	21.0	44
	F_4	181	75	81	0.93	20.0	46	130	54	83	0.65	20.2	44
	$x_{av.}$	170^{aA}	69^{aA}	85^{aB}	0.81^{aB}	20.6^{aB}	45^{aA}	$133b^{A}$	$54b^{A}$	84^{aB}	0.64^{aB}	20.3^{aB}	45^{aA}
Nawra	F_0	299	89	99	0.90	22.1	59	221	82	67	1.23	18.2	63
	F_1	190	61	104	0.59	22.7	51	178	61	85	0.72	20.5	55
	F_4	325	104	86	1.21	20.6	61	287	83	95	0.87	21.7	62
	$x_{av.}$	271^{aA}	85^{aA}	96^{aB}	0.90^{aB}	21.8^{aB}	57^{aA}	229^{aB}	75^{aA}	82^{aB}	0.94^{aAB}	20.1^{aB}	60^{aB}

Symbols: W – deformation energy (baking value), P – maximum pressure parameter (corresponds to the maximal resistance or the dough during deformation), L – length of curve (indicates the extensibility of the dough until the breaking point), P/L – curve configuration ratio, G – index of swelling, Ie – elasticity index;
a, b, c – mean values in lines marked by the same letter are not significantly different ($p = 0.05$);
A, B – mean values in columns marked by the same letter are not significantly different ($p = 0.05$).

Table 8

Farinographic evaluation of flour depending on variety, grain size and milling scheme

Variety	Fraction	Total flour					I break flour				
		WA (%)	R (min)	S (min)	Rm (FU)	LJ (–)	WA (%)	R (min)	S (min)	Rm (FU)	LJ (–)
Sukces	F_0	60.8	1.7	1.4	100	27	57.7	1.2	1.2	90	22
	F_1	59.3	1.7	1.6	100	28	–*	–*	–*	–*	–*
	F_4	60.6	2.2	2.6	80	42	57.7	1.4	1.5	80	26
	$x_{av.}$	60.2^{aA}	1.9^{aA}	1.9^{aA}	93^{aA}	32^{aA}	57.7^{bA}	1.3^{aA}	1.4^{aA}	85^{aA}	24^{aA}
Tonacja	F_0	59.8	3.3	4.2	100	58	56.3	1.3	2.0	100	31
	F_1	58.9	2.2	3.7	100	58	56.2	1.4	1.1	110	20
	F_4	60.3	2.2	4.1	100	57	57.5	1.4	1.7	120	26
	$x_{av.}$	59.7^{aA}	2.6^{aA}	4.0^{aB}	100^{aA}	58^{aB}	56.7^{bA}	1.4^{bA}	1.6^{bA}	110^{aB}	26^{bA}
Nawra	F_0	60.0	2.2	2.6	70	38	59.0	1.4	1.3	70	23
	F_1	57.2	1.8	2.0	70	32	–*	–*	–*	–*	–*
	F_4	61.8	1.7	4.6	60	56	60.2	2.2	3.9	50	45
	$x_{av.}$	59.7^{aA}	1.9^{aA}	3.1^{aAB}	67^{aB}	42^{aAB}	59.6^{aB}	1.8^{aA}	2.6^{aA}	60^{aAB}	34^{aA}

Symbols: WA – water absorption, R – dough development, S – dough stability, Rm – degree of viscosity, LJ – quality index;
* – not measured because amounts of flour was too small;
a, b, c – mean values in lines marked by the same letter are not significantly different ($p = 0.05$);
A, B – mean values in columns marked by the same letter are not significantly different ($p = 0.05$).

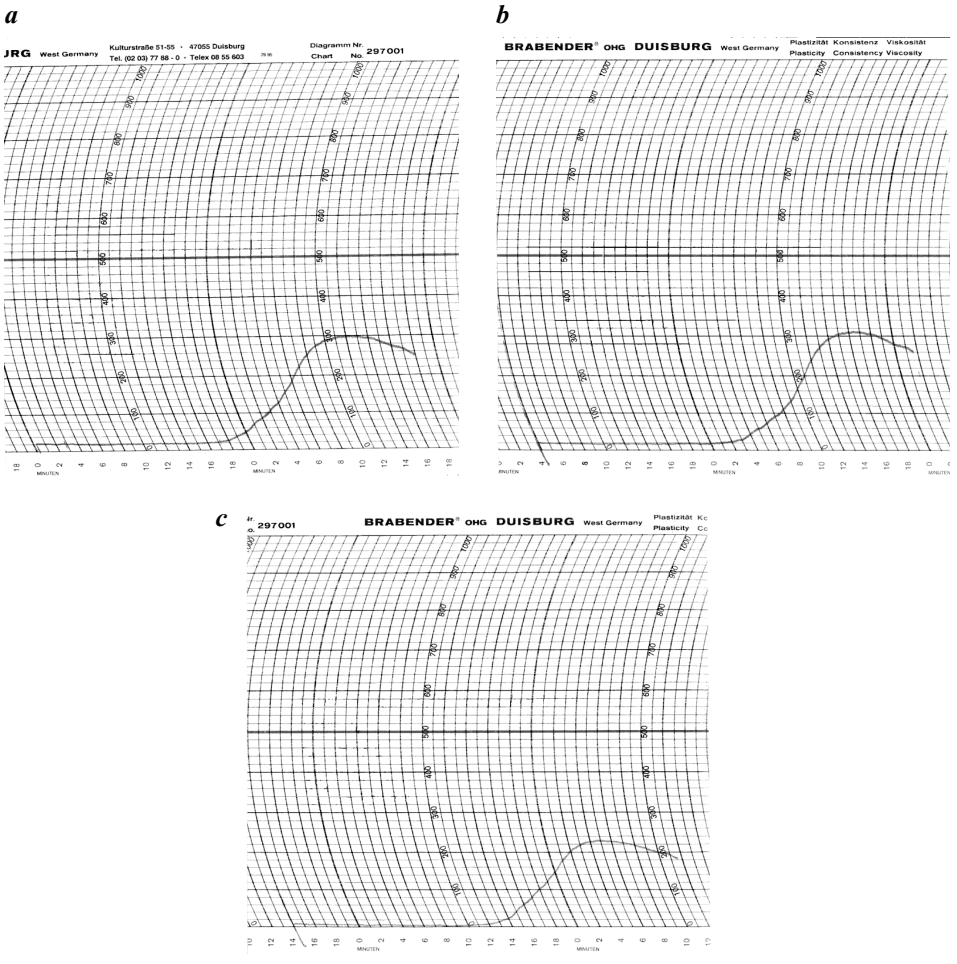

Fig. 4. Viscosity and temperature of gelatinization of flour obtained from Tonacja wheat grains: *a* – total flour, *b* – I break flour, *c* – III break flour + III reduction flour

Variety differences, grain size and the milling scheme are clearly documented by the alveographic evaluation (Table 7). The W value was significantly dependent on variety, grain size and type of flour. Likewise in the farinographic evaluation, dough made of I break flour from smaller grains was characterized by the lowest values of W, P, L, P/L and G and were statistically different compared to the total flour.

The presented characteristics of flour, considering such variables as: variety, grain size, milling scheme, were used to determine correlations. The coefficients of correlations calculated between pairs of varieties and

quality of all types of flour examined demonstrated significantly high values of correlation coefficients between the Sukces and Tonacja varieties, the Sukces and Nawra varieties as well as the Tonacja and Nawra varieties and protein content of flour (Table 9). The sedimentation index was highly correlated only in the flour of the Sukces and Tonacja varieties. The amylographic evaluation did not demonstrate any correlations. Likewise, most of the indicators in the farinographic evaluation had low correlation coefficients. In contrast, in the alveographic evaluation, high correlation coefficients were reported more frequently in the flour of the Sukces:Tonacja and the Sukces:Nawra varieties. High correlations, in turn, were observed between total flour and I break flour in terms of protein content sedimentation index, water absorption, dough softening, maximum viscosity, final temperature of starch gelatinization and parameters of alveographic evaluation: W, P and I_e.

Table 9

Correlation coefficients between pairs of varieties and flour types

Discriminant	Variety			Type of flour		
	Sukces : : Tonacja	Sukces : : Nawra	Tonacja : : Nawra	MO : MIś	MO : MIIIś + MIIIw	MIś : MIIIś + MIIIw
B	0.99*	0.97*	0.98*	0.96*	0.86*	0.91*
WS	0.95*	0.24	0.18	0.93*	–0.18	–0.13
Alveographic evaluation						
W	0.71	0.96*	0.60	0.95*	–	–
P	0.88*	0.93*	0.71	0.90*	–	–
L	0.28	0.07	0.63	0.61	–	–
P/L	0.90*	0.64	0.63	0.75*	–	–
G	0.28	0.08	0.64	0.63*	–	–
I_e	0.73	0.97*	0.75	0.97*	–	–
Viscosity and temperature of gelatinization						
MaxL	–0.59	0.31	0.12	0.99*	–	–
Tp	0.00	0.40	–0.24	0.59	–	–
Tk	0.37	–0.37	–0.43	0.97*	–	–
Farinographic evaluation						
WA	0.95*	0.34	0.38	0.80*	–	–
R	0.54	0.09	0.47	–0.39	–	–
S	0.52	0.78	0.18	0.64	–	–
Rm	–0.56	0.84	–0.88	0.92*	–	–
LJ	0.56	0.85	0.26	0.41	–	–

Symbols: MO – total flour, MIś – I break flour, MIIIś + MIIIw – III break flour + III reduction flour, B – protein content, WS – value of Zeleny's sedimentation test, W – deformation energy, P – maximum pressure parametr, L – lenght of cureie, P/L – curve configuration ratio, G – index of swelling, I_e – elasticity index, Max L – maximal viscosity, Tp – initial temperature gelatinization, Tk – final temperature gelatinization, WA – water absorption, R – dough development, S – dough stability, Rm – degree of viscosity, LJ – quality index;

* – correlation coefficients significant at $p = 0.05$

Conclusion

The results of this study confirm the possibility of modeling the protein content in wheat flour on the three tested variables: variety, grain size and flour of I break and flour of III break + III reduction. The quality traits of I break flour with the lowest protein content (regardless of the lower rheological properties of dough) are the subject of further studies that are aimed at determining whether the proteomic characteristics of these proteins also meet the conditions required for reducing their food intolerance. Meeting this requirement would be the basis for applying such flour in the production of cereal products recommended in diets of people sensitive to the presence of α gliadins. Nevertheless, when applying controlled milling, there is no possibility to obtain flour that would meet the conditions of the allowable content of α-gliadins in the diets of children and adults suffering from celiac disease.

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PROXIMATE COMPOSITION AND FATTY ACID PROFILE OF MUSCLES FROM THE SIBERIAN STURGEON (*ACIPENSER BAERI* BRANDT) X GREEN STURGEON (*ACIPENSER MEDIROSTRIS* AYRES) HYBRID

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K e y w o r d s: proximate components, fatty acids, white muscle, red muscle, dorsal part, ventral part, sturgeon.

A b s t r a c t

The aim of this study was to compare the proximate composition and fatty acid profile of the white (*m. lateralis magnus*) and red (*m. rectus lateralis*) muscle as well as of the dorsal and ventral part of the white muscle from the Siberian sturgeon x green sturgeon hybrid. The analysis of proximate components showed that the white and red muscle differed in fat content (6.05% vs. 9.67%) and in water content (77.72% vs. 74.13%), but had comparable concentrations of protein and ash. An identical relationship was observed while comparing the dorsal and ventral part of the white muscle: the latter contained more fat (7.38% vs. 5.14%) and less water (76.44% vs. 78.72%). The qualitative fatty acid profile of the above body parts of the hybrid was almost the same, differences were found only in the concentrations of some fatty acids and their groups. The red muscle and the ventral part had a higher fat content, and so contained relatively more monounsaturated fatty acids MUFAs (54.86% vs. 44.94% and 45.56% vs. 41.45%) and less polyunsaturated fatty acids PUFAs (20.48% vs. 29.07% and 28.27% vs. 32.29%), which resulted in a lower contribution of *n*-3 PUFAs as well as in lower concentrations of LC *n*-3 PUFAs, i.e. 20:5*n*-3 (eicosapentaenoic acid, EPA) and 22:6*n*-3 (docosahexaenoic acid, DHA), compared with the white muscle and the dorsal part, respectively.

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SKŁAD PODSTAWOWY I PROFIL KWASÓW TŁUSZCZOWYCH MIĘŚNI HYBRYDA JESIOTRA SYBERYJSKIEGO (*ACIPENSER BAERI* BRANDT) Z JESIOTREM SACHLIŃSKIM (*ACIPENSER MEDIROSTRIS* AYRES)

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S ł o w a k l u c z o w e: składniki podstawowe, kwasy tłuszczowe, mięsień biały, mięsień czerwony, część grzbietowa, część brzuszna, jesiotr.

A b s t r a k t

Celem badań było porównanie zawartości składników podstawowych i profilu kwasów tłuszczowych mięśni bocznego wielkiego (biały) i podskórnego bocznego (czerwony) oraz części grzbietowej i brzusznej mięśnia białego hybryda jesiotra syberyjskiego z jesiotrem sachalińskim. Analiza zawartości składników podstawowych wykazała, że mięśnie biały i czerwony różniły się zawartością tłuszczu (6,05% wobec 9,67%) oraz wody (77,72% wobec 74,13%), natomiast zawartość białka oraz popiołu mieściła się w zbliżonym przedziale wartości. Identyczną zależność stwierdzono, porównując części grzbietową i brzuszną mięśnia białego. Więcej tłuszczu (7,38% wobec 5,14%) i mniej wody (76,44% wobec 78,72%) zawierała ta ostatnia. Jakościowy profil kwasów tłuszczowych wymienionych części ciała hybryda pozostawał taki sam, różnice stwierdzono w zawartości niektórych kwasów tłuszczowych i ich grup. W mięśniu czerwonym oraz części brzusznej zawierających więcej tłuszczu stwierdzono relatywnie więcej monoenowych kwasów tłuszczowych MUFA (54,86% wobec 44,94% i 45,56% wobec 41,45%), a mniej polienowych kwasów tłuszczowych PUFA (20,48% wobec 29,07% i 28,27% wobec 32,29%). Spowodowało to zmniejszenie udziału *n*-3 PUFA i niższą w nich zawartość LC *n*-3 PUFA, czyli 20:5*n*-3 (ikozapentaenowy, EPA) i 22:6*n*-3 (dokozapentaenowy, DHA) w porównaniu odpowiednio z mięśniem białym i częścią grzbietową.

Introduction

Members of the family Acipenseridae have always been highly valued for their excellent eating quality. The decrease in the population size of sturgeons has contributed to an increasing interest in their farming under controlled environmental conditions (CHLEBANOV, BILLARD 2001, WILLIOT et al. 2001, PIKITCH et al. 2005). Experimental work on sturgeon breeding makes use of hybridization as a method for improving their functional characteristics. It has been demonstrated that sturgeon hybrids are superior to the parent lines in terms of survival and growth rates, feed conversion ratios and (which is most important to potential consumers) market value, i.e. meat yield and quality (BURTSEV 1969, BURTSEV et al. 1987, GERSHANOVICH, BURTSEV 1987, DOROFEEVA et al. 1982, JANKOWSKA et al. 2005). However, professional literature on the subject provides scant information on sturgeon hybrids and the quality

of their meat. This concerns also the Siberian sturgeon (*Acipenser baeri* Brandt) x green sturgeon (*Acipenser medirostris* Ayres) hybrid, produced for the first time in 1995. When raised in water reservoir tanks, this hybrid is characterized by a faster growth rate and a higher feed conversion ratio than the parental species (KOLMAN et al. 1997). The only available report on the quality of meat from this hybrid shows that the color (salmon-pink) of its fillet differs from the color (white-gray) of the fillet obtained from the Siberian sturgeon. In addition, the hybrid has relatively higher concentrations of polyunsaturated fatty acids (JANKOWSKA et al. 2002).

The aim of this study, which is a continuation of our previous research, was to compare the proximate composition and fatty acid profile of the white and red muscle as well as of the dorsal and ventral part of the white muscle from the Siberian sturgeon x green sturgeon hybrid.

Materials and Methods

The experimental materials comprised Siberian sturgeon x green sturgeon hybrids, cultured in a concrete tank, under natural thermal conditions. The hybrids came from a group aged 6+ years. The fish stayed in the tank over winter, without feed. In the spring, when the ice cover subsided, the fish were given pelleted feed for the trout, Aller – 45/15, containing 45% of protein, 21% of carbohydrates and 15% of fat. At first the ration was small, due to the low nutrient requirements of sturgeons, and then it was gradually increased, along with the increase in water temperature. When the fish were harvested for analysis, water temperature ranged between 19 and 22°C, i.e. it was optimal for the growth of the majority of sturgeon species (KOLMAN 1999). Over that period the daily ration was equal to 0.8 to 1.0% of fish body weight, in accordance with the relevant feeding standards (KOLMAN 1998).

Six individuals were taken for analysis. The fish were decapitated by a straight cut, eviscerated, filleted and skinned. *Musculus rectus lateralis* and *musclusus lateralis magnus* were separated from the right fillet. The left fillet (after the separation of *m. rectus lateralis*) was divided along the horizontal septum of connective tissue into the dorsal and ventral part. The muscles, passed through a 3 mm plate of a grinder, served as analytical material and were used to determine the concentrations of proximate components and the fatty acid profile.

Proximate components

Water content was determined by sample drying to constant mass at 105°C. Total protein content was determined by the Kjeldahl method, using the 6.25 multiplier. Fat content was determined by the Soxhlet method, with petroleum benzin as a solvent. Ash content was determined by sample mineralization at 550 to 600°C (*Official Methods*... 1975).

Fatty acid composition

A quantitative and qualitative analysis of the fatty acid composition was made following cold extraction of muscular lipids (FOLCH et al. 1957). Methylation of fatty acids was carried out using a chloroform:methanol:sulfuric acid mixture (100:100:1) (PEISKER 1964). Chromatographic separation was performed on a HP 6890 gas chromatograph with a flame-ionization detector (FID), a capillary column 30 m in length, 0.32 mm in inner diameter, liquid phase Supelcowax 10, film thickness 0.25 μm. Separation conditions: carrier gas – helium, flow rate – 1 $ml \cdot min^{-1}$; detector temperature – 250°C, injector temperature – 225°C, column temperature – 185°C. Signals from the detector were recorded with a 1mV full-scale Philips recorder, at a chart speed of 10 $mm \cdot min^{-1}$.

Statistical analysis

The values given are means ± S.E.M. Differences between means of the tested traits were calculated by one-factor analysis of variance (ANOVA). Student-Newman-Keuls test was applied. Statistically significant differences were determined at $P \leq 0.01$. Computations were done using Statistica 6.0 PL software.

Results

White and red muscle

The analysis of proximate components showed that the white and red muscle differed significantly ($p \leq 0.01$) in the content of fat and water. The fat content of the white muscle amounted to 6.05% and was by 38% lower in

comparison with the red muscle. The differences in fat content were compensated by water content, which was 77.72% and 74.13% in the white and red muscle, respectively. Both muscles had comparable concentrations of protein and minerals (Table 1).

Table 1

Concentrations of basic components in the white and red muscle (%) (mean ± SEM)

Component	White muscle	Red muscle
Water	77.72 ± 0.30^a	74.13 ± 0.42^b
Protein	15.20 ± 0.24^a	15.10 ± 0.26^a
Fat	6.05 ± 0.33^a	9.67 ± 0.41^b
Ash	0.94 ± 0.01^a	0.90 ± 0.02^a

Values in lines followed by different letters differ significantly at $p \leq 0.01$

The qualitative fatty acid profile of the white and red muscle was identical. The relative concentrations of some fatty acids and their groups were as follows: the muscles did not differ ($p \leq 0.01$) in the content of saturated fatty acids (SFAs) whose total concentrations were similar. The white muscle had lower ($p \leq 0.01$) concentrations of three monounsaturated fatty acids (MUFAs), i.e. 18:1cis9 (oleic acid), 20:1*n*-9 (gadoleic acid) and 22:1n-11 (cetolic acid). In consequence, the total MUFA content of the white muscle was lower ($p = 0.01$), compared with the red muscle (44.94% vs. 54.86%). Differences were also observed in the relative concentrations of two *n*-3 polyunsaturated fatty acids, namely 20:5*n*-3 (eicosapentaenoic acid, EPA) and 22:6*n*-3 (docosahexaenoic acid, DHA). Their content was higher ($p \leq 0.01$) in the white muscle. It follows that this muscle had also a higher ($p \leq 0.01$) total concentration of *n*-3 PUFAs (23.11% vs. 15.37% in the red muscle). No differences were found ($p \leq 0.01$) in the levels of particular *n*-6 fatty acids (*n*-6 PUFAs) or their total content. The muscles differed ($p \leq 0.01$) in the *n*-3/*n*-6 ratio, which was 4.31 in the white muscle and 3.01 in the red muscle (Table 2).

Dorsal and ventral part of the white muscle

The analysis of proximate components showed that the dorsal and ventral part of the white muscle differed significantly in the content of fat and water. The fat content of the ventral part was by 43% higher ($p \leq 0.01$) in comparison with the dorsal part, where it amounted to 5.14%. Statistically significant differences ($p \leq 0.01$) were also observed in water content, which was lower in

Table 2

Fatty acid profile (% of total fatty acids) of the white and red muscle (mean ± SEM)

Fatty acid	White muscle	Red muscle
14:0	4.90 ± 0.11	4.63 ± 0.11
15:0	0.49 ± 0.02	0.39 ± 0.05
16:0	18.81 ± 0.44	17.81 ± 0.39
18:0	1.66 ± 0.08	1.74 ± 0.08
20:0	0.15 ± 0.01	0.12 ± 0.00
Σ SFA	26.01 ± 0.50	24.69 ± 0.67
14:1	0.25 ± 0.01	0.23 ± 0.01
16:1	6.56 ± 0.24	5.49 ± 0.24
18:1 cis 9	24.77 ± 0.60^a	30.78 ± 1.16^b
18:1 cis 11	2.82 ± 0.13	2.83 ± 0.60
20:1 *n*-9	6.16 ± 0.26^a	9.49 ± 0.22^b
22:1 *n*-11	3.82 ± 0.12^a	5.17 ± 0.20^b
22:1 *n*-9	0.56 ± 0.04	0.87 ± 0.03
Σ MUFA	44.94 ± 0.81^a	54.86 ± 1.12^b
18:2 *n*-6	4.39 ± 0.30	4.19 ± 0.20
20:2 *n*-6	0.39 ± 0.03	0.44 ± 0.04
20:4 *n*-6	0.58 ± 0.02	0.48 ± 0.03
Σ *n*-6 PUFA	5.36 ± 0.31	5.11 ± 0.24
18:3 *n*-3	1.24 ± 0.02	0.99 ± 0.04
18:4 *n*-3	1.39 ± 0.02	0.91 ± 0.04
20:4 *n*-3	0.95 ± 0.01	1.01 ± 0.05
20:5 *n*-3	6.33 ± 0.26^a	3.91 ± 0.19^b
22:5 *n*-3	1.92 ± 0.09	1.53 ± 0.13
22:6 *n*-3	11.88 ± 0.27^a	7.02 ± 0.24^b
Σ *n*-3 PUFA	23.11 ± 0.86^a	15.37 ± 0.71^b
Σ PUFA	29.07 ± 1.01^a	20.48 ± 0.68^b
n-3/*n*-6	4.31 ± 0.58^a	3.01 ± 0.30^b

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids

Values in lines followed by different letters differ significantly at $p \leq 0.01$

the ventral part. No such differences were found in the concentrations of protein and minerals (Table 3).

The fatty acid composition of lipids of the dorsal and ventral part of the white muscle, presented in Table 4, shows that both parts contained the same fatty acids. SFAs were dominated by palmitic acid (16:0), MUFAs by oleic acid (18:1cis9), while PUFAs by docosahexaenoic acid (22:6*n*-3, DHA).

Table 3
Concentrations of basic components in the dorsal and ventral part of the white muscle (%) (mean ± SEM)

Component	Dorsal part	Ventral part
Water	78.72 ± 0.28^a	76.44 ± 0.36^a
Protein	15.18 ± 0.22^a	15.24 ± 0.25^a
Fat	5.14 ± 0.30^a	7.38 ± 0.41^b
Ash	0.96 ± 0.01^a	0.94 ± 0.01^a

Values in lines followed by different letters differ significantly at $p \leq 0.01$

Table 4
Fatty acid profile (% of total fatty acids) of the dorsal and ventral part of the white muscle (mean ± SEM)

Fatty acid	Dorsal part	Ventral part
14:0	4.98 ± 0.10	4.89 ± 0.09
15:0	0.49 ± 0.20	0.44 ± 0.02
16:0	18.80 ± 0.45	18.79 ± 0.50
18:0	1.69 ± 0.09	1.70 ± 0.09
20:0	0.20 ± 0.01	0.35 ± 0.01
Σ SFA	26.16 ± 0.54	26.17 ± 0.60
14:1	0.20 ± 0.01	0.20 ± 0.01
16:1	6.20 ± 0.21	6.58 ± 0.20
18:1 cis 9	22.15 ± 0.60^a	24.49 ± 0.87^b
18:1 cis 11	2.59 ± 0.10	2.77 ± 0.12
20:1 *n*-9	6.01 ± 0.20^a	7.13 ± 0.24^b
22:1 *n*-11	3.80 ± 0.08	3.99 ± 0.10
22:1 *n*-9	0.50 ± 0.02	0.40 ± 0.02
Σ MUFA	41.45 ± 0.89^a	45.56 ± 0.99^b
18:2 *n*-6	4.60 ± 0.32	4.24 ± 0.30
20:2 *n*-6	0.40 ± 0.01	0.40 ± 0.01
20:4 *n*-6	0.58 ± 0.01	0.60 ± 0.02
Σ *n*-6 PUFA	5.58 ± 0.34	5.24 ± 0.24
18:3 *n*-3	1.20 ± 0.02	1.20 ± 0.02
18:4 *n*-3	1.40 ± 0.02	1.45 ± 0.02
20:4 *n*-3	0.98 ± 0.01	1.00 ± 0.01
20:5 *n*-3	6.80 ± 0.26^a	5.28 ± 0.24^b
22:5 *n*-3	1.80 ± 0.08	2.01 ± 0.10
22:6 *n*-3	14.63 ± 0.28^a	12.09 ± 0.30^b
Σ *n*-3 PUFA	26.81 ± 0.80^a	23.03 ± 0.88^b
Σ PUFA	32.39 ± 1.12^a	28.27 ± 1.11^b
n-3/*n*-6	4.80 ± 0.57	4.39 ± 0.60

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids
Values in lines followed by different letters differ significantly at $p \leq 0.01$

The dorsal and ventral part had comparable ($p \leq 0.01$) concentrations of 16:0 and the other SFAs, which resulted in a similar ($p \leq 0.01$) total content of fatty acids of this group. A significant ($p \leq 0.01$) difference was recorded between the quantities of 18:1 cis 9 and DHA. The concentration of 18:1 cis 9 was by 9.5% lower, whereas the concentration of DHA was by 21% higher in the dorsal part, compared with the ventral part. Both parts of the white muscle differed ($p \leq 0.01$) also in the quantities of 20:1*n*-9 (by 15.7% higher in the ventral part) and 20:5*n*-3 (by 29% higher in the dorsal part). Moreover, the dorsal part contained less MUFAs (41.45% vs. 45.56%) and more PUFAs (32.39% vs. 28.27%). Both parts of the fillet differed ($p \leq 0.01$) also in the total concentrations of *n*-3 PUFAs, but not in the total content of *n*-6 PUFAs or in the ratio between these two groups of fatty acids.

Discussion

In fish white muscles differ from red muscles in chemical composition, which is related to the specific character of metabolic processes. In white muscles energy is supplied primarily as a result of glycogen decomposition, while in red muscles the main source of energy are lipids (KOŁAKOWSKI 1986). A higher lipid content of red muscles has been confirmed in such species as *Sardinella maderensis, Sardinella aurita, Cephalopholis taeniops* (NJINKOUÉ et al. 2002), haddock (*Melanogrammus aeglefinus* L.) (NANTON et al. 2003), Atlantic salmon (*Salmo salar*) (AURSAND et al. 1994), white sucker (*Catostomus commersoni*) (MAI, KINSELLA 1979), African sharptooth catfish (*Clarias gariepinus* Burchell) (HOFFMAN et al. 1995) and rainbow trout (*Oncorhynchus mykiss*) (INGEMANSSON et al. 1991). Depending on the species, the lipid content of the red muscle has been found to be 2.5- to 5-fold higher, compared to the white muscle. In our study the difference in fat content between both muscles of the tested hybrid was smaller. The higher protein content of white muscles, reported for some fish species, was not observed, either (HOFFMAN et al. 1995, CARPENE et al. 1998). A correlation was found between the levels of fat and water, whereas protein concentration was comparable in both muscles.

The difference in fat content between the dorsal and ventral part of the fillets, observed in this study, has been previously described by other authors (EINEN et al. 1998, NICKELL, BROMAGE 1998, NORDVEDT, TUENA 1998, DIAS et al. 2001, REGOST et al. 2001, 2003, TESTI et al. 2006). In has also been demonstrated that in the turbot (*Psetta maxima*) (REGOST et al. 2001, REGOST et al. 2003) this relationship is affected neither by the concentration nor by the kind of fat in the diet. However, in the European catfish (*Silurus glanis* L.) the difference in fat content between the dorsal and ventral part of the fillet

has been observed only in individuals fed a natural diet, and not in those fed intensively an artificial diet (JANKOWSKA et al. 2005). In the tested hybrid, fed artificial feed, both parts of the white muscle contained various amounts of fat.

Differences in the fatty acid composition of the white and red muscle were related to various relative concentrations of MUFAs and PUFAs, at a comparable level of SFAs. Different concentrations of PUFAs in these muscles resulted in a different contribution of *n*-3 PUFAs, EPA and DHA. An identical relationship was observed by INGEMANSSON et al. (1991) in the rainbow trot. Also the red muscle of the haddock (*Melanogrammus aeglefinus* L.) contained more MUFAs than the white muscle (NANTON et al. 2003). HOFFMAN et al. (1995) recorded a higher MUFA content of the dark muscle and a higher USFA content of the light muscle (higher concentrations of DHA and EPA) in the African sharptooth catfish, as well as no differences between these muscles in SFA content. In the majority of freshwater fish species phospholipids contain more PUFAs, similar amounts of SFAs and less MUFAs, compared to neutral lipids (HENDERSON, TOCHER 1987). Therefore, the various concentrations of total lipids (and probably also phospholipids and triacylglycerols) in the red and white muscle of the tested hybrid could be the reason for differences in MUFA content. This relationship could also result from the fact that in fish mitochondrial β-oxidation of MUFAs is more intensive in red muscles than in white muscles, and so the former contain more fatty acids of this kind (NANTON et al. 2003).

Different fat levels as well as different proportions between neutral and polar lipids could be also the reason for different relative concentrations of MUFAs and PUFAs in the dorsal and ventral part of the white muscle, observed in the hybrid. The dorsal part, with a lower fat content, contained less MUFAs and more PUFAs, in comparison to the dorsal part. It should be stressed that the different PUFA content was a consequence of various quantities of long-chain *n*-3 polyunsaturated fatty acids (LC *n*-3 PUFAs), i.e. EPA and DHA. It has been demonstrated in various fish species that phospholipids being components of cell membranes and granules are characterized by a high content of these fatty acids (SÉROT et al. 1998, ORBAN et al. 2000, PENG et al. 2003, OGATA et al. 2004). Also RUIZ-GUTIERREZ et al. (1997) reported that the dorsal part of the gempylid fish (*Ruvettus pretiosus*) contains more PUFAs and less MUFAs. Data identical to those presented in this study were also obtained while comparing the fatty acid profile of the dorsal and ventral part in the European catfish fed a natural diet. Similarly as in the sturgeon, differences in PUFA content in this species resulted from various quantities of *n*-3 PUFAs, whereas the concentrations of *n*-6 PUFAs in both fillet parts remained at a comparable level. According to TESTI et al. (2006), the European sea bass (*Dicentrarchus labrax* L.), gilthead sea bream (*Sparus*

aurata L.) and rainbow trout differ in MUFA and PUFA concentrations in the dorsal and ventral part of the fillet, which is a consequence of a higher content of highly unsaturated fatty acids (*n*-3 PUFA) in the former.

Conclusions

Differences in proximate composition between the red and white muscle as well as between the dorsal and ventral part of the white muscle of the Siberian sturgeon and green sturgeon hybrid concerned two components, i.e. fat and water. Both the white muscle and its dorsal part had a lower fat content compensated by a higher fat content. The qualitative fatty acid profile of the above body parts of the hybrid was almost the same, differences were found only in the concentrations of some fatty acids and their groups. The white muscle and the dorsal part had a lower fat content, and so contained relatively less monounsaturated fatty acids (MUFAs) and more polyunsaturated fatty acids (PUFAs), which resulted in a higher contribution of *n*-3 PUFAs as well as in higher concentrations of LC *n*-3 PUFAs, i.e. EPA and DHA, compared with the red muscle and the ventral part.

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EFFECTS OF THE ADDITION OF SAGE AND SODIUM ISOASCORBATE ON THE QUALITY AND SHELF LIFE OF POULTRY MEATBALLS DURING COLD STORAGE

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K e y w o r d s: sage, sodium isoascorbate, storage stability, poultry meatballs, lipid oxidation, microbiology, sensory quality.

A b s t r a c t

The effects of sage and sodium isoascorbate on oxidative and hydrolytic changes, as well as the microbiological and sensory quality of poultry meatballs, were studied during cold storage. The addition of sage more effectively inhibited oxidative changes in meat products, whereas sodium isoascorbate was an efficient inhibitor of hydrolysis of the fat fraction. Sage contained in semi--finished products reduced the counts of mesophilic bacteria, psychrophilic bacteria and coli rods. During the storage the addition of sage inhibited the growth of coli roads and sulphate reducing *Clostridium* sp. Sodium isoascorbate showed antimicrobial effect against psychrophilic bacteria. The additive of sage and sodium isoascorbate decreased water activity in the products.

The sensory quality of meatballs deteriorated during storage. A warmed-over flavour (WOF) and sour flavour developed at a later stage of storage meatballs containing sage or sodium isoascorbate in comparison with the control samples. After 15 days the products with additives received higher scores for sensory properties than the control samples.

WPŁYW DODATKU SZAŁWII I IZOASKORBINIANU SODU NA JAKOŚĆ I TRWAŁOŚĆ WYROBÓW GARMAŻERYJNYCH Z MIĘSA DROBIOWEGO W CZASIE CHŁODNICZEGO PRZECHOWYWANIA

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S ł o w a k l u c z o w e: szałwia, izoaskorbinian sodu, stabilność przechowalnicza, klopsy drobiowe, utlenianie tłuszczów, mikrobiologia, jakość sensoryczna.

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Abstrakt

Badano wpływ szałwii oraz izoaskorbinianu sodu na zmiany oksydacyjne i hydrolityczne oraz jakość mikrobiologiczną i sensoryczną klopsów z mięsa drobiowego w czasie chłodniczego przechowywania. Dodatek szałwii skuteczniej hamował zmiany oksydacyjne, a izoaskorbinianu sodu – proces hydrolizy frakcji tłuszczowej badanych wyrobów. Szałwia i izoaskorbinianu sodu spowodowały obniżenie aktywności wody w wyrobach doświadczalnych. Odnotowano korzystny wpływ dodatku szałwii w półproduktach na zmniejszenie liczby bakterii mezofilnych, psychrofilnych oraz pałeczek z grupy coli. W czasie przechowywania szałwia hamowała wzrost *Clostridium* sp. i bakterii z grupy coli, a izoaskorbinian sodu – wzrost liczby bakterii psychrofilnych.

W czasie przechowywania klopsów obniżała się ich jakość sensoryczna. Smak WOF później pojawił się w wyrobach z dodatkiem szałwii i izoaskorbinianu sodu niż w kontrolnych. Po 15 dniach przechowywania korzystniejsze noty uzyskały wyroby z dodatkiem szalwii i izoskorbinianu sodu niż kontrolne.

Introduction

Technological processes destroy tissue structure and facilitate oxygen access to all food components. Fats are among food ingredients most exposed and susceptible to oxidation. Lipid oxidation is the most common reason for food spoilage, and a primary degradation reaction for fats. This process can take place spontaneously or with the active participation of microorganisms and enzymes. Oxidative reactions are responsible for a decline in the quality of food, including deterioration of taste, aroma, texture and consistency, as well as a decrease in nutritive value (KANNER 1994). The kind of compounds formed during lipid oxidation affects the organoleptic properties of foods. Not all compounds produced during fat metabolism are taste-detectable. Peroxides and hydroperoxides do not change the organoleptic properties of fats. The presence of low-molecule volatile substances, such as aldehydes, ketones and free fatty acids causes itself by the taste and aroma typical of rancid fat.

The simplest method to prevent lipid oxidation is to eliminate oxidation--causing factors from the environment. In addition, fats can be protected by properly selected antioxidants (POKORNY 1991). A tendency can be observed currently to replace synthetic antioxidants by natural ones, although the former are less expensive, readily available, and characterized by good quality and stronger antioxidative properties. This is due to the fact that toxicological analyses revealed a detrimental effect of synthetic antioxidants on food products. They may contribute to the development of many serious diseases, including cancer.

Antioxidants can be found, among others, in seasonings and spices added to food products to alter their organoleptic properties. The group of herbs that has been studied most extensively is the family Labiate, whose members contain phenolic compounds with antioxidative properties.

Sage contain the larges amounts of antioxidants (GU and WENG 2001, LU and FOO 2001). The antioxidant effect of crumbled sage leaves added to meat products described, among by MADSEN et al. (1996), KARPIŃSKA et al. (2001) and KORCZAK et al. (1988).

The spices show antimicrobial or bacteriostatic properties thanks to the compounds present in them. According to DELAMARE et al. (2007), SAĞDI et al. (2002) and TEPE et al. (2005) sage extract inhibited the development of microorganisms.

The aim of the present study was to determine the effects of sage and sodium isoascorbate (commonly used in the meat industry) on sensory and microbiological quality and shelf-life of poultry meatballs during cold storage.

Materials and Methods

Materials

The experimental material comprised poultry meatballs made of: thigh muscles of B-6 turkey-toms (80%), wheat roll soaked in water (13%), beaten eggs (5%), potato flour (2%) and salt (1% in relation to the whole mass). Three kinds of meatballs were made: without additives, herbs or spices (control; A), with sodium isoascorbate in the amount of 0.4 $g \cdot kg^{-1}$ meat pulp (B), and with sage in the amount of 0.1 $g \cdot kg^{-1}$ (C). Meat was ground in a meat grinder, type MMU-10Z with a 4 mm mesh, and then mixed in a multifunctional food processor (Bauknecht). Meatballs weighing 80 g ± 1 g, about 1 cm in thickness and about 8 cm in diameter, were formed of smooth pulp and placed in a convection-steam oven, type BECK FCV 4 EDS, with a measuring probe. Steam and hot air were used for heat treatment (air temperature – 200°C, steam saturation – 30%). The treatment was continued until a 82°C inside the product was achieved. Ready-made meatballs were vacuum-packed, with a MULTIVAC A 300 packaging unit, into bags of five-layer PE-LD/adh/PA/ADH/PE-PD film (total thickness – 0.08 mm, PA layer thickness – 0.024 mm, oxygen permeability – 40 $cm^3 \cdot m^{-2}$ 24 h^{-1} bar^{-1}, water vapour permeability – 10 g m^{-2} 24 h^{-1} bar^{-1}. The samples were stored at 3°C ± 1°C for 15 days.

Methods

The isolation of fat used for the determinations of fatty acid composition was performed according to the method described by FOLCH et al. (1957). The content of fatty acid methyl esters was determined by the GC method after methylation with a chloroform/methanol/sulphuric acid (100:1000:1) mixture (PEISKER 1964). Peaks of methyl esters were identified by comparison of their retention times of standard peaks of the mixture of known composition (Applied Science Corporation). Analyses were performed with a HP 6890 GC machine equipped with a 30 m x 0.32 mm capillary column. Supelcowax 10-0.25 μm was used as a liquid phase. The content of malondialdehyde (MDA) was assayed by the method developed by Tarladgis and modified by PIKUL et al. (1989). In order to determine pH, 10 g of a sample and 90 cm^3 of distilled water were homogenized for 60 s. The pH of the samples was measured using an ATC PICCOLO 2 pH-meter.

The acid value was determined in the fat extracted from the products in accordance with the Polish Standard PN-EN ISO 660 (*Animal and vegetable fats and oils*... 2005).

Water activity was read (NOVASINA, type AWC 203-C) after equilibration at 20°C.

The counts of the following groups of microorganisms were determined in the products: mesophilic aerobic bacteria on nutrient agar (incubation at 30°C for 72 h), psychrotrophilic bacteria on nutrient agar (incubation at 6.5°C for 7 days), yeast and moulds on YGC (Merck) (incubation at 25°C for 4 days), coli rods on VRBL-Agar (Merck), incubation at 30°C for 48 h. The presence sulphate reducing *Clostridium* sp. of spore-forming rods was examined after heating of adequate dilutions at 80°C for 15 minutes on meat-liver agar medium after 48 h incubation at 37°C.

The meat products were subjected to a sensory evaluation by the flavour profile method (MEIGAARD et al. 1999).

The sensory panel consisted of five panellists trained in accordance with the Polish Standard ISO 11035 (*Identification and selection of descriptors*... 1999). All judges were trained to be familiar with the flavour attributes to be measured (meaty, typical of poultry meat, typical of roasted meat, aromatic, spicy, WOF, sour). Each attribute was evaluated using a scale ranging from one (lack of perception) to five (very strongly noticeable perception). Standard samples with very strongly noticeable perception of WOF were prepared from poultry patties cooked in boiling water until a temperature of 82°C was reached inside the product, cooled at room temperature, packed into polyethylene bags and stored at 4°C for three days. Using a standardized lexicon of meat descriptors for WOF (LOVE 1988), the judges described the standard samples

off-flavours with the terms "boiled fish" or "stale", and off-odours with the terms "painty" or "cardboardy".

The sensory evaluation was performed on coded samples immediately after thermal processing and during storage. At the beginning of each session, the panel was presented with the reference samples for the extremes of scales of the flavour attributes measured. Each samples was evaluated in triplicate. The intensity of particular attributes was determined on a 5-point scale: 0-lack of sensation; 1 – hardly noticeable sensation; 2 – slightly noticeable sensation; 3 – moderately noticeable sensation; 4 – strongly noticeable sensation; 5 – very strongly noticeable sensation.

Raw samples and thermally processed meatballs were used to determine: the fatty acid composition, pH, water activity, AV and MDA, and to perform a sensory evaluation. The pH, water activity, AV and MDA were determined during storage, at three-day intervals. A microbiological evaluation was conducted on raw samples, thermally processed samples and samples stored for 15 days.

All analyses were performed in nine replications. The results obtained in the study were analyzed statistically using Statistica 7.1 software (Statsoft, Inc). The significance of differences was estimated with the Tukey's test at a significance level of $p < 0.05$.

Results and Discussion

The susceptibility of meat products to oxidative changes is affected not only by the quantity, but also by the quality of fat contained in ready-made goods, and especially the concentration of polyenoic acids. An analysis of fatty acid composition in the fat extracted from poultry meatballs showed a high concentration of unsaturated acids – 68.23 to 69.83% (Table 1). The highest content of these acids was found in the samples containing sage, and the lowest – in the control samples. The concentration of polyenoic acids is important from the nutritional perspective, but on the other hand food products containing large amounts of these acids are more prone to oxidation. The mean concentration of polyenoic acids in poultry meatballs was 33.33% (control samples), 34.58% (samples with sodium isoascorbate) and 35.04% (samples with sage). Linoleic acid had the highest proportion (29.60-30.78%) in the group of polyenoic acids. It was demonstrated that the samples with additives contained significantly more linoleic and arachidonic acids than the control samples, and the samples with sage had the highest content linoleic acid content. The level of monoene acids was also high in poultry meatballs (34.59-34.90%). Oleic acid dominated in this group (31.08-31.26%). Similarly as

polyenoic acids, oleic acid helps to reduce the serum levels of cholesterol and low-density lipoproteids. At the same time this acid is less prone to oxidation. Palmitoleic acid accounted for 2.82-2.99%, and the other acids of this group were present in quantities below 1%.

Table 1

The content (% weight) of selected fatty acids in the fat of poultry meatballs

Fatty acids (%)		Type of samples		
		reference	with sodium isoascorbate	with sage
C16:0	$\bar{x}$	21.60^{c}	20.80^{b}	20.35
	S(x)	0.046	0.028	0.390
C16:1	$\bar{x}$	2.94^{b}	2.82^{a}	2.99^{b}
	S(x)	0.062	0.026	0.036
C18:0	$\bar{x}$	8.82^{c}	8.65^{b}	8.50^{a}
	S(x)	0.053	0.050	0.020
C18:1	$\bar{x}$	31.26^{a}	31.09^{a}	31.08^{ba}
	S(x)	0.170	0.026	0.030
C18:2	$\bar{x}$	29.60^{a}	30.78^{b}	30.65^{b}
	S(x)	0.056	0.095	0.072
C18:3	$\bar{x}$	2.38^{a}	2.40^{a}	2.61^{b}
	S(x)	0.026	0.020	0.060
C20:4	$\bar{x}$	1.21^{a}	1.22^{a}	1.59^{b}
	S(x)	0.030	0.030	0.040
Σ SFA	$\bar{x}$	31.77^{b}	30.83^{a}	30.16^{a}
	S(x)	0.332	0.280	0.056
Σ USFA	$\bar{x}$	68.23^{a}	69.17^{b}	69.83^{c}
	S(x)	0.276	0.044	0.046
Σ monoenoic acids	$\bar{x}$	34.90^{b}	34.59^{a}	34.79^{b}
	S(x)	0.174	0.030	0.025
Σ polyenoic acids	$\bar{x}$	33.33^{a}	34.58^{b}	35.04^{c}
	S(x)	0.106	0.026	0.026

a, b, c – mean values marked the same letters in rows are not significantly different at $p < 0.05$

Malondialdehyde content is one of indicators of oxidative changes in lipids. A statistical analysis of results confirmed a significant effect of sodium isoascorbate and sage on the inhibition of lipid oxidation (Figure 1). Sage inhibited lipid oxidation more effectively than sodium isoascorbate. During storage the concentration of malondialdehyde in the samples with sage was at a similar level (0.13-0.15 mg · kg^{-1}). In the products with sodium isoascorbate the amount of MDA increased during the first six days, from 0.22 mg · kg^{-1} to 0.30 mg · kg^{-1}, and then remained at a stable level of 0.28-0.30 mg · kg^{-1}. The MDA content of the control samples increased significantly after three

days of storage, from 0.49 to 0.69 mg · kg^{-1}, to change slightly only with further storage. After 15 days MDA concentration in the control samples amounted to 0.77 mg · kg^{-1} and was significantly higher than at the beginning of the experiment. The antioxidative properties of sage were also reported by KARPIŃSKA et al. (2001), KORCZAK et al. (1988), MADSENET et al. (1996).

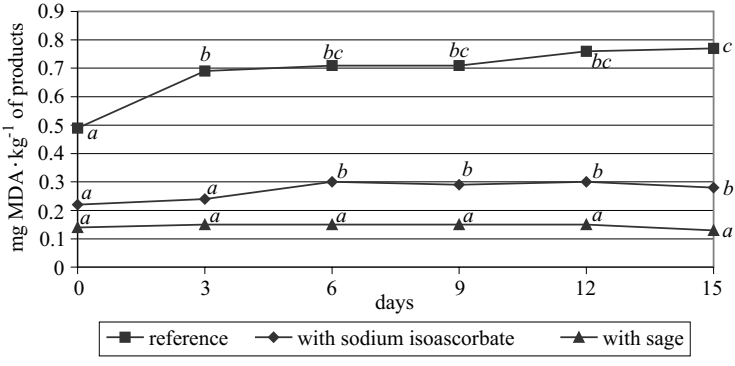

Fig. 1. The content of malondialdehyde in poultry meatballs with different antioxidant during refrigerated storage: *a, b, c* – mean values marked with the same letters are not significantly different at $p < 0.05$

Hydrolytic changes are caused primarily by water, but also – to a lower degree – by light and air. These processes can also occur under the influence of the enzyme lipase found in animal tissue. As a result of hydrolysis, free fatty acids are liberated from glycerides. In order to determine the concentration of free fatty acids in poultry meatballs, acid value was measured.

Significant hydrolytic changes were observed in the lipids of the tested products (Figure 2). The rate of these changes was slower in the samples with additives than in the control samples. An analysis of results showed that hydrolytic changes were less intensive in the products containing a synthetic antioxidant (2.24-2.98 mg NaOH · kg^{-1}) than in those with sage (2.78-3.41 mg NaOH · kg^{-1}). In the samples with sodium ascorbate a significant increase in the acid value was recorded after three days of storage only, whereas in the samples with sage the acid value was increasing until the 9th day of storage. In the control samples the acid value range was 3.10-3.69 mg NaOH · kg^{-1}, and its significant changes were observed until the 6th day of the experiment. Other authors (KARPIŃSKA et al. 2001, FERNANDEZ-FERNANDEZ et al. 2001, 2002) reported a rising tendency in the acid value in meat products during cold storage. The results obtained by KARPIŃSKA et al. (2001) indicated that changes in the acid value were slighter in poultry meatballs containing sage, compared with the control samples and those with a mixture of spices.

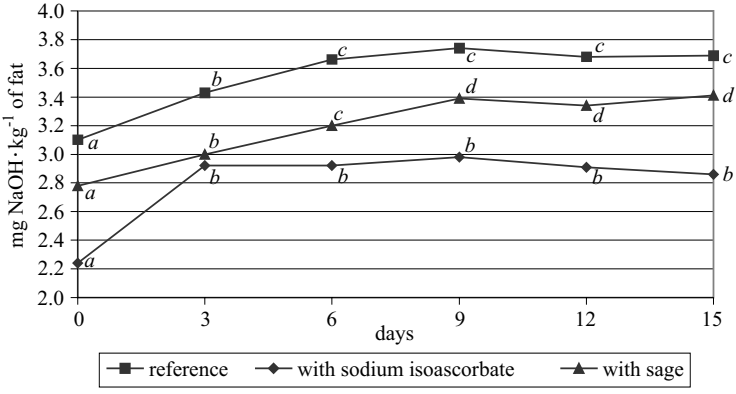

Fig. 2. Acid value (AV) of fat extracted from poultry meatballs with different antioxidants during refrigerated storage: *a, b, c, d* – mean values marked with the same letters are not significantly different at $p < 0.05$

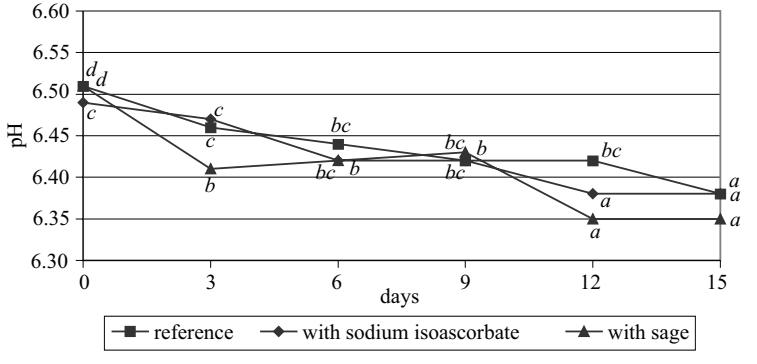

Fig. 3. The pH value of poultry meatballs with different antioxidants during refrigerated storage: *a, b, c, d* – mean values marked with the same letters are not significantly different at $p < 0.05$

Acidity is one of the parameters affecting microorganism development in food. The values of pH (Figure 3) in poultry meatballs immediately after heat treatment were at a similar level (6.49-6.51). During storage the pH of the samples gradually decreased. The pH of the control and sage-containing samples decreased significantly already at the first stage of storage (3 days), and the pH of the samples with sodium isoascorbate – after six days. After 15 days of storage the pH of all samples was similar and ranged from 6.35 to 6.38. A statistical analysis of results showed that the samples significantly differed in pH after three and twelve days of storage, and that the level of this discriminant was significantly lower in the samples with sage. A decrease in

pH enables effective control of microorganism growth. In the present experiment the pH reduction could be caused by an increase in the count of lactic acid bacteria over storage, resulting from secondary infection of meatballs during packaging. This observation is consistent with results obtained by other authors (ANIFANTAKI et al. 2002, LIN and LIN 2002, METAXOPOLUOS et al. 2001, PEXARA et al. 2002), who reported a gradual decrease in the pH of vacuum – or modified atmosphere-packed meat products under conditions of prolonged cold storage.

Water activity is critical for water availability for microorganism growth and chemical reactions in foods. The microbiological stability of a product can be predicted based on the value of a_w. A substantial effect of spices and additives, as well as storage time, on water activity was observed in the study (Figure 4). The values of water activity were significantly lower in the samples with additives than in the control ones. The lowest level of this discriminant was recorded in the samples with sodium isoascorbate. This tendency was also reported by NASSU et al. (2003), who observed lower values of water activity in fermented goat meat sausages containing rosemary than in the control samples. During storage water activity decreased in the samples with sodium isoascorbate and sage, which reduced their susceptibility to spoilage. A significant increase in water activity was recorded in the control samples at the beginning of storage, which could be the reason for intensive microflora growth, leading to worse final quality of the products. Later on the value of this discriminant decreased, similarly as in the other samples. Reduced water activity in cold-stored vacuum-packed meat products was also observed by FERNANDEZ-FERNANDEZ et al. (2001), LIN and LIN (2002) and METAXOPOULOS et al. (2001).

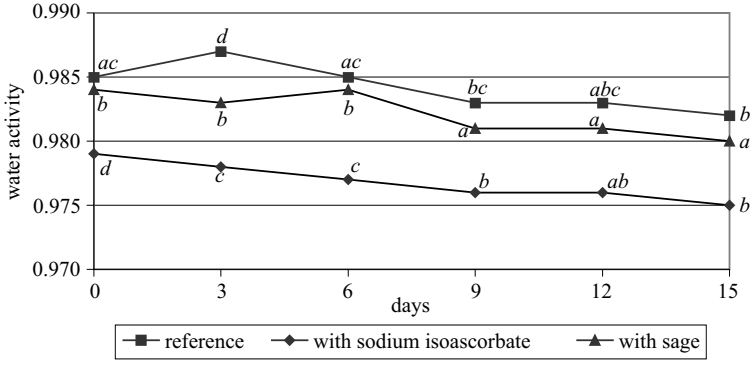

Fig. 4. Water activity of poultry meatballs with different antioxidants during refrigerated storage: *a, b, c, d* – mean values marked with the same letters are not significantly different at $p < 0.05$

The initial count of mesophilic bacteria was 10^3 – 10^5 cfu · g^{-1} of the sample (semi-finished products) and 10^2 – 10^4 cfu · g^{-1} of the sample (heat-processed products). The lowest bacterial count was recorded in the semi-finished products with sage (10^3 cfu · g^{-1}) and heat-processed products with sodium isoascorbate (10^2 cfu · g^{-1}). Heat treatment enabled a partial destruction of mesophilic bacteria (Table 2), which were destroyed to the highest degree in the samples with sodium isoascorbate. During storage intensive development of mesophilic bacteria (to 10^6 cfu · g^{-1}) was observed in the sage-containing samples only. It seems that their source was the microflora contained in sage. SMITH and ALVAREZ (1988) did not find any significant changes in the mesophilic bacteria count during storage of vacuum-packed cooked turkey breast rolls.

The count of psychrophilic bacteria in semi-finished products was 10^2 – – 10^4 cfu · g^{-1}; the lowest bacterial count was recorded in the samples with sage. It can confirm antibacterial effect the sage against psychrophilic bacteria. In a study performed by ANIFANTAKI et al. (2002) the count of psychrophilic bacteria was lower in frankfurters containing rosemary and oregano than in control frankfurters, which according to these authors suggests the antimicrobiological properties of herbs and spices.

Following heat treatment, the count of psychrophilic bacteria remained at a similar level in the samples with sodium isoascorbate and sage, and increased in the control samples. After 15 days of storage the bacterial count was similar in the control and sage-containing samples, and decreased in those with a synthetic antioxidant. It can confirm antimicrobial effect of sodium isoascorbate against psychrophilic bacteria. According to LIN and LIN (2002), the time of cold storage had no significant effect on the count of psychrophilic bacteria in vacuum-packed sausages.

Products before thermal processing contained 10^2 – 10^3 cfu · g^{-1} of yeast. Heat treatment resulted in a complete destruction of the yeast population. No fungal growth was observed during storage.

The presence of coli rods in food products is considered an indicator of their sanitary status. Due to their putrefactive properties, some *E. coli* strains may cause food spoilage (JIANGHONG-MENG et al. 1994).

Coli rods were not found in semi-finished products containing sage. They were present in the quantity of $1.0 \cdot 10^5$ cfu · g^{-1} and $1.3 \cdot 10^4$ cfu · g^{-1} in the samples with sodium isoascorbate and in the control samples respectively. This could be caused by food contamination in the preparatory phase, and confirms the inhibitory effect of the compounds contained in sage on coli rods growth. Coli rods were not found in the meatballs after heat treatment, at a detection level of 10 cfu · g^{-1}. During storage the count of coli rods increased considerably in the control samples (to $8.7 \cdot 10^2$ cfu · g^{-1}) and in the samples with sodium isoascorbate (to $5.2 \cdot 10^2$ cfu · g^{-1}).

Table 2

The effect of thermal procesing and storage on the microbiological quality of poultry meatballs

Type of samples		Total mesophilic bacteria (cfu · g^{-1})	Total psychrotrophilic bacteria (cfu · g^{-1})	Yeast (cfu · g^{-1})	Moulds (cfu · g^{-1})	Coli rods (cfu · g^{-1})	The presence of sulphate (IV) reducing *Clostridium* sp.
Reference	raw	$2.6 \cdot 10^5$	$2.0 \cdot 10^3$	$3.2 \cdot 10^3$	ND	$1.3 \cdot 10^4$	in 0.01 g
	after thermal procesing	$2.1 \cdot 10^4$	$1{,}3 \cdot 10^4$	ND	ND	<10	in 0.001 g
	after 15 days storage	$7.3 \cdot 10^2$	$1.3 \cdot 10^4$	ND	ND	$8.7 \cdot 10^2$	in 0.01 g
With sodium isoascorbate	raw	$2.1 \cdot 10^5$	$1.3 \cdot 10^4$	$3.9 \cdot 10^2$	ND	$1.0 \cdot 10^5$	in 0.01 g
	after thermal procesing	$3.2 \cdot 10^2$	$9.0 \cdot 10^3$	ND	ND	<10	in 0.001 g
	after 15 days storage	$2.5 \cdot 10^2$	$9.6 \cdot 10^2$	ND	ND	$5.2 \cdot 10^2$	in 0.01 g
With sage	raw	$2.3 \cdot 10^3$	$9.6 \cdot 10^2$	$4.1 \cdot 10^2$	ND	<10	in 0.01 g
	after thermal procesing	$4.3 \cdot 10^3$	$2.3 \cdot 10^3$	ND	ND	<10	in 0.01 g
	after 15 days storage	$1.6 \cdot 10^6$	$2.0 \cdot 10^3$	ND	ND	<10	ND

ND – none detected

Sulphate reducing *Clostridium* sp. were found in 0.01 g of before processing products. They were not destroyed by heat treatment. After 15 days of storage they were present in control samples and in the samples with sodium isoascorbate. The sage showed antibacterial effect against these bacteria.

Taste and aroma sensations belong to major qualitative features of meat products. Their role still increases when they do not correspond with consumers; preferences. The most important problem is the development of undesirable warmed-over flavour (WOF) during cold storage of heat-treated meat products. The meat products were subjected to a sensory evaluation by the flavour profile method. The results of this evaluation are illustrated in Figures 5-7. It was found that immediately after the thermal process the samples differed in sensory quality. The samples with sage received lower scores for such attributes as "meaty", "typical of poultry" and "typical of roasted meat", and higher for "aromatic" and "spicy". The organoleptic properties of meatballs changed over the storage period. The intensity of the following flavour attributes: "meaty", "typical of poultry" and "typical of roasted meat", "aromatic" and "spicy" decreased, and negative marks (sour, WOF) appeared.

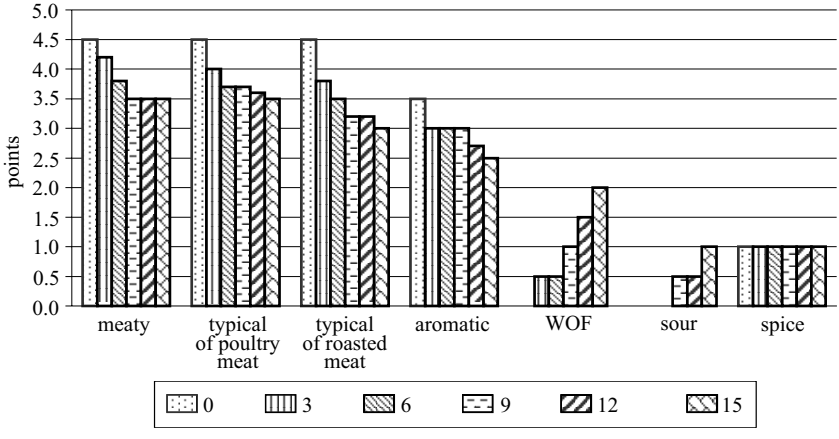

Fig. 5. The flavour profile of control poultry meatballs during refrigerated storage

According to KARPIŃSKA et al. (2000, 2001), prolonged cold or deep-freeze storage of deli poultry products is accompanied by deterioration of their sensory quality. These authors found that the intensity of a "meaty" flavour decreased sooner in samples without herbs or spices, and that such samples were described as sour or WOF within a shorter period of time.

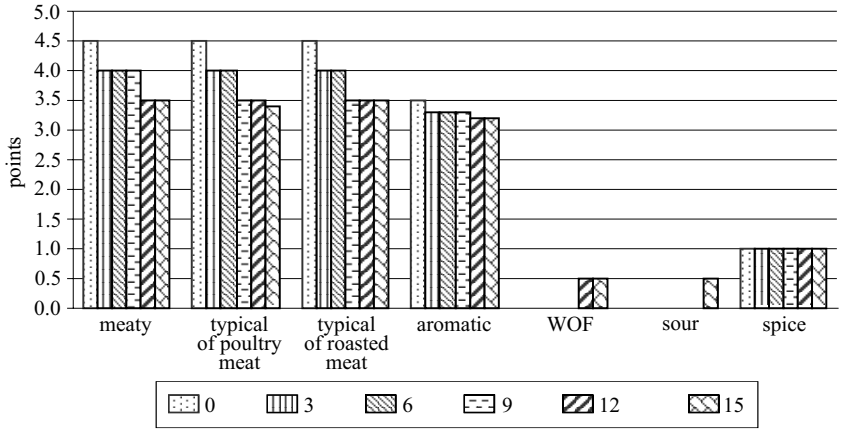

Fig. 6. The flavour profile of poultry meatballs with sodium isoascorbate during refrigerated storage

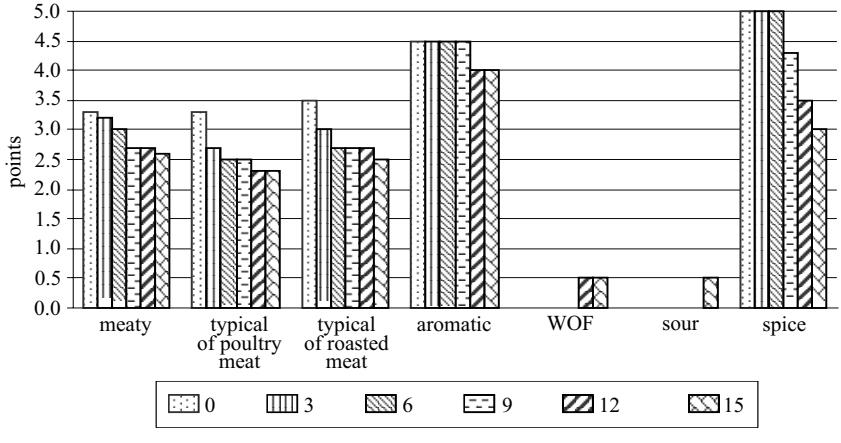

Fig. 7. The flavour profile of poultry meatballs with sage during refrigerated storage

The results of the present study show that the addition of sage and sodium isoascorbate inhibited the development of WOF in the experimental products. The warmed-over flavour was recorded first in the control samples (after 3 days), and latest of all in the sage-containing samples, and its intensity increased as the time of cold storage was prolonged. This tendency is consistent with the findings of WU et al. (2000) and AHN et al. (2002), who demonstrated that the intensity of WOF in deli pork and beef products increased along with prolonged cold storage.

Conclusions

It was demonstrated that the additives used in the experiment enabled to reduce oxidative changes in poultry meatballs. Sage had stronger antioxidative properties than sodium isoascorbate. Significant hydrolytic changes were observed in the lipid fraction of the tested products, and the rate of these changes was slower in the samples with additives. Sodium isoascorbate was a more effective inhibitor of hydrolytic changes than sage. The coli rods count before storage was below 10 per g of the product. After 15 days of storage the count of coli rods increased to $8.7 \cdot 10^2$ and $5.2 \cdot 10^2$ cfu in the control samples and in the samples with sodium isoascorbate respectively. Sulphate reducing *Clostridium* sp. were found in 0.01 g of stored the control samples and the samples with sodium isoascorbate. The additive of sage inhibited the growth of coli roads and sulphate reducing *Clostridium* sp. Slight changes in the sensory properties of the products were observed over storage. The intensity of the following flavour attributes: "meaty", "typical of poultry" and "typical of roasted meat", "aromatic" and "spicy" decreased, and negative marks (sour, WOF) appeared. After 15 days of storage the control samples were characterized by the worst sensory quality.

Translated by Aleksandra Poprawska

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THE EFFECT OF SAGE EXTRACT AND A MIXTURE OF SAGE EXTRACT AND SODIUM ISOASCORBATE ON OXIDATIVE AND HYDROLYTIC PROCESSES AS WELL AS ON SENSORY QUALITY OF POULTRY MEATBALLS

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K e y w o r d s: sage extract, storage, hydrolytic changes, lipid oxidation, sensory evaluation, poultry meatballs.

A b s t r a c t

The aim of the present study was to determine the effect of sage extract and a mixture of sage extract and sodium isoascorbate on oxidative and hydrolytic processes as well as on the sensory quality of poultry meatballs.

It was found that sage extract, applied alone or combined with sodium isoascorbate, significantly inhibited lipid oxidation after 15 days of cold storage. It was also demonstrated that the rate of hydrolytic and oxidative changes was slower in the experimental products containing a mixture of sage extract and sodium isoascorbate, as compared to those with sage extract alone, which may suggest that there is an interaction between these substances. The products with additives had a better sensory quality during cold storage than the control samples. In the former the intensity of flavor attributes (meaty, typical of poultry meat, typical of roasted meat, aromatic) decreased at a slower rate. A warmed-over flavor (WOF) and a sour flavor developed at a later stage of storage in poultry meatballs containing sage extract or sage extract + sodium isoascorbate, in comparison with the control samples.

Both sage extract and a mixture of sage extract and sodium isoascorbate can be added to meat products in order to preserve their high quality during storage.

WPŁYW DODATKU EKSTRAKTU SZAŁWII I JEGO MIESZANKI Z IZOASKORBINIANEM SODU NA PROCESY OKSYDACYJNE I HYDROLITYCZNE ORAZ JAKOŚĆ SENSORYCZNĄ KLOPSÓW Z MIĘSA DROBIOWEGO

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S ł o w a k l u c z o w e: ekstrakt z szałwii, przechowywanie, zmiany hydrolityczne, utlenianie lipidów, ocena sensoryczna, klopsy drobiowe.

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Abstrakt

Badano wpływ dodatku ekstraktu szałwii i jego mieszanki z izoaskorbinianem sodu na procesy oksydacyjne i hydrolityczne oraz jakość sensoryczną klopsów z mięsa drobiowego.

Wykazano, że po 15 dniach chłodniczego przechowywania zarówno ekstrakt szałwii, jak i jego mieszanka z izoaskorbinianem sodu w wysokim stopniu hamowały procesy utleniania lipidów. Wykazano, że w wyrobach doświadczalnych z dodatkiem mieszanki ekstraktu szałwii i izoaskorbinianu sodu odnotowano mniejsze tempo zmian hydrolitycznych i oksydacyjnych niż w próbkach z samym ekstraktem, co może świadczyć o współdziałaniu tych substancji. Lepszą jakość sensoryczną w czasie chłodniczego przechowywania zachowywały wyroby doświadczalne z dodatkami niż kontrolne. Odnotowano w nich mniejsze tempo obniżania natężenia wyróżników smaku mięsnego, typowego dla mięsna drobiowego i pieczonego, oraz aromatycznego. Stwierdzono, że zarówno w próbkach z dodatkiem ekstraktu, jak i jego mieszanki z izoaskorbinianem sodu, później był wyczuwalny smak WOF i kwaśny w porównaniu z próbkami kontrolnymi.

Zarówno ekstrakt szałwii, jak i jego mieszankę z izoaskorbinianem sodu można dodawać do produktów mięsnych w celu zabezpieczenia ich jakości w czasie chłodniczego przechowywania.

Introduction

Heat treatment makes meat more susceptible to oxidation, since the antioxidant enzymes contained in meat undergo denaturation and lose their activity (LEE et al. 1996). Oxidative rancidity is the main reason for quality deterioration in raw and cooked meats during chill and deep-freeze storage (RAHARJO, SOFOS 1993). Progressing oxidative changes may lead to organoleptic changes, followed by the development of a rancid flavor, thus discouraging consumers from buying the product. WOF is more intensive and develops faster in stored heat-processed meat than in raw meat (HAN et al. 1995). The products formed as a result of fat oxidation may cause destructive changes in vitamins and reduce the nutritional value of foods (KANNER 1994). In addition, they are believed to be responsible for numerous diseases, such as neoplastic diseases, gastric diseases or atherosclerosis (JACOB 1995). Biological compounds with double bonds, including *n*-3 polyunsaturated fatty acids, are particularly susceptible to oxidation. Poultry meat contains fat rich in unsaturated fatty acids, so it is more susceptible to oxidation and less stable than other kinds of meat.

The shelf-life of foodstuffs is one of the most important quality attributes. Various additives are used to improve the keeping quality of foods, including antioxidants. Antioxidants can delay oxidation or slow down its rate in oxidation-susceptible products. Synthetic antioxidants are universally used for meat processing, but due to the fact that they may be dangerous to human health (DECKER, MEI 1996), researchers have recently turned to alternative, natural sources of these compounds. Antioxidants can be found, among others, in seasonings and spices. The group of herbs that has been studied most extensively is the family *Labiatae* whose members, e.g. sage, contain phenolic

compounds with strong antioxidant properties (CUVELIER et al. 1994). The antioxidant effect of crumbled sage leaves added to meat products was described, among others, by MADSEN et al. (1996), KARPIŃSKA et al. (2001) and KORCZAK et al. (1988), whereas the antioxidant activity of sage extract was studied by ABD-EL-ALIM et al. (1999) and MCCARTHY et al. (2001a, b).

The aim of the present study was to determine whether sage extract is more effective with respect to the inhibition of lipid oxidation during cold storage of vacuum-packed meatballs when applied alone or when combined with sodium isoascorbate. The effect of both additives on the sensory quality of the products was also estimated.

Materials and Methods

Materials

The experimental material comprised poultry meatballs made of: thigh muscles of B-6 turkey-toms (80%), wheat roll soaked in water (13%), beaten eggs (5%), potato flour (2%) and salt (1% in relation to the whole mass). Three kinds of meatballs were made: without additives (control; A), with sage extract in the amount of 0.1% (B), and with sage extract + sodium isoascorbate, in the amount of 0.05% each (C).

Sage extract was obtained from dried sage leaves. Prior to extraction with ethanol, sage leaves were steam-distilled for two hours. Next sage leaves were filtered off (the leave extract was not analyzed) and dried at 60°C. The moisture content of dried sage was determined. Then sage leaves were milled in a WZ1 laboratory mill for 30 s. 50 g of dried material was extracted with 250 cm^3 of 96% ethanol at 60°C for two hours, stirring constantly. The extraction was repeated, the extracts were combined and filtered off. Ethanol was evaporated in a vacuum evaporator, and the residue was diluted with ethanol to a volume of 50 cm^3 (1 g of d.m./1 cm^3 of ethanol).

Meat was ground in a meat grinder, type MMU-10Z with a 4 mm mesh, and next mixed in a multifunctional food processor (Bauknecht). Meatballs weighing 80 g ±1 g, about 1 cm in thickness and about 8 cm in diameter, were formed of smooth pulp and placed in a convection-steam oven, type BECK FCV 4 EDS, with a measuring probe. Steam and hot air were used for heat treatment (air temperature – 200°C, steam saturation – 30%). The treatment was continued until a temperature of 82°C inside the product was achieved. Ready-made meatballs were vacuum-packed, with a MULTIVAC A 300 packaging unit, into bags of five-layer PE-LD/adh/PA/ADH/PE-PD film (total thickness – 0.08 mm,

PA layer thickness – 0.024 mm, oxygen permeability – 40 $cm^3 \cdot m^{-2} \cdot 24h^{-1} \cdot bar^{-1}$, water vapor permeability – 10 $g\ m^{-2} \cdot 24\ h^{-1}$). The samples were stored at 3°C ± 1°C for 15 days.

Methods

The extraction of fat used for the determination of the fatty acid profile was performed according to the method described by FOLCH et al. (1957). The content of fatty acid methyl esters was determined by the GC method after methylation with a chloroform/methanol/sulfuric acid (100:1000:1) mixture (PEISKER 1964). Peaks of methyl esters were identified by comparing their retention times to the standard peaks of a mixture of known composition (Applied Science Corporation). The analysis was performed with a HP 6890 GC machine equipped with a 30 m x 0.32 mm capillary column. Supelcowax 10-0.25 μm was used as a liquid phase. The acid value was determined in the fat extracted from the products in accordance with the Polish Standard PN-EN ISO 660 (*Animal and vegetable fats and oils* ... 2005). The malondialdehyde (MDA) content was assayed by the method developed by Tarlangis and modified by PIKUL et al. (1989). The MDA concentration in the control samples and in the samples with additives provided the basis for calculating lipid oxidation inhibition, as follows:

$$\text{Inhibition } (\%) = \frac{a - b}{a} \cdot 100,$$

a – MDA content of the control samples,
b – MDA content of the samples with antioxidants.

The meat products were subjected to a sensory evaluation by the flavor profile method (MEIGAARD et. al. 1999). The sensory panel consisted of five panelists trained in accordance with the Polish Standard ISO 11035 (*Identification and selection of descriptors* ... 1999). All judges were trained to be familiar with the flavor attributes to be measured (meaty, typical of poultry meat, typical of roasted meat, aromatic, spicy, WOF, rancid). Each attribute was evaluated using a scale ranging from one (lack of perception) to five (very strongly noticeable perception). Standard samples with very strongly noticeable perception of WOF were prepared from poultry patties cooked in boiling water until a temperature of 82°C was reached inside the product, cooled at room temperature, packed into polyethylene bags and stored at 4°C for three

days. Using a standardized lexicon of meat descriptors for WOF (LOVE 1988), the judges described the standard samples off-flavors with the terms "boiled fish" or "stale", and off-odors with the terms "painty" or "cardboardy".

The sensory evaluation was performed on coded samples immediately after thermal processing and during storage. At the beginning of each session, the panel was presented with the reference samples for the extremes of scales of the flavor attributes measured. Each samples was evaluated in triplicate. The intensity of particular attributes was determined on a 5-point scale: 0-lack of perception; 1 – hardly noticeable perception; 2 – slightly noticeable perception; 3 – moderately noticeable perception; 4 – strongly noticeable perception; 5 – very strongly noticeable perception.

AV, fatty acid content and MDA were determined in raw samples. AV, MDA and sensory quality were determined in meatballs after thermal processing and during storage, at three-day intervals.

All analyses were performed in nine replications. The results were verified statistically using Statistica ver. 6 software (StatSoft, Inc.). The significance of differences was estimated with the Tukey's test at a significance level of $p < 0.05$.

Results

Poultry meatballs were characterized by high concentrations of unsaturated fatty acids (67.93% to 68.50%, Table 1). The susceptibility of meats to oxidation is related primarily to the presence of polyene acids, greatly affected by oxidative changes. ELMORE et al. (1999) demonstrated that larger amounts of lipid degradation products, such as unsaturated aldehydes, ketones and alcohols, are formed in beef which contains high concentrations of *n*-3 polyunsaturated fatty acids. According to these authors, the most important role is played by aldehydes, responsible for deterioration of meat taste. The concentration of polyene acids (Table 1) in poultry meatballs ranged between 33.10% and 33.33%, and was comparable to the concentration of monoene acids (34.64% to 35.40%, Table 1), which are less susceptible to oxidation due to the presence of only one double bond in their structure.

The malondialdehyde content is the main indicator of the amounts of secondary products of lipid oxidation. The concentration of this compound (Figure 1) in semi-finished products was significantly higher in the control samples (0.24 kg^{-1} of the product) than in the samples with sage extract (0.07 $mg \cdot kg^{-1}$) and sage extract + sodium isoascorbate (0.06 $mg \cdot kg^{-1}$). The additives inhibited lipid oxidation during meatball preparation. It was found that during heat treatment the malondialdehyde content increased significantly

Table 1

The content of fatty acids in poultry meatballs

Fatty acids (%)	Type of samples		
	reference	with extract of sage	with mixture extract of sage and sodium isoascorbate
Total saturated	$31.77^{a} \pm 0.332$	$32.07^{a} \pm 0.460$	$31.50^{a} \pm 0.632$
Total unsaturaterd	$68.23^{a} \pm 0.346$	$67.93^{a} \pm 0.455$	$68.50^{a} \pm 0.626$
Total monounsaturated	$34.90^{a} \pm 0.061$	$34.64^{a} \pm 0.006$	$35.40^{b} \pm 0.884$
Total polyunsaturated	$33.33^{a} \pm 0.975$	$33.29^{a} \pm 0.450$	$33.10^{a} \pm 0.695$

a, b – mean values marked with the same letters in rows are not significantly different at $p < 0.05$

only in the control samples (Figure 1). During cold storage the concentration of this compound increased substantially in all samples, first in the control ones (after three days) and later in meatballs with additives (six to nine days). The level of MDA was relatively low in the experimental products containing sage extract or sage extract + sodium isoascorbate (0.1 to 0.2 mg · kg^{-1}), and higher in the control samples (0.2 to 0.6 mg · kg^{-1}).

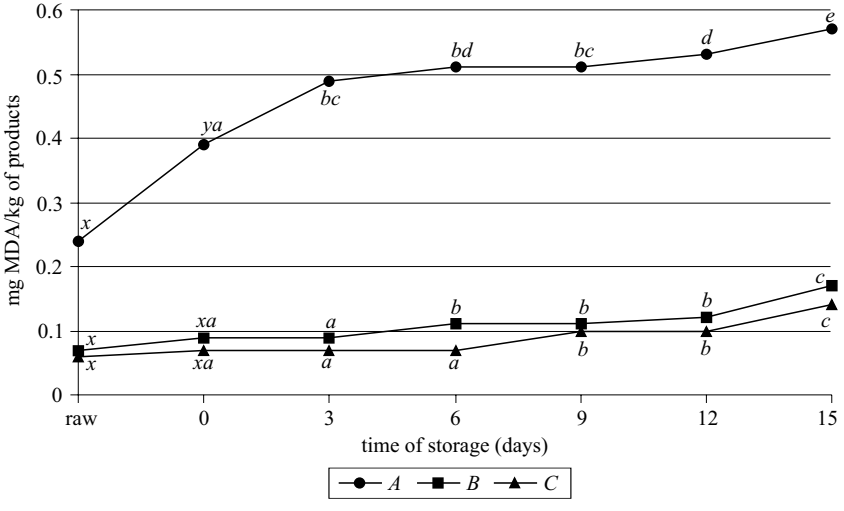

Fig. 1. The content of malondialdehyde in raw and after thermal processing poultry meatballs during refrigerated storage samples: *A* – reference, *B* – with extract of sage, *C* – with mixture extract of sage and sodium isoascorbate

x, y – mean values marked with the same letters for raw and after cooking "0 time of storage" samples are not significantly different at $p < 0.05$

a, b, c, d - mean values marked with the same letters for samples during storage are not significantly different at $p < 0.05$

The rate of lipid oxidation was inhibited to a higher degree (Table 2) in the products containing a mixture of sage extract and sodium isoascorbate, as compared to those with sage extract alone (75.30% to 86.40% vs. 69.97% to 82.30%), which may suggest a synergistic action of these substances. The ability of both additives to inhibit lipid oxidation displayed a falling tendency during prolonged storage, but after 15 days it was still relatively high, i.e. 69.97% and 75.30% in samples A and B respectively.

Table 2
Lipid oxidation inhibition (%) by extract of sage and mixture extract of sage and sodium isoascorbate during refrigerated storage

Time of storage (days)	Extract of sage	With mixture extract of sage and sodium isoascorbate
Raw meatballas	$71.80^{xA} \pm 3.00$	$76.00^{xB} \pm 5.18$
0	$77.77^{ybA} \pm 2.97$	$81.20^{ybB} \pm 1.54$
3	$82.30^{cA} \pm 1.40$	$86.40^{cB} \pm 1.35$
6	$79.07^{bA} \pm 0.83$	$86.23^{cB} \pm 2.11$
9	$78.93^{bcA} \pm 0.92$	$79.60^{bA} \pm 1.39$
12	$77.37^{bA} \pm 1.69$	$81.77^{bB} \pm 0.83$
15	$69.97^{aA} \pm 1.78$	$75.30^{aB} \pm 1.55$

x, y — mean values marked with the same letters for raw and after cooking "0 time of storage" samples are not significantly different at $p < 0.05$;
a, b, c, d – mean values marked with the same letters in columns for samples during storage are not significantly different at $p < 0.05$;
A, B – mean values marked with the same letters in rows are not significantly different at $p < 0.05$.

McCarthy et al. (2001b) added 0.05% of sage extract to meatballs made from fresh and frozen pork, which were then stored at 4°C. These authors found that sage extract inhibited lipid oxidation more effectively in meatballs prepared from frozen pork. According to Buckley et al. (1995), the cell structure of meat is destroyed by ice cubes during deep-freezing, which increases its susceptibility to oxidation. Abd-El-Alim et al. (1999) added sage extract to meatballs made from chicken meat and pork, which enabled to inhibit lipid oxidation both during chill and deep-freeze storage. Wong et al. (1995) added sage extract to beef homogenates, and demonstrated that it was a potent inhibitor of lipid oxidation.

Significant hydrolytic changes (Table 3) were observed in the fat of stored poultry meatballs. The rate of these changes was slower in the samples with additives than in the control samples. In the control products the acid value increased significantly over the first six days of storage, whereas in the products containing sage extract a considerably increase in the acid value was

recorded after three and 15 days. In the samples with a mixture of sage extract and sodium isoascorbate the acid value increased significantly only at the first stage of cold storage (after three days), which could result from the interaction of both substances. FERNANDEZ-FERNANDEZ et al. (2002) and KARPIŃSKA et al. (2001) reported a rising tendency in the acid value in meat products during cold storage. KARPIŃSKA et al. (2001) noted that the rate of changes in the acid value was slower in products containing crumbled sage leaves, as compared with the control samples.

Table 3

Acid value (AV) in fat extracted from poultry meatballs with different antioxidants during refrigerated storage

Time of storage (days)	Reference samples	Samples with extract of sage	Samples with mixture extract of sage and sodium isoascorbate
Raw meatballas	$4.55^{yC} \pm 0.06$	$4.02^{yB} \pm 0.14$	$3.60^{yA} \pm 0.06$
0	$4.10^{xaC} \pm 0.03$	$3.53^{xaB} \pm 0.03$	$3.39^{xaA} \pm 0.06$
3	$4.43^{cB} \pm 0.03$	$3.72^{bA} \pm 0.021$	$3.65^{bA} \pm 0.05$
6	$4.66^{dB} \pm 0.03$	$3,75^{bA} \pm 0.05$	$3.79^{bA} \pm 0.01$
9	$4.74^{dC} \pm 0.01$	$3.72^{bA} \pm 0.01$	$3.85^{bB} \pm 0.02$
12	$4.68^{dB} \pm 0.02$	$3.76^{bA} \pm 0.02$	$3.80^{bA} \pm 0.03$
15	$4.75^{dC} \pm 0.02$	$4.05^{cB} \pm 0.04$	$3.89^{bA} \pm 0.01$

x, y – mean values marked with the same letters for raw and after cooking "0 time of storage" samples are not significantly different at $p < 0.05$,
a, b, c, d – mean values marked with the same letters in columns for samples during storage are not significantly different at $p < 0.05$,
A, B, C – mean values marked with the same letters in rows are not significantly different at $p < 0.05$.

Taste and aroma sensations belong to major qualitative features of meat products. The most important problem is the development of an undesirable warmed-over flavor (WOF) in deli products, especially those made from poultry meat. WOF is easily perceptible during cold storage of heat-treated meat products. The results of a sensory assessment of poultry meatballs reflect the qualitative changes that occurred during storage. The sensory quality of the products was determined by the flavor profile method, and the results of this evaluation are illustrated in Figures 2 to 4. Immediately after the thermal process the control samples received higher scores for such flavor attributes as "meaty", "typical of poultry meat" and "typical of roasted meat", and lower for "aromatic" and "spicy", in comparison with sage-containing samples. The intensity of the following flavor attributes: "meaty", "typical of poultry meat", "typical of roasted meat" and "aromatic" decreased over the storage period, to a higher degree in the control samples than in those containing antioxidants.

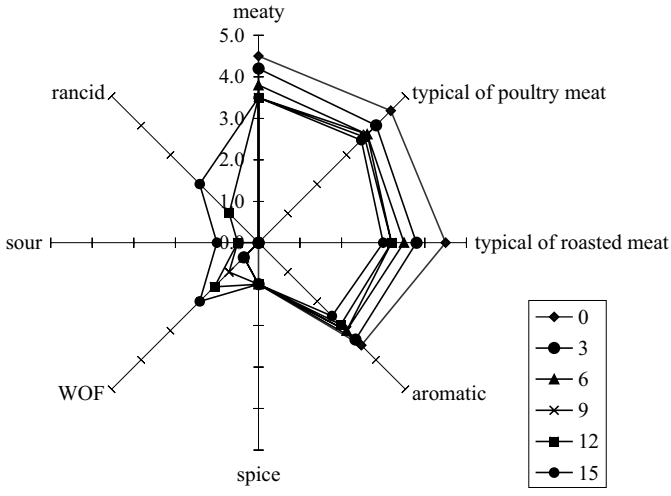

Fig. 2. The flavour profile of control poultry meatballs during refrigerated storage

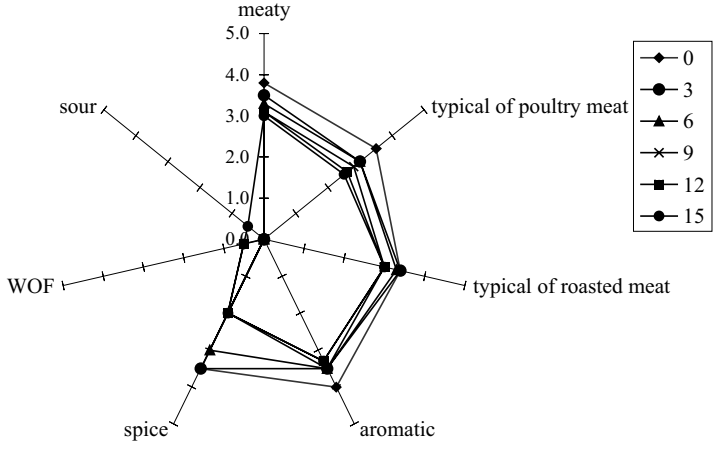

Fig. 3. The flavour profile of poultry meatballs with extract of sage during refrigerated storage

It was found that the addition of sage extract and a mixture of sage extract and sodium isoascorbate inhibited the development of WOF in the experimental products. A warmed-over flavor was recorded in the control samples already after 3 days, and its intensity increased as the time of cold storage was prolonged. In meatballs containing sage extract or sage extract and sodium isoascorbate, WOF was slightly perceptible after nine days of storage. A sour

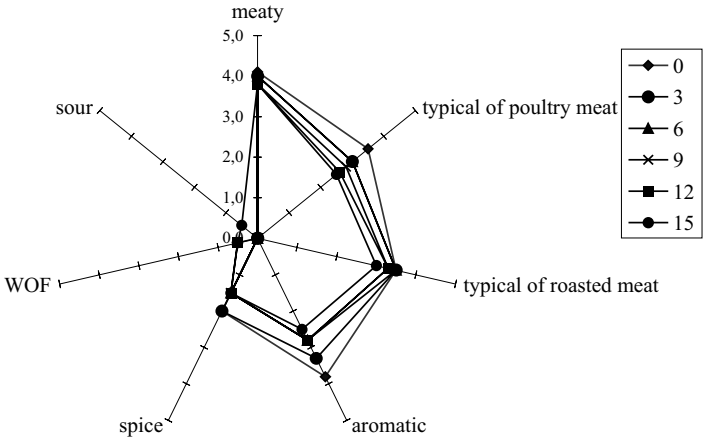

Fig. 4. The flavour profile of poultry meatballs with mixture extract of sage and sodium isoascorbate during refrigerated sorage

flavor was recorded after nine and 15 days in the control and experimental samples respectively. According to GRAY and PEARSON (1987), a rancid flavor may be perceptible in meat products in which the value of TBA is 0.5 to 2.0. In the present study such a value of TBA was observed only in control meatballs, characterized by a rancid flavor towards the end of storage (12 to 15 days). Due to the fact that flavoring substances were eliminated from sage leaves partly only, the intensity of a spicy flavor was higher in sage-containing meatballs than in the control samples and those with sage extract and sodium isoascorbate over the entire storage period.

KARPIŃSKA et al. (2000, 2001) reported that prolonged cold or deep-freeze storage of deli poultry products is accompanied by deterioration of their sensory quality. In samples containing herbs and spices (sage, a mixture of pepper, paprika, sage, garlic and marjoram, and rosemary extract) the sensory quality deteriorated at a slower rate, and WOF and a sour flavor developed later than in the control samples. AHN et al. (2002) and WU et al. (2000) also demonstrated that the intensity of WOF in deli products made from pork and beef increased gradually as the time of cold storage was extended.

Conclusions

It was found that sage extract, applied alone or combined with sodium isoascorbate, inhibited oxidative changes in poultry meatballs. The rate of lipid oxidation was inhibited to a higher degree in the products containing a mixture

of sage extract and sodium isoascorbate, as compared to those with sage extract alone, which may suggest a synergistic action of these substances. After 15 days of cold storage the ability of both sage extract and sage extract + sodium isoascorbate to inhibit lipid oxidation was still high, i.e. 69.97% and 75.30% respectively. The additives inhibited also hydrolytic changes in experimental meatballs. The rate of these changes was slower in the products containing a mixture of sage extract and sodium isoascorbate, in comparison with those with sage extract.

The products with additives had a better sensory quality during cold storage than the control samples. In the former the intensity of flavor attributes (meaty, typical of poultry meat, typical of roasted meat, aromatic) decreased at a slower rate. A warmed-over flavor (WOF) and a sour flavor developed at a later stage of storage in poultry meatballs containing sage extract or sage extract + sodium isoascorbate, in comparison with the control samples. A rancid flavor was not recorded in samples with additives.

The result of the study show that both sage extract and a mixture of sage extract and sodium isoascorbate can be added to meat products in order to preserve their high quality during storage.

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THE EFFECT OF HYDROTHERMAL TREATMENT OF CARROT ON THE TRANSFORMATIONS OF SELECTED CHEMICAL COMPONENTS AND QUALITY OF PUREE

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K e y w o r d s: carrot, hydrothermal treatment, chemical compounds, characteristics of puree.

A b s t r a c t

This study aimed at determining the effect of various types of hydrothermal treatment of carrot on the transformations of their dietetic and bioactive components. Carrot of the Bangor, Fayette and Nektarina varieties were used in the study. Among the hydrothermal parameters used were steam, water (100°C) and 0.05% citric acid solution (100°C), and the carrot were treated as whole roots or in cubes. It was shown that treatment with steam caused the lowest level of loss of soluble components, such as reducing sugars and the components of soluble dietary fibre (SDF), statistically significant in comparison with other variants ($p < 0.05$). Treating carrot cubes increased the intensity of transformations of the analysed components. Multidirectional transformations of α- and β-carotene were found to take place. Not only did their overall level decreased, but they were also transformed to the cis- form. Other observed tendencies included interaction with pectins and releasing carotenoids from compounds with insoluble dietary fibre. In the case of carotenoids, the most favourable variant was the treatment of whole carrot roots with 0.05% solution of citric acid. The loss of α- and β-carotene in this variant amounted to: 8.22%-17.64% and 17.33%-23.45%, respectively. The varied effect of the hydrothermal treatment of carrot on the quality of puree, as shown in this study, presents the possibility of selecting the optimal variant, depending on the planned final product.

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WPŁYW WARUNKÓW OBRÓBKI HYDROTERMICZNEJ MARCHWI NA ZMIANY WYBRANYCH SKŁADNIKÓW CHEMICZNYCH I JAKOŚĆ PRZECIERÓW

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S ł o w a k l u c z o w e: marchew, obróbka hydrotermiczna, składniki chemiczne, charakterystyka przecierów.

A b s t r a k t

Określono wpływ różnych sposobów obróbki hydrotermicznej marchwi odmian: Bangor, Fayette i Nektarina na przemiany składników dietetycznych i bioaktywnych. Marchew poddawano obróbce w całości lub w postaci kostki, stosując parę wodną (103°C), wodę (100°C) oraz 0,05% roztwór kwasu cytrynowego (100°C). Wykazano, że obróbka parą wodną powodowała najmniejsze straty rozpuszczalnych składników marchwi, jak cukry redukujące oraz komponenty frakcji rozpuszczalnej błonnika pokarmowego (SDF), istotne statystycznie w porównaniu z pozostałymi wariantami ($p < 0,05$). Poddanie obróbce marchwi w postaci kostki zwiększało intensywność przemian analizowanych składników. Stwierdzono wielokierunkowe przemiany α- i β-karotenu. Oprócz zmniejszenia ogólnej ich zawartości, obserwowano transformację do formy cis, wykazano tendencję do interakcji z pektynami, a jednocześnie uwalnianie karotenoidów z połączeń z frakcją nierozpuszczalną błonnika pokarmowego. W przypadku karotenoidów wariantem najbardziej korzystnym była obróbka w 0,05% roztworze kwasu cytrynowego całych korzeni marchwi. Stwierdzono, że straty α- i β-karotenu w odniesieniu do surowca wynosiły odpowiednio: 8.22%-17.64% i 17.33%-23.45%. Wykazany w badaniach zróżnicowany wpływ sposobu obróbki hydrotermicznej marchwi na jakość otrzymanych przecierów daje możliwość wyboru optymalnego wariantu w zależności od projektowanego wyrobu gotowego.

Introduction

Carrot puree is a semi-finished product widely used in juice and beverage production and as a component of dinner courses and desserts for children. The quality of puree, determined by such features as dietetic and bioactive component content, sensory qualities or rheological properties, is largely determined by carrot variety and by the conditions of the hydrothermal treatment which precedes the process of rubbing. Transformations within pectins, dietary fibre and carotenoids are considered to be the most important (SHARPLESS et al. 1995, REDONDO et al. 1997, BOROWSKA et al. 2003, ZADERNOWSKI et al. 2003).

Hydrothermal treatment is conducted according to various methods, with varied values of temperature and time. According to REDONDO et al. (1997), thermolabile polysaccharides (pectins) are affected to the largest extent by hydrothermal treatment. Pectin transformations in carrots caused by heating

concern mainly the changes in the branched area and β-elimination, which leads to an increase in the proportion of the soluble fraction (GREVE et al. 1994). A high, 2.5-fold increase in the content of the fraction was found after boiling carrot (PENNER, KIM 1991, PHILLIPS, PALMER 1991). Cellulose, hemicellulose and lignins was found to be less susceptible to change (MASSIOT et al. 1992, BOROWSKA et al. 2000). Transformations of polysaccharides are accompanied by changes in texture, one of the reasons being a loss of pectins; ability to form gel structures. These transformations may result in the separation of puree components (MASSIOT et al. 1992, VAN BUREN, PITIFER 1992, KATO et al. 1997).

Thermal treatment is accompanied by multidirectional transformations of carotenoids. Mild heating may lead to binding of α- and β-carotene with pectins, particularly in unesterified ones (SIMS et al. 1993, SHARPLESS et al. 1995). More extreme hydrothermal treatment, particularly at an increased pressure, may lead to isomerisation and to an increase in the content of cis β-, α-carotene and β-cryptoxanthin, with vitamin A activity reduced by about 50% as compared to the *trans* forms (HERRMANN 1995, REDONDO et al. 1997). According to CHEN et al. (1995), the formation of cis-isomers is accompanied by deterioration of carrot juice colour.

Consequently, the correct choice of the thermal treatment parameters is important for the technological processes and for the nutritional quality of the products. Our earlier studies (BOROWSKA et al. 2004) concerned the effect of variable conditions of the thermal treatment of carrots on its sensory and texture quality. The aim of this study was to assess the changes of chemical compounds in carrot such as acid detergent fibre, dietary fibre, pectins, α- and β-carotene during various types of hydrothermal treatment and the characteristics of obtained puree.

Materials and Methods

The material used in the study were puree obtained from carrot of the Bangor, Fayette and Nektarina varieties, grown at the Experimental Farm in Żelazna near Skierniewice. The raw material was collected at the beginning of October. Morphological characteristics of the examined carrot varieties was described in the doctoral dissertation (KOWALSKA 2002). The carrots were washed, peeled and subjected to hydrothermal treatment. Hydrothermal treatment was applied for either whole carrot roots of carrot cubes (15 x 15 x 10 mm), with the use of water, water with the addition of citric acid or steam (in a convection-type steam furnace) as a hydrothermal medium. A description of experimental variants is presented in Table 1. After hydrothermal treatment,

the carrot were rubbed in a laboratory device (HR 2831 Philips) and ground on a colloidal mill (MZ-80/R Fryma). The puree obtained in this way was used in chemical analyses (variant 1-6). Raw carrots, ground in a laboratory robot (HR 2831 Philips), were used as a reference sample (variant 0).

Table 1

The conditions of hydrothermal treatment of carrot

Variant	Form of carrot	Hydrothermal medium	Temperature (°C)	Time (min)
1	cubes	steam	103	45
2	whole	steam	103	50
3	cubes	water	100	30
4	whole	water	100	55
5	cubes	0.05% solution of citric acid	100	50
6	whole	0.05% solution of citric acid	100	80

The following were determined in the puree: content of extract (PN-90-A--75101/02), reducing and total sugars according to Lane-Eynona (PN-90-A--75101/07), acid detergent fibre (ADF), including lignin and cellulose (AOAC 1990), total dietary fibre (TDF), including the insoluble (IDF) and soluble fraction (SDF), according to Asp et al. (1983), pectins, including the fraction soluble in water, fraction soluble in oxalates and protopectin according to the method proposed by YU et al. (1996). The degree of pectin esterification was determined according to MCCREADY (1970). Among the bioactive compounds, α- and β-carotene was determined by the HPLC technique according to CHEN et al. (1995). In the Bangor variety, the amount of α- and β-carotene was determined, which is bound with the insoluble fraction of dietary fibre and pectins.

The carotenoids were separated using a Hewlett-Packard HPLC 1050 system with diode array detector. The separation conditions were as follows: Vydac 5 μm 218TP54 column 250 mm x 4.6 mm and precolumn Zorbax 300 SB-C18 12.5 mm x 4.6 mm, 5 μm; temperature 25°C; mobil phase, methanol-methylene chloride (99:1 v/v) with methanol-methylene chloride (45:55 v/v) as sample solvent; flow rate, 1 $cm^3 \cdot min^{-1}$; detection wavelength, 450 nm; data integration with ChemStation (HP) program. The standards of α- and β-carotene from Sigma were used.

In order to determine the quantities of α-and β-carotene bound with dietary fiber, its insoluble fraction was obtained according to ASP et al. (1983). The pectin fraction was extracted according SABIR et al. (1976). Both fractions were rinsed with 30 cm^3 of water-ethanol (1:1) mixture, containing 1.5 cm^3 2M HCl.

Carotenoids were extracted from samples prepared in this way, according to the method given by CHEN et al. (1995).

The experiment was repeated and chemical analysis were carried out in triplicate. The results were elaborated statistically employing a one-factor analysis of variance. The significance of differences between mean values of the results was estimated by the Duncan test. A statistical analysis was made at the significance level of $p < 0.05$.

Results and Discussion

Extract, sugars

The puree obtained in the experiment contained varied amounts of extract, ranging from 6.37% to 11.20% (Table 2). Regardless of the variety, statistically significant differences were found ($p < 0.05$) between puree obtained after treating carrot with steam (variant 1, 2), and that obtained after the hydrothermal treatment in water or 0.05% citric acid solution (variant 3, 4, 5, 6). The form of the raw material was also shown to have affected the extract content in the puree. The highest extract, ranging from 10.72% to 11.20% was found in the puree obtained after treating whole carrot roots with steam. These puree contained more extract than the raw material. Treating diced carrots resulted in more intense washing out of the extract components as a result of increasing the size of surface in contact with the hydrothermal

Table 2

Extract and sugar content in raw carrot and in puree

Variant	Extract (%)			Reducing sugars ($g \cdot 100\ g^{-1}$)			Total sugars ($g \cdot 100\ g^{-1}$)		
	Bangor	Fayette	Nektarina	Bangor	Fayette	Nektarina	Bangor	Fayette	Nektarina
0	10.62^{aA}	9.93^{abdA}	8.87^{aB}	4.24^{aA}	3.01^{aB}	2.02^{aC}	8.01^{aA}	6.59^{aB}	6.71^{aB}
1	11.20^{aA}	11.07^{aA}	10.72^{bA}	4.99^{bA}	4.45^{bB}	2.23^{bC}	5.16^{bA}	4.48^{bB}	4.22^{beB}
2	10.73^{aA}	10.48^{abA}	9.70^{cB}	4.63^{cA}	4.20^{cB}	2.22^{bC}	5.39^{bcA}	4.69^{cB}	3.97^{bB}
3	6.37^{bA}	6.60^{cA}	6.62^{dA}	2.26^{dA}	2.42^{dB}	1.41^{cC}	5.72^{cdA}	4.90^{dB}	4.12^{bdB}
4	7.90^{cA}	9.77^{bdB}	8.73^{aA}	2.54^{eA}	$2.97a^{eB}$	2.01^{aC}	5.83^{dA}	4.91^{dB}	5.05^{cdefB}
5	6.82^{bA}	6.70^{cA}	7.55^{eB}	2.02^{dA}	2.82^{fB}	1.52^{dC}	5.75^{dA}	4.91^{dB}	4.84^{bfB}
6	8.57^{cA}	8.98^{dA}	8.62^{aA}	2.54^{eA}	2.93^{eB}	2.00^{aC}	5.93^{dA}	5.01^{eB}	5.00^{cdefB}

Notes:
0 – raw material; 1 – cubes of carrot, steam (103°C, 45 min); 2 – whole carrot, steam (103°C, 50 min.); 3 – cubes of carrots, water (100°C, 30 min); 4 – whole carrot, water (100°C, 55 min); 5 – cubes of carrot, 0.05% solution of citric acid (100°C, 50 min); 6 – whole carrot, 0.05% solution of citric acid (100°C, 80 min.);
Different letters (*a, b, c.*) in the same column and different letters (*A, B, C*) in the row indicate significant differences, $p < 0.05$.

medium. It was also shown that the effect of a carrot variety was much smaller than that of the conditions of treatment and was statistically insignificant for most samples ($p < 0.05$).

Similar relationships were observed for directly reducing sugars (Table 2). Their highest amounts, higher than in the raw material, were found in the puree portions which were obtained after treating carrots with steam (variant 1, 2). A loss of reducing sugars was observed in carrots treated with water (variant 3) or 0.05% solution of citric acid (variant 5) as cubes and were partly caused by their washing out to the hydrothermal medium. In most variants of the experiments, the differences in reducing sugar contents within individual varieties were statistically significant ($p < 0.05$). Smaller differences for specific variants of the experiment were observed for total sugars, determined according to the method proposed by Lane-Eynona (PN-90-A-75101/07) – Table 2.

Among the analysed puree, the highest extract and the total reducing sugars content (11.20% and 4.99 g · 100 g^{-1}, respectively) was found in the puree obtained from the Bangor variety carrot in variant 1. It should be noted that the raw carrot of this variety contained the highest amounts of extract and the highest amounts of sugars among the varieties covered by the study. These results are well correlated with the sensory assessment, which reveals that in both the raw material and the puree obtained from the Bangor variety, the sweet taste was felt with a greater intensity (BOROWSKA et al. 2004). The results obtained by other researchers indicate that an increase in the concentration of simple sugars as a result of the hydrothermal treatment of carrot is caused primarily by transformations in pectins, particularly in the "hairy region", and to a lesser extent – by transformations of cellulose (MASSIOT et al. 1992, REDONDO et al. 1997). This fact is corroborated by the changes in fibre and pectin fraction in the experiment in question (Tables 3, 4, 5).

Table 3

Fibre content (ADF) in raw carrot and in puree

Variant	Total (ADF) (g · 100 g^{-1})			Cellulose (g · 100 g^{-1})			Lignin (g · 100 g^{-1})		
	Bangor	Fayette	Nektarina	Bangor	Fayette	Nektarina	Bangor	Fayette	Nektarina
0	1.42^{aA}	1.79^{aB}	1.17^{aC}	1.36^{aA}	1.58^{aB}	1.11^{aC}	0.06^{aA}	0.21^{acB}	0.06^{aA}
1	0.59^{bA}	0.87^{bB}	0.41^{bC}	0.54^{bA}	0.68^{bB}	0.36^{bC}	0.05^{bA}	0.19^{bB}	0.05^{aA}
2	0.70^{cA}	1.10^{cB}	0.51^{cC}	0.64^{cA}	0.90^{cB}	0.46^{cC}	0.06^{abA}	0.20^{abB}	0.05^{aA}
3	0.80^{dA}	1.12^{cB}	0.55^{cC}	0.74^{dA}	0.90^{cB}	0.50^{cC}	0.06^{abA}	0.22^{cdB}	0.05^{aA}
4	1.37^{eA}	1.42^{dA}	0.84^{dB}	1.31^{eA}	1.20^{dA}	0.78^{dB}	0.06^{aA}	0.22^{cdB}	0.06^{aA}
5	1.10^{fA}	1.26^{cB}	0.85^{dC}	0.95^{fA}	1.04^{cB}	0.79^{dC}	0.06^{aA}	0.22^{cdB}	0.06^{aA}
6	1.37^{eA}	1.29^{cA}	1.05^{eB}	1.31^{eA}	1.08^{cB}	0.99^{eB}	0.06^{aA}	0.21^{abdB}	0.06^{aA}

Explanations see Table 2

Table 4

Dietary fibre (DF) content in raw carrot and in puree

Variant	Total (DF)			Insoluble fraction (IDF)			Soluble fraction (SDF)		
	($g \cdot 100\ g^{-1}$)			($g \cdot 100\ g^{-1}$)			($g \cdot 100\ g^{-1}$)		
	Bangor	Fayette	Nektarina	Bangor	Fayette	Nektarina	Bangor	Fayette	Nektarina
0	4.57^{aA}	4.69^{acA}	4.86^{aB}	2.99^{aA}	3.04^{aA}	2.85^{aB}	1.58^{aA}	1.65^{aA}	2.01^{aB}
1	4.38^{bA}	4.11^{bB}	4.67^{bC}	1.55^{bA}	1.73^{bB}	1.46^{bC}	2.83^{bA}	2.38^{bB}	3.21^{bC}
2	4.40^{bA}	4.28^{bB}	4.77^{abC}	1.74^{cA}	2.01^{cB}	2.21^{cC}	2.66^{cA}	2.27^{bdB}	2.56^{cC}
3	4.10^{dA}	4.35^{abB}	4.28^{cB}	2.48^{dA}	2.35^{dB}	2.40^{dB}	1.62^{aA}	2.00^{cB}	1.88^{aC}
4	4.53^{aA}	4.68^{acA}	4.90^{aA}	2.51^{dA}	2.43^{dA}	2.51^{dA}	2.01^{dAB}	2.26^{bdB}	2.40^{dCB}
5	4.31^{bA}	4.46^{abcA}	5.09^{dB}	2.33^{eA}	2.36^{dA}	2.39^{dA}	1.98^{dA}	2.10^{cdA}	2.69^{cB}
6	4.60^{aA}	4.81^{cB}	5.09^{dC}	2.40^{fA}	2.41^{dA}	2.49^{dA}	2.20^{eA}	2.40^{bB}	2.60^{cC}

Explanations see Table 2

Acid detergent fibre (ADF), dietary fibre (DF)

The analysis of transformations in the acid detergent fibre revealed that the hydrothermal treatment conducted according to all of these variants (1-6) caused a statistically significant ($p < 0.05$) decrease in its concentration in puree as compared with the raw material (Table 3). ADF content in the puree obtained in the experiment ranged from 0.59 $g \cdot 100\ g^{-1}$ to 1.37 $g \cdot 100\ g^{-1}$ for the Bangor variety, from 0.87 $g \cdot 100\ g^{-1}$ to 1.42 $g \cdot 100\ g^{-1}$ for the Fayette variety and from 0.41 $g \cdot 100\ g^{-1}$ do 1.05 $g \cdot 100\ g^{-1}$ for the Nektarina variety. The most strong changes were observed when the carrot were treated with steam, while the least significant changes took place during the treatment with 0.05% solution of citric acid. These changes were largely affected by the form of the raw material subjected to treatment. Higher losses of ADF, even by as much as about 54%, were observed in samples treated in the form of cubes, and were largely restricted to the cellulose fraction.

The cellulose fraction dominated both in the raw material and in the puree (Table 3). Its content in the puree ranged from 0.36 $g \cdot 100\ g^{-1}$ to 1.31 $g \cdot 100\ g^{-1}$. The transformations in this fraction were affected to the greatest extent by the treatment with steam. The effect of the treatment with 0.05% solution of citric acid was not so large. As a result of the transformations of cellulose, simpler soluble forms migrated to the treating medium. The grinding of carrots increased the intensity of the transformations of this fraction by increasing the surface in contact with the treating medium. Such transformations of cellulose were substantiated in the studies conducted by PENNER, KIM (1991).

The lignin fraction was highly stable (Table 3). The differences in the concentration of this fraction between raw material and puree within the varieties were in most cases statistically insignificant ($p < 0.05$). However, such

Table 5

Characteristics of pectins in raw carrot and in puree

Variant	Total pectin ($g \cdot 100\ g^{-1}$)			Water soluble pectin ($g \cdot 100\ g^{-1}$)			Oxalate soluble pectin ($g \cdot 100\ g^{-1}$)			Protopectin fraction ($g \cdot 100\ g^{-1}$)		
	Bangor	Fayette	Nektarina	Bangor	Fayette	Nektarina	Bangor	Fayette	Nektarina	Bangor	Fayette	Nektarina
0	1.23^{aA}	1.20^{aB}	0.94^{aC}	0.05^{aA}	0.04^{aB}	0.04^{aB}	0.24^{aA}	0.34^{aB}	0.28^{aC}	0.94^{aA}	0.82^{aB}	0.61^{aC}
1	0.63^{bA}	0.61^{bB}	0.52^{bC}	0.36^{bA}	0.31^{bB}	0.24^{bC}	0.14^{bA}	0.18^{bB}	0.12^{bC}	0.12^{bA}	0.11^{bA}	0.06^{bB}
2	0.70^{cA}	0.66^{cB}	0.57^{cC}	0.35^{bA}	0.30^{cB}	0.24^{bC}	0.14^{bA}	0.19^{bB}	0.13^{bcA}	0.21^{cA}	0.17^{cB}	0.09^{cC}
3	0.55^{dA}	0.59^{dB}	0.54^{dA}	0.21^{cA}	0.22^{dB}	0.20^{cA}	0.16^{cA}	0.20^{cB}	0.14^{cC}	0.18^{dA}	0.17^{cA}	0.13^{dB}
4	0.58^{eA}	0.61^{bB}	0.56^{cC}	0.20^{cA}	0.24^{eB}	0.22^{dC}	0.16^{cA}	0.20^{cB}	0.20^{dB}	0.21^{cA}	0.17^{cB}	0.14^{dC}
5	0.61^{fA}	0.58^{eB}	0.59^{eC}	0.21^{cA}	0.20^{fA}	0.22^{dB}	0.17^{dA}	0.20^{cB}	0.20^{dB}	0.23^{eA}	0.17^{cB}	0.17^{eB}
6	0.67^{gA}	0.64^{fB}	0.60^{fC}	0.23^{dA}	0.25^{gB}	0.27^{eC}	0.20^{eA}	0.22^{dB}	0.22^{eB}	0.24^{eA}	0.18^{dB}	0.17^{eC}

Explanations see Table 2

differences were shown to exist between the varieties. The highest lignin content (almost 3 times as high as in the other varieties), technologically adverse, was found in the puree obtained from the Fayette variety.

The concentration of the total dietary fibre (DF) in the analysed puree samples was more levelled than was the case for ADF and ranged from 4.10 g · 100 g^{-1} to 5.09 g · 100 g^{-1} (Table 4). This level was close to that found for the raw material. The effect of varied parameters of the hydrothermal treatment was shown by a separate analysis of the insoluble (IDF) and soluble (SDF) fractions. Compared with the raw material, the puree samples contained smaller amounts of the insoluble fraction and higher amounts of the soluble one. The largest changes were observed for the IDF fraction in the puree obtained from diced carrots. The puree obtained in variant 1 were significantly different ($p < 0.05$) from all the others. The changes in the insoluble fraction are mainly linked to the transformations of cellulose, hemicellulose and protopectins, while the transformations of the soluble fraction were observed mainly in pectins, arabinoxylans and β-glucans (MASSIOT et al. 1992, REDONDO et al. 1997). The transformations of the dietary fibre polysaccharides are significant as they determine not only the rheological properties of puree, but also affect their nutritional quality (SVANBERG et al. 1995).

Pectins

The hydrothermal treatment of carrots brought about statistically significant transformations ($p < 0.05$) of all the determined fractions of pectins as compared to the raw material (Table 5). These transformations resulted in changes in texture, a loss of turgor and softening (BOROWSKA et al. 2004). In each variant and for each carrots variety, the amount of water-soluble fraction was found to increase, with an observed decrease in the concentration of protopectins and oxalate-soluble pectins. For example, the concentration of water-soluble pectins in raw carrot of the Bangor variety was as low as 0.05 g · 100 g^{-1}, while that found in puree ranged from 0.20 g · 100 g^{-1} (variant 4) to 0.36 g · 100 g^{-1} (variant 1). The proportions were reversed in the case of protopectins. The protopectin fraction dominated in the raw material and its concentration equalled 0.94 g · 100 g^{-1}, whereas in puree samples it ranged from 0.12 g · 100 g^{-1} (variant 1) to 0.24 g · 100 g^{-1} (variant 6). Of the treatment types used in the experiment, the most intense transformations were observed as a result of the action of steam. The puree samples, obtained in variant 1, 2 differed significantly from the others ($p < 0.05$) in terms of the concentrations of the pectin fractions discussed. When discussing the effect of a carrot form on the transformations in question, a higher intensity of transformations in diced

carrots should be stressed. This is indicated by a higher loss of protopectins under the treatment by various hydrothermal media (variant 1, 3, 5).

According to MASSIOT et al. (1992), boiling carrots for 10 min. increases the amount of soluble pectins by 25%, a fact believed to be caused mainly by the hydrolysis of protopectin. According to REDONDO et al. (1997) and ANDERSON, CLYDESDALE (1980), a loss in total pectin content is observed both during the treatment by hot water and by steam in an autoclave, which the authors see as caused by migration of the galacturonic acid to the hydrothermal medium. PLAT et al. (1988) and MASSIOT et al. (1992) observed a multi-fold increase in the amounts of rhamnose, arabinose and glucose after blanching, which is short-time heating, of carrots. Another way of transformations is a degradation of pectins to shorter fragments of polygalacturonic acid of higher solubility (GREVE et al. 1994). Our results confirm such directions of changes, indicating an increase in extract and reducing sugars concentration in the puree samples obtained in variant 1 and 2 (steam) – Table 2.

Another feature determined was the degree of pectin esterification (Figure 1). This index ranged from 7.5% (variant 1, Fayette variety) to 36.7% (variant 6, Bangor variety) and was significantly different for the analysed puree samples ($p < 0.05$). The highest degree of pectin deesterification for each carrot variety was observed for variant 1 and 2. It was also shown that the transformations were more intense for the diced carrots. The degree of pectin esterification was the lowest (3-7 times lower than in the raw material) in the

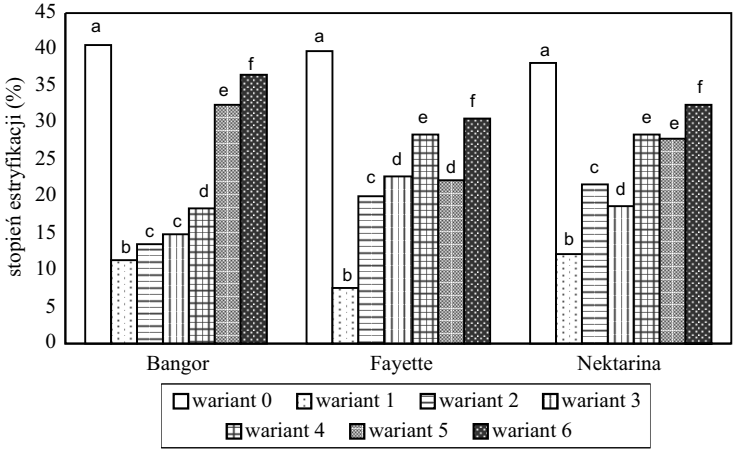

Fig. 1. Esterification degree of pectins in raw carrot and in puree: 0 – raw material; 1 – cubes of carrot, steam (103°C, 45 min), 2 – whole carrot, steam (103°C, 50 min), 3 – cubes of carrots, water (100°C, 30 min), 4 – whole carrot, water (100°C, 55 min), 5 – cubes of carrot, 0.05% solution of citric acid (100°C, 50 min); 6 – whole carrot, 0.05% solution of citric acid (100°C, 80 min); different letters indicate significant differences, $p < 0.05$

puree samples obtained from carrots treated with steam. The least intense transformations took place under the treatment of whole carrot roots by 0.05% solution of citric acid.

Pectin transformations are important both from a technological and nutritional point of view. Pectin deesterification caused by hydrothermal treatment leads to a change in their properties, such as solubility, swelling ability and the ability to create colloidal systems (SAJJAANANTAKUL et al. 1989). Extreme conditions of hydrothermal treatment may result in pectin depolymerisation and the formation of low-weight molecules without the ability to create gels (PAGAN, IBARZ 1999). It has also been shown that the lower level of pectin esterification, the more prone they are to bind to carotenoids, which restricts their bioavailability in puree juices (SIMS et al. 1993).

α- and β-carotene

Carotenoid transformations during the hydrothermal treatment were multidirectional and depended on the type of a hydrothermal medium (indirectly – on the temperature and time of the treatment) and on the raw material form (Table 6, Figures 2, 3). Analysing the carotenoids with the HPLC technique pointed to β-carotene as the dominant compound in the raw material (60-70% of the total carotenoids), followed by α-carotene (25-30%). Small amounts of lutein and 13-cis-β-carotene were also found. In the carrot Bangor variety included in the study, 68.28% of α-carotene and 55.93% of β-carotene occurred in the form bound to the insoluble dietary fibre. The soluble fraction, represented mainly by pectins, was found to contain 8.28% of α-carotene and 9,03% of β-carotene.

Table 6

α- and β-carotene content in raw carrot and in puree

Variant	α-carotene ($mg \cdot 100\ g^{-1}$)			β-carotene ($mg \cdot 100\ g^{-1}$)		
	Bangor	Fayette	Nektarina	Bangor	Fayette	Nektarina
0	2.90^{aA}	4.49^{aA}	5.12^{aC}	10.69^{aA}	24.25^{aB}	20.01^{aC}
1	1.93^{bA}	3.44^{bB}	3.25^{bC}	6.98^{bA}	15.11^{bB}	14.01^{bC}
2	1.99^{cA}	3.68^{cC}	3.51^{cC}	7.01^{bA}	16.12^{cB}	16.11^{cB}
3	2.04^{dA}	3.95^{dB}	4.39^{dC}	7.55^{cA}	17.04^{dB}	18.59^{dC}
4	2.15^{eA}	3.91^{dB}	4.63^{eC}	7.89^{dA}	18.48^{eB}	19.24^{eC}
5	2.22^{fA}	4.00^{deB}	4.23^{fC}	8.01^{eA}	19.88^{fB}	21.15^{fC}
6	2.52^{gA}	4.11^{eB}	4.69^{eC}	8.51^{fA}	20.00^{fB}	22.01^{gC}

Explanations see Table 2

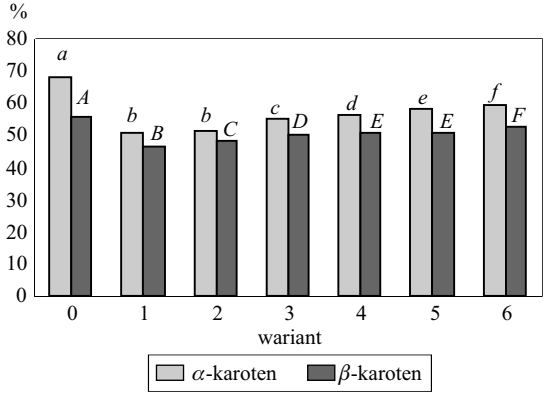

Fig. 2. Proportion of α- and β-carotene bound to insoluble fraction of dietary fibre (Bangor variety) Explanations see Fig. 1

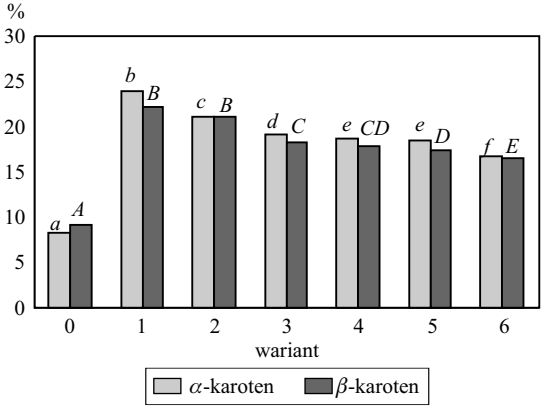

Fig. 3. Proportion of α- and β-carotene bound to pectins (Bangor variety) Explanations see Fig. 1

Most of the puree samples differed significantly ($p < 0.05$) in terms of α-and β-carotene content (Table 6). Like with other analysed components, more intense transformations were observed in the diced material. The hydrothermal treatment resulted in a decrease in β-carotene; smaller changes were observed in the case of α-carotene. These changes were brought about both by a migration to the hydrothermal medium, and by the isomerisation to the cis-form. The highest increase – 2-2.5 times – in the concentration of cis β-carotene was observed when carrots were treated with steam. A high susceptibility of β-carotene to isomerisation to the cis-form is emphasised in the studies conducted by BORCHGREVNIK, CHARLEY (1996).

Interactions (caused by heating) between α- and β-carotene on the one hand and IDF and pectins on the other, were analysed for the Bangor variety (Figures 2, 3). Each puree sample obtained from this variety (variant 1-6) was found to contain lower amounts, as compared to the raw material and a statistically significant ($p < 0.05$) amount of carotenoids bound to IDF. The most intense transformations took place during the treatment of diced carrots by steam and the weakest when whole carrot roots were treated with 0.05% solution of citric acid. Reverse relationships were observed in the case of pectins. Hydrothermal treatment, especially with steam, favoured their binding with α-and β-carotene. The results suggest that the fact may be linked to deesterification of pectins during a hydrothermal treatment and to an increase in their susceptibility to binding carotenoids. These results corroborate those obtained in our earlier study on the Caropak, Simba and Fayette varieties, with whole carrot roots being subjected to hydrothermal treatment (BOROWSKA et al. 2003). The highest degree of binding carotenoids by pectins and releasing carotenoids from their compounds with the IDF fraction was observed during the action of steam on carrots. The possibility of this kind of interaction is indicated by SIMS et al. (1993). According to those authors, such transformations may result in a deterioration in juice colour.

Conclusions

1. Hydrothermal treatment of carrot applied before rubbing had significantly influence on the changes in dietetic compounds such as fibre, pectins and carotenoids.

2. It was shown that the changes of analysed compounds in carrot were depended on the type of a hydrothermal medium (steam, water, 0.05% solution of citric acid) and also on the raw material form (whole roots, cubes). More intense changes of compounds were observed during the treatment of diced carrots.

3. The characteristics of the obtained puree showed that steam had the most conservative effect on the soluble compounds in carrot puree extracts.

4. Hydrothermal treatment had effect on interaction fibre with carotenoids and pectins with carotenoids. The obtained puree were characterised by lower amounts of α- and β-carotene compared to the raw material.

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MICROBIOLOGICAL QUALITY OF HIGH-PRESSURE PRESERVED STUFFING AND PROCESSED MEATS

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K e y w o r d s: pressurization, meat-fat stuffing, microbiological quality.

A b s t r a c t

The aim of this study was to determine the effects of high-pressure technology on the microbiological quality of pork meat-fat stuffing corned with different amounts of table salt, and sausages made from this stuffing. Vacuum-packed stuffing and stuffing in barrier casings were brined with 1% and 2% NaCl and pressurized at 200, 300 and 500 MPa for 10 minutes. Pressurization changed pH levels in meat-fat stuffing. The majority of bacterial groups were not affected by the varied quantity of table salt added to the stuffing. Pressurization at 300 and 500 MPa of meat-fat stuffing contributed to a reduction in the counts of mesophilic aerobes and psychrotrophic bacteria by 2 to 5 log cycles, and limited their growth over 7-day cold storage. Pressurization at 500 MPa of fine ground and semi-coarse ground sausages scalded at 55°C for 30 minutes enabled to reduce total bacterial counts below 0.95 log cycle as well as to maintain them at this level during 7-day cold storage.

JAKOŚĆ MIKROBIOLOGICZNA FARSZÓW I WĘDLIN UTRWALONYCH TECHNIKĄ WYSOKICH CIŚNIEŃ

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S ł o w a k l u c z o w e: ciśnieniowanie, farsze mięsno-tłuszczowe, jakość mikrobiologiczna.

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Abstrakt

Badano wpływ technologii wysokich ciśnień na jakość mikrobiologiczną wieprzowych farszów mięsno-tłuszczowych, peklowanych z 1 i 2% dodatkiem soli kuchennej, oraz kiełbas wyprodukowanych z tych farszów. Farsze w osłonkach barierowych lub zapakowane próżniowo poddawano ciśnieniu 200, 300, 500 MPa przez 10 minut, co przyczyniło się do zmiany ich odczynu. Na liczbę większości badanych grup bakterii nie wpływał zróżnicowany dodatek soli kuchennej. Poddawanie farszów mięsno-tłuszczowych ciśnieniu 300 i 500 MPa spowodowało redukcję mezofilnych bakterii tlenowych i bakterii psychrotrofowych o 2-5 cykli logarytmicznych, a także wpłynęło na mniejszy wzrost ich liczby w czasie 7-dniowego chłodniczego przechowywania. Paskalizacja w 500 MPa wędlin parzonych w obniżonej temperaturze (55°C przez 30 min), drobno i średnio rozdrobnionych, przyczyniła się do redukcji liczby oznaczanych bakterii do poziomu poniżej 0,95 cyklu log, który nie uległ zmianie w czasie 7-dniowego chłodniczego przechowywania.

Introduction

High-pressure technology, one of modern physical methods for food preservation and modification, has been gaining an increasing interest among food producers in recent years. High-pressure technology (to 1000 MPa) can successfully replace thermal processing and is used primarily for extending the shelf-life of food products. Compared to heating, pressurization has more advantages since it protects the natural freshness of foodstuffs causing no undesirable changes. In the case of meat raw materials and processed products, pressurization not only preserves them, but also contributes to improving the functional properties of muscle proteins, including among others gelling without or with a small amount of table salt, the ability to form stable water-lipid-protein emulsions as well as a higher water-holding capacity (CREHANI et al. 2000, GARRIGA et al. 2004, HUGAS et al. 2002, JANKOWSKA et al. 2000, JUNG et al. 2003, KALCHAYANAND et al. 1998, KORZENIOWSKI et al. 1999, OTERO, SANZ 2003, SCHRÖBERL et al. 1998, TYSZKIEWICZ 1997). Pressures above 400 MPa allow to reduce microbial counts to a degree comparable to that observed while heating at temperatures higher than 100°C (HEINZ, BUCKOW 2004).

It has been demonstrated (SHIGEHISA et al. 1991) that pressurization of ground pork at 400 MPa for 10 minutes enables to reduce, by 6 log cycles, the counts of such microorganisms as *Escherichia coli, Campylobacter jejuni, Pseudomonas aeruginosa, Salmonella Typhimurium*, *Yersinia enterocolitica, Saccharomyces cerevisiae, Candida utilis* and other. The counts of *Micrococcus luteus, Staphylococcus aureus* and *Enterococcus faecalis* can be reduced to a comparable extent when pressurized at 500 or 600 MPa. The possibility to inactivate spore-forming *Bacillus cereus* under the above conditions has not been confirmed (CHEFTEL, CULIOLI 1997). However, it has been found that high pressures (above 300 MPa) inactive Gram-negative bacteria first, fol-

lowed by yeasts (above 400 MPa) and Gram-positive bacteria. It has also been reported that some bacterial strains do not undergo complete inactivation when pressurized. They respond by stress and appear to be dead, but after a repair phase, taking place during 3 to 9 days of storage of high-pressure preserved meat at low temperatures, they are able to recover and proliferate again (CHEFTEL, CIULIOLI 1997).

The aim of this study was to determine the effects of high-pressure technology on the microbiological quality of pork meat-fat stuffing and sausages made from this stuffing. The influence of pressurization on such characteristics of stuffing as pH and free water content, important for bacterial growth, was also tested.

Materials and Methods

Pork stuffing was made using meat from *musculus longissimus dorsi* (80%) and back fat (20%). Pork was chopped into 3 cm x 3 cm cubes and corned for 24 hours in a salt brine solution composed of 0.12 g/kg sodium nitrite, 10 g/kg sodium lactate and 1 or 2% table salt. Corned pork was passed through a 3 mm plate of a grinder and ground until a final temperature of 10°C was achieved. Next the stuffing was chilled to 4°C. During grinding water was added (in the form of ice) in the amount of 10% of meat-fat pulp. The stuffing was packed in barrier casings or vacuum-packed in polyethylene casings, forming bars about 50 g in weight and 10 cm in length. The pressurization process was conducted at the High-Pressure Research Center, Polish Academy of Sciences in Warsaw. Pressure was generated by a direct method, with a piston driven by a low-pressure pump, in a 2.5 liter chamber, using distilled water with propylene glycol (1:1) as a transfer medium. The stuffing was pressurized at 200, 300 and 500 MPa for 10 minutes, at 20°C (±3). The stuffing in casings served as a control sample.

The following determinations were made in samples of pressurized and control stuffing: pH (with a combined electrode coupled to a HAH SENSION^{TM1} pH-meter, at 18°C), water-holding capacity – by the Grau-Hamm method (HAMM 1972, KLETTNER et al. 1999), table salt content in accordance with the Polish Standard PN-ISO 1841-22 (2002). The microbiological assessment was performed 24 hours after pressurization and after 7 days of cold storage at 4°C, as well as after heat treatment (at 55°C for 30 minutes) of cold-stored stuffing, based on the counts of mesophilic aerobes, psychrotrophic bacteria, acidifying bacteria, coliforms and coagulase-positive staphylococci – PN-A-82055 (2002). Analytical samples (10 g) were transferred

into 90 cm^3 of a physiological solution and homogenized in a stomacher. 1:10 dilutions were used to prepare additional serial tenfold dilutions, which were cultured and incubated under appropriate conditions. The counts of particular microbial groups are given as logarithms of the numbers of colony-forming units (cfu) per g of the tested sample. Four experimental series were carried out. Results were verified statistically, calculating means, standard deviations and significance of differences by Duncan's test. Computations were done using Microsoft Exel 4.0 and Statistica 6.0 PL software.

Non-pressurized stuffing corned with 1 and 2% of table salt was used to produce model fine ground sausages. The stuffing was packed into barrier casings, forming bars 50 g in weight and 10 cm in length, and then scalded at 55°C for 20 minutes and chilled. Model semi-coarse ground sausages were made from experimental stuffing (20%), pork of the first quality class passed through a 20 mm plate of a grinder (20%) and pork of the second quality class passed through a 10 mm plate of a grinder (60%). The raw material was corned with the same amounts of curing additives as the model stuffing, plus 2% NaCl. The ingredients were mixed and the stuffing was molded into natural casings. The bars were left for 30 minutes at 18°C, and next smoked for 15 minutes at 40°C and for 20 minutes at 60°C, scalded for 35 minutes at 55°C and chilled until a temperature of 10°C was achieved inside the bar.

Both types of sausages were pressurized at 500 MPa for 10 minutes. The microbiological evaluation of pressurized and non-pressurized sausages was performed after 7 days of cold storage, determining the same bacterial groups as in the stuffing.

Results and Discussion

The average pH levels in corned pork stuffing were 5.38 and 5.35 (for 1% and 2% addition of NaCl, respectively). High pressures, ranging between 200 and 500 MPa, caused an increase in pH, directly proportional to the pressure applied. Changes in pH were affected neither by the table salt content of pork stuffing, nor by packaging method (Table 1). The pH increase in pressurized meat and meat products is related to changes in the configuration of protein chains, exposing basic imidazole groups (JANKOWSKA et al. 2000, TYSZKIEWICZ 1997).

Free water was present only in non-pressurized stuffing containing 1% NaCl. Pressurization had no significant effect on the increase in free water content, but the differences caused by the addition of table salt were still observed. There was no correlation between the amount of free water and pressure levels (Table 2).

Table 1

Changes in pH levels in high-pressure processed pork stuffing

Stuffing packaging	NaCl (%)	Pressure (MPa)			
		0.1	200	300	500
Barrier casing	1	5.38^{a}	5.46^{a}	5.51^{ab}	5.55^{b}
Vacuum packaging, polyethylene casing	1	5.38^{a}	5.48^{a}	5.55^{b}	5.59^{c}
Barrier casing	2	5.36^{a}	5.55^{b}	5.56^{b}	5.55^{b}
Vacuum packaging, polyethylene casing	2	5.35^{a}	5.42^{b}	5.48^{c}	5.52^{c}

a, b, c – values in lines followed by different letters differ significantly at $p < 0.05$

Table 2

Changes in the free water content of high-pressure processed pork stuffing

Stuffing packaging	NaCl (%)	Pressure (MPa)			
		0.1	200	300	500
Barrier casing	1	0.38^{a}	0.54^{b}	0.58^{b}	0.50^{b}
Vacuum packaging, polyethylene casing e	1	0.38^{a}	0.53^{b}	0.58^{b}	0.51^{b}
Barrier casing	2	0.01^{a}	0.34*b*	0.28^{b}	0.29^{b}
Vacuum packaging, polyethylene casing	2	0.01^{a}	0.35^{b}	0.30^{b}	0.30^{b}

a, b – values in lines followed by different letters differ significantly at $p < 0.05$

Coagulase-positive staphylococci were not found in the stuffing at any stage of the experiment. The counts of the other microbial groups in raw, non-pressurized meat-fat stuffing (stored for 48 hours) depended on the type of packaging. In the stuffing packed in barrier casings the count of mesophilic aerobes was by 0.7 to 2.7 log cycles lower (Table 3). A less significant difference was observed in the counts of psychrotrophic bacteria (by 0.2 to 0.5 log cycle) – Table 4) and acidifying bacteria (0.4 to 0.8 log cycle) – Table 5). In the group of coliforms this difference was as high as 2.5 log cycles (Table 6). The majority of bacteria were not affected by the varied quantity of table salt added to the stuffing. The only exception were mesophilic aerobes whose count was higher, by almost two log cycles, in vacuum-packed stuffing containing 2% NaCl (Table 3). During cold storage of meat-fat stuffing the count of mesophilic aerobes increased by 1.2 to 1.8 log cycles, but remained at an elevated level only in vacuum-packed stuffing containing 1% NaCl (Table 3). The count of psychrotrophic bacteria increased by 1.1 to 1.72 log cycles in stuffing packed in barrier casings, and by 2.28 to 1.93 log cycles in vacuum-packed stuffing (Table 4). Much less marked changes were noted in the case of acidifying bacteria in cold-stored stuffing, since the increase in their count did not exceed one log cycle (Table 5). Cold storage contributed to a reduction in the number

Table 3
Changes in the counts of mesophilic aerobes in high-pressure processed pork stuffing (log cfu · g^{-1})

Stuffing packaging	NaCl (%)	Pressure (MPa)			
		0.1	200	300	500
Barrier casing	1	5.78^a	5.41^a	3.30^b	<0.95^c
Vacuum packaging, polyethylene casing	1	6.48^a	5.69^a	5.08^b	2.53^c
Barrier casing	2	5.56^a	5.36^a	3.26^b	<0.95^c
Vacuum packaging, polyethylene casing	2	8.30^a	5.94^b	5.08^c	3.68^d
After 7 days of storage					
Barrier casing	1	6.93^a	7.08^a	5.38^b	1.90^c
Vacuum packaging, polyethylene casing	1	8.30^a	7.57^a	5.85^b	1.68^c
Barrier casing	2	7.28^a	7.23^a	5.08^b	2.04^c
Vacuum packaging, polyethylene casing	2	8.00^a	7.68^a	5.04^b	2.48^c
Thermally processed, after 7 days of storage					
Barrier casing	1	2.40^a	2.70^b	2.48^a	<0.95^c
Vacuum packaging, polyethylene casing	1	n.d.	n.d.	n.d.	n.d.
Barrier casing	2	3.32^a	3.36	2.18^b	<0.95^c
Vacuum packaging, polyethylene casing	2	n.d.	n.d.	n.d.	n.d.

n.d. – not determined
a, b, c, d – values in lines followed by different letters differ significantly at $p < 0.05$

Table 4
Changes in the counts of psychrotrophic bacteria in high-pressure processed pork stuffing (log cfu · g^{-1})

Stuffing packaging	NaCl (%)	Pressure (MPa)			
		0.1	200	300	500
Barrier casing	1	5.78^a	5.41^a	3.30^b	<0.95^c
Vacuum packaging, polyethylene casing	1	6.00^a	5.98^a	3.48^b	1.48^c
Barrier casing	2	5.56^a	5.36^a	3.26^b	<0.95^c
Vacuum packaging, polyethylene casing	2	6.11^a	5.89^a	3.30^b	<0.95^c
After 7 days of storage					
Barrier casing	1	6.93^a	7.08^a	5.38^b	1.90^c
Vacuum packaging, polyethylene casing	1	8.32^a	7.53^a	5.51^b	<0.95^c
Barrier casing	2	7.28^a	7.23^a	5.08^b	2.04^c
Vacuum packaging, polyethylene casing	2	8.04^a	7.30	4.93^c	<0.95^d
Thermally processed, after 7 days of storage					
Barrier casing	1	2.40^a	2.50^a	2.48^a	<0.95^c
Vacuum packaging, polyethylene casing	1	n.d.	n.d.	n.d.	n.d.
Barrier casing	2	3.32^a	3.36^a	2.18^b	<0.95^c
Vacuum packaging, polyethylene casing	2	n.d.	n.d.	n.d.	n.d.

n.d. – not determined
a, b, – values in lines followed by different letters differ significantly at $p < 0.05$

Table 5
Changes in the counts of acidifying bacteria in high-pressure processed pork stuffing (log cfu · g^{-1})

Stuffing packaging	NaCl (%)	Pressure (MPa)			
		0.1	200	300	500
Barrier casing	1	3.78^a	3.56^a	3.28^b	$<0.95^c$
Vacuum packaging, polyethylene casing	1	4.23^a	3.34^b	2.85^c	1.48^d
Barrier casing	2	3.26^a	3.11^a	2.98^a	$<0.95^b$
Vacuum packaging, polyethylene casing	2	4.04^a	3.89^a	2.75^b	$<0.95^c$
After 7 days of storage					
Barrier casing	1	4.50^a	4.88^b	3.00^c	$<0.95^d$
Vacuum packaging, polyethylene casing	1	4.00^a	3.71^b	3.54^b	$<0.95^c$
Barrier casing	2	4.00^a	4.46^b	4.26^b	3.30^c
Vacuum packaging, polyethylene casing	2	5.20^a	2.32^b	$<0.95^c$	$<0.95^c$
Thermally processed, after 7 days of storage					
Barrier casing	1	2.18^a	2.79^b	1.00^c	$<0.95^c$
Vacuum packaging, polyethylene casing	1	n.d.	n.d.	n.d.	n.d.
Barrier casing	2	2.76^a	2.79^a	2.18^b	$<0.95^c$
Vacuum packaging, polyethylene casing	2	n.d.	n.d.	n.d.	n.d.

n.d. – not determined
a, b, c, d – values in lines followed by different letters differ significantly at $p < 0.05$

Table 6
Changes in the counts of coliforms in high-pressure processed pork stuffing (log cfu · g^{-1})

Stuffing packaging	NaCl (%)	Pressure (MPa)			
		0.1	200	300	500
Barrier casing	1	3.40	<1	<1	n.f.
Vacuum packaging, polyethylene casing	1	<1	<1	<1	n.f.
Barrier casing	2	2.53	n.f.	n.f.	n.f.
Vacuum packaging, polyethylene casing	2	<10	<1	n.f.	n.f.
After 7 days of storage					
Barrier casing	1	1.0	<1	n.f.	n.f.
Vacuum packaging, polyethylene casing	1	<1	<1	n.f.	n.f.
Barrier casing	2	2.20	<1	n.f.	n.f.
Vacuum packaging, polyethylene casing	2	<1	<1	n.f.	n.f.
Thermally processed, after 7 days of storage					
Barrier casing	1	n.f.	n.f.	n.f.	n.f.
Vacuum packaging, polyethylene casing	1	n.f.	n.f.	n.f.	n.f.
Barrier casing	2	n.f.	n.f.	n.f.	n.f.
Vacuum packaging, polyethylene casing	2	n.f.	n.f.	n.f.	n.f.

n.f. – not found

Table 7
Changes in the counts of mesophilic aerobes, psychrotrophic and acidifying bacteria in fine ground and semi-coarse ground sausages (log cfu · g^{-1})

Type of sausage	NaCl (%)	Pressure (MPa)	
		0.1	500
Mesophilic aerobes			
Fine ground sausage	1	5.83^a	2.59^b
Fine ground sausage	2	4.70^a	4.94^a
Semi-coarse ground sausage	2	3.88^a	2.40^b
Psychrotrophic bacteria			
Fine ground sausage	1	2.59^a	<0.95^b
Fine ground sausage	2	4.94^a	<0.95^b
Semi-coarse ground sausage	2	2.40^a	<0.95^b
Acidifying bacteria			
Fine ground sausage	1	2.60^a	<0.95^b
Fine ground sausage	2	4.04^a	<0.95^b
Semi-coarse ground sausage	2	2.54^a	<0.95^b

a, b, – values in lines followed by different letters differ significantly at $p < 0.05$

of coli rods below one log cycle (Table 6). Heat processing of cold-stored stuffing packed in barrier casings reduced the size of all bacterial populations: mesophilic aerobes and psychrotrophic bacteria to a level of 2.40 to 3.32 log cycle · g^{-1} of sample (Table 3), acidifying bacteria to 2.18-2.76 log cycles · g^{-1} of sample (Table 5). Coliforms were completely eliminated (Table 6).

Compared to the changes observed in non-pressurized stuffing, a pressure of 200 MPa had no considerable effect on the counts of mesophilic aerobic, psychrotrophic and acidifying bacteria, except for vacuum-packed stuffing with 2% addition of table salt, in which the count of mesophilic aerobes reduced by about 0.5 log cycle (Table 3). In comparison with the control stuffing (cold-stored and thermally processed), heat treatment of pressurized (200 MPa) and cold-stored stuffing caused a slight decrease in the counts of mesophilic aerobic and acidifying bacteria in the variant with 1% NaCl, but had no significant effect on the population size of psychrotrophic bacteria. Pressurization of pork stuffing at 300 MPa reduced the counts of all microbial groups, compared with non-pressurized stuffing (Tables 3, 4, 5, 6). The population size of mesophilic aerobes decreased by 2.48 to 2.30 log cycles in stuffing packed in barrier casings, and by 1.40 to 3.22 log cycles in stuffing vacuum-packed in polyethylene casings. The decrease was even greater in the case of psychrotrophic bacteria – 2.48 to 2.30 and 2.52 to 2.81 log cycles, respectively. The slightest changes were observed in the count of acidifying

bacteria. During cold storage of stuffing pressurized at 300 MPa the population size of most microbial groups increased, but to a lower extent than in non-pressurized stuffing stored under identical conditions. Heating of cold-stored stuffing enabled to reduce bacterial counts, but this decrease was not always higher than in the control stuffing.

As expected, the greatest changes in bacterial counts were recorded following pressurization at 500 MPa. Compared to non-pressurized stuffing, the count of mesophilic aerobes decreased by 4.83 to 4.61 log cycles and by 3.95 to 4.62 log cycles in stuffing packed in barrier casings and in vacuum-packed stuffing, respectively (Table 3). More significant changes were observed in the count of psychrotrophic bacteria, which reduced by 4.83 to 4.61 and by 4.52 to 5.16 log cycles, depending on the type of packaging (Table 4). As for acidifying bacteria, a pressure of 500 MPa reduced their count to a lesser degree (2.83 to 2.31 and 2.75 to 3.09 log cycles, respectively), but finally contributed to a decrease in their population size below one log cycle (Table 5). Bacterial counts increased over cold storage of stuffing pressurized at 500 MPa, but in comparison with non-pressurized stuffing the population size of mesophilic aerobes decreased by 5.03 to 5.24 and by 6.62 to 5.52 log cycles (Table 3), whereas that of psychrotrophic bacteria – by 5.03 to 5.24 and 7.37 to 7.09 log cycles, respectively (Table 4).

The analysis of experimental data shows that significant changes in the bacterial microflora of meat-fat stuffing brined with 1 and 2% NaCl take place at a pressure of at least 300 MPa. However, it would be difficult to compare the present results with reference data, since the latter most often concern ground meat only. CARLEZ et al. (1993) reported that a pressure of 200 MPa applied to ground beef at 20°C for 20 minutes was sufficient to reduce the count of *Pseudomonas fluorescens* by 5 log cycles, thus permitting the reduction of their population size below one log cycle. These authors also demonstrated that pressurization at a lower (4°C) or higher (35°C) temperature, a comparable reduction in microbial counts may be achieved already at a pressure of 170 MPa. In the case of *Citrobacter freundii* (fecal bacteria) and *Listeria innocua* such a reduction was possible only at 250 and 300 MPa, respectively.

Among the pressure ranges analyzed in the study, the most significant decrease in bacterial counts was observed at 500 MPa. Therefore, only this pressure was applied in order to determine the effects of high-pressure technology on the bacterial microflora of scalded sausages. A microbiological analysis of processed meats was performed after 7-day cold storage, following pressurization (Table 7). It was found that pressurization had a stabilizing influence on the count of mesophilic aerobes as well as on the keeping quality of sausages during cold storage. The population size of mesophilic aerobic bacteria reduced by 1.5 log cycle in semi-coarse ground sausages and by 2 log

cycles in fine ground sausages, regardless of the addition of table salt. The counts of psychrotrophic and acidifying bacteria decreased below one log cycle in semi-coarse ground sausages and in fine ground sausages corned with 2% NaCl.

Conclusions

1. High pressures (200 to 500 MPa) caused an increase in the pH of meat-fat stuffing, but did not affect free water content.

2. The counts of mesophilic aerobic, psychrotrophic and acidifying bacteria as well as coliforms in non-pressurized corned meat-fat stuffing, determined immediately after the completion of the production process and after 7 days of cold storage, depended on the type of packaging and were found to be lower in the case of barrier casings. The majority of bacterial groups were not affected by the varied quantity of table salt (1 or 2%) added to the stuffing.

3. Pressurization at 500 MPa of pork meat-fat stuffing contributed to a significant reduction in the counts of the tested microbial groups (even by 5 log cycles), and inhibited their growth over cold storage.

4. Pressurization at 500 MPa of fine ground and semi-coarse ground sausages scalded at 55°C for 30 minutes enabled to reduce total bacterial counts below 0.95 log cycle as well as to maintain them at this level during 7-day cold storage.

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CADMIUM AND LEAD CONTENTS IN HUMAN MILK, COW'S MILK AND INFANT FORMULAS

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Key words: cadmium, lead, human milk, cow's milk, infant formulas.

Abstract

Cadmium and lead contents were determined in human milk samples collected from four regions of Poland, as well as in cow's milk UHT and infant formula samples. Determinations were performed by flame atomic absorption spectrometry after dry digestion. The highest contents of cadmium (0.0110 mg · dm^{-3}) and lead (0.0632 mg · dm^{-3}) were found in human milk of the women living in Silesia – an industrial region of Poland. The lowest Cd content (0.0035 mg · dm^{-3}) was determined in samples of human milk collected from Gdańsk, whereas the lowest Pb content (0.0270 mg · dm^{-3}) was found in human milk samples from Olsztyn. The average heavy metals contents in all human milk samples were 0.0072 mg · dm^{-3} for Cd and 0.0452 mg · dm^{-3} for Pb.

Provisional tolerable weekly intake (PTWI) is equal to 0.007 mg · kg^{-1} of body weight – for cadmium and 0.025 mg · kg^{-1} of body weight for lead. The calculated weekly intake was: for cadmium – 0.0353 mg · kg^{-1} b. w. and for lead – 0.221 mg · kg^{-1} b. w. PTWI was exceeded (in case of human milk feeding) about 161% and 49% for Pb and Cd, respectively. However in case of infant formulas, PTWI was exceeded about 77% and 22% for Pb and Cd, respectively.

The average content of Pb (0,089 mg · dm^{-3}) in cow's milk was four times higher than the acceptable level.

ZAWARTOŚĆ KADMU I OŁOWIU W MLEKU KOBIECYM, KROWIM I MODYFIKOWANYM MLEKU DLA NIEMOWLĄT

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Słowa kluczowe: kadm, ołów, mleko kobiece, mleko krowie, mleko modyfikowane dla niemowląt.

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Abstrakt

W próbkach mleka kobiecego zebranego w 4 regionach Polski oraz w próbkach mleka krowiego UHT i w odżywkach dla niemowląt oznaczono zawartości kadmu i ołowiu. Zastosowano metodę spektrofotometrii absorpcji atomowej. Najwyższą zawartość kadmu (0,0110 mg · dm^{-3}) i ołowiu (0,0632 mg · dm^{-3}) stwierdzono w mleku kobiet zamieszkałych na Śląsku – przemysłowym regionie Polski. Najniższą zawartość kadmu (0,0035 mg · dm^{-3}) odnotowano w próbkach mleka kobiecego zebranego w Gdańsku, a najniższą zawartość ołowiu (0,0270 mg · dm^{-3}) – w próbkach mleka kobiecego zebranego w Olsztynie. Średnia zawartość metali ciężkich we wszystkich próbkach mleka kobiecego wynosiła: Cd – 0,0072 mg · dm^{-3}, Pb – 0,0452 mg · dm^{-3}.

Tymczasowe dopuszczalne tygodniowe pobranie (PTWI) kadmu wynosi 0,007 mg · kg^{-1} m.c., ołowiu – 0,025 mg · kg^{-1} m.c., obliczone tygodniowe pobranie wynosiło odpowiednio 0,0353 mg · · kg^{-1} m.c., 0,221 mg · kg^{-1} m.c. W przypadku karmienia mlekiem kobiecym, PTWI zostało przekroczone o ok. 161% dla kadmu i ok. 49% dla ołowiu, a w przypadku żywienia odżywkami dla niemowląt – odpowiednio o ok. 77% i 22%.

Średnia zawartość ołowiu (0,089 mg · dm^{-3}) w mleku krowim 4-krotnie przekraczała dopuszczalną normę.

Introduction

The development of industry has brought about substantial pollution of the soil, water and air with various harmful substances, including heavy metals. Metals such as cadmium and lead can penetrate into the human body, mainly through the alimentary and respiratory systems. Cadmium and lead, present in the environment, can penetrate into food. The results of multiple studies confirm their occurrence in food of plant and animal origin (BULIŃSKI et al. 1992, JĘDRZEJCZAK 1992, KREŁOWSKA-KUŁAS et al. 1999, RODRIGUEZ RODRIGUEZ et al. 1999, WOJCIECHOWSKA-MAZUREK 2000, ŻMUDZKI, SZKODA 2000). What is particularly alarming is the fact that heavy metals are found in food that is intended for infants (EKLUND, OSKARSSON 1999, FRKOVIC et al. 1997, PALMINGER et al. 1995, JĘDRZEJCZAK, SZTEKE 1991, MARZEC, ZARĘBA 2003, ROCHEL et al. 1998, TRIPATHI et al. 1999). The body of a small child is particularly susceptible to harmful effects of these compounds due to the immaturity of its enzymatic detoxication systems.

Elements such as cadmium and lead belong to a group of toxic compounds having teratogenic and mutagenic effects on the human body (DUDEK 1993). A high level of these metals in the body results in damage and disorders in the functions of the nervous, hematogenic and alimentary systems, of the heart muscle, liver and kidneys, and it can even cause damage to the brain (WROŃSKA-NOFER, HAŁASEK 1993). Children's health is endangered by lead as early as in their foetal life, since the placenta is not a barrier protecting against this element. On the other hand, severe lead poisoning causes pain, muscle tremors, hallucinations, memory dysfunctions, convulsions, paralysis and coma (TRACZYK, SZPONAR 1995).

Milk and milk products are widely used in child nutrition, and breast milk is the first food for a newborn infant. Therefore, it is highly important to determine the level of heavy metals and other harmful elements or chemical compounds due to the threat they pose to infant health.

The aim abjective of the research was to determine the content of cadmium and lead in human milk obtained from women living in four regions of Poland and to compare the results obtained with the level determined for UHT cow's milk and infant formulas.

Materials and Methods

Samples of human milk were collected in hospitals in Olsztyn, Białystok, Gdańsk and in the Silesia region (Katowice, Zabrze, Tychy, Jaworzno). Human milk was collected both from women inhabiting farming and industrial areas. Olsztyn and Białystok are located in the areas of north-east of Poland an agricultural region with a small area occupied with industry known as the Green Lungs of Poland. Gdansk is located in the north of Poland, on the Baltic Sea. This is area, with some shipyard and mining industries (Gdańsk Refinery). Silesia is a region situated in the southern part of Poland. This is the most urbanized and industrialized area of the country (mining, metallurgical and power industries) and highly endangered with industrial pollution.

The research human milk samples were collected from January 1999 to February 2000. Samples of milk were obtained between day three to five after childbirth from women aged between 17 and 43 (average 27). The average weight of women was 64 kg (range 45-98), and the average weight of infant was 3.39 kg (range 1.97-4.98).

There were examinations of two types of the most popular infant milk formulas and also of UHT cow's milk carried out containing 3.2% fat. The research comprised, in total, 78 samples of human milk, 6 samples of infant formulas and 16 samples of cow's milk.

Dry mineralization (RUTKOWSKA 1981) and the method described by ŻMUDZKI (ŻMUDZKI, SZKODA 2000) were applied to determine the content of cadmium and lead in the material under examination. Samples of human milk, cow's milk and infant formulas were dried in quartz crucibles at about 80°C, charred on electric plates and mineralized in an electric oven to obtain white ash in the temperature that did not exceed 450°C. The ash was dissolved in 5 M nitric acid and transferred to a measuring flask. The following reagents were added, one by one: phenol red, ammonium citrate, ammonia water, 2% solution of APDC (ammonium pyrrolidine dithiocarbonate) and MIBK (methyl isobutyl ketone), previously saturated with deionized water. The solution was

then shaken for one minute. The content of cadmium and lead was determined by flame atomic absorption spectrophotometry, with the use of Unicam 939 AA spectrometer equipped with an ADAX data station; background correction (Deuterium lamp) and a suitable hallow cathode lamps (Pb and Cd). Applied method was validated. Following references materials were used: BCR No 63 (skim milk powder-natural levels) and BCR No 150 (skim milk powder-lower levels). The recovery level of Cd was 104.5% and 112.8%, and of Pb was 110% and 96%.

In order to assess the influence of factors analysed on the value of the qualities under examination, an analysis of variance was applied (test F) for homogenous experiments. Additionally, the standard error of the mean (SEM) was calculated. Homogenous groups (not significantly different means) were determined with a q – Student-Newman-Keuls test. (STANISZ 1998).

Results and Discussion

Table 1 presents the results of cadmium and lead concentrations in human milk depending on the region inhabited by the women under investigation. The highest content of cadmium was found in human milk obtained from women inhibited Silesia region (0.0110 $mg \cdot dm^{-3}$ of milk). In human milk samples from Białystok, the content of cadmium was lower by 26%, and in human milk samples from Olsztyn – by 54% in comparison to Pb content in human milk samples from Silesia. The lowest cadmium concentration was found in human milk samples collected in Gdańsk (by 68% in comparison to Cd content in human milk samples from Silesia). A similar tendency was observed in the case of lead in human milk from the regions under examination. Statistically significant differences were established between the content of cadmium in the samples of human milk obtained from women living in Silesia and the content of cadmium in samples obtained in Gdańsk and Olsztyn. As regards lead occurring in human milk, there were no statistically significant differences in the content of this element in relation to the region inhabited by the women. The occurrence of high levels of heavy metals in industrialized areas indicates higher pollution of the natural environment. Silesia is an area definitely more endangered with greater contamination of the environment due to the occurrence of industrial plants emitting pollution. The results of other authors have also shown the occurrence of high levels of heavy metals (Pb, Cd) in the milk obtained from industrial areas. For example Wiechuła et al. reported, similar content of cadmium in human milk from the area of Tarnowskie Góry and Miasteczko Śląskie amounted to on average 0.0082 $mg \cdot dm^{-3}$ (WIECHUŁA et al. 2000).

Table 1

Content of cadmium and lead in human milk, depending on the area inhabited by women ($mg \cdot dm^{-3}$ of milk)

Region	Statistical measures	Cd	Pb
Olsztyn $n = 24$	min	0.0006	0.0075
	max	0.0120	0.0808
	mean	0.0051^{B}	0.0270^{a}
	SEM	0.0006	0.0038
Białystok $n = 15$	min	0.0009	0.0020
	max	0.0200	0.1540
	mean	0.0081^{B}	0.0596^{a}
	SEM	0.0081	0.0159
Gdańsk $n = 15$	min	0.0007	0.0020
	max	0.0089	0.0586
	Mean	0.0035^{B}	0.0311^{a}
	SEM	0.0006	0.0046
Silesia $n = 24$	min	0.0007	0.0081
	max	0.0393	0.1515
	mean	0.0110^{A}	0.0632^{a}
	SEM	0.0015	0.0175

Explanations: *A, B* – means marked with various letters are significantly different at $p \leq 0.01$, *a* – no significant differences at $p \leq 0.05$

Table 2 presents the results of Cd and Pb content in human milk, cow's milk and infant formulas. While analysing the results, no statistically significant differences were found between the content of these metals and individual types of milk. However, it was observed that the average amount of cadmium in samples of human milk – 0.0072 $mg \cdot dm^{-3}$ was higher then in other types of milk under examination. The content of cadmium in infant formulas and cow's milk was lower than the amount determined in human milk: 18% and 54%, respectively.

Table 2

Content of cadmium and lead in human milk, UHT cow's milk and infant formulas ($mg \cdot dm^{-3}$ of milk)

Milk	Statistical measures	Cd	Pb
Human $n = 78$	min	0.0006	0.0020
	max	0.0393	0.1540
	mean	0.0072^{a}	0.0452^{a}
	SEM	0.0007	0.0073
UHT cow's $n = 16$	min	0.0006	0.0067
	max	0.0062	0.4880
	mean	0.0033^{a}	0.0890^{a}
	SEM	0.0008	0.0066
Infant formulas $n = 6$	min	0.0025	0.0135
	max	0.0086	0.0421
	mean	0.0059^{a}	0.0306^{a}
	SEM	0.0010	0.0048

Explanations: *a* – no significant differences at $p \leq 0.05$

The highest amount of lead was found in cow's milk samples – 0.0890 mg · dm^{-3} of milk. The lead level in human milk samples was twice – and in infant formula samples, even three times – lower than the level determined for cow's milk.

Study carried out by Jędrzejczak and Szteke showed in infant formulas the average cadmium and lead contents of 0.0007 mg · dm^{-3} and 0.008 mg · dm^{-3} respectively (JĘDRZEJCZAK, SZTEKE 1991). In the current study, the concentration of the examined metals was an order of magnitude higher.

RODRIGUEZ et al. found in human milk 0.0027 mg · dm^{-3} of Cd and 0.0083 mg · dm^{-3} of Pb, on average. The authors also included in their research pasteurized cow's milk, and measured 0.0043 mg · dm^{-3} of Cd and 0,0103 mg · dm^{-3} of Pb in it, on average. On the other hand, they found 0.0038 mg · dm^{-3} of Cd and 0.0083 mg · dm^{-3} of Pb in infant formulas (RODRIGUEZ RODRIGUEZ et al. 1999).

The maximum acceptable lead level in cow's milk and in infant preparations amounts to 0.02 mg · kg^{-1} of the product, according to the Commission Regulation (EC) No 466/2001 of March 8, 2001 setting maximum levels for certain contaminants in foodstuffs (Official Journal of the European Communities L 77/1).

According to the recommendations of FAO/WHO, the provisional tolerable weekly intake (PTWI) of a given toxic element from all sources, without harmful effects to the health, is 0.025 mg · kg^{-1} of body weight for lead and 0.007 mg · kg^{-1} of body weight – for cadmium. At an average weight of infants amounting to 3.39 kg, PTWI equals 0.0848 mg for Pb and 0.0237 mg for Cd. In the first week of life, infants are recommended to receive seven 100 ml portions of milk formula – which gives 4.900 ml in a week. Such an amount of infant formula contains, on average, 0.1499 mg of Pb and 0.0289 mg of Cd, which exceeds PTWI for Pb and Cd by 77% and 22%, respectively. By comparing human milk in the same way, it has been established that PTWI is exceeded for Pb by 161% and for Cd – by 49%.

Conclusions

The mean Pb content determined in milk of women living in Olsztyn (n=24) and Gdańsk (n=15) were higher, than the maximum acceptable lead level in products dedicated to infants by 35% and 56%, respectively. The maximum tolerable level of lead was exceeded by approximately three times in human milk samples obtained from Silesia and Białystok.

The average lead content in all samples of human milk (0.0452 mg · dm^{-3}) exceeded the maximum level of this metal in food for infant by 126%.

On the other hand, it has been found that the maximal acceptable lead level in infant formulas was exceeded by 53%, while in cow's milk the acceptable level was exceeded even four times.

Currently, the highest acceptable levels of cadmium content in milk have not been established. Therefore, it is not possible to compare the results obtained with the legal regulations currently in force.

A high content of cadmium and lead in infant formulas and in human milk poses a potential threat to infant health. However human milk contain all indispensable substances required for development of infants and is recommend for infants during the first 6 months of live.

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HISTOPATHOLOGICAL EXAMINATION OF GILLS OF ROACH *RUTILUS RUTILUS* (L.) FROM SELECTED LAKES OF NORTHEASTERN POLAND

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K e y w o r d s: roach, gills, *Myxobolus*, trematodes, histopathological examination.

A b s t r a c t

The study aimed at investigating the morphology of gills in roach *Rutilus rutilus* (L.) caught from lakes Ukiel, Wulpińskie, Dgał Wielki and Warniak in Warmia Lake District, characterized by different degree of eutrophication and pollution. The following lesions were found in the gills: hyperemia and extravasations of blood, infiltration of lymphoid cells, deformation of secondary lamelles towards clavate shape, adhesions of transverse blades and excessive accumulation of mucus and exfoliated epithelial cells on transverse gill lamelles. In few cases necrosis of transverse gill lamelles was found. The largest number of roach with damaged gills was found among those caught from lakes Dgał Wielki and Warniak. Parasites of genus *Myxobolus,* monogeneans (17 species) of genera *Dactylogyrus, Gyrodactylus* and *Paradiplozoon,* digenetic trematodes (metacercariae of *Posthodiplostomum cuticola),* and four species of *Crustacea* and *Hirudinea* were found on gills.

BADANIE HISTOPATOLOGICZNE SKRZELI PŁOCI *RUTILUS RUTILUS* (L.) POCHODZĄCYCH Z WYBRANYCH JEZIOR PÓŁNOCNO-WSCHODNIEJ POLSKI

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S ł o w a k l u c z o w e: płoć, skrzela, *Myxobolus,* przywry, badanie histopatologiczne.

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Abstrakt

Celem badań było prześledzenie morfologii skrzeli u płoci *Rutilus rutilus* (L.), odłowionych w jeziorach Ukiel, Wulpińskie, Dgał Wielki i Warniak na Pojezierzu Warmińskim. Jeziora te charakteryzowały się różnym stopniem eutrofizacji i zanieczyszczenia. W skrzelach stwierdzono przekrwienie i wynaczynienia krwi, nacieki komórek limfocytarnych, zniekształcenie blaszek i przyjmowanie kształtu buławkowatego, zrosty blaszek poprzecznych oraz nadmierne gromadzenie się śluzu i złuszczonych komórek nabłonka na blaszkach poprzecznych skrzeli. U nielicznych ryb wykazano martwicę blaszek poprzecznych skrzeli. Najwięcej uszkodzeń w skrzelach miały ryby odławiane z jezior Dgał Wielki i Warmiak.

Na skrzelach stwierdzono pasożyty z rodzaju *Myxobolus,* przywry monogenetyczne (17 gatunków) z rodzajów *Dactylogyrus, Gyrodactylus* i *Paradiplozoon,* przywry digenetyczne (metacerkarie *Posthodiplostomum cuticola)* oraz po 4 gatunki *Crustacea* i *Hirudinea.*

Introduction

Gills of fish are exposed to direct influence of potential pathogenic factors in the water of the lake. At the same time, the gills are highly sensitive to influence of toxic, contagious or parasitic agents. Damaged gills are unable to perform sufficiently their respiratory function in fish and the fish become more susceptible to influence of unfavorable factors present in the lake water. Fish in fishponds and lakes are exposed to bacterial infections and parasitic infestations (VALTONEN and KOSKIVAARA 1994, MARTINS et al. 1999, ROUBAL 1999). In case of *Myxobolus* presence, focal inflammatory granulomas develop in gills leading to changes in gill blade shapes and cellular infiltrations in transverse lamelles. Presence of various parasites in roach caught from the studied lakes was described in the paper by DZIKA (2003). Histological changes in gills of pike-perch from lake Balaton caused by *Myxosporea* (*Hemneguya)* were described by MOLNAR (1998), while HAAPARANTA et al. 1994 described changes in gills of perch from Finnish lakes. OLSON and PIERCE (1997) and BLAZER and GRATZEK (1985) repeated changes in gills of *Oncorhynchus mykiss* caused by metacercariae of trematodes. WŁASOW et al. (1997) described the histological changes in gills and other organs of *Oncorhynchus mykiss* caused probably by *Myxosporea.* Also the lesions of spherical sporoze in carp reared in ponds with different water temperatures (POJMAŃSKA et al. 1998). Also HAAPARANTA et al. 1996 described changes in gills of roach and perch caught in the Finnish lakes representing different degrees of eutrophication and pollution. In young fish the changes in gills were accompanied by changes in spinal curvature and regressive changes in muscular fibers caused by infestation with *Posthodiplostomum cuticola* (PROST 1994). Pollutions of water reservoirs with, e.g. pesticides and heavy metals, damage fish gills as described by LICHTENFELS et al. 1996. Under laboratory conditions the reaction of gills to presence

of different metals at concentrations higher than under natural conditions was also studied (ALLERENO et al. 1999).

This study aimed at investigating morphological changes and identification of presence of parasites in gills of roach caught from lakes Ukiel, Wulpińskie, Dgał Wielki and Warniak in Mazury Lake district of northeastern Poland.

Material and Methods

Gills of roach *Rutilus rutilus* L. were collected for histopathological examination during post mortem of fish caught from lakes Ukiel, Wulpińskie, Dgał Wielki and Warniak, that were characterized in Table 1. The numbers of examined fish originated from individual lakes are given in Table 2.

Table 1

Morphometric and trophic parameters of lakes studied

	Dgał Wielki Lake	Warniak Lake	Ukiel Lake	Wulpińskie Lake
Drainage basin	Mamry, Węgorapa	Dgał, Mamry, Węgorapa	Kortówka – Łyna	Giłwa – Pasłęka
Longitude	21°47.6'	21°48.2'	20°24.9'	20°22.8'
Latitude	54°06.6'	54°07.4'	53°47.2'	53°42'
Elevation above sea level (m)	120.1	120.4	104.4	105.8
Surface area (ha)	95.4	38.4	412	706.7
Maximum depth (m)	17.6	3.7	43	54.6
Average depth (m)	5.3	1.2	10.6	10.9
Water volume (in thousands m^3)	4995.9	456.7	43611.5	76990.3
pH	8.7[a]	7.2-9.6[a]	8.1[a]	8.3[a]
Maximum length (m)	1300	1000	5360	8321
Maximum width (m)	1125	500	1715	2330
Length of coastal line (m)	5188	2625	22550	29800
Mictic type	dimictic	polymictic	dimictic	dimictic
Fisheries type	bream	tench-pike	bream	bream
Range of macrophytes (m)	5	3	5	4
Phytolitoral	48.5 ha 51.5% area	31.3 ha 81.5% area	small	very poor
Trophic type	weakly eutrophicated	strongly eutrophicated	strongly eutrophicated Olsztyn and Lupstych parts. weakly eutrophicated other parts of the lake	strongly eutrophicated eastern part

a – and of summer stagnation

Table 2

Characteristics of material from four lakes

Lake	Time of catch	Sample tot./hist.	Length (cm)	Weight (g)	Age of fish
Warniak	9/1998-11/1999 A	251/197	12.5-30	15-240.7	2+ – 11+
Dgał Wielki	9/1998-11/1999 A	277/207	11-33	15.61-430	2+ – 11+
Ukiel	11/1998-12/1999 B	213/213	15-31	35-380	4+ – 11+
Wulpińskie	11/1998-12/1999 B	202/202	12-25.5	35-180	4+ – 10+

Explanations: tot. – number of fish, hist. – sampled for histology. A – no sample in Dec. – March, B – no sample in Jan. – March

The gills were fixed in neutralized (pH =7.4) 10% formalin immediately after killing and embedded in the paraffin blocks. From each block from 4 to 7 sections were obtained and haematoxylin and eosin (HE) stained. The preparations were inspected under light microscope using different magnifications, particularly for identification of presence of parasites – *Protozoa* and *Metazoa*. For comparison of morphological changes in gills of fish from different lakes, the following classification of gill lesions was implemented: 0 – normally structured organ, 1 – adhesion of two neighboring gill lamelles, 2 – adhesion of 3 to 4 neighboring gill lamelles and presence of increased quantity of mucus on the surface, 3 – adhesion of 5 and more neighboring gill lamelles and presence of increased quantity of mucus on the surface, 4 – clavate shape of gills and hyperemia, 5 – hyperemia and extravasations of blood in transverse lamelles, 6 – Epithelial necrosis with infiltration of lymphoid cells between lamelles, 7 – presence of *Myxobolus,* 8 – presence of *Posthodiplostomum cuticola.*

Results and Discussion

Quantitative tabulation of morphological lesions found in gills is given as Table 3.

Morphological damages to gills were focal in nature, i.e. they involved a number of neighboring gill lamelles. Blood vessels in gills, and in particular in transverse lamelles, were hyperemic, some were broken and small extravasations in gills or hematoma in gill arch developed (Figure 1). Those lesions were found in from 29.44% fish from lake Warniak to 38.64% in fish from lake Dgał Wielki. In a not very numerous group of fish the gills were covered in large volumes of mucus (Figure 2) and their tips took clavate shape. Those lesions were most frequently observed in roach from lake Warniak (18.28%) and the least frequently in those from lake Ukiel (5.63%) while in lake

Table 3
Morphological changes in gills of fish from lakes Ukiel, Wulpińskie, Dgał Wielki and Warniak

No	Histopathological change	Ukiel (%)	Wulpińskie (%)	Dgał Wielki (%)	Warniak (%)
0	Organ normal	56.8	58.41	47.34	53.29
1	Adhesions of 2 gill blades and presence of increased quantities of mucus on surface	0.47	0.00	1.44	1.01
2	Adhesions of 3-4 gill blades and presence of increased quantities of mucus on surface	4.69	3.96	4.81	6.56
3	Adhesions of 5 and more gill blades and presence of increased quantities of mucus on surface	0.00	0.99	0.00	1.01
4	Clavate shape of gill blades covered with large quantities of mucus	5.63	0.00	13.52	18.27
5	Hyperemia and extravasations of blood in transverse blades	36.61	36.63	38.64	29.44
6	Epithelial necrosis with infiltration of lymphoid cells	12.67	13.36	23.18	20.30
7	Presence of *Myxobolus plasmodia*	6.10	5.90	5.10	5.30
8	Presence of *Posthodiplostomum cuticola*	5.63	24.70	27.07	20.32

Wulpińskie such lesions were not recorded. Plasmodia of *Myxobolus* (Figure 3), metacercariae of *Postodilostomum cuticula* (Figure 4) and monogenetic trematodes of genus *Paradiplozoon* (Figure 5) were found in histological preparations of gills. Monogenetic trematodes of genera: *Dactylogyrus, Gyrodactylus, Diplozoon, Paradiplozoon*, crustaceans of genera *Ergasilus, Caligus* and leeches of genera: *Piscicola, Hemiclepsis, Caspiobdella* were also found on the gills. The percentage share of individual parasitic taxa on gills of roach differed between lakes studied. Monogenean, crustaceans and leeches were most numerous in lakes Dgał Wielki and Warniak (DZIKA 2003).

Their presence influenced the morphology of gill blades. Adhesions of 3-4 lamelles that were subject to major deformation, thickening were most frequent; additionally transverse lamelles adhered to one another and were subject to hyperemia. Adhesions of 2 lamelles and of more than 5 lamelles were highly infrequent in the examined fish. Infiltrations of lymphoid cells and hyperemia of lamelles were also recorded. The surfaces of such gills were covered in excess mucus and exfoliated epithelial cells. In a rather large group of fish examined focal coagulative necrosis of epithelium covering the gills was found accompanied by infiltrations of lymphoid cells and presence of large volumes of mucus on gill surface. Such lesions applied to from 12.67% of fish in lake Ukiel to 23.18% of fish from lake Dgał Wielki; no such lesions were recorded in fish caught from lake Wulpińskie.

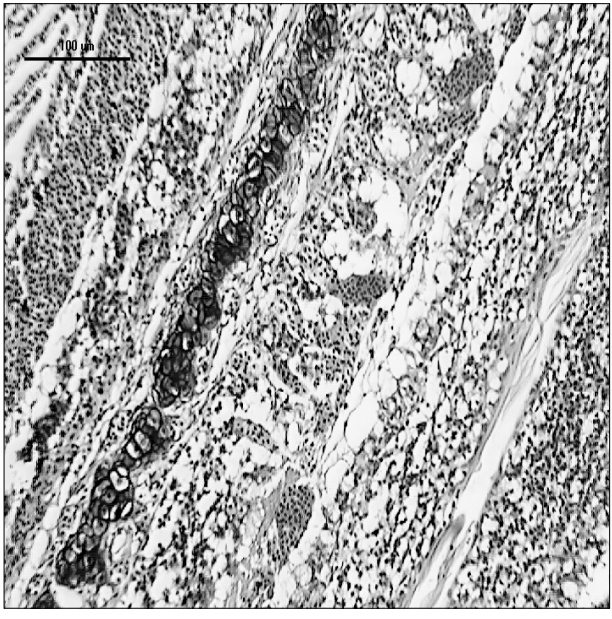

Fig. 1. Hyperemia and extravasations of blood in gills. Staining HE

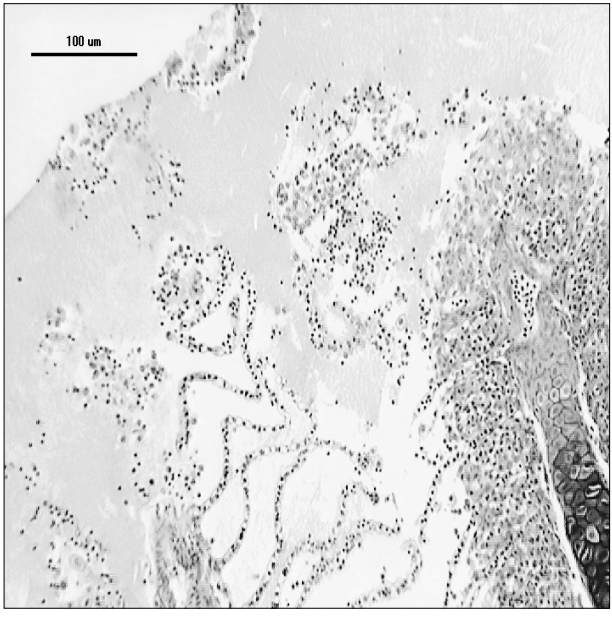

Fig. 2. Large quantity of mucus on surfaces of gill lamelles. Staining HE

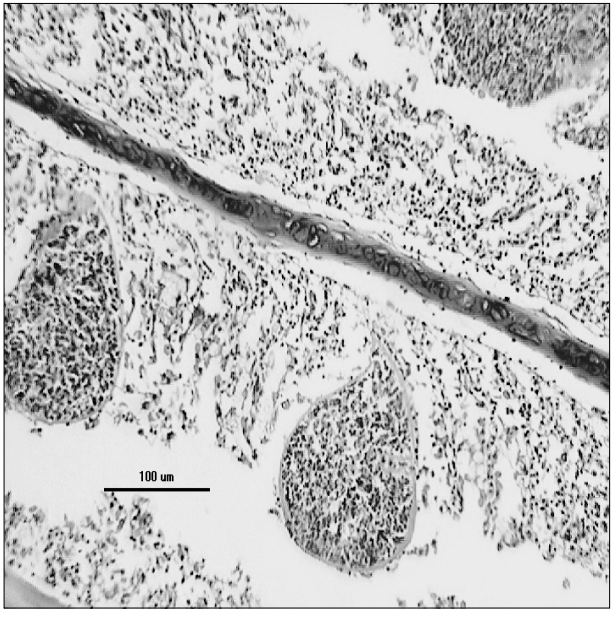

Fig. 3. Presence of *Myxobolus* plasmodia in gill lamelles. Staining HE

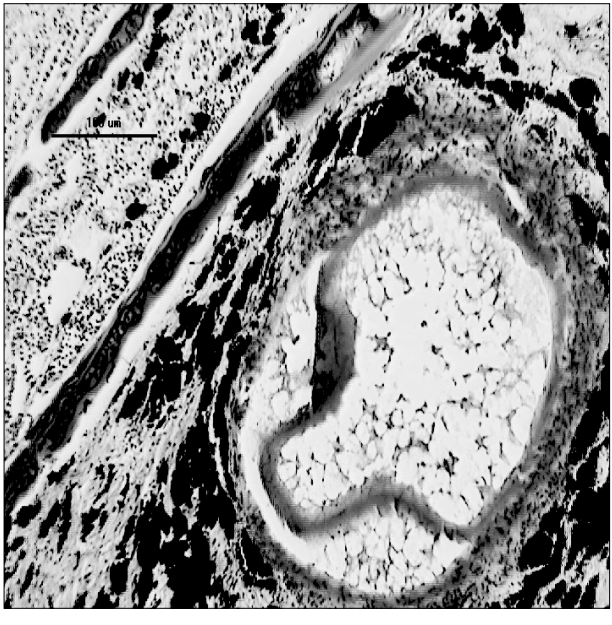

Fig. 4. *Posthodiplostomum cuticola* in the gill arch – melanin capsule around the parasite is visible. Staining HE

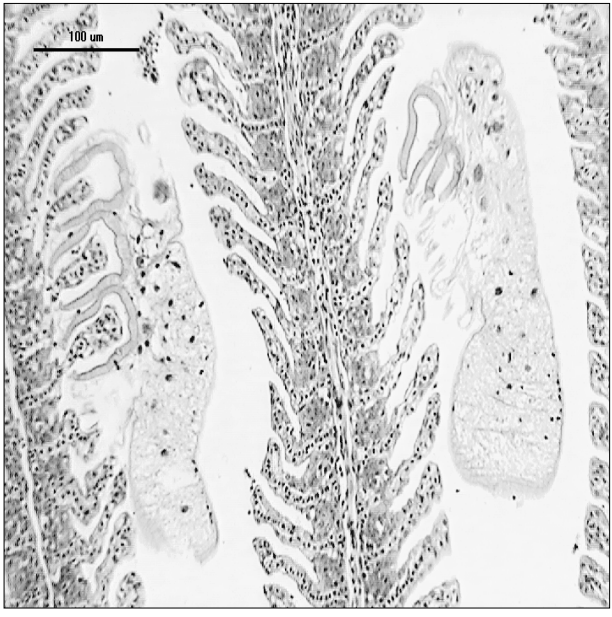

Fig. 5. *Paradilozoon* in gills of roach. Staining HE

Presence of *Myxobolus* plasmodia in gills of similar percentage of the examined fish from all lakes, with minor extent differences, deserves noticing.

Much larger differences were recorded as concerns extensity of infestation with *Posthodiplostomum cuticola*, (27.07%) that was most extensive in fish caught from lake Dgał Wielki and the least in Ukiel lake – 5.63% (DZIKA 2003).

The morphological lesions found in the gills of roach were regressive and inflammatory in nature and were probably caused by the direct influence of unfavorable factors in the water and the presence of parasites. Development of morphological damages to gills was supported by acid pH of the water as in such lakes concentrations of potentially toxic metals increases. Even minor morphological damages of gill blades obstruct acid-base homeostasis and exchange of gases in fish. Epithelium of gill transverse lamelles plays an important role in those processes. Epithelium of gills consists of different cells that participate in exchange of gases, produce mucus and take active part in transportation of necessary elements. The reaction of gills to appearing damaging factors may lead to adaptation that is expressed by increased production of mucus to create a protective barrier. Mucus generating cells are expanded or multiply. Such processes were recorded in own studies in roach caught from four lakes. It may be considered that the mucus accumulating of the surfaces of gill blades makes breathing of the fish slightly more difficult; nevertheless excessive quantities of mucus are accompanied by hyperemia

of transverse blades' vessels and, most often, small extravasations of blood. Also the breathing epithelium of gill blades is damaged; its cells get exfoliated in some places while in other places they multiply. That leads to thickening of the lamelles, swelling and development of epithelium necrosis leading to major deformations of gill lamelles. The inflammatory process accompanied by infiltration of lymphocytes, macrophages and leukocytes develops as a consequence of damage to transverse lamelles. Those processes lead to adhesion of transverse lamelles and next excess growth of connective tissue cells; inflammatory granulomas and ulcers develop. The described morphological changes were found in the examined roach caught from all four lakes. The extent, however, of morphological changes of retrogressive nature was small; inflammatory processes, particularly in fish where presence of parasites was established, were more pronounced. Own studies confirmed their frequent presence in roach. Parasites may appear in gills temporarily or they may position there permanently and have a negative influence on functioning of gills. Those observations are confirmed by findings of other authors describing numerous species of parasites in gills, e.g. LOM and DYKOVÁ (1984) report frequent presence of *Emeria sp.* in gills of various fish species. During infestation with *Myxobolus,* histopathological examination shows deformation and changes in the structure of blood vessels of gills. Also during infestation with *Dactylogyrus* gills are pale, covered with large volumes of mucus while endings of gill lamelles grow excessively and extravasations are observed among cells (PROST, 1994). Infestations with *Gyrodactylus* cause exudations, small foci of necrosis and increased secretion of mucus while in *Diplozoon* excessive growth and swelling of gill lamelles (JARA and CHODYNIECKI 1999).

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THE EFFECT OF IMMUNOSTIM-PLUS ON THE LEVEL OF TOTAL PROTEIN, γ-GLOBULINS, LYSOZYME, SOME ACUTE PHASE PROTEINS AND CYTOKINES IN RATS' BLOOD SERUM AFTER ACUTE CARBON TETRACHLORIDE INTOXICATION

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K e y w o r d s: rats, carbon tetrachloride, Immunostim-plus, total protein, γ-globulins, acute phase proteins, cytokines.

A b s t r a c t

Rats which had undergone carbon tetrachloride poisoning (CCl_4) were treated with the Immunostim-plus preparation (produced by Herbapol, Lublin), which possesses antioxidative, immunomodulating and hepatoprotective properties and consists of dry extract of Siberian gingseng and schizandra.

The tests proved that the preparation administered to rats in a dose of 4 $mg \cdot kg^{-1}$ for 10 days regulated the disturbed protein metabolism in the animals but did not remove completely the consequences of the inflammatory process in the liver induced by CCl_4.

WPŁYW PREPARATU IMMUNOSTIM-PLUS NA POZIOM BIAŁKA CAŁKOWITEGO, γ-GLOBULIN, LIZOZYMU, NIEKTÓRYCH BIAŁEK OSTREJ FAZY ORAZ CYTOKIN W SUROWICY KRWI SZCZURÓW PO OSTRYM ZATRUCIU CZTEROCHLORKIEM WĘGLA

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S ł o w a k l u c z o w e: szczury, czterochlorek węgla, Immunostim-plus, białko całkowite, γ-globuliny, białka ostrej fazy, lizozym, cytokiny.

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Abstrakt

U szczurów po zatruciu czterochlorkiem węgla (CCl_4) stosowano preparat Immunostim-plus (Herbapol, Lublin) o właściwościach antyoksydacyjnych, immunomodulujących i hepatoprotekcyjnych, składający się z suchego ekstraktu żeńszenia syberyjskiego i cytryńca chińskiego.

Wykazano, że preparat podawany szczurom przez 10 dni w dawce 4 $mg \cdot kg^{-1}$ regulował zaburzoną u zwierząt przemianę białkową, lecz nie usuwał w pełni następstw procesu zapalnego wątroby spowodowanego przez CCl_4.

Introduction

The hepatotoxic effect of carbon tetrachloride (CCl_4) on animals has long been recognized (BORZUCHOWSKA et al. 1980, ISSELBACHER et al. 1960, KUŹNA-GRYGIEL 1960). Animals which have received this compound develop an inflammatory damage of hepatocyctes (BORZUCHOWSKA et al. 1980, KUŹNA-GRYGIEL 1960). One of the consequences observed in impaired areas is the reduction and sometimes even complete disappearance of the activity of oxidation-reduction enzymes (KUŹNA-GRYGIEL 1960) and, as the changes progress, the activity of aminotransferases in blood increases (BORZUCHOWSKA et al. 1980, YAO et. al. 1994, YU et. al. 2004). The occurrence of those disorders is largely connected with the fact that while the liver metabolizes CCl_4 a highly reactive trichloromethyl is produced, which initiates peroxidation of lipids and unsaturated fatty acids. Membrane structures become damaged and certain enzymatic disorders occur (CHAVAPIL et.al. 1973). Other hepatic functions, such as metabolism of proteins, fats and carbohydrates, synthesis of plasma proteins and clotting factors as well as detoxifying activities (ISSELBACHER et. al. 1960) can also be disturbed.

Later on, stemming from the above disorders, cirrhosis of the liver that resembles macrondular cirrhosis in humans, may develop (KUŹNA-GRYGIEL 1960). The molecular mechanism of those changes is complex and depends on mutual interactions between liver cells, growth factors and pro- and anti-inflammatory cytokines (YU et al. 2004). Under the effect of CCl_4, hepatocytes intensify the synthesis of tumour necrosis factor (TNF-α), nitrogen oxide (NO), transforming growth factors (TGF-α and TGF-β) and interleukins (Il). The latter include Il-1, Il-6 and Il-10 (ROBAK 1995, WEBER et al. 2003, YU et al. 2004), which modulate the immunological response via intensification of the synthesis of acute phase proteins and induction of immunological mechanisms, such as production of antibodies, differentiation of T cells, synthesis of prostaglandins, hormone release factors and stimulation of the production of other cytokins (ROBAK 1995).

Several pharmaceuticals, including herbal preparations, are used in the therapy of impaired liver functions. They include Sylmarin (FERENCI et al.

1989, MUZES et al. 1990), Oxymatrine (YU et al. 2004), Matrine (ZHANG et al. 2001, ZHU et al. 2002) and others.

The aim of the present study has been to determine the effect of Immunostim-plus (produced by Herbapol, Lublin) which consists of 155 mg of Eleutherococci rad. Extr. Siccum (*Eleuthreococcus senticosus*, Siberian gingseng) and 100 mg of Schizandrae fruc. extr. siccum (*Schizandra chinensis*, schizandra) and which possesses antioxidative, immunomodulating and hepatoprotective properties on the level of total protein, γ-globulins, lysozyme, C-reactive protein (CRP), ceruloplasmin (Cp), Il-1β and TNF-α in the blood serum of rats after CCl_4 intoxication.

Material and Methods

The tests were carried out on 18 healthy adult Wistar/Han. Rats, weighing 263 ± 18 g, derived from own breeding (Pathophysiology Unit University of Warmia and Mazury). The rats were maintained under natural light conditions in rooms with gravitational ventilation. They were fed *ad libitum* with a granulated feed for rats called LSM (Łomna) and had free access to water.

Prior to the tests the animals were divided at random into three groups, each consisting of 6 animals. Group I was the control (C) while the other two groups: II (CCl_4) and III (CCl_4 +Ip) were the experimental ones, in which the rats received a single dose of spectrally pure carbon tetrachloride, dissolved in olive oil at a ratio of 1 : 1 (CCl_4 158.24 containing at least 99.5% of CCl_4; produced by POCh Gliwice) which was introduced intragastrically by probe. Four days after CCl_4 administration, animals from group III for 10 consecutive days intragastrically received an aqueous solution of Immunostim-plus (Herbapol, Lublin) (Ip) in a dose of 4 $mg \cdot kg^{-1}$ body weight. The remaining rats, groups I and II, received by probe the same volume of physiologic saline.

On the 15th day of the experiment, when the administration of the preparation had been terminated, all the animals were given halothane anaesthesia (Narcotan – Leciva, Prague) and maximum amount of blood was collected from each animal via heart puncture until complete exsanguination. Immediately after the collection, the blood was centrifuged for 15 minutes at 3000 rev./min and the serum thus separated was frozen at -22°C.

The following were determined in the serum: total protein level with the biuret method using Diagnostic Kits and Reagents, Protein Total Reagent (Sigma), γ-globulins using poliethylene glycol 10 000 (Sigma), lysozyme using *Micrococcus lysodeikticus* (Sigma) according to PARRA (1965), CPR – with the ELISA spectrophotometric micromethod and a CRP determination kit (Sigma), Cp – with the spectrophotometric method according to RICE (1986) as

modified by SIWICKI and ANDERSON (1993), and Il-1β and TNT-α using kits produced by Endogen (USA).

The results of the determinations were processed statistically using t-Student test (Statgraphic Statistical Graphic System software). The mean values ($x \pm$ SEM) and significance levels ($p \leq 0.01$ and $p \leq 0.05$) were computed of the results obtained for the experimental groups versus the controlone.

All the tests involving the animals were carried out in compliance with the guidelines of the Act on experiments on animals and the recommendations of the Local Ethics Commission concerning Experiments on Animals, at the University of Warmia and Mazury in Olsztyn (permit no 42 N).

Results

In the blood serum of the rats which had received CCl_4 (group II), compared to the control (group I), statistically significant (s.i.) decrease in the total protein concentration by 5.2 $g \cdot l^{-1}$ ($p \leq 0.01$) and increase in the CRP level by 7.2 $mg \cdot l^{-1}$ (by 5.2 $g \cdot l^{-1}$, $p \leq 0.05$) and TNF-α by 6.3 $pg \cdot ml^{-1}$ (by 5.2 $g \cdot l^{-1}$, $p \leq 0.01$) were determined. The remaining blood serum indices, such as the level of γ-globulins, Cp and Il-1β tended to decrease.

In the animals from group III which, after being administered CCl_4, were treated days with Immunostim-plus for 10, the tests revealed that the levels of CRP and TNF-α were higher than in the control group by 5 $mg \cdot l^{-1}$ ($p \leq 0.01$) and 5.7 $pg \cdot ml^{-1}$ ($p \leq 0.05$) respectively, while the respective concentrations of Cp and Il-1β were lower by 18.7 $UI \cdot l^{-1}$ ($p \leq 0.01$) and 54 $pg \cdot ml^{-1}$ ($p \leq 0.05$). The values of the other indices, such as total protein, γ-globulin and lysozyme were lower than in the control group.

Comparisons between group III (CCl_4 + Ip) and II (CCl_4) showed that the Cp level in group III compared to that in group II was depressed by 16.7 $UI \cdot l^{-1}$ ($p \leq 0.01$), whereas the concentration of lysozyme and TNF-α tended to decrease. In contrast, total protein and γ-globulins tended to increase their concentrations.

Results and Discussion

In a few hours following the administration of CCl_4 the rats developed symptoms of acute intoxication which were manifested by the dejected behaviour of the animals, bristled hair, loss of appetite, increased thirst and excretion of blood-stained urine. Among the animals which showed such symptoms one rat died (group III – CCl_4 +Ip). In the following days the

Table 1
Level of total protein, γ-globulins, lysozyme, CRP, Cp, Il-1β and TNF-α in blood serum of rats ($x \pm$ SEM)

Group/ /index	Total protein ($g \cdot l^{-1}$)	γ-globulins ($g \cdot l^{-1}$)	Lysozyme ($mg \cdot l^{-1}$)	CRP ($mg \cdot l^{-1}$)	CP ($UL \cdot l^{-1}$)	Il-1β ($pg \cdot ml^{-1}$)	TNF-α ($pg \cdot ml^{-1}$)
Group I (C) $n = 6$	62.9 ± 0.86	14.4 ± 1.38	3.29 ± 0.26	17.5 ± 0.93	128.8 ± 2.73	39.9 ± 1.20	38.9 ± 0.95
Group II (CCl_4) $n = 6$	57.7^{A} ± 1.31	12.0 ± 1.55	3.13 ± 0.38	24.7^{a} ± 2.58	126.8 ± 2.90	36.3 ± 1.26	45.2^{A} ± 1.05
Group III (CCl_4+Ip) $n = 5$	60.9 ± 1.07	13.91 ± 0.85	2.73 ± 0.26	22.5^{A} ± 1.04	110.1^{AB} ± 1.96	34.5^{A} ± 0.63	44.6A ± 0.39

Comparisons:
Group I (C) vs groups II (CCl_4) and III (CCl_4+Ip) *A* ($p \leq 0.01$), *a* ($p \leq 0.05$);
Group II (CCl_4) vs group III (CCl_4+Ip) *B* ($p \leq 0.01$).

intoxication symptoms were gradually disappearing, so that after 4 days the behaviour of those rats did not differ visually from the control animals. Since that moment, i.e. day 5, intragastrical administration of Immunostim-plus to rats group III began. The rats received 4 $mg \cdot kg^{-1}$ body weight of the preparation daily. The treatment continued for 10 consecutive days and the daily dose applied corresponded to 1 capsule (300 mg) of the preparation administered to a human of the body weight equal to 70 kg.

Although the time which elapsed from the intoxication with CCl_4 to the blood tests was 14 days, its consequences, which could have been diagnosed on the basis of the blood serum indices, pertained. In group II (CCl_4) the total protein level versus the controlone was statistically significantly depressed. This implies impaired protein metabolism in the rats' organisms caused by some damage to the liver and possibly also the kidneys. The increased levels of CRP and TNF-α in group III suggests that there was an inflammatory condition in the organism and, on the other hand, that the immunologic processes were intensified (KOSTRO et al. 2000, 2002, ROBAK 1995).

Immunostim-plus applied in group III caused increase in the total protein level and decrease in Il-1-β. Elevated concentration of protein in blood serum should be interpreted as a positive effect of the preparation on protein metabolism, whereas increased concentration of pro-inflammatory cytokin Il-1β may indicate that the inflammation was receding. This, however, is contradicted by the persistently higher, compared to the controlone, level of CRP and TNF-α as well as lower Cp concentration. On the one hand, the levels obtained by those indices confirm that there was an inflammatory state in the

organism (increased TNF-α and decreased CRP) and, on the other hand, that there were certain reparatory processes at work (increased CRP) (KOSTRO et al. 2000).

In conclusion, it should be said that the Immunostim-plus preparation, tested in the present experiment did not lead to complete normalization of the liver functions in the rats which had been intoxicated with CCl_4. This may have been due to the dose of the preparation, possibly insufficient, or the time period of the application, which may have been too short. Nonetheless, the regulatory effect of Immunostim-plus on protein metabolism which was confirmed by our tests enables us to hypothesise that the preparation can be used to treat less severe pathogenic conditions of the liver. The hypothesis, however, requires further studies.

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ZEARALENONE INTOXICATION OF GAME ANIMALS

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K e y w o r d s: zearalenone, roe-deer, red deer, wild board, Warmia and Mazury.

A b s t r a c t

Large game animals (roe deer, red deer and wild boars) were harvested in hunting grounds located in the Warmia and Mazury District. Blood samples were collected (from the heart or jugular vein) immediately after the animals had been shot. Plasma was separated from whole blood to determine the levels of zearalenon (ZEA) and α-zearalenol (α-ZOL). ZEA and α-ZOL were found in all animal species examined in the study, including 11.7% of the red deer, 33.3% of the roe deer and 56.2% of the wild boars. The plasma levels of ZEA and α-ZOL ranged from 1.0 to 83.0 ng · ml^{-1}, and from 0.3 to 11.0 ng · ml^{-1} respectively. The highest concentrations of these mycotoxins were recorded in wild boars – on average 5.1 ng · ml^{-1} of α-ZOL and 16.7 ng · ml^{-1} of ZEA. The lowest amounts of these compounds were observed in young wild boars (weaners), and the largest – in the oldest ones. The presence of both forms of mycotoxins in the blood samples of game animals indicates that their feeding grounds, such as crop fields, are infested with mould fungi (mainly of the genera *Fusarium* and *Penicillium*). Such a high percentage of animals (especially wild boars) whose blood contains mycotoxins constitutes a serious health hazard to wild game consumers.

ZATRUCIE ZWIERZĄT ŁOWNYCH ZEARALENONEM

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S ł o w a k l u c z o w e: zearalenon, sarna, jeleń, dzik, Warmia i Mazury.

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Abstrakt

W obwodach łowieckich położonych na Warmii i Mazurach pozyskiwano grubą zwierzynę łowną (sarny, jelenie i dziki), z której, natychmiast po odstrzale, pobierano krew (z serca lub żyły szyjnej). Z krwi oddzielano osocze, w którym oznaczano ilość zearalenonu (ZEA) i α-zearalenolu (α-ZOL).

U wszystkich analizowanych gatunków zwierząt, w tym u 11,7% jeleni, 33,3% saren i 56,2% dzików stwierdzono występowanie ZEA i α-ZOL. Poziom ZEA w plazmie wynosił od 1,0 do 83,0 ng · ml^{-1}, a α-ZOL od 0,3 do 11,0 ng · ml^{-1}. Najwyższe stężenie badanych mikotoksyn stwierdzono u dzików. Średnio wynosiło ono 5,1 ng · ml^{-1} α-ZOL i 16,7 ng · ml^{-1} ZEA. Najniższy poziom badanych związków zanotowano u młodych dzików warchlaków, najwyższy u dzików najstarszych. Obecność obu form mikotoksyny we krwi zwierząt łownych wskazuje jednoznacznie na przyjmowanie przez te zwierzęta karmy pochodzącej z pól, porażonych grzybami pleśniowymi (głównie z rodzajów *Fusarium* i *Penicillium*). Duża liczba zwierząt, u których stwierdzono występowanie mikotoksyn (szczególnie dzików), stwarza zagrożenie zdrowotne dla ewentualnych konsumentów dziczyzny.

Introduction

Zearalenon (ZEA, $C_{18}H_{22}O_5$, synonym: F-2 toxin) is a secondary metabolite produced by many microscopic fungi of the genus *Fusarium*. These moulds develop on plants and produce mycotoxins (secondary metabolism products) during the growing season. These mycotoxins belong to low molecular weight aromatic (seldom aliphatic) hydrocarbons. As low weight molecules, mycotoxins are resistant to environmental factors and are not immunogenic (PEJSAK 1997).

The presence of ZEA has been proved in samples of wheat, barley, oat and rye, as well as maize grain (KUIPER-GOODMAN et al. 1987, TAKAHASHI-ANDO et al. 2002, ZALEWSKI, ŁOGIN 1999) and grass hay. According to PERKOWSKI et al. (1999), in Poland ZEA was detected in 50% of wheat samples (mean concentration – 33 μg · kg^{-1},), 47% of barley samples (63 μg · kg^{-1}) and 50% of oat samples (120 μg · kg^{-1}). Analysis of grain obtained from wheat ears infested with fungi of the genus *Fusarium* showed that 75% of the samples contained this metabolite, and that its mean concentration was 140 μg · kg^{-1}.

First reports suggesting zearalenon intoxication of animals come from 1927, and concern pigs – the species that was later found to be extremely sensitive to this toxin. ZEA, a non-steroid mycotoxin, exhibits strong estrogenic effects. However, these effects are over 100-fold weaker when compared with the activity of 17 β-estradiol (MCNUTT et. al. 1928, NOGOWSKI et al. 1994). Consumption of foodstuffs and feedstuffs contaminated with zearalenon by humans and animals causes zearalenon mycotoxicosis manifested as disorders that affect the reproductive system. In such cases the main metabolites detected in blood samples are α-zearalenol (α-ZOL) in monogastric animals, and β-zearalenol (β-ZOL) in ruminants. Detailed data on the effects of mycotoxins can be found in previous review papers devoted to this topic (FRAYSSINET et al. 1997, PEJSAK 1997, SCUDAMORE, WATSON 1998).

Available literature provides no information on the effects of mycotoxins on game animals. Since roe deer, red deer and wild boars have easy access to crop fields, it seems that the actual health impacts of ZEA should be assessed in this group of animals.

The aim of the present study was to estimate the risk of zearalenon intoxication following ingestion of contaminated feed in roe deer, red deer and wild boars by determination of the levels of zearalenon and α-zearalenol in blood samples.

Material and Methods

The experiment was performed on a group of game animals composed of 15 red deer (7 males, 8 females), 17 roe deer (8 males, 9 females) and 32 wild boars (14 males, 18 females). Blood samples for laboratory analysis were collected from the jugular vein (or from heart) immediately after the animals had been shot. 70% of wild boards harvested in winter. The roe deer (female) and red deer hunting on 1.X to 30.XII.

The levels of ZEA and α-ZOL were determined in blood samples according to the method described elsewhere (OBREMSKI et al. 2003). ZEA and α-ZOL were isolated from blood plasma, incubated with β-glucuronidase (from Helix Pomatia, type H-2, enzymatic activity of 100 000 IU · ml^{-1}, pH 5.0, G 7017 Sigma-Aldrich Ltd.) and extracted with gradient grade methanol (LiChrosolv™, No 1.06 007, Merck-Hitachi, Germany) on immunoaffinity columns (Zearala--Test™ Zearalenone Testing System, G1012, VICAM, Watertown, USA). A quantitative analysis was carried out by high performance liquid chromatography, using a fluorescence detector (Hewlett Packard 1100, FLD G1321A) at excitation wavelength λ_{Ex} = 218 nm and emission wavelength λ_{Em} = 438 nm.

Results

Tables 1 and 2 present the characteristics of game animal species analyzed in the study, and the results of tests for the presence of ZEA and α-ZOL in the blood plasma from wild boars.

A total of 64 experimental animals were examined in the study. The mycotoxins (ZEA, α-ZOL) were recorded in 11.7% of the red deer, 33.3% of the roe deer and in 56.2% of the wild boars. The plasma levels of ZEA varied greatly and depended on the species and – in the case of wild boars – also on the age of animals (Table 2). From among 17 red deer, ZEA was detected in two stags only, at a concentration of 3 and 10 ng · ml^{-1}. In roe deer (5 adults) the

Table 1

Characteristics of game animal species analyzed in the study

Species	Sex		Age and body weight of animals					
	male	female	0.5-1 year	kg *x*	2-3 years	kg *x*	>3 years	kg *x*
Roe-deer	7	8	5	13	4	18	6	19.5
Red-deer	8	9	3	51	3	73	11	122.0
Wild boar	14	18	17	24	9	54	6	78

Table 2

Plasma levels of ZEA and α-ZOL in wild boars (a), roe-deer (b) and red deer (c) harvested in Warmia and Mazury

a)

No	Sex	Body weight (kg)	α-zearalenol ng · ml^{-1} of plasma	Zearalenon ng · ml^{-1} of plasma
1.	m	23	7	26
2.	m	22	3	–
3.	m	24	–	–
4.	m	26	–	–
5.	m	27	–	20
6.	m	31	–	–
7.	m	22	–	–
8.	f	23	–	–
9.	f	21	–	–
10.	f	21	–	–
11.	f	22	–	–
12.	f	24	–	–
13.	f	25	2.0	1.7
14.	f	19	–	9.0
15.	f	19	–	–
16.	f	28	3.3	2.0
17.	f	28	1.7	1.0
1.	m	50	8.0	13.0
2.	m	53	–	11.0
3.	m	61	2.7	1.6
4.	m	62	6.6	0.3
5.	f	53	–	–
6.	f	49	–	–
7.	f	60	–	–
8.	f	56	11.0	83.0
9.	f	60	6.0	–
1.	m	91	–	40.0
2.	m	96	–	37.0
3.	m	60	6.6	–
4.	f	83	–	–
5.	f	76	5.6	12.0
6.	f	62	3.0	–

b)

No	Sex	Body weight (kg)	α-zearalenol ng · ml^{-1} of plasma	Zearalenon ng · ml^{-1} of plasma
1	f	17	6.7	–
2	f	18	–	7.0
3	f	19	3.2	–
4	f	19	10.0	–
5	m	17	–	6.6
6–15	m–6	16–22	–	–
	f–4	13–20	–	–

c)

No	Sex	Body weight (kg)	α-zearalenol ng · ml^{-1} of plasma	Zearalenon ng · ml^{-1} of plasma
1	m	131	–	10.0
2	m	103	3.0	–
3–17	m–6	57–164	–	–
	f–9	51–90	–	–

The chromatographic separation of mycotoxins isolated from blood of game animals presented in Figure 1.

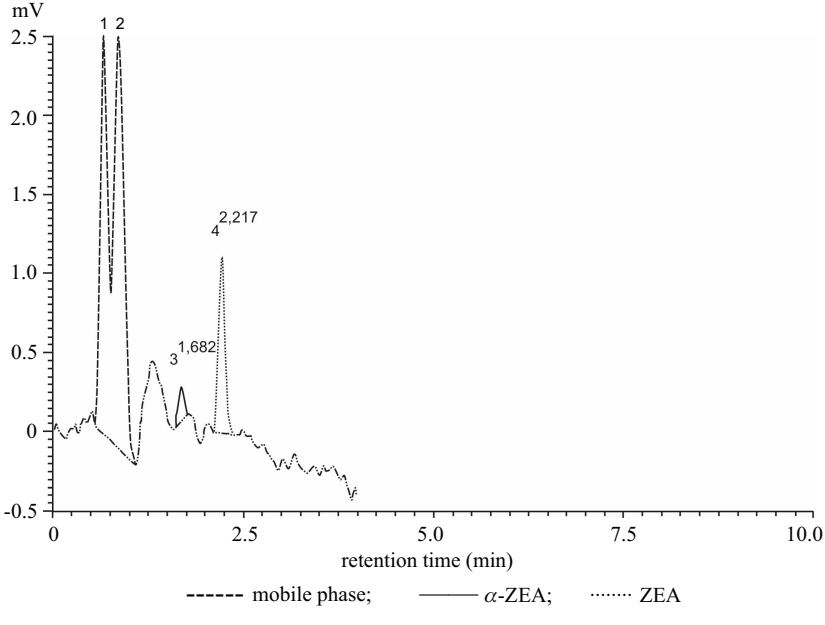

Fig. 1. The chromatographic separation of mycotoxins isolated from blood of game animals

plasma levels of ZEA and α-ZOL ranged from 3 to 7 ng · ml^{-1} and from 3.2 to 10.0 ng · ml^{-1} respectively. In the group of wild boars the plasma levels of these mycotoxins were quite different. In weaners (aged 0.5 – 1 years) ZEA and α-ZOL were detected in 41.1% of the population (concentration range 1.0 – 26.0 ng · ml^{-1}). In young (aged 1-2 years) and older (3 years of age and older) wild boars, one or both forms of the mycotoxins were recorded in 66.6% of the animals. Their concentrations ranged widely, between 0.3 and 83.0 ng · ml^{-1} of plasma.

Discussion

Zearalenon is a phytosteroid, but also a mycotoxin – an undesirable substance. ZEA is believed to have relatively low toxicity. It affects primarily the reproductive system and reproductive processes (CURTUI et al. 2001). The effects of ZEA observed in laboratory animals included, among others, the enlargement of the uterus, infertility, changes in the color of the vulva, and bleeding from the reproductive organs. Due to its biological effects and activity stimulating cell proliferation in estrogen-dependent tissues, ZEA is used as a feed component, since it positively affects body weight gains in slaughter animals (NOGOWSKI 1994). Tests have also proved the presence of zearalenon in the blood of humans (GAJĘCKI 2002, TOMASZEWSKI et al. 1998), pigs and fur-bearing animals (OBREMSKI et al. 2005a).

The presence of zearalenon and α-zearalenol in the blood samples taken from game animals indicates that their feeding grounds are attacked by fusariosis, or that they receive poor-quality feed in the summer and winter. It also confirms the occurrence of zearalenon mycotoxicosis within this population, which may be at the subclinical stage at a given moment. Considerable differences in the prevalence of zearalenon mycotoxicosis in particular species may be related to their different feed preferences. The red deer and roe deer prefer green forage (meadow grass, winter crops), seeds and lignified parts of some tree species. The wild boar prefers seeds of forest trees (beech, oak), seeds of field crops during the growing season, and residues of root crops and maize in the fall and winter, which are frozen and have high levels of mycotoxins (ZALEWSKI, ŁOGIN 1999).

Zearalenon posioning does not necessarily cause disorders of the reproductive system. According to LUNDH and LUNDGREN (1991), ZEA has a significant effect on some biochemical changes related to general metabolism, thus disturbing the respiratory chain with oxidative phosphorilation. Our previous observations showed that the presence of zearalenon in the blood samples (0.5-4.6 ng · ml^{-1}) and liver sections (60-183 ng · g^{-1}) from rabbits was a conse-

quence of serious retrogressive changes in numerous organs, i.e. the lungs, liver, kidneys or testes, accompanied by symptoms of kidney fibrosis and atrophy of lymphatic follicles in the spleen. This indicated long-term intoxication; weakened rabbits were more susceptible to diseases and showed reproductive disturbances (OBREMSKI et al. 2005a). YANG et al. (1995) observed similar symptoms in minks during experimental zearalenon mycotoxicosis. There is presence of ZEA in liver and muscles of domestic pigs and cow's milk and muscles. (AUMAITRE 1999, HOSHY 1999). Roe-deer and red deer same as domestic cattle are ruminants, adsorption and transformation of ZEA will be similar to domestic cattle with high probability.

Due to the presence of a phenolic ring, zearalenon can bind to cellular estrogen receptors in the uterus, vagina, ovaries and oviduct (KATZENELLENBOGEN 1996). This enhances cell proliferation and the synthesis of RNA and proteins in reproductive cells (BOYD et al. 1978, OBREMSKI et al. 2004, 2005b). Metabolic disorders results in various clinical signs of ZEA intoxication, which in pigs include swelling and reddening of the vulva, a decrease in the weight of the ovaries, enlargement of the uterus (BLANEY et al. 1984, CURTUI 2001, VANYI et al. 1994), changes in *libido*, infertility, phantom pregnancies, enlargement of the mammary glands, lactation disorders (ETIENNE, JEMMALI 1982, GAJĘCKI 2002), splayleg in piglets, or prolapse of the vaginal walls and of the anus (SYDENHAM et al. 1988). Although not previously documented, the above symptoms could be probably observed also in wild boars, as the domestic pig and the wild boar are closely related.

Conclusion

1. Game animals (roe deer, red deer, wild boars) that live in north-eastern Poland are threatened by zearalenon mycotoxicosis, which occurs in almost 50% of the population.

2. From among the three game species examined in the study, the risk of ZEA and α-ZOL intoxication is the highest in wild boars.

3. The presence of mycotoxins in the blood of game animals, especially wild boars, constitutes a possible health hazard to wild game consumers.

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DERMANYSSUS GALLINAE INVASION IN THE LAYER HOUSE

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K e y w o r d s: *Dermanyssus gallinae*, young layers, layer house.

A b s t r a c t

The invasion of *Dermanyssus gallinae* was studied in a four-storied layer house populated by 100 000 pullet chicks kept in cages arranged in rows. In order to monitor the levels of red mite infestation, tube traps were placed three times, at two week intervals, 0.8 m and 1.8 m above the floor. At 8 weeks the largest numbers of *Dermanyssus gallinae* females, eggs and larvae were found in traps placed 0.8 m above the floor. Later on red mites invaded cages on higher stories, and by 12 weeks the greatest numbers of females and nymphs were recorded in traps placed 1.8 m above the floor. Such a distribution pattern of red mites is related to temperature and air humidity in the laying hen house, which during the first 10 to 12 weeks are higher in cages located on lower stories. The ratio between the number of parasites found in tube traps and their total population in the house suggests that *Dermanyssus gallinae* may constitute a serious epizootic threat to laying hens.

PRZEBIEG INWAZJI *DERMANYSSUS GALLINAE* W WYCHOWALNI KUR NIOSEK

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S ł o w a k l u c z o w e: *Dermanyssus gallinae*, młode kury, wychowalnia kur.

A b s t r a k t

Badania przebiegu inwazji *Dermanyssus gallinae* prowadzono w wychowalni kur niosek, zasiedlonej 100 tys. piskląt. Młode kurki umieszczono na 4 kondygnacjach w systemie klatkowym. W celu oceny inwazji ptaszyńca rozstawiano 3-krotnie rurki-pułapki, w odstępach 2-tygodniowych, na

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wysokości 0,8 i 1,8 m od posadzki. Największą liczbę samic pasożyta oraz jaj i larw stwierdzono w 8. tyg. chowu na wys. 0,8 m od posadzki.

W miarę upływu czasu inwazja ptaszyńca przenosiła się na wyższe kondygnacje. W 12. tyg., na wys. 1,8 m, stwierdzono największą liczbę samic i nimf. Na takie rozmieszczenie ptaszyńców w wychowalni może mieć wpływ temperatura i wilgotność, które są wyższe na dole klatek w pierwszych 10-12 tyg. chowu. Biorąc pod uwagę liczbę pasożytów w rurkach, przeliczoną na ich populację w całej wychowalni, można sądzić, że stanowią one poważne zagrożenie epizootyczne dla przyszłych kur niosek.

Introduction

For the first 17 weeks laying hen chicks are usually raised in a battery cage system in buildings. Cages are arranged in rows on four stories. Such layer houses are equipped with automatic feeders and drinkers, as well as with conveyor belt for excrement disposal. A high density of birds in a small space creates specific indoor climatic conditions, conducive to invasions of various parasites, mainly *Dermanyssus gallinae* (red mite) (CENCEK et al. 2000, 2002). *Dermanyssus gallinae* females lay large numbers of eggs which need 8 days to mature to the adult stage.

Red mite infestation is relatively easy to recognize, since fast-running *Dermanyssus gallinae* females, larvae and nymphs can be found in residue from the floor or conveyor belts (CHAUVE 1998, CHIRICO and TAUSON 2002, LUNDH et al. 2005). However, the invasion is very difficult to control (ROMANIUK and SOKÓŁ 2003, 2005, SOON-IL et al. 2004). Heavy infestation of this parasite is often associated with increased anxiety, anemia, skin damage or even death, especially in chicks (KILPINEN et al. 2005, KIRKWOOD 1967). In addition, high infestation levels inhibit the growth and development of birds (PILARCZYK et al. 2004, WÓJCIK et al. 2000).

Red mites can cause heavy losses when present in high densities. Therefore, the aim of the present study was to assess *D. gallinae* infestation levels in a layer house at the second stage of hen raising. The results can provide some insight into this problem, thus contributing to effective red mite control in laying hen houses.

Materials and Methods

The study was performed in a four-storied layer house populated by 100 000 pullet chicks kept in cages arranged in rows. The birds were fed and cared for (including veterinary checks) in accordance with the relevant welfare standards and recommendations for such husbandry systems. *Dermanyssus gallinae* invasion was not controlled before the pullets were brought to the

farm and during the time they stayed in cages. 40 chicks were put into a cage, on the third story of each row. As their body weight increased, the birds were placed on higher and lower stories. By 8 weeks they occupied all stories in the house. At that time (May 27, 2005) paper tube traps (20 cm in length, 1 cm in diameter) were placed under the feeders, 0.8 m and 1.8 m above the floor, in order to monitor the levels of red mite infestation. The traps were collected at 12-day intervals, put into jars and closed, and then replaced with the new ones. At the laboratory the traps were chilled in the freezer. Next each trap was cut longitudinally and the contents was emptied onto a Petri dish. *D. gallinae* eggs, larvae, nymphs and adults were counted under a binocular tube, Olympus SZX12.

Results and Discussion

It was found that the dynamics of *D. gallinae* invasion in the layer house varied and was probably related to the indoor climatic conditions. The greatest numbers of females, eggs and larvae were recorded in tube traps collected first, i.e. at 8 weeks, 0.8 m above the floor. This suggests that at that time the parasite had the optimal growth conditions at this level. At 10 weeks the abundance of females and larvae in traps placed 1.8 m above the floor was low. However, by 12 weeks the traps at 1.8 m contained over 45 *D. gallinae* females and about 9 larvae, i.e. 1.6-fold more as compared with those located 0.8 m above the floor. No parasite eggs were identified in traps collected at 12 weeks (Table 1).

Table 1

Occurrence of *Dermanyssus gallinae* in the layer-house at the first stage of layer rearing

Date of analysis	Week	Developmental stages of *Dermanyssus gallinae* – number per 100 mg of residue					
		imagoes		eggs		larvae and nymphs	
		0.8	1.8	0.8	1.8	0.8	1.8
27.05.05	8	37.7	0.2	35.8	0.4	8.9	0
10.06.05	10	18.8	1.0	21.8	0	4.7	0.3
22.06.05	12	28.9	45.3	15.8	0	5.3	8.8

Parasitosis caused by *Dermanyssus gallinae* poses a serious problem affecting laying hens and other birds. CENCEK et al. (2002) described a case of red mite infestation that resulted in high mortality rates in broiler ducks.

The present study shows that the intensity of *D. gallinae* invasion in layer houses is dependent on a variety of factors. During the first 10 weeks the parasite has good conditions for growth at a level of 0.8 m above the floor, whereas by 12 weeks the number of red mites and their developmental stages increases on higher stories (1.8 m). The absence of eggs and the presence of larvae and nymphs in tube traps collected at 12 and 14 weeks indicate that they moved from lower to higher stories. Such a distribution pattern of red mites is related to temperature and air humidity in the laying hen house, which during the first 10 to 12 weeks are higher in cages located on lower stories. ROMANIUK and SOKÓŁ (2005) demonstrated that the growth of a *D. gallinae* population on a layer farm is affected by temperature, air humidity, the use of high-density cages and the lack of parasite control agents that would prove effective under commercial farm conditions. WÓJCIK et al. (2000) stressed the scale of economic losses caused by high densities of *Dermanyssus gallinae* on laying hen farms. They found that despite good husbandry conditions the invasion of red mites resulted in increased mortality, lower egg production efficiency and a shorter egg production cycle. According to these authors, both indoor climatic conditions and the technical condition of the house have a significant effect on the prevalence of red mites.

The ratio between the number of parasites found in tube traps and their total population in the house suggests that *Dermanyssus gallinae* may constitute a serious epizootic threat to laying hens. Therefore, it is recommended that pullets are dusted with acaricide prior to placement in a thoroughly cleaned and disinfected house. This is an important component of *D. gallinae* prevention programs, which can minimize the spread of red mites in layer houses when accompanied by strict sanitary procedures.

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