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EFFECTS OF GROWTH REGULATORS, APPLIED ALONE OR IN COMBINATION WITH MAGNESIUM SULFATE, ON NITROGEN AND MAGNESIUM ECONOMY IN SPRING TRITICALE PLANTS

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K e y w o r d s: spring triticale, nitrogen, magnesium, growth regulators.

A b s t r a c t

The effects of growth regulators, applied alone or in combination with magnesium sulfate, on nitrogen and magnesium economy in spring triticale plants were studied in a three-year field experiment. Growth regulators: benzylaminopurine (BAP), α-naphthylacetic acid (NAA), 3-indolebutyric acid (IBA), triacontanol (TRIA), and gibberellic acid (GA3) were applied to leaves, alone or in combination with a 5% aqueous solution of magnesium sulfate, twice during the growing season of spring triticale – at the ear formation stage and before flowering.

The growth regulators and their mixtures with magnesium sulfate differentiated the nitrogen and magnesium content of spring triticale. Gibberellic acid combined with magnesium sulfate significantly decreased nitrogen concentration in culms. α-naphthylacetic acid and benzylaminopurine applied with magnesium sulfate significantly decreased the nitrogen content of leaves, compared to treatments with IBA, TRIA and GA_3. All growth regulators (except for TRIA) applied alone caused an increase in the nitrogen content of triticale grain, whereas the addition of magnesium sulfate to the growth regulators (except for IBA) reduced nitrogen concentration in grain. A significant impact of growth regulators combined with magnesium sulfate was observed in the case of flag leaves, where magnesium concentration increased by about 17%. Triticale grain contained significantly less magnesium following the application of IBA, and the addition of magnesium sulfate to this growth regulator caused an increase in magnesium levels. A reverse effect was recorded for the BAP+Mg combination.

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WPŁYW REGULATORÓW WZROSTU ORAZ ICH MIESZANEK Z SIARCZANEM (VI) MAGNEZU NA GOSPODARKĘ AZOTEM I MAGNEZEM PSZENŻYTA JAREGO

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S ł o w a k l u c z o w e: pszenżyto jare, azot, magnez, regulatory wzrostu.

A b s t r a k t

W trzyletnim doświadczeniu polowym badano wpływ regulatorów wzrostu i ich mieszanek z siarczanem (VI) magnezu na gospodarkę azotem i magnezem pszenżyta jarego. Regulatory: benzyloaminopurynę (BAP), kwas α-naftylooctowy (NAA), kwas indolilo-3-masłowy (IBA), triacontanol (TRIA) oraz kwas giberelinowy (GA_3) stosowano dolistnie, samodzielnie lub w mieszance z 5% wodnym roztworem siarczanu (VI) magnezu, dwukrotnie w okresie wegetacyjnym pszenżyta – w fazie kłoszenia oraz tuż przed kwitnieniem.

Regulatory wzrostu i ich mieszanki z siarczanem (VI) magnezu różnicowały zawartość azotu i magnezu w pszenżycie jarym. Po zastosowaniu mieszanki kwasu giberelinowego z siarczanem magnezu stwierdzono istotny spadek zawartości azotu w źdźble pszenżyta w porównaniu z działaniem tego regulatora w czystej postaci. Kwas α-naftylooctowy oraz benzyloaminopuryna z magnezem istotne ograniczały ilość azotu w liściach w porównaniu z obiektami z IBA, TRIA i GA_3. Ziarno pszenżyta charakteryzowało się wyższą zawartością tego pierwiastka po zastosowaniu substancji wzrostowych (oprócz TRIA), natomiast dodatek siarczanu (VI) magnezu do regulatorów (poza IBA) ją ograniczał. Istotne działanie mieszanek regulatorów z siarczanem magnezu uwidoczniło się w liściu flagowym, w którym stwierdzono ok. 17% zwiększenie ilości magnezu. Ziarno pszenżyta zawierało istotnie mniej tego pierwiastka po zastosowaniu IBA, natomiast dodatek siarczanu magnezu do tego regulatora powodował zwiększenie zawartości magnezu. Odwrotny efekt współdziałania uzyskano w obiekcie, w którym zastosowano BAP+Mg.

Introduction

Rational fertilization systems include the supply of major nutrients (NPK), but also magnesium microelements applied to soil or leaves (CHWIL 2001, CZUBA 1993a,b). Under conditions of intensive plant production, characterized by high nutrient demand, magnesium deficiency may result in yield decline and deteriorate its quality (BARŁÓG, GRZEBISZ 1994). Adequate magnesium availability in light soils enables to increase its concentration in cereal crops (SIENKIEWICZ 1994). According to KOC et al. (1994), grain is most susceptible to magnesium deficiency.

Fertilization efficiency is limited by the biological capabilities of plants. Biological barriers to the optimum crop productivity may be partially removed by the application of growth regulators (WIERZBOWSKA, NOWAK 1999). Research results show that growth regulatory can significantly modify

crop yield and mineral balance (AUFHAMER, FEDEROLF 1992, NOWAK, WIERZBOWSKA 1991).

The aim of this study was to determine which of the tested growth regulators, applied alone or in combination with magnesium sulfate, had the most beneficial effect on nitrogen and magnesium economy in spring triticale plants.

Materials and Methods

A three-year two-factorial field experiment was conducted during the years 1999-2001 at the Experimental Station in Tomaszkowo near Olsztyn. Spring triticale cv. Gabo was grown on brown soil of quality class IIIb, on microplots covering an area of 2 m^2. The soil had a slightly acid reaction (pH = 6.3 in 1 mol KCl dm^{-3}) and was relatively abundant in available phosphorus and potassium, but poor in available magnesium.

Weather conditions over the experimental period are presented in Table 1. The average temperature during the growing seasons (13.86°C) was close to the mean multiannual temperature of the 1970-2000 period (13.82°C). Rainfall total was higher in some months (June 1999, August 2000, July and September 2001), so the precipitation total for the growing seasons (70.3 mm) was by about 15% higher than the mean precipitation for a thirty-year period (61.2 mm). Therefore, it may be assumed that the present three-year field experiment was carried out under humidity and temperature conditions typical of the region of Warmia and Mazury, and the obtained results may be considered reliable.

The response of spring triticale to five growth regulators: benzylaminopurine (BAP), α-naphthylacetic acid (NAA), 3-indolebutyric acid

Table 1

Meteorological data provided by the Meteorological Station in Tomaszkowo near Olsztyn

Month	Mean daily temperature (°C)				Precipitation total (mm)			
	years			1970-2000	years			1970-2000
	1999	2000	2001		1999	2000	2001	
April	8.4	4.4	7.2	6.9	99.3	19.6	54.9	36.1
May	10.9	13.2	12.8	12.7	75.8	53.5	33.2	51.9
June	17.2	15.6	13.9	15.9	113.5	34.8	77.9	79.3
July	19.5	15.3	20.0	17.7	44.3	98.7	148.6	73.8
August	16.9	17.1	18.1	17.2	73.4	110.8	53.0	67.1
September	14.8	12.7	11.4	12.5	14.0	49.6	110.4	59.0

(IBA), triacontanol (TRIA), and gibberellic acid (GA_3), applied alone or in combination with a 5% aqueous solution of magnesium sulfate ($MgSO_4 \cdot 7H_2O$), was studied.

Experimental design:

Treatments:	Concentration of growth regulators (mg dm^{-3})
1. Control	H_2O
2. IBA	20
3. IBA + Mg	20
4. TRIA	0.3
5. TRIA + Mg	0.3
6. BAP	40
7. BAP + Mg	40
8. NAA	50
9. NAA + Mg	50
10. GA_3	40
11. GA_3 + Mg	40

The growth regulators and their mixtures with magnesium sulfate were applied to leaves twice during the growing season of spring triticale – at the ear formation stage and before flowering. The experimental plants were sprayed with growth regulator solutions until visibly wet, using 300 dm^3 liquid per hectare. The control plants were sprayed with distilled water.

The effects of growth regulators and their combinations with magnesium sulfate were studied in relation to a constant level of NPK fertilization: nitrogen – $100_{(40 + 60)}$ kg N per ha (ammonium salpeter), phosphorus – 20 kg P per ha (triple superphosphate), potassium – 74.7 kg K per ha (57% potash salt). Phosphorus and potassium were applied before sowing, whereas nitrogen was applied at two rates, before sowing and as top-dressing. The remaining agricultural practices were carried out in accordance with spring triticale requirements.

Triticale plants, 20 of each plot, were collected at the full maturity stage. They were divided into organs and weighed, and biometric measurements were taken. Averaged samples were ground and mineralized wet in concentrated sulfuric acid, with hydrogen peroxide as an oxidizer. Total nitrogen was determined by the Kjeldahl method, and magnesium by atomic absorption spectrometry (AAS).

Results were verified statistically by a two-factorial analysis of variance in a randomized block design. Experimental factor *a* was the type of growth regulator, and experimental factor *b* was a combination of a growth regulator with magnesium sulfate. The least significant difference was determined at

a significance level $p = 0.05$. Means of three years provided the basis for discussing the results.

Results and Discussion

The effects of five growth regulators and their mixtures with magnesium sulfate on the nitrogen content of the aboveground parts of spring triticale plants are presented in Table 2. Growth regulators applied alone reduced nitrogen concentration in triticale culms, compared to the control treatment. The highest (approx. 17%) decrease in nitrogen content was observed as a result of spraying with gibberellic acid (GA_3). Magnesium sulfate added to growth regulators enhanced the decrease, which was confirmed by a statistical analysis in the GA_3+Mg treatment. A decrease in the nitrogen content of flag leaves was also recorded following the application of growth regulators and their mixtures, except for gibberellic acid combined with magnesium sulfate and α-naphthylacetic acid (NAA) applied alone. The other leaves responded differently to the tested growth regulators. Spraying with triacontanol (TRIA) and TRIA+Mg as well as with gibberellic acid (GA_3) and GA_3+Mg caused an increase (by approx. 9%) in nitrogen concentration, compared to the control treatment. The application of α-naphthylacetic acid (NAA) and benzylaminopurine (BAP) with magnesium sulfate resulted in a significant decrease in the nitrogen content of leaves, compared to treatments with IBA, TRIA and GA_3. NOWAK and CZAPLA (1996) demonstrated that BAP affected an increase in the nitrogen content of buckwheat leaves, most probably in consequence of nitrogen metabolism activation. In this experiment the analyzed growth regulators and their mixtures with magnesium sulfate (except for BAP and BAP+Mg) increased nitrogen concentration in triticale glumes, compared to the control treatment. Grain harvested from plots sprayed with α-naphthylacetic acid was most abundant with nitrogen – it contained by 7% nitrogen more than grain harvested from the control plots. CZAPLA et al. (2000) and ZAHIR et al. (2001) also observed an increase in nitrogen levels in triticale grain and rice seeds, respectively, under the influence of growth regulators. WIERZBOWSKA and NOWAK (1999) reported that kinetin and auxin increased the nitrogen content of wheat grain by about 4%, in comparison with the control treatment. In our study growth regulators applied with magnesium sulfate contributed to a decrease in the total nitrogen content of triticale grain, except for the IBA+Mg combination. CZAPLA and NOGALSKA (2000a) found that triacontanol (TRIA) combined with magnesium sulfate reduced nitrogen concentration in oat grain.

Table 2

Nitrogen content in above-grove triticale organs

Combination	Content in g per kg DM				
	culm	leaves		glumes	grain
		flag	other		
Control	5.93	10.27	10.03	7.84	17.73
IBA	5.23	9.80	10.50	8.35	17.87
IBA+Mg	5.09	8.77	10.22	8.82	18.06
TRIA	5.65	10.13	11.08	8.73	17.92
TRIA+Mg	5.04	9.99	10.73	8.91	17.44
BAP	5.74	9.38	9.71	7.70	17.89
BAP+Mg	5.13	10.13	9.15	7.56	17.67
NAA	5.18	10.83	9.05	9.24	18.99
NAA+Mg	5.13	8.96	10.31	8.35	17.73
GA_3	4.92	9.94	10.87	8.31	18.19
GA_3+Mg	3.45	10.92	10.97	8.40	17.45
Mean					
Growth regulators	5.34	10.02	10.24	8.47	18.17
Mixtures	4.76	9.75	10.28	8.41	17.67
NIR 0.05 – LSD 0.05 a	r.n.	r.n.	1.24	r.n.	r.n.
b	0.64	r.n.	r.n.	r.n.	r.n.
$a \times b$	r.n.	r.n.	r.n.	r.n.	r.n.

Legend: a – growth regulator, b – mixture growth regulators with magnesium sulphate, $a \times b$ – interaction

After the application of growth regulators, especially in combination with magnesium sulfate, nitrogen content usually increased in grain and glumes, but decreased in culms (Figure 1). Nitrogen levels in triticale grain increased following the application of growth regulators applied with magnesium sulfate, with the exception of the TRIA+Mg combination. Studies on barley also showed that the nitrogen content of grain increased after spraying with growth regulators and their combinations with magnesium sulfate (NOGALSKA and CZAPLA 2002). NOWAK and WIERZBOWSKA (1991) achieved a 5% increase in nitrogen concentration in wheat kernels sprayed with cytokinins and auxins, while the nitrogen content of flag leaves decreased by over 50%. Changes in the nitrogen content of flag leaves and the remaining triticale leaves, noted in the present experiment, were ambiguous.

The magnesium content of spring triticale plants was also studied in this experiment (Table 3). All tested growth regulators (except for BAP) and their mixtures with magnesium sulfate caused a decrease in magnesium concentration in culms, compared to the control treatment. Following the application of growth-promoting substances, with the exception of 3-indolebutyric acid, the magnesium content of flag leaves increased on average by 15%.

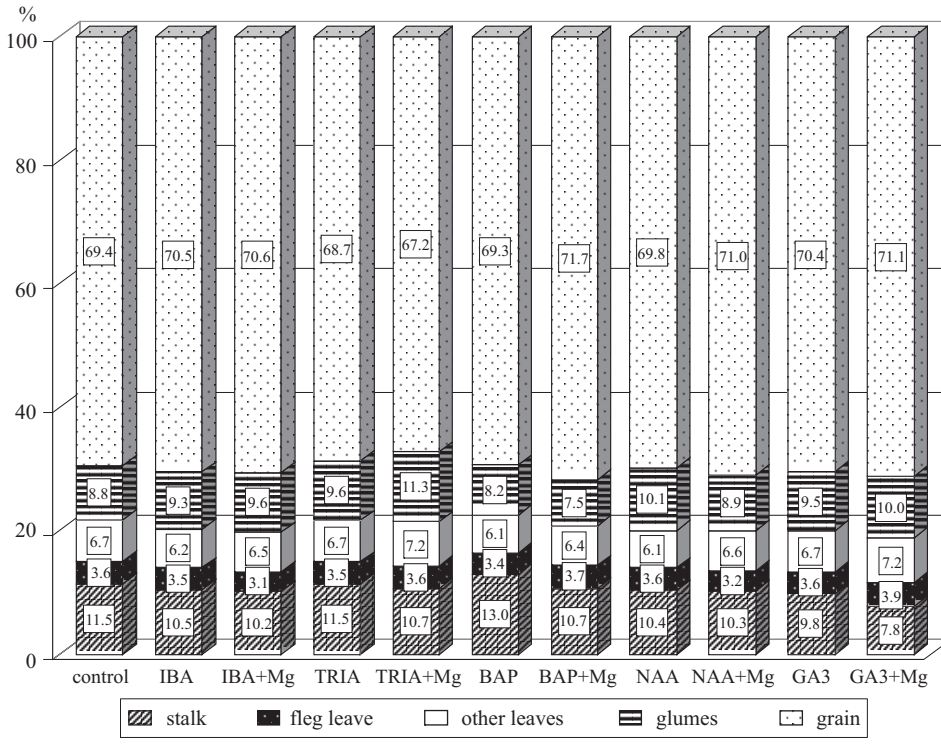

Fig. 1. Nitrogen perticipation in spring triticale organs (1999-2001)

The addition of magnesium to growth regulators affected a significant increase in magnesium levels, on average by 17% in flag leaves, compared to plots sprayed with pure growth regulators. The remaining leaves of spring triticale generally had a lower magnesium content than the control plants. The addition of magnesium sulfate to growth regulators caused an increase in the magnesium content of leaves, on average by 7.3%, compared to treatments without magnesium sulfate. CZAPLA and NOGALSKA (1999) demonstrated that benzylaminopurine combined with magnesium sulfate caused an over fivefold increase in the magnesium content of leaves. In our study the magnesium content of glumes was at a level comparable to that observed in the control plots. Triticale grain harvested from plots treated with IBA and NAA contained less magnesium than grain harvested from the control plots. Spraying with IBA caused a significant decrease in magnesium concentration, while IBA combined with magnesium sulfate enabled to increase the magnesium content of grain, which indicates an interaction of IBA with magnesium sulfate. According to NOGALSKA and CZAPLA (2001), 3-indolebutyric acid applied with magnesium sulfate increased magnesium concentration in buckwheat seeds.

An opposite trend was observed in this study, since the BAP+Mg interaction reduced the magnesium content of grain by 5.5%, compared with BAP applied alone. A positive effect of the interaction between growth regulators and magnesium sulfate was reported by CZAPLA and NOGALSKA (2000b). In the experiment conducted by these authors the NAA+Mg combination caused an increase in the magnesium content of spring barley grain.

Table 3

Magnesium content in above-grove triticale organs

Combination	Content in g per kg DM				
	culm	leaves		glumes	grain
		flag	other		
Control	0.27	0.34	0.60	0.61	1.08
IBA	0.20	0.30	0.54	0.55	1.01
IBA+Mg	0.22	0.37	0.56	0.59	1.06
TRIA	0.23	0.36	0.57	0.63	1.10
TRIA+Mg	0.22	0.45	0.62	0.60	1.07
BAP	0.27	0.36	0.56	0.65	1.10
BAP+Mg	0.25	0.42	0.63	0.64	1.04
NAA	0.22	0.39	0.55	0.53	1.06
NAA+Mg	0.22	0.43	0.61	0.57	1.07
GA_3	0.23	0.33	0.51	0.63	1.10
GA_3+Mg	0.20	0.39	0.54	0.64	1.07
Mean					
Growth regulators	0.23	0.35	0.55	0.60	1.08
Mixtures	0.22	0.41	0.59	0.61	1.06
NIR 0.05 – LSD 0.05 a	r.n.	r.n.	r.n.	r.n.	0.03
b	0.64	0.04	r.n.	r.n.	r.n.
$a \times b$	r.n.	r.n.	r.n.	r.n.	0.05

Explanations as in Table 2

In most cases, growth regulators applied alone had a positive effect on magnesium concentration in triticale grain, as compared to the control treatment (Figure 2). An increase in the magnesium content of grain as a result of the application of a growth regulator combined with magnesium sulfate was recorded in the NAA+Mg treatment. Triticale glumes collected in these plots contained the lowest amount of magnesium. In the majority of treatments triticale glumes and culms responded by a decrease in magnesium levels to the combination of growth regulators with magnesium sulfate. A reverse effect was noted in the case of flag leaves and the remaining leaves.

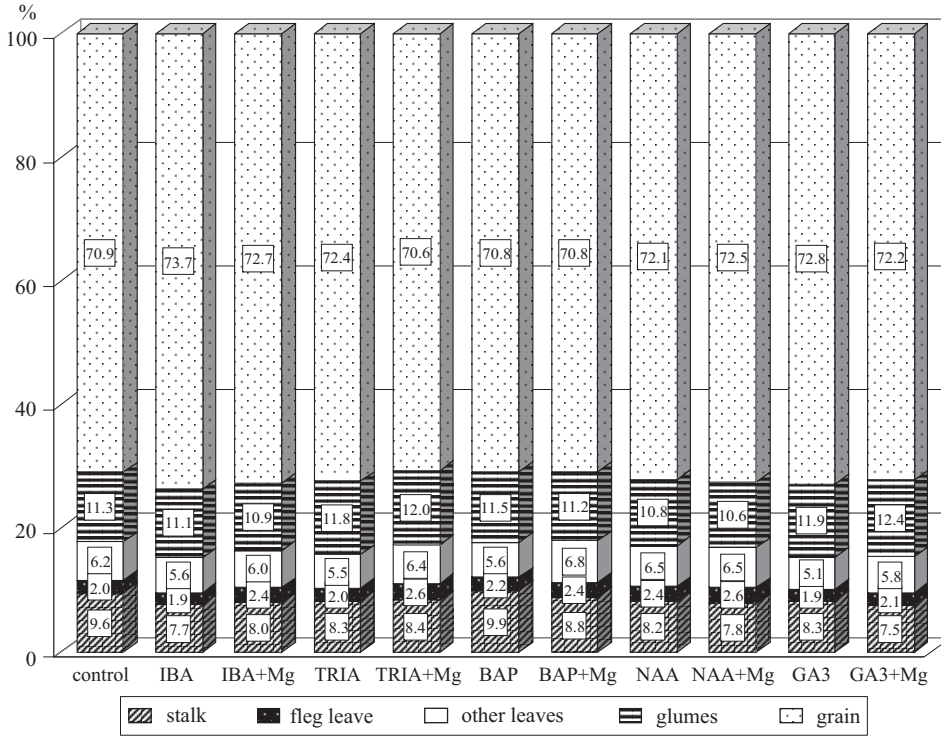

Fig. 2. Magnesium perticipation in spring triticale organs (1999-2001)

Conclusions

1. Gibberellic acid combined with magnesium sulfate significantly decreased nitrogen concentration in culms. α-naphthylacetic acid and benzylaminopurine applied with magnesium sulfate significantly decreased the nitrogen content of leaves, compared to treatments with IBA, TRIA and GA_3. Growth regulators applied alone generally increased, whereas the addition of magnesium sulfate decreased nitrogen concentration in spring triticale grain.

2. After the application of growth regulators, especially in combination with magnesium sulfate, nitrogen content increased in grain and glumes, but decreased in culms.

3. Growth regulators combined with magnesium sulfate caused a significant increase in magnesium concentration in flag leaves. Triticale grain contained significantly less magnesium following the application of IBA, and the addition of magnesium sulfate to this growth regulator caused

an increase in magnesium levels. A reverse effect was recorded for the BAP+Mg combination.

4. Growth promoters usually contributed to an increase in magnesium levels in triticale grain, while their mixtures with magnesium sulfate caused an opposite response.

Translated by ALEKSANDRA POPRAWSKA

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SOIL NOXIOUS MACROFAUNA SHAPED BY LONG TERM PROPER CROP ROTATION

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K e y w o r d s: Proper crop rotation, crop shift, soil macrofauna, *Elateridae*, *Tipulidae*, *Agrotinae*, *Scarabaeidae*, polyphagous insects.

A b s t r a c t

In the recent years, polyphagous pests which belong to soil macrofauna have clearly demonstrated their adverse influence. Possible reasons include simplification of agronomic practices applied in the cultivation of most crops and large areas of fallow land which have appeared in agrocenoses. Our study, based on a permanent, static field experiment, has proved that a proper crop rotation system carried out over a number of years can largely contribute to the reduction of noxious species which inhabit the soil environment. The contribution of those species to the total soil macrofauna was 16% and likewise their density was low. Proper crop rotation has eliminated risk caused by larvae of *Elateridae*, *Tipulidae*, *Agrotinae*, and *Scarabaeidae*.

SZKODLIWA MAKROFAUNA GLEBY UKSZTAŁTOWANA WIELOLETNIM WŁAŚCIWYM ZMIANOWANIEM

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S ł o w a k l u c z o w e: właściwe zmianowanie, płodozmian, makrofauna glebowa, *Elateridae, Tipulidae, Agrotinae*, *Scarabaeidae,* polifagi.

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Abstrakt

W ostatnich latach uwidoczniły się w agrocenozach zagrożenia ze strony szkodników wielożernych należących do makrofauny glebowej. Przyczyną tego są uproszczenia agrotechniczne, stosowane w uprawie większości roślin oraz pojawienie się odłogów. Badania przeprowadzone w oparciu o trwałe statyczne doświadczenie polowe wykazały, że właściwe zmianowanie roślin uprawnych, stosowane przez szereg lat, przyczynia się w znacznym stopniu do redukcji gatunków szkodliwych zasiedlających środowisko glebowe. Udział tych gatunków w strukturze makrofauny wynosił 16%, niskie było również ich zagęszczenie. Właściwe zmianowanie roślin uprawnych, stosowane przez wiele lat, minimalizuje zagrożenia powodowane przez larwy *Elateridae, Tipulidae, Agrotinae* i *Scarabaeidae.*

Introduction

Soil use has essential influence on its biological life. In the past ten years, threats caused by noxious macrofauna, and especially polyphagous insects represented by *Elateridae*, *Agrotinae*, *Scarabaeidae* and *Tipulidae*, to agrocenoses have become drastically evident (MRÓWCZYŃSKI, SOBKOWIAK 1999, WALCZAK, JAKUBOWSKA 2001, KOWALSKA, WIERZBOWSKI 2002, SĄDEJ et al. 2003, TREPASHKO et al. 2006). Species representing the two former orders are particularly dangerous due to their abundant occurrence and the fact that one generation of their larvae feed on plants for several seasons.

The main reasons are the simplification of agronomic practices used for the cultivation of most crops, prevalence of cereal crops, monocultures and appearance of fallow lands in agrocenoses.

Considering the above, a study has been completed on noxious macrofauna in grey-brown podzolic soil which had been cultivated under proper crop rotation consisting of traditional shift of crops. The aim was to establish the structure of the pest insect assemblages, its abundance, species composition and potential threat.

Material and Methods

The research on macrofauna was carried out at the Chair of Phytopathology and Entomology of the University of Warmia and Mazury in 2002-2004. It was based on a long-term static field experiment established by the Chair of Environmental Chemistry of the UWM in Olsztyn. The experiment was set up on grey-brown podzolic soil at the Experimental Station in Bałcyny in 1972. In compliance with Good Agricultural Practice the experiment consisted of a 7-year rotation system which comprised potato, spring barley, winter oilseed rape, winter wheat + winter rye aftercrop, winter aftercrop + corn, spring

barley, winter wheat. In 2002 the fifth crop rotation series began. Plant protection treatments were restricted to the bare minimum.

The study covered the whole area (0.26 ha). Assessment of the macrofauna was performed on the material obtained from grey-brown pozdolic soil collected with cylinders 95 mm in diameter and 160 mm in height. Samples were taken six times in each season. On each occasion, 24 samples were collected along the diagonal of the object. The selection of the equipment depended on the varied row spacing patterns for each of the crops and the specific character of a strict experiment. Zooedaphone was obtained using sieves and Tullgren apparatuses (GÓRNY, GRÜM 1981).

Results and Discussion

The structure of the analysed group of insects was as follows: 16% of noxious macrofauna and 84% of useful macrofauna including 46% of earthworms (Figure 1). Earthworms were shown in the diagram as a separate group because they belong to the type *Annelida*, class *Oligochaeta* and order *Lumbricoidea*. Thus, they are a distant systematic group and their positive role in soil environment is different from that played by useful entomofauna.

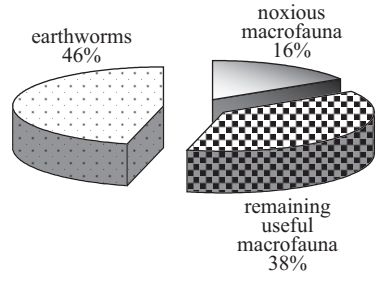

Fig. 1. Structure of the macrofauna in grey-brown podzolic soil

The numerical ratios between the above groups are certainly a product of the crop rotation system carried out in the experiment for 34 years. Apart from creating optimum conditions for the growth and development of crops, another important aim of proper crop shift is the reduction of the threat brought about by agrophagous animals.

In total, 148 specimens representing noxious macrofauna were acquired, including imago forms (25.1%), larvae (69.9%) and pupas (5%). The specimens belonged to three orders of insects: *Coleoptera* beetles, *Lepidoptera* butterflies and *Diptera* dipterans (Table 1).

Table 1

Species composition and number of noxious macrofauna

Group, order	Family	Species	Number	%
Insecta Coleoptera	*Elateridae*	*Agriotes obscurus* L.	4* 31**	3.0 21.0
		Selatosomus aeneus L.	1* 5**	0.8 3.5
		Athous niger L.	2* 4**	1.6 2.5
	Scarabaeidae	*Melolontha melolontha* L.	4* 8**	2.5 5.0
		Amphimallon solstitialis L.	1* 2**	0.8 1.6
		Phyllopertha horticola L	0* 1**	0.0 0.8
	Tenebrionidae	*Pedinus* Latr.	0* 6**	0.0 4.0
	Chrysomelidae	*Gohyoctera* sp.	1* 4**	0.8 2.5
	Silphidae	*Silpha triscis* L.	1* 3**	0.8 2.0
Lepidoptera	*Agrotinae*	*Agrotis segetum* Schiff.	0* 5**	0.8 3.0
Diptera	*Tipulidae*	*Tipula scripta* Meig.	0* 11** 5***	0.0 7.5 3.0
	Bibionidae	*Bibio pomonae* F.	0* 17** 3***	0.0 11.6 2.0
Different			22* 7**	14.0 4.9
Total			148	100

* imago **larva ***pupa

The beetles collected from the soil samples belonged to five families: click beetles *Elateridae*, which made up over 32% of this group, with the dominant species dark elaterid beetle *Agriotes obscurus* L. and sporadically present *Selatosomus aeneus* L. and *Athous niger* L., scarabids *Scarabidaeidae* (ca 11%), which were dominated by May beetle *Melolontha melolontha* L. and sporadically present European June beetle *Amphimallon solstitialis* L. and garden chafer *Phyllopertha horticola* L.; darkling beetles *Tenebrionidae* (4%); leaf beetles *Chrysomelidae* (ca 3) and carrion beetles *Silphidae* (2%). The latter were represented by single species. *Lepidoptera* butterflies were present as caterpillars of turnip moth *Agrotis segetum* Schiff., of the family *Agrotinae*,

and they made up ca 4% of the whole group. Dipterians obtained from the soil samples were mainly larvae; pupas were found only sporadically. They belonged to two families: crane flies *Tipulidae* with the species *Tipula scripta* Meig. (ca 11%) and march flies *Bibionidae* with the species *Bibio pomonae* F. (ca 14%). Among the other insects collected from the soil samples, adult specimens prevailed (14%) whereas larvae were in minority (4.9%).

The density of noxious macrofauna varied over the years (Figure 2). In 2002 the species which potentially threatened the crops occurred in highest numbers – on average there were 5.1 specimens per m^{-2}. In the following year their number evidently declined (2.9 specimens per m^{-2}). In 2004 the average number of noxious insects was 4.2 individuals per m^{-2}. The former of the three figures given was derived from the most extensive set of means.

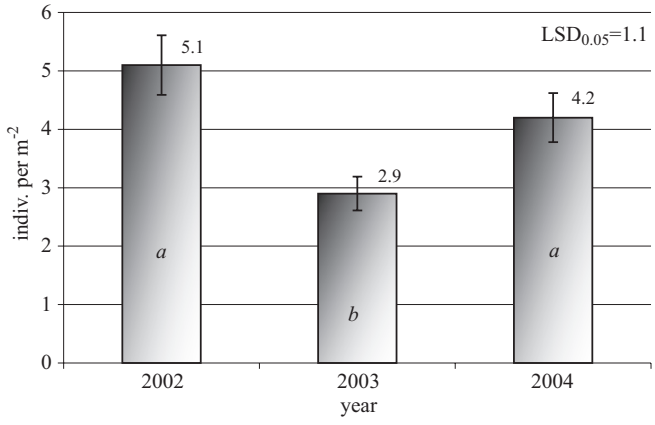

Fig. 2. The average number of noxious macrofauna (indiv. per m^{-2})

The statistical analysis showed a significant difference in the numbers of noxious macrofauna between the second year under study and the other time periods. Between the first and the third year no such differences were observed. Variation in the abundance of noxious macrofauna, and in particular its much lower number in 2003, was obviously a result of relatively intensive agronomic treatments which were performed while growing potatoes. Such treatments are part of the agronomic plant protection method, which aims at reducing abundance of agrophagous insects. It is generally known that bulb and root plants are an excellent forecrop, leaving a good field for subsequent crops. As regards turnip moths of the family *Agrotinae* as well as scarabids of the family *Scarabaeidae*, their numbers varied also because of the cyclic nature of their occurrence (WALCZAK, JAKUBOWSKA 2001).

According to Pruszczyński (2004), the noxiousness threshold for *Elateridae* is 11 larvae/m^{-2}, for *Tipulidae* it is 10 larvae/m^{-2}, for *Agrotinae* it is 6 larvae/$^{-2}$ and for *Scarabaeidae* from 3 to 6 grubs per m^{-2}. In contrast, our study showed that at the highest density of noxious macrofauna, the total number of those insects was 5.1 specimens/m^{-2}. The data included in table 1 proves that less than 70% of the whole macrofauna consisted of larval forms of noxious species, with Elateridae larvae being dominant.

The relatively low density of phytophagous species determined at the test object was certainly resulting from the proper rotation farming practice.

The evident dominance of useful entomofauna, which consisted mainly of the zoophages antagonistic to phytophagous insects is an additional factor which guarantees that the latter will be less dangerous to crops.

Conclusions

1. The macrofauna which can be potentially noxious to crops grown on grey-brown podzolic soil consists mainly of click beetles *Elateridae* with the dominant species dark elaterid beetle *Agriotes obscurus* L., March flies *Bibionidae* with the dominant species *Bibio pomonae* F., scarabids *Scarabaeidae* with European June beetle *Amphimallon solstitialis* L. and crane flies *Tipulidae*, which were dominated by the species *Tipula scripta* Meig.

2. Relatively small contribution of noxious species to the total macrofauna present in the analysed soil environment as well as its low densities in the subsequent seasons examined during the three-year tests enable us to conclude that proper crop rotation carried out over many years eliminated threats caused by noxious insects.

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COMPARISON OF ENERGETICAL AND ECONOMICAL EFFECTIVENESS OF DIFFERENT TECHNOLOGIES OF PEA PRODUCTION

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K e y w o r d s: pea, production technology, energy consumption, varible costs.

A b s t r a c t

The purpose of the study was to determine and comparison the energy and economic efficiency of three technologies pea production differing first of all nitrogen fertilization and plant protection. The result of a three-year field experiment carried out in 2001-2003 were presented in the paper. The experiment was conducted in Experimental Station at Bałcyny on soil of good wheat complex. It was shown that medium-input technology was characterized the last energy consumption per unit and the highest index of energy efficiency. In the high-input technology energy consumption per unit was 24% higher compared with the medium-input technology. The most favoruable value of direct surplus was achived also in the medium-input technology. It was noted that the direct surplus quotient per 1 pln variable costs was slightly lower than achived in the low-input technology.

PORÓWNANIE ENERGETYCZNEJ I EKONOMICZNEJ EFEKTYWNOŚCI RÓŻNYCH TECHNOLOGII PRODUKCJI GROCHU SIEWNEGO

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Abstrakt

Celem badań było porównanie energetycznej i ekonomicznej efektywności produkcji grochu siewnego, uprawianego w trzech technologiach (niskonakładowej – *A*, średnionakładowej – *B*, wysokonakładowej – *C*) różniących się przede wszystkim poziomem nawożenia azotem i ochroną roślin. Doświadczenie polowe przeprowadzono w latach 2001-2003 na glebie kompleksu pszennego dobrego w Zakładzie Produkcyjno-Doświadczalnym „Bałcyny". Najniższą jednostkową energochłonnością produkcji odznaczała się technologia średnionakładowa, w której uzyskano również najkorzystniejszy wskaźnik efektywności energetycznej. Technologia ta charakteryzowała się także najwyższą opłacalnością – otrzymano w niej największą nadwyżkę bezpośrednią z 1 ha. W technologii wysokonakładowej energochłonność jednostkowa produkcji grochu była większa o 24% niż w technologii średnionakładowej. Nadwyżka bezpośrednia na 1 zł kosztów zmiennych w technologiach *A* i *B* była zbliżona.

Introduction

The percentage of energy used for production purposes in the agricultural sector in Poland is only around 5%, the increase in energy use in this sector will be at least in proportion to the increase of the energy consumption of the whole economy even if this relation was maintained at a relatively constant level (ZALEWSKI 2000, NIKODEM 2003). It is noteworthy that technological progress, expressed first of all by an increase in the use of material means of production, is the source of food overproduction and, at the same time, the factor of the growth of its energy consumption. This fact provokes to ask a question about the purposefulness of using more intense production technologies both in the context of economical use of energy and the efficiency of its processing (WOŚ 1995). The latter relation in the case of plants grown for food purposes is important due to the fact that food also constitutes one of the elements of energy "input" in the energy cumulated account.

The aim of this investigation was to determine the level and structure of energy input and an energetical and economical evaluation of the technology production of pea seeds, varied in terms of the level of nitrogen fertilization and the degree and method of plant protection.

Methods

The experiment was carried out at the Production and Experimental Establishment at Bałcyny near Ostróda. Energy consumption of three technologies of cultivation of four pea cultivars has been evaluated in a three-year experiment carried out in the years 2001-2003. Methods of establish and detailed experience conditions and yields were presented in earlier paper (SZWEJKOWSKA 2004).

The variation of cultivations of sowing pea was established along a three--degree scale at the low, medium and high input level.

Technology low input – *A* – without nitrogen fertilization, with a mechanical method of weed control (harrowing); seed dressing, weed, pest and disease chemical control and desiccation were not used.

Technology medium input – *B* – included the application of nitrogen 30 kg ha^{-1}, seed dressing (Funaben T in a dose 400 g 100 kg of seeds, mechanical protection against weeds (3-fold harrowing – obliquely in relation to rows – and chemical protection against pests (Decis 2.5 EC in a dose 0.3 l ha^{-1}); chemical protection against weeds and diseases or desiccation were not used.

Technology high input – *C* – included the application of nitrogen 60 kg ha^{-1}, seed dressing, additional leaf feeding, desiccation, chemical protection against diseases, pests and weeds control. Againts weeds (Bladex 50 WP + Basagran 480 SL in a dose 2.0 kg ha^{-1} + 1.25 l ha^{-1}) were used, Funaben T dressing in a dose 400 g 100 kg of seeds, Bravo 500 SC in a dose 1.5 l ha^{-1} and Dithane 75 WG in a dose 2 kg ha^{-1}) were used against diseases, Cyperkill Super 25 EC in a dose 0.1 l ha^{-1}, Decis 2.5 EC in a dose 0.3 l ha^{-1} – against pests, and desiccation (Reglone Turbo 200 SL in a dose 2.0 l ha^{-1}).

Three variants of pea production technology were subject to energy evaluation. The evaluation of cultivation effectiveness was carried out on the basis of mean yields four cultivars in the years under study.

Four energy streams were distinguished in the account of input energy effectiveness: labour force, energy carriers (diesel oil), materials (mineral fertilizers, sowing material, herbicides), machines and tools (WIELICKI 1989). Means of production, labour input and tractive force expenditures incurred for the soil cultivation, sowing, protection measures and harvesting were converted to MJ, using appropriate energy consumption indices applied in the energy account of plant production (ANUSZEWSKI 1987, WÓJCICKI 1983). The following energy-consumption indices were adopted: human labour – 40 MJ rbh^{-1}, energy carriers – 48 MJ kg^{-1}, nitrogen fertilizers (N) – 77 MJ kg^{-1}, phosphatic fertilizers (P_2O_5) – 15 MJ kg^{-1}, potassic fertilizers (K_2O) – 10 MJ kg^{-1}, sowing material – 24 MJ kg^{-1}, herbicides – 300 MJ kg^{-1} of active substance. The amount of the energy input was determined on the basis of the author's direct measurements according to cultivation variants applied in production fields with the use of machinery and equipment typical of agricultural production: U1224+U043/1 (first ploughing), U1224+Tur 120B (deep ploughing), U4514+U212/2 (harrowing light harrow), U1224+U216 (harrowing heavy harrow), U4514+N-035 RNW-3 (fertilization), U4514+S043/3C (sowing), U4514+Pilmet 815 (chemical plant protection, foliar fertilization and desycation), Deutz Fahr 4085 HTS (seed harvest). The energy effectiveness index, resulting from the proportion between the energy value of pea

seeds and the expenditures incurred for its production, was the effectiveness meter. The cumulated energy profit and unit energy consumption index were also used for the energy evaluation (ZAREMBA 1986, WIELICKI 1989, KLEPACKI 1990).

The economic assessment was done according to the so-called direct surplus methodology as a category resulting from the difference between the yield value and the variable (material) costs incurred for production of that yield. Variable costs included real consumption of material sowing, mineral fertilization, chemical means of plant protection and energy carriers (*Produkcja i rynek zbóż.* 2002). Calculated also direct surplus quotient per 1 zł variable costs. Costs of industraial means of production were estimated according to the market prices during the 4th year quarter of 2006. The paper presents results for mean yield of examined technologies.

Results

Energy efficiency of pea production technologies

The level of energy input for the studied technologies ranged from 9832 MJ ha^{-1} in the low input variant of technology production to 18 329 MJ ha^{-1} in the high input variant (Table 1). The structure of energy input per particular agrotechny element was varied. The highest position in the structure of energy input in the low and medium input technology was occupied by sowing and sowing material – 62.8% and 49.5% of total energy input, respectively. In the high input technology, the relative share of energy used for sowing and sowing was lower and amounted to 34.8%. In this technology, the highest relative energy expenditure was included in mineral

Table 1

Input of energy for pea production (MJ ha^{-1}), according to agrotechnical measures

Agrotechnical measures	Technology variants		
	A	*B*	*C*
Soil cultivation	1566	1566	1566
Sowing and sowing material	6178	6373	6373
Mineral fertilization	1388	3698	7456
Weed control	227	642	994
Chemical diseases control	0	0	259
Chemical pests control	0	129	929
Harvesting	474	474	751
Total	9832	12 882	18 329

fertilization (40.7%), its share being significantly higher than in the medium- and low-input technology – 28.7% and 14.1%, respectively.

Energy used for pre-sowing cultivation of soil constituted from 8.2 to 15.9% of total energy input for the whole agrotechny. In the intensive technology, chemical protection of pea (measures limiting the occurrence of weeds, diseases and pests) took 11.9%, and in the medium-input technology – 6.0% of the input structure. On the other hand, this share decreased to 2.3% in the low input technology with pea protection only against weeds. Energy input incurred for harvest in the low and medium input variants remained at a similar level and amounted to a 4.8% and 3.7% share in the input structure.

Considering the structure of cumulated energy input according to the streams of energy, the highest position is occupied by materials (with a share of 74.2% in the lowest variant through 78.4% in the medium-input variant up to 80.6% in the high-input technology (Figure 1). Such a high percentage of the share of this stream was determined mostly by expenditures incurred for mineral fertilizers and sowing material. The second position in terms of the amount of input was occupied by energy carriers. The percentage of this energy stream ranged from 13.4% to 18.8%.

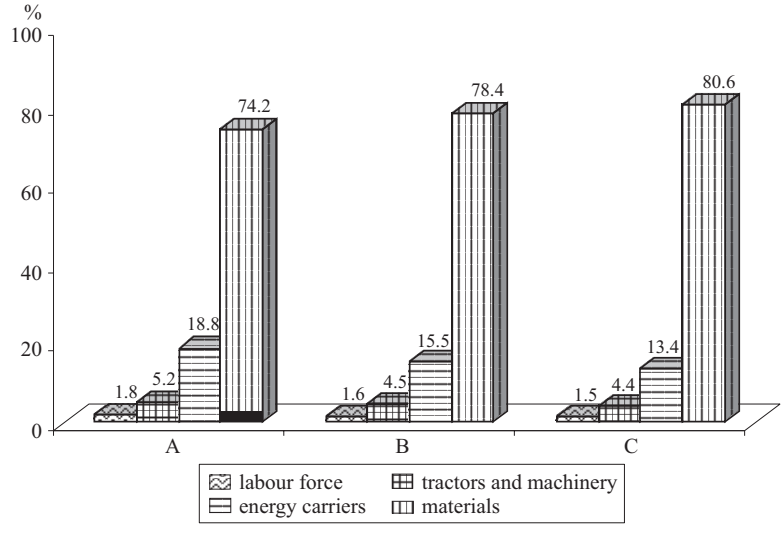

Fig. 1. The structure of energy input for pea production differing by variant technology

The highest cumulated energy profit was in the high input technology and amounted to 101.1% of energy profit in the medium input technology and as much as 147.2% energy obtained in the low input technology (Table 2).

The energy consumption of the production of 1 dt of pea seeds was the highest in technology *C*. A decrease in energy input for pea production made it possible to produce 1 dt of seeds with energy input which was 17.6% lower in technology *A*. The medium input technology was the most energy-saving one.

Table 2
Selected elements of energy evaluation of pea production differentiated by technology intensity

Description	Technology variants		
	A	*B*	*C*
Energy outlay (MJ ha^{-1})	9832	12 882	18 329
Energetic value of yield (MJ ha^{-1})	60 000	85 200	92 160
Gain of cumulative energy (MJ ha^{-1})	50 167	72 318	73 831
Energy consumption per unit (MJ dt^{-1})	393.3	362.9	477.3
Index of energy efficiency	6.10	6.61	5.03

The most favourable energy effectiveness index was in the medium-input technology. The increase in the yield energy value in the high input technology did not compensate the increased input (particularly for mineral fertilization) incurred for pea seed production. A more favourable energy effectiveness index (resulting from lower energy input) was also obtained in the low input technology of pea seed production in comparison with the high input technology.

Economic efficiency of pea production technologies

The variable costs of pea cultivation varied from 647.3 PLN ha^{-1} (*A* variant) to 1 678.6 PLN ha^{-1} (*C* variant) – Table 3. The largest part of variable costs in the low input technology (*A*) sowing material took place, next energy carriers and mineral fertilizers. In the middle input technology (*B*) the sowing material constituted nearly 29% of the variable costs, the cheapest were pesticides. In the high input technology (*C*) NPK fertilizers incurred the highest costs (28.3%). Very expensive were also herbicides, sowing material and petrol, respectively 17.7, 16.4 and 16.0%. Significant part of variable costs dessication took place. The cheapest were pesticides.

The highest direct surplus obtained in *B* technology (Table 4). The value of this distinguishing feature in *A* and *C* technologies was comparable,

Table 3

Variable costs of 1 ha pea production

Description	*A*		*B*		*C*	
	(pln ha^{-1})	(%)	(pln ha^{-1})	(%)	(pln ha^{-1})	(%)
Sowing material	275.0	42.5	275.0	28.6	275.0	16.4
Mineral fertilization (NPK)	170.0	26.3	230.0	24.0	475.0	28.3
Weed control	0.0	0.0	214.0	22.3	296.8	17.7
Chemical diseases control	0.0	0.0	0.0	0.0	132.6	7.9
Chemical pests control	0.0	0.0	22.4	2.3	34.9	2.1
Desiccation	0.0	0.0	0.0	0.0	195.5	11.6
Energy carriers	202.3	31.2	218.9	22.8	268.7	16.0
Total	647.3	100.0	960.3	100.0	1 678.6	100.0

Table 4

Index of economic valuation cultivation technologies of pea

Description	*A*	*B*	*C*
Variable costs (pln per ha)	647.3	960.3	1 678.6
Yield value (pln per ha)	1875.0	2662.5	2880.0
Direct surplus (pln per ha)	1227.7	1702.2	1201.4
Direct surplus quotient per 1 pln variable costs	1.9	1.8	0.7

although in high input technology yield value was over 53% higher. The direct quotient per 1 zl variable costs in *A* and *B* technology indicates very nearing profitability compared technologies.

Discussion

In agricultural economics, energy evaluation of production processes is mostly determined by means of the cumulated calculation method. KOZIC and WIELICKI (1996), KSIĘŻAK et al (1998) claim that due to the essential value of the research, the calculation method based on an analysis of the production process (technical method), used in this investigation, is particularly useful. SZEMPLIŃSKI (2003), while carrying out research on the energy effectiveness of various methods of the production of spring oat fodder seed, proved that the level of mineral fertilization differentiated the structure of energy input and, most importantly, it was found out that technological variants with lower doses of mineral fertilization ensured a higher energy effectiveness of the crop.

On the other hand, JANKOWSKI and BUDZYŃSKI (2003), BUDZYŃSKI et al. (2004) found out that relinquishment of protection against pests increased unit energy consumption of rapeseed production by an average of 36%, and reduced the energy effectiveness index by around 20-25%. An increase in the cumulated energy input for fertilization of protected and unprotected rape was – according to cited authors – compensated by an increase in the crop energy. In the case of this investigation, the latter thesis was only partially confirmed with reference to technology treated as a whole. The most favourable energy effectiveness index was obtained in the case of the medium-input technology with the use of mineral fertilization but without chemical control of weeds and diseases.

Analyzing the findings both of this investigation and research on the energy effectiveness of pea crop carried out by other authors, it should be emphasized that they are burdened with an element of relativity resulting from the fact that although the yield abilities of pea (denominator of the energy effectiveness calculation) are very high, its cropping is often low and instable (BOCHNIARZ 1988, KSIĘŻAK et al. 1998). According to KSIĘŻAK et al (1998) the differentiation of sowing pea technologies wasn't significant influence on seed yield level. The high input technology was less effective in comparison with economical technology (medium input). Higher labour outlay and direct costs in high-input technology weren't covered by increasing level of seed yield. The profitability coefficient was the most advantageous in the medium and low input technologies. Similar results obtained KSIĘŻAK et al (1997) comparing different technologies of faba bean. The medium and low input technologies were more effective than high input technology.

An increase in the input for plant production to achieve better productivity is justifiable only when the effect increase surpasses the input increase. This concerns both input and effects expressed as a value, but also as an energetic dimension.

Conclusions

This research on the energy consumption of various pea cultivation technologies had, first of all, a cognitive purpose and as such allowed to formulate the following conclusions:

1. The medium-input technology of pea cultivation turned out to be the least energy-consuming. The most profitable was also medium-input technology of sowing pea production, where obtained the highest direct surplus.

2. The medium-input technology ensured obtaining the most favourable energy effectiveness index. The least favourable index of this effectiveness was

obtained with the use of the high-input technology. This was connected with a significant burden with energy contained in material input.

3. The yield value increment (by 7.5%) caused by using high-input technology did not cover the variable costs increase. The direct surplus in high-input technology was lower by 29.4% than in medium input technology.

The evaluation of production technology based on the criterion of energy consumption and energy effectiveness is mostly of cognitive importance now. Due to limited sources of energy it may become at least equal to the criterion of economic effectiveness in the near future.

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THE EFFECT OF NICKEL ON THE AMMONIFICATION PROCESS IN SOIL*

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K e y w o r d s: L-aspartic acid, L-arginine, DL-alanine, nickel, soil.

A b s t r a c t

The effect of soil contamination with nickel on the ammonification process was determined in a laboratory experiment. The experiment involved samples of soil collected from the humus arable horizon. Under natural conditions, it was proper brown soil of granulometric composition of heavy loamy sand and proper brown soil of granulometric composition of light silty loam.

The experiment was carried out in three replications. Soil samples were contaminated with nickel in the form of two compounds: $NiCl_2 \cdot 6H_2O$ and $NiSO_4 \cdot 7H_2O$ in the following doses: 0, 100, 200, 300, 400 mg Ni^{2+} kg^{-1} soil. Nitrogen was introduced in the amount of: 0, 250 mg N kg^{-1} soil in the form of L-aspartic acid, L-arginine and DL-alanine. The soil prepared in such a way was incubated for 6, 12, 18, 24, 48 and 72 hours. Afterwards, following thorough mixing, the humidity of soil was brought up to 60% capillary water capacity.

The results of the experiments demonstrated that the effect of nickel on the ammonification process depended on the type of ammonified organic compound, the type of soil, a dose of metal and the type of nickel compound. Contamination of soil with the amount of nickel between 100 and 400 mg Ni^{2+} kg^{-1} had an inhibitory effect on the ammonification process. Nickel chloride was a stronger inhibitor of ammonification than nickel sulfate. The amount of ammonified nitrogen was larger in light silty loam than in heavy loamy sand. Nickel had lower inhibitory effect on this process in a heavier soil type than in a lighter one. Soil contamination with nickel compounds contributed to lowering soil pH.

WPŁYW NIKLU NA PRZEBIEG PROCESU AMONIFIKACJI W GLEBIE

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S ł o w a k l u c z o w e: kwas L-asparaginowy, L-arginina, DL-alanina, nikiel, gleba.

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A b s t r a k t

W doświadczeniu laboratoryjnym określono wpływ zanieczyszczenia gleby (próbki pobrano z poziomu orno-próchnicznego) niklem na przebieg procesu amonifikacji. W stanie naturalnym była to gleba brunatna właściwa o składzie granulometrycznym piasku gliniastego mocnego oraz brunatna właściwa o składzie granulometrycznym gliny lekkiej pylastej.

Doświadczenie wykonano w sześciu powtórzeniach. Próbki glebowe zanieczyszczono niklem w postaci dwóch związków – $NiCl_2 \cdot 6H_2O$ i $NiSO_4 \cdot 7H_2O$ w następujących dawkach: 0, 100, 200, 300, 400 mg Ni^{2+} kg^{-1} gleby. Azot wprowadzono w ilości: 0, 250 mg N na kg^{-1} gleby w postaci kwasu L-asparaginowego, L-argininy oraz DL-alaniny. Tak przygotowaną glebę inkubowano przez 6, 12, 18, 24, 48 i 72 godziny. Następnie po dokładnym wymieszaniu, doprowadzono jej wilgotność do 60% kapilarnej pojemności wodnej.

Wykazano, że wpływ niklu na przebieg procesu amonifikacji zależał od rodzaju amonifikowanego związku organicznego, gatunku gleby, dawki metalu i rodzaju związku niklu. Zanieczyszczenie w ilości od 100 do 400 mg Ni^{2+} kg^{-1} wpłynęło inhibicyjnie na proces amonifikacji. Silniejszym inhibitorem amonifikacji był chlorek niż siarczan niklu. Ilość zamonifikowanego azotu była większa w glinie lekkiej pylastej niż w piasku gliniastym mocnym. Nikiel w mniejszym stopniu wpływał hamująco na ten proces w glebie cięższej niż w lżejszej. Zanieczyszczenie związkami niklu przyczyniło się do obniżenia pH gleby.

Introduction

Heavy metals introduced to the environment are bound in soil and can reduce nitrogen uptake by plants or influence the processes organic compound mineralization (OMAR et al. 1999, ANTIL et al. 2001). Accumulation of heavy metals in soil inhibits humification and the processes of organic substance biodegradation; consequently, the amount of humic acids and chelate compounds with metals is reduced. This causes a decrease in the inactivation of metals in soil environment and increases their effects on the biocoenosis (GORLACH 1995, SŁABA, DŁUGOŃSKI 2002). Additionally, they provide early symptoms of changes taking place in the soil, long before the changes in chemical composition and physical properties of soil occur (DICK 1994, KISS 1999).

Organic nitrogen, to be available for plants, must be mineralized to ammonia and nitrates (BARABASZ 1992). Major factors limiting the intensity of the process include: type of soil, pH, C_{org}: N ratio, contamination with various heavy metals, temperature and soil humidity (DENI, PENNINCKX 1999, PRZYBULEWSKA et al. 2003b, WYSZKOWSKA, KUCHARSKI 2004). Organic substances in the soil create complex chelate-type compounds with metals, of low molecular weight organic substances, such as organic acids, polysaccharides, amino acids and polyphenols (ALLOWAY, AYRES 1999, KARCZEWSKA 2002). Typically, complex compounds of metals with organic acids demonstrate high solubility (KARCZEWSKA 2003). Such metals include nickel, which may be found in a form bound to the organic substance, to a large extent in the form

of mobile chelates. However, quite frequently and particularly in mineral soils, this metal is absorbed by Fe and Mn hydroxides, but it also may remain in easily soluble forms (WĘGLARZY 2001). Nickel, in light sandy soils, is found in much lower amounts than in loamy soils. Increased amounts of this metal were also found in some organic soils, as well as in soils formed from alkaline igneous or volcanic rocks (TERELAK, PIOTROWSKA 1997, WĘGLARZY 2001).

The objective of the conducted research was to determine the effect of soil contamination with nickel on the ammonification process. As results from previous research by WYSZKOWSKA et al. (2006a), this element also inhibits the process of nitrification.

Materials and Methods

The effect of soil contamination with nickel on the ammonification process was determined in an experiment. The study involved samples of two types of soil, collected from the humus arable horizon. Under natural conditions, it was proper brown soil, formed from heavy loamy sands and proper brown soil, formed of light silty loam. Detailed characteristics of these soils are presented in Table 1.

Table 1

Some physicochemical properties of the soil in the experiments

Type of soil	Granulometric composition (mm)			pH_{KCl}	Hh	S	C_{org} (g kg^{-1})
	1-0.1	0.1-0.02	<0.02		(mmol(+) · kg^{-1}soil)		
hls	66	17	17	6.90	11.25	89.30	7.50
lsl	42	32	26	7.00	8.77	159.00	11.15

hls – heavy loamy sand, lsl – light silty loam, Hh – hydrolitic acidity,
S – sum of exchangeable base cations, C_{org} - organic carbon content

The study was carried out in six replications, by placing portions of 50 g of air-dry soil in 100 cm^3 capacity beakers. Soil samples were contaminated with nickel in the form of two compounds: $NiCl_2 \cdot 6H_2O$ and $NiSO_4 \cdot 7H_2O$ using the following doses: 0, 100, 200, 300, 400 in mg Ni^{2+} kg^{-1} soil. Afterwards, nitrogen was added in the form of the following amino acids: L-aspartic acid, L-arginine and DL-alanine, in the amount of: 0, 250 mg N kg^{-1} soil and after thorough mixing, the humidity of soil was brought up to 60% capillary water capacity. The prepared soil was incubated for 6, 12, 18, 24, 48 and 72 hours.

At specific hours, the content of $NH_4^+$and NO_3^- ions, as well as pH, were determined in 1% extract of the water solution of K_2SO_4. Ammonium ions were determined with the use of Nessler's reagent, and nitrated – with phenol disulfonic acid. A detailed procedure of extraction and determination of nitrogen forms has been provided by WYSZKOWSKA et al. (2006b).

The amount of ammonified nitrogen was calculated on the basis of the results obtained (WYSZKOWSKA 2002). They were statistically analysed using Duncan's multiple range test, applying the five factor variance analysis. The statistical analysis was accomplished with Statistica software (StatSoft, Inc. 2003).

Results and Discussion

Nickel caused a reduction in the ammonification rate for the following amino acids: L-aspartic acid, L-arginine and DL-alanine (Table 2). The negative effect of this metal depended on the concentration in which it occurred, the type of ammonified organic compound and the type of soil used in the experiment. The rate of the ammonification process of examined amino acids was varied. As regards heavy loamy sand uncontaminated with nickel, 82.30% of L-arginine was ammonified already at hour 48, as compared with only 66.63% of DL-alanine and 52.08% of L-aspartic acid. A similar tendency was found for light silty loam, in which L-arginine was also ammonified in the largest degree, and L-aspartic acid – to the lowest degree.

Table 2

The effect of nickel on the amount of ammonified nitrogen, in %

Ni dose mg kg^{-1} of soil	Type of nickel compound											
	$NiCl_2 \cdot 6H_2O$						$NiSO_4 \cdot H_2O$					
	Time of soil incubation, in hours											
	6	12	18	24	48	72	6	12	18	24	48	72
1	2	3	4	5	6	7	8	9	10	11	12	13
Heavy loamy sand												
L-aspartic acid												
0	4.94	4.73	14.49	22.96	52.08	59.77	4.94	4.73	14.49	22.96	52.08	59.77
100	2.03	1.57	2.24	12.32	48.24	54.18	5.32	4.61	0.28	11.75	40.45	54.95
200	1.41	1.18	0.43	11.90	16.19	44.43	4.99	4.47	0.50	7.47	3.94	51.57
300	1.38	0.73	0.61	8.77	18.10	43.73	4.47	4.00	0.42	7.16	1.20	47.67
400	0.45	0.41	0.87	6.49	15.46	35.47	3.85	3.81	0.32	4.39	0.19	37.59
r	-0.89	-0.86	-0.75	-0.91	-0.89	-0.98	-0.84	-0.90	-0.71	-0.90	-0.91	-0.97

cont. table 2

1	2	3	4	5	6	7	8	9	10	11	12	13
L-arginine												
0	12.16	41.79	43.15	65.01	82.30	77.45	12.16	41.79	43.15	65.01	82.30	77.45
100	9.60	25.45	30.07	40.10	68.41	75.16	10.70	28.37	26.34	46.90	75.37	75.71
200	7.97	17.48	22.09	26.92	73.29	73.05	10.00	21.52	18.06	25.17	70.16	72.30
300	7.34	14.58	16.05	16.80	72.02	69.55	9.09	17.63	14.94	16.92	62.21	68.96
400	6.84	11.00	11.13	12.49	68.86	66.52	8.33	13.90	10.22	14.48	53.91	64.15
r	-0.95	-0.94	-0.98	-0.96	-0.66	-0.99	-0.99	-0.96	-0.94	-0.96	-0.99	-0.99
DL-alanine												
0	8.02	7.59	7.13	18.77	66.63	72.66	8.02	7.59	7.13	18.77	66.63	72.66
100	7.49	4.63	2.64	9.31	65.59	70.36	8.36	6.19	2.27	7.65	65.20	72.43
200	6.57	3.84	0.43	7.02	42.78	66.09	8.34	5.37	0.65	3.37	43.13	69.04
300	6.01	3.11	0.49	2.17	19.44	64.19	7.85	5.47	0.49	1.88	28.87	57.04
400	5.61	3.25	0.60	1.86	10.54	58.84	7.54	5.26	0.37	1.39	14.11	54.28
r	-0.99	-0.88	-0.84	-0.94	-0.97	-0.99	-0.68	-0.87	-0.84	-0.89	-0.98	-0.94
Light silty loam												
L-aspartic acid												
0	8.30	10.05	26.67	42.00	57.56	54.63	8.30	10.05	26.67	42.00	57.56	54.63
100	8.63	4.71	22.17	39.75	52.28	51.21	7.82	8.10	18.68	41.63	55.31	53.81
200	2.27	2.14	16.56	31.80	51.56	52.87	6.89	3.28	13.71	26.58	52.64	52.95
300	1.59	0.72	9.49	27.94	51.41	53.21	7.27	1.99	10.29	17.14	52.19	54.10
400	0.95	0.52	4.42	15.74	49.35	51.01	6.94	0.24	5.63	9.41	46.44	52.59
r	-0.91	-0.92	-0.99	-0.97	-0.89	-0.55	-0.85	-0.98	-0.99	-0.98	-0.96	-0.71
L-arginine												
0	6.23	15.53	33.57	56.15	76.43	80.89	6.23	15.53	33.57	56.15	76.43	80.89
100	6.74	14.75	27.36	45.87	72.30	77.10	7.35	12.87	27.81	41.20	75.44	77.25
200	3.09	8.17	19.00	31.09	69.70	77.54	7.38	9.93	25.31	31.01	75.87	69.65
300	2.09	5.37	14.78	24.89	67.68	72.75	8.02	7.11	15.28	25.39	73.00	64.66
400	1.12	3.36	10.71	20.60	65.17	68.37	8.09	4.87	13.35	20.51	65.42	60.85
r	-0.94	-0.97	-0.99	-0.98	-0.99	-0.96	0.93	-0.99	-0.98	-0.97	-0.85	-0.99
DL-alanine												
0	4.73	3.37	4.79	16.74	74.02	61.80	4.73	3.37	4.79	16.74	74.02	61.80
100	4.55	1.02	3.11	11.88	66.40	59.25	5.48	2.15	4.89	12.74	67.69	59.46
200	2.11	0.18	1.90	8.59	61.81	58.75	5.22	0.52	2.01	8.10	62.19	53.60
300	1.43	0.13	0.84	5.19	56.88	56.39	6.12	0.61	0.97	5.26	54.41	51.70
400	0.50	0.35	0.50	4.11	50.04	52.03	5.41	0.32	0.42	5.89	45.89	48.92
r	-0.97	-0.80	-0.98	-0.98	-0.99	-0.96	0.63	-0.91	-0.95	-0.94	-0.99	-0.98
*LSD$_{0.01}$	a – 0.23; b – 0.15; c – 0.15; d – 0.18; e – 0.26; $a \cdot b$ – 0.33; $a \cdot c$ – 0.33; $a \cdot d$ – 0.41; $a \cdot e$ – 0.58; $b \cdot c$ – 0.21; $b \cdot d$ – 0.26; $b \cdot e$ – 0.36; $c \cdot d$ – 0.26; $c \cdot e$ – 0.36; $d \cdot e$ – 0.45; $a \cdot b \cdot c$ – 0.47; $a \cdot b \cdot d$ – 0.58; $a \cdot b \cdot e$ – 0.81; $a \cdot c \cdot d$ – 0.58;$a \cdot c \cdot e$ – 0.81; $a \cdot d \cdot e$ – 1.00; $b \cdot c \cdot d$ – 0.36; $b \cdot c \cdot e$ – 0.51; $b \cdot d \cdot e$ – 0.63; $c \cdot d \cdot e$ – 0.63; $a \cdot b \cdot c \cdot d$ – 0.81; $a \cdot b \cdot c \cdot e$ – 1.15; $a \cdot b \cdot d \cdot e$ – 1.41; $a \cdot c \cdot d \cdot e$ – 1.41; $b \cdot c \cdot d \cdot e$ – 0.89; $a \cdot b \cdot c \cdot d \cdot e$ – 1.99											

* LSD (least statistical difference) for: a – nickel dose, b – nickel compound, c – type of soil, d – source of nitrogen, e – day of analysis;
r – coffelation coeffictiens significant difference for: $p < 0.01$; $n = 30$

The process of ammonification of all tested compounds was disturbed by soil contamination with nickel. The degree of this destructive activity was directly related to the amount of nickel occurring in the soil (Table 3) and to a larger extent occurred in heavy loamy sand than in light silty loam. However, regardless of the nickel dose and the type of soil, the smallest disturbances were found in L-arginine transformation (inhibition of the ammonification process by 34.2%), and the largest – of L-aspartic acid (inhibition of the ammonification process by 45.8%). A higher disturbance of ammonification,

Table 3

Inhibition of the ammonification process by nickel, in %

Ni dose mg kg^{-1} of soil	Type of nickel compound											
	$NiCl_2 \cdot 6H_2O$						$NiSO_4 \cdot H_2O$					
	Time of soil incubation, in hours											
	6	12	18	24	48	72	6	12	18	24	48	72
1	2	3	4	5	6	7	8	9	10	11	12	13
heavy loamy sand												
without nitrogen												
100	9.58	8.00	24.47	21.14	21.48	24.22	15.80	5.48	6.48	8.43	8.57	13.40
200	15.52	12.43	28.45	29.02	29.37	31.60	17.23	8.04	10.60	12.77	14.98	26.14
300	19.54	16.13	33.17	32.52	35.82	39.50	19.25	12.87	13.50	15.56	22.25	29.29
400	25.05	22.02	38.90	38.29	40.30	41.14	24.42	17.17	18.03	19.03	27.47	31.22
r	0.99	0.99	0.99	0.99	0.99	0.97	0.95	0.99	0.99	0.99	0.99	0.91
L-aspartic acid												
100	27.65	27.14	54.87	36.67	9.52	11.60	6.11	1.89	52.90	33.36	17.75	7.96
200	36.12	33.07	63.18	40.83	58.60	25.98	9.53	4.60	54.14	46.52	73.01	15.44
300	38.91	39.01	64.91	50.58	57.39	28.81	14.85	11.47	55.86	48.42	78.84	21.24
400	49.58	45.33	66.79	58.93	62.40	39.87	22.83	15.76	58.44	57.21	81.63	34.70
r	0.98	0.99	0.92	0.99	0.81	0.97	0.98	0.99	0.99	0.96	0.84	0.98
L-arginine												
100	13.41	33.54	29.36	33.43	19.04	7.88	10.35	27.29	31.46	22.26	9.98	5.21
200	24.22	50.22	44.20	51.99	15.54	11.49	14.55	41.40	46.84	51.25	16.22	11.20
300	29.12	56.63	55.83	65.76	17.88	16.64	20.04	49.99	52.96	62.47	25.32	15.25
400	33.93	64.77	65.76	72.42	21.76	20.07	26.04	58.16	62.26	66.28	34.41	20.58
r	0.98	0.98	0.99	0.98	0.52	0.99	0.99	0.99	0.98	0.93	0.99	0.99
DL-alanine												
100	4.21	20.95	36.37	36.51	4.05	5.97	1.86	9.70	26.00	36.07	1.86	1.39
200	13.25	28.35	49.65	47.05	33.41	12.26	2.71	16.25	36.54	51.19	30.01	7.84
300	18.98	34.96	52.55	63.60	63.12	16.03	6.93	18.22	39.30	57.05	48.86	21.82
400	24.46	37.28	55.91	67.10	74.87	22.32	11.65	21.87	42.92	60.08	67.87	25.28
r	0.99	0.98	0.93	0.97	0.98	0.99	0.97	0.97	0.95	0.94	0.99	0.98

cont. table 3

1	2	3	4	5	6	7	8	9	10	11	12	13
Light silty loam												
Without nitrogen												
100	14.72	12.48	15.94	12.37	9.98	5.26	5.26	1.46	1.40	3.84	2.46	2.48
200	9.90	18.92	22.14	23.04	20.81	22.56	8.27	2.28	6.15	5.88	5.50	6.83
300	12.82	23.02	23.70	25.91	29.88	35.60	16.54	5.12	8.87	9.41	15.44	16.40
400	14.96	28.21	27.06	30.14	32.24	40.42	21.99	7.37	11.59	16.91	14.36	26.25
r	0.20	0.99	0.97	0.96	0.97	0.97	0.99	0.98	0.99	0.96	0.91	0.99
L-aspartic acid												
100	10.14	22.25	12.73	6.58	11.21	4.16	6.50	4.65	12.01	0.41	5.31	0.05
200	29.51	34.16	26.96	22.54	15.64	8.54	11.91	18.70	24.23	23.38	9.32	2.67
300	33.97	41.14	41.64	29.36	18.82	12.94	16.26	24.36	32.36	38.68	13.14	4.81
400	37.72	45.53	53.35	49.00	21.93	17.33	21.20	30.91	42.91	53.06	19.24	10.24
r	0.92	0.98	0.99	0.99	0.99	0.99	0.99	0.98	0.99	0.99	0.99	0.98
L-arginine												
100	10.69	9.03	15.65	16.79	5.79	3.60	1.29	6.53	8.33	19.60	0.67	2.68
200	21.10	28.90	32.64	37.91	11.31	8.04	3.41	14.10	14.72	32.45	1.12	10.79
300	27.12	38.21	40.51	46.25	15.80	16.03	7.10	22.67	32.98	40.32	6.66	17.98
400	32.42	46.32	48.95	52.76	18.85	21.34	10.90	29.47	37.48	48.59	13.56	24.20
r	0.99	0.97	0.98	0.96	0.99	0.99	0.99	0.99	0.97	0.99	0.95	0.99
DL-alanine												
100	12.43	19.25	18.48	18.42	10.41	3.33	0.96	5.76	0.43	11.15	7.02	2.17
200	18.69	27.74	27.64	32.59	17.92	9.71	4.44	11.89	13.70	23.05	13.18	10.03
300	23.87	31.54	32.38	42.15	25.25	16.69	7.39	14.11	19.33	31.77	23.51	15.34
400	29.44	35.40	36.33	47.24	32.51	23.08	14.70	17.06	23.41	34.97	31.40	21.70
r	0.99	0.98	0.98	0.98	0.99	0.99	0.98	0.97	0.96	0.97	0.99	0.99
*LSD$_{0.01}$	a – 0.25; b – 0.17; c – 0.17; d – 0.25; e – 0.30; $a \cdot b$ – 0.35; $a \cdot c$ – 0.35; $a \cdot d$ – 0.49; $a \cdot e$ – 0.60; $b \cdot c$ – 0.25; $b \cdot d$ – 0.35; $b \cdot e$-0.01; $c \cdot d$ – 0.35; $c \cdot e$ – 0.43; $d \cdot e$ – 0.60; $a \cdot b \cdot c$ – 0.49; $a \cdot b \cdot d$ – 0.69; $a \cdot b \cdot e$ – 0.85; $a \cdot c \cdot d$ – 0.69; $a \cdot c \cdot e$ – 0.85; $a \cdot d \cdot e$ – 1.20; $b \cdot c \cdot d$ – 0.49; $b \cdot c \cdot e$ – 0.60; $b \cdot d \cdot e$ – 0.85; $c \cdot d \cdot e$ – 0.85; $a \cdot b \cdot c \cdot d$ – 0.98; $a \cdot b \cdot c \cdot e$ – 1.20; $a \cdot b \cdot d \cdot e$ – 1.70; $a \cdot c \cdot d \cdot e$ – 1.70; $b \cdot c \cdot d \cdot e$ – 0.1.20; $a \cdot b \cdot c \cdot d \cdot e$ – 2.41											

*LSD (least statistical difference) for: a – nickel dose, b – nickel compound, c – type of soil, d – source of nitrogen, e – day of analysis;
r – coffelation coeffictiens significant difference for: $p < 0.01$; $n = 24$

particularly of L-aspartic acid, was found in heavy loamy sand than in light silty loam. The most intense inhibition of ammonification process, in both tested types of soil, was found at hour 18 of incubation and then successively decreased, reaching the lowest level at hour 72. To recapitulate, it should be stated that soil contamination with nickel compounds strongly disturbed the

ammonification process. Stronger negative results were brought about by nickel chloride than by nickel sulfate (Table 3). The medium inhibition of ammonification by nickel chloride was 45.4%, while in case of sulfate – 37.4%. The above described disturbances to a larger extent occurred in heavy loamy sand than in light silty loam. This is also proved by higher in the case of heavy loamy sand in comparison to light silty loam (Table 4), negative correlation coefficients between nickel doses and mineral nitrogen content ($N\text{-}NO_3$ and $N\text{-}NH_4$). However, they were the highest in soils with no addition of amino acids. Significant negative correlation coefficients were also found between a nickel dose and soil pH. In the case of these two variables, the highest correlation coefficients were also found in the soil that was not fertilized with nitrogen. Both nickel chloride and nickel sulfate significantly reduced soil pH (Table 5). They acidified the lighter soil (heavy loamy sand) more strongly than the heavier soil (light silty loam). Perhaps this element additionally influenced the fact that the ammonification process in heavy loamy sand was slightly more strongly inhibited by nickel than in the case of light silty loam. All amino acids significantly affected soil pH. However, their activity was varied: while L-aspartic acid and DL-alanine resulted in pH reduction, L-arginine increased it. In soils with the addition of amino acids, pH increased with the growth of the amount of ammonified nitrogen.

Table 4

Coefficients of correlation between a nickel dose and the content of mineral forms of nitrogen and soil pH

Variable	Ni	$N\text{-}NH_4$	$N\text{-}NO_3$	$N\text{-}NO_3 + N\text{-}NH_4$	pH
1	2	3	4	5	6
Heavy loamy sand					
Without nitrogen					
Ni		-0.34**	-0.47**	-0.39**	-0.61**
$N\text{-}NH_4$	-0.34**		0.83**	0.99**	-0.01
$N\text{-}NO_3$	-0.47**	0.83**		0.91**	-0.01
$N\text{-}NO_3 + N\text{-}NH_4$	-0.39**	0.99**	0.91**		-0.01
pH	-0.61**	-0.01	0.01	-0.01	
L-aspartic acid					
Ni		-0.52**	-0.27**	-0.34**	-0.45**
$N\text{-}NH_4$	-0.52**		0.1	0.24**	0.13
$N\text{-}NO_3$	-0.27**	0.1		0.99**	0.78**
$N\text{-}NO_3 + N\text{-}NH_4$	-0.34**	0.24**	0.99**		0.78**
pH	-0.45**	0.13	0.78**	0.78**	

cont. table 4

1	2	3	4	5	6
L-arginine					
Ni		-0.50**	-0.31**	-0.34**	-0.28**
$N\text{-}NH_4$	-0.50**		0.26**	0.32**	0.34**
$N\text{-}NO_3$	-0.31**	0.26**		0.99**	0.86**
$N\text{-}NO_3 + N\text{-}NH_4$	-0.34**	0.32**	0.99**		0.86**
pH	-0.28**	0.34**	0.86**	0.86**	
DL-alanine					
Ni		-0.47**	-0.24**	-0.26**	-0.22**
$N\text{-}NH_4$	-0.47**		0.57**	0.61**	0.62**
$N\text{-}NO_3$	-0.24**	0.57**		0.99**	0.96**
$N\text{-}NO_3 + N\text{-}NH_4$	-0.26**	0.61**	0.99**		0.96**
pH	-0.22**	0.62**	0.96**	0.96**	
light silty loam					
without nitrogen					
Ni		-0.42**	-0.40**	-0.52**	-0.40**
$N\text{-}NH_4$	-0.42**		-0.09	0.97**	-0.43**
$N\text{-}NO_3$	-0.40**	-0.09		0.16**	0.42**
$N\text{-}NO_3 + N\text{-}NH_4$	-0.40**	-0.43**	0.42**	-0.32**	-0.32**
pH	-0.40**	-0.43**	0.42**	-0.32**	
L-aspartic acid					
Ni		-0.45**	-0.24**	-0.28**	-0.19**
$N\text{-}NH_4$	-0.45**		0.06	0.16**	0.03
$N\text{-}NO_3$	-0.24**	0.06		0.99**	0.97**
$N\text{-}NO_3 + N\text{-}NH_4$	-0.28**	0.16**	0.99**		0.97**
pH	-0.19**	0.03	0.97**	0.97**	
L-arginine					
Ni		-0.47**	-0.23**	-0.26**	-0.28**
$N\text{-}NH_4$	-0.47**		0.52**	0.58**	0.48**
$N\text{-}NO_3$	-0.23**	0.52**		0.99**	0.94**
$N\text{-}NO_3 + N\text{-}NH_4$	-0.26**	0.58**	0.99**		0.93**
pH	-0.28**	0.48**	0.94**	0.93**	
DL-alanine					
Ni		-0.58**	-0.15**	-0.19**	-0.22**
$N\text{-}NH_4$	-0.58**		0.28**	0.36**	0.30**
$N\text{-}NO_3$	-0.15**	0.28**		0.99**	0.95**
$N\text{-}NO_3 + N\text{-}NH_4$	-0.19**	0.36**	0.99**		0.95**
pH	-0.22**	0.30**	0.95**	0.95**	

r – correlation coeffictiens significant difference for: ** $p < 0.01$; * $p < 0.05$; $n = 2880$

Table 5

pH changes (in 1% K_2SO_4) during the ammonification process

Ni dose mg kg^{-1} of soil	Type of nickel compound											
	$NiCl_2 \cdot 6H_2O$						$NiSO_4 \cdot H_2O$					
	Time of soil incubation, in hours											
	6	12	18	24	48	72	6	12	18	24	48	72
1	2	3	4	5	6	7	8	9	10	11	12	13
heavy loamy sand												
without nitrogen												
0	7.23	6.90	6.83	6.90	6.90	6.90	7.23	6.90	6.83	6.90	6.90	6.90
100	7.13	6.70	6.67	6.80	6.80	6.80	6.93	6.77	6.70	6.80	6.80	6.87
200	7.03	6.70	6.60	6.80	6.70	6.73	6.83	6.73	6.70	6.73	6.80	6.80
300	7.03	6.63	6.60	6.70	6.70	6.70	6.73	6.70	6.60	6.70	6.77	6.73
400	6.93	6.63	6.50	6.63	6.60	6.67	6.73	6.70	6.60	6.67	6.70	6.70
r	-0.97	-0.87	-0.94	-0.97	-0.97	-0.97	-0.91	-0.89	-0.93	-0.97	-0.95	-0.99
L-aspartic acid												
0	6.87	6.97	6.33	6.40	7.33	7.73	6.87	6.97	6.33	6.40	7.33	7.70
100	6.77	6.93	6.00	6.30	7.13	7.67	6.23	6.00	5.77	6.20	7.03	7.73
200	6.67	6.90	5.73	6.23	6.70	7.60	6.10	5.83	5.70	6.07	6.63	7.57
300	6.67	6.57	5.63	6.13	6.33	7.50	6.07	5.87	5.70	6.00	6.07	7.50
400	6.43	6.10	5.50	5.90	5.63	7.20	6.07	5.77	5.47	5.80	5.77	7.10
r	-0.95	-0.90	-0.97	-0.97	-0.98	-0.93	-0.82	-0.80	-0.88	-0.99	-0.99	-0.89
L-arginine												
0	7.50	7.20	7.23	7.33	7.97	7.90	7.50	7.20	7.23	7.33	7.97	7.90
100	7.40	7.20	7.30	7.10	7.90	7.90	7.30	7.17	7.20	7.20	7.93	7.70
200	7.20	7.10	7.17	7.10	7.83	7.80	7.20	7.13	7.20	7.10	7.87	7.73
300	7.10	7.10	7.10	7.10	7.80	7.80	7.10	7.13	7.20	7.10	7.70	7.70
400	7.10	7.10	7.10	7.10	7.57	7.73	7.03	7.10	7.17	7.10	7.50	7.63
r	-0.96	-0.87	-0.85	-0.71	-0.94	-0.95	-0.98	-0.97	-0.89	-0.87	-0.95	-0.84
DL-alanine												
0	6.63	6.80	6.87	7.10	7.47	7.73	6.63	6.80	6.87	7.10	7.47	7.73
100	6.60	6.70	6.83	7.00	7.40	7.70	6.70	6.80	6.80	6.90	7.43	7.77
200	6.60	6.70	6.73	6.93	7.13	7.67	6.63	6.73	6.80	6.80	7.30	7.70
300	6.57	6.67	6.70	6.80	6.97	7.67	6.60	6.70	6.70	6.80	7.17	7.60
400	6.53	6.67	6.63	6.80	6.90	7.63	6.60	6.67	6.70	6.77	7.10	7.47
r	-0.97	-0.87	-0.99	-0.97	-0.98	-0.97	-0.65	-0.97	-0.95	-0.89	-0.98	-0.91

cont. table 5

1	2	3	4	5	6	7	8	9	10	11	12	13
light silty loam												
without nitrogen												
0	7.10	6.97	6.90	6.90	6.90	6.80	7.10	6.97	6.90	6.90	6.90	6.80
100	7.10	6.87	6.83	6.87	6.80	6.80	7.20	6.90	6.90	6.80	6.80	6.80
200	7.10	6.87	6.80	6.80	6.80	6.70	7.00	6.83	6.80	6.80	6.80	6.80
300	7.10	6.80	6.80	6.80	6.73	6.67	7.00	6.80	6.80	6.80	6.80	6.80
400	7.00	6.80	6.80	6.70	6.67	6.67	7.00	6.80	6.80	6.77	6.80	6.70
r	-0.71	-0.93	-0.85	-0.96	-0.97	-0.93	-0.71	-0.95	-0.87	-0.83	-0.71	-0.71
L-aspartic acid												
0	5.80	6.37	6.77	7.10	7.60	7.70	5.80	6.37	6.77	7.10	7.60	7.70
100	5.80	6.23	6.67	7.07	7.57	7.70	5.60	6.33	6.80	6.87	7.53	7.57
200	5.67	6.13	6.50	6.87	7.50	7.70	5.60	6.20	6.60	6.60	7.50	7.50
300	5.67	6.03	6.23	6.80	7.50	7.60	5.53	6.10	6.33	6.50	7.50	7.50
400	5.63	6.00	6.13	6.50	7.47	7.60	5.50	5.93	6.10	6.23	7.50	7.50
r	-0.92	-0.98	-0.99	-0.96	-0.96	-0.87	-0.91	-0.98	-0.95	-0.99	-0.85	-0.85
L-arginine												
0	7.17	7.20	7.20	7.37	7.67	7.80	7.17	7.20	7.20	7.37	7.67	7.80
100	7.10	7.13	7.10	7.20	7.60	7.70	7.10	7.17	7.13	7.20	7.63	7.60
200	7.00	7.10	7.03	7.07	7.60	7.63	7.10	7.10	7.10	7.07	7.63	7.57
300	7.00	7.07	7.00	7.00	7.60	7.57	7.00	7.10	7.07	7.00	7.60	7.57
400	7.00	7.03	7.00	7.00	7.53	7.57	7.00	7.00	7.00	7.00	7.50	7.57
r	-0.89	-0.99	-0.93	-0.94	-0.89	-0.96	-0.95	-0.96	-0.99	-0.94	-0.90	-0.78
DL-alanine												
0	6.80	6.90	7.00	7.00	7.50	7.63	6.80	6.90	7.00	7.00	7.50	7.63
100	6.73	6.80	6.93	7.00	7.47	7.60	6.80	6.87	6.97	6.93	7.47	7.57
200	6.70	6.80	6.90	6.93	7.40	7.57	6.77	6.83	6.90	6.83	7.40	7.50
300	6.70	6.77	6.90	6.83	7.30	7.57	6.70	6.80	6.90	6.77	7.30	7.50
400	6.67	6.73	6.87	6.80	7.10	7.53	6.67	6.77	6.83	6.77	7.17	7.43
r	-0.94	-0.93	-0.94	-0.96	-0.95	-0.97	-0.96	-0.99	-0.97	-0.96	-0.97	-0.97
*LSD$_{0.01}$	a – 0.01; b – 0.01; c – 0.01; d – 0.01; e – 0.01; $a \cdot b$ – 0.01; $a \cdot c$ – 0.01; $a \cdot d$ – 0.02; $a \cdot e$ – 0.02; $b \cdot c$ – 0.01; $b \cdot d$ – 0.01; $b \cdot e$ – 0.01; $c \cdot d$ – 0.01; $c \cdot e$ – 0.01; $d \cdot e$ – 0.02; $a \cdot b \cdot c$ – 0.02; $a \cdot b \cdot d$ – 0.03; $a \cdot b \cdot e$ – 0.03; $a \cdot c \cdot d$ – 0.03; $a \cdot c \cdot e$ – 0.03; $a \cdot d \cdot e$ – 0.04; $b \cdot c \cdot d$ – 0.02; $b \cdot c \cdot e$ – 0.02; $b \cdot d \cdot e$ – 0.03; $c \cdot d \cdot e$ – 0.03; $a \cdot b \cdot c \cdot d$ – 0.04; $a \cdot b \cdot c \cdot e$ – 0.04; $a \cdot b \cdot d \cdot e$ – 0.06; $a \cdot c \cdot d \cdot e$ – 0.06; $b \cdot c \cdot d \cdot e$ – 0.06; $a \cdot b \cdot c \cdot d \cdot e$ – 0.09											

* LSD (least statistical difference) for: a – nickel dose, b – nickel compound, c – type of soil, d – source of nitrogen, e – day of analysis;
r – coffelation coeffictiens significant difference for: $p < 0.01$; $n = 30$

The negative effect of nickel on the ammonification process is, from the point of view of soil fertility, a very unfavourable phenomenon. Nitrogen found in organic compounds is almost inaccessible for plants. To be used by them, it must be mineralized (Barabasz 1992), and this is determined by the following factors: granulometric composition of soil, pH, organic substance content, C_{org} : N ratio, contamination of soil with various heavy metals, temperature and humidity (Antil et al. 2001, DENI, PENNINCKX 1999, OMAR, ISMAIL 1999, PRZYBULEWSKA et al. 2003b, WYSZKOWSKA, KUCHARSKI 2004).

The destabilizing effect of nickel on the ammonification process, just as it is the case with other metals (BENBI et al. 1996, WYSZKOWSKA 2002), was mainly related to its negative influence on the growth and development of soil microorganisms, as well as to an inhibitory effect on soil enzymes. A stronger inhibiting activity of nickel was observed in the lighter soil (heavy loamy sand) than in the heavier soil (light silty loam). Obviously, this is directly related to more favourable physicochemical properties of loamy soil in comparison with sandy soil, which results, among others, from an abundance of organic and mineral colloids (WYSZKOWSKA, KUCHARSKI 2001, PRZYBULEWSKIEJ et al. 2003a).

The ammonification rate was determined not only by the type of a nickel compound, its dose, and the soil type, but also by chemical properties of amino acids themselves. The process of ammonification for L-aspartic acid – considered to be one of acid amino acids – was much slower than for L-arginine, which is considered to be one of the basic amino acids. DL-alanine demonstrated an intermediate ammonification rate. Chemical properties of amino acids had their effect on the change of soil reaction, which is not neutral to the activity of ammonification bacteria (BARABASZ 1992, BARABASZ et al. 2002).

Conclusions

1. Soil contamination with increasing doses of nickel had a negative effect on the ammonification process of L-aspartic acid, L-arginine and DL-alanine. Nickel applied in doses of 400 mg $Ni^{2+} \cdot kg^{-1}$ inhibited the ammonification of L-aspartic acid by 26%, L-arginine by 22%, and DL-alanine by 23%.

2. Nickel chloride proved to be a stronger inhibitor of ammonification than nickel sulfate.

3. Applied nickel compounds contributed to the reduction of soil pH, which had a visible effect on the ammonification process, since acid reaction influences the activation and mobility of heavy metals.

4. A higher disturbance of ammonification, particularly of L-aspartic acid, was found in heavy loamy sand than in light silty loam.

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THE EFFECT OF A MICROBIOLOGICAL AND A DISINFECTING PREPARATION ON THE PHYSICAL AND CHEMICAL PROPERTIES OF LITTER AND THE RESULTS OF BROILER CHICKEN BREEDING

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K e y w o r d s: broiler chickens, litter, temperature, humidity, reaction, ammonia, production output.

A b s t r a c t

The object of the study was to conduct an assessment of the effect that a disinfecting preparation Lubisan® and a microbiological preparation Biosan-GS® have on the chemical and physical properties of litter and the results of chicken breeding. 360 broiler chickens, Cobb 500 meat type, were used as experimental material; they were divided into three even groups: control (*C*) and two experimental groups: one in which the microbiological preparation Biosan-GS® was used (*B*), and the other – where the effect of disinfectant Lubisan® was assessed (*L*). The addition of a microbiological or disinfecting preparation has been shown to improve its physical and chemical properties. A significantly lower level of relative humidity ($p \leq 0.01$) in litter treated with the examined preparations was measured during the last two weeks of study. The ammonia concentration in the experimental litter was also lower than in the control; in week four of the experiment, the difference was significant ($p \leq 0.05$), whereas in week five a highly significant ($p \leq 0.01$) difference was found to exist between groups *C* and *L*. In addition, the bodyweight of chickens was higher in the rooms where the preparations were used. Statistically significant differences appeared in week three of the breeding period ($p \leq 0.05$) and the difference between group *L* and control in week three and four was highly significant ($p \leq 0.01$).

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WPŁYW PREPARATU MIKROBIOLOGICZNEGO I DEZYNFEKUJĄCEGO NA WŁAŚCIWOŚCI FIZYKOCHEMICZNE ŚCIÓŁKI ORAZ WYNIKI ODCHOWU KURCZĄT BROJLERÓW

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S ł o w a k l u c z o w e: kurczęta brojlery, ściółka, temperatura, wilgotność, odczyn, amoniak, wyniki produkcyjne.

A b s t r a k t

Celem badań była ocena wpływu preparatu dezynfekcyjnego Lubisan® i mikrobiologicznego Biosan-GS® na właściwości fizykochemiczne ściółki oraz wyniki odchowu kurcząt brojlerów. Doświadczenie przeprowadzono na 360 kurczętach brojlerach typu mięsnego Cobb 500. Podzielono je na trzy jednakowe grupy: kontrolną (*C*) i doświadczalne, w których do ściółki dodawano preparat mikrobiologiczny Biosan-GS® (*B*) lub dezynfekcyjny Lubisan® (*L*). Wykazano, że dodatek tych preparatów poprawia fizykochemiczne właściwości ściółki. W 2 ostatnich tyg. badań stwierdzono istotnie niższą wilgotność względną ($p \leq 0{,}01$) w ściółkach grup *B* i *L*. Stężenie amoniaku było w nich także niższe w porównaniu z grupą *C*, przy czym w 4. tyg. badań pomiędzy pomieszczeniami doświadczalnymi a kontrolnym różnica była istotna ($p \leq 0{,}05$), natomiast w 5. tyg. stwierdzono wysoko istotną różnicę ($p \leq 0{,}01$) pomiędzy grupami *C* i *L*. Masa ciała kurcząt odchowywanych w pomieszczeniach, w których stosowano preparaty była również wyższa. Statystyczne różnice tego parametru występowały od 3. tyg. odchowu ($p \leq 0{,}05$), przy czym w 3. i 4. tyg. pomiędzy grupą *L* i kontrolną *C* różnica była wysoko istotna ($p \leq 0{,}01$).

Introduction

One of the factors which guarantees bird welfare is the proper and efficient controlling of environmental conditions. The microclimate in poultry breeding facilities is largely determined by the physical and chemical properties of litter. Such factors as temperature, humidity, pH and the duration of use affect the amount and activity of microorganisms, both in the litter itself and in the air in the breeding rooms (HIMATHONGKHAM, REIMANN 1999, SZEJNIUK, KLUCZEK 1999). Studies into the microflora of breeding rooms for various animal species have shown that the amount of bacteria is the largest in hen houses (SZEJNIUK, KLUCZEK 1999). There can be saprophytes, but also pathogens or bacteria which are responsible for enzymatic decomposition of organic matter to ammonia, carbon dioxide, hydrogen sulphide, methane and many other gaseous and odorous substances (TYMCZYNA 1993, TYMCZYNA et al. 1995). With a relative humidity of litter of 40-60%, the release of ammonia is the most intensive (WATHES et al. 2004). In addition, the high reaction of litter can

largely accelerate decomposition of ureic acid to ammonia (TYMCZYNA 1993, AL HOMIDAN et al. 2003). NAHM (2003) found ammonia to be completely expelled from faeces of cattle and poultry at pH 8; a reaction of less than 4 completely inhibits the process. With an extended time of litter use, the amount of droppings increases. Accumulation of excretion brings about intensive development of thermophilic bacteria, which participate in the degradation of nitrogen compounds to ammonia (TYMCZYNA, SABA 1987, TYMCZYNA 1993). PRATT et al. (2004) and KANONIUK et al. (2004), tried to determine the dynamics of nitrogen level fluctuations in bird droppings and its emission to the atmosphere and found the transformations of ammonium nitrogen to depend on the temperature of incubation. The higher the temperature, the earlier the transformations – and, consequently, ammonia emission to the atmosphere – are observable. According to NAHM (2003), it is necessary to keep the temperature below 10°C to prevent the release of ammonia; however, keeping this temperature in broiler breeding facilities is not physiologically favourable to birds. In order to slow down the process of bacteria-induced ammonification and the production of noxious gases and their emission to the atmosphere, a temperature of 25°C in the birds; environment should not be exceeded. According to AL HOMIDAN et al. (2003), only a slight increase in air temperature by 1-2 degrees can significantly raise the ammonia level in a hen house.

As it is necessary to keep the climatic parameters in breeding facilities at an appropriate level, various methods are sought to improve the properties of litter. It is possible to achieve this by adding some admixtures to litter, with a view to drying it, decreasing the pH value, reducing the emission of noxious gases and inhibiting the growth of bacteria and parasites.

This study aimed at assessing the effect of the disinfectant by the name of Lubisan® and the microbiological preparation Biosan-GS® on the physical and chemical properties of litter and the results of broiler chicken breeding.

Materials and Methods

360 broiler chickens, Cobb 500 meat type, were used as experimental material; they were divided into three even groups: control (*C*) and two experimental groups: one in which the microbiological preparation Biosan-GS® was used (*B*), and the other – where the effect of disinfectant Lubisan® was assessed (*L*). According to the producer (PWiZBiIG BIOGEN), the biopreparation consists of a starting nutrient and a properly selected mixture of non-pathogenic bacteria. Thanks to a wide range of effects, the preparation brings about a decomposition of harmful substances (ammonia, nitrites, hydrogen

sulphide, indole, skatole, mercaptans), improves the profile of fermentation processes by reducing putrefactive and alcohol fermentation, inhibits the development of pathogenic bacteria and destroys eggs and resting forms of insects and endoparasites. The producer of Lubisan® (STARVET) states that the preparation is intended for disinfection and sanitation of rooms for all animals; it contains chloramine T, inorganic compounds and essential oils, and has as strong bacterio-, viru-, fungi- and larvicidal effect. It also inhibits the release of noxious gases (ammonia, hydrogen sulphide) and absorbs moisture.

According to the producers; recommendations, the preparations were applied in the following manner:

– Biosan-GS® spread onto the litter at 5 g per 1 m^2 before the birds were added, and then, at the same quantity, every fortnight when the birds were inside,

– Lubisan was spread onto the floor at 100 g per m^2 before the litter was laid out, and then 50 g of the preparation was applied per 1 m^2 of the litter every day during the period of birds; breeding.

Throughout the period of chicken breeding, the temperature and relative humidity of the air outdoors and in the experimental rooms was controlled by continuous measurement (every 10 minutes for 24 hours ad day) with the use of a LB-520 minilogger, manufactured by LAB-EL.

Everyday at 7.00 a.m., 1.00 p.m. and 9.00 p.m., the temperature and relative humidity on the internal layer of the litter was measured with a Dramiński digital tester of hay and straw humidity, and the concentration of ammonia on the litter surface was measured with a MiniTOX 3 multi-gas meter. The relative humidity (%) of the external litter layer was analysed twice a week by the method of skin-drying (DOBRZAŃSKI, MAZURKIEWICZ 1994); the litter reaction was measured with an agricultural digital pH-meter for measuring the reaction of soil and liquids, manufactured by Dramiński, after making a suspension of the litter in distilled water (10 g of litter for 25 ml of water).

Throughout the period of chicken breeding, fodder consumption by the group and bodyweight of all the birds was checked every 7 days, and the chicken's deaths were recorded systematically.

The experiment results were analysed by a single-factor analysis of variance in orthogonal or non-orthogonal systems. The significance of differences between the mean values of the examined factors were calculated with Duncan's test.

Results and Discussion

Macro- and microclimatic conditions

Due to the fact that the experiments were conducted in winter and then in spring, the weather conditions around the experiment site varied. The mean air temperature during the whole six week cycle was -0.5°C (Figure 1); in the first week the mean temperature was -3.66°C (minimum -16.2°C), while in the last one it was 3.40 (maximum 17.4°C). However, the temperatures in the breeding rooms met the requirements of conditions for broiler chickens (J. of Laws 2003, No. 167, item 1629). In the first week, the mean temperature of the air in each group was about 26°C, and in the following weeks it was gradually reduced to below 20°C in week six (Figure 1).

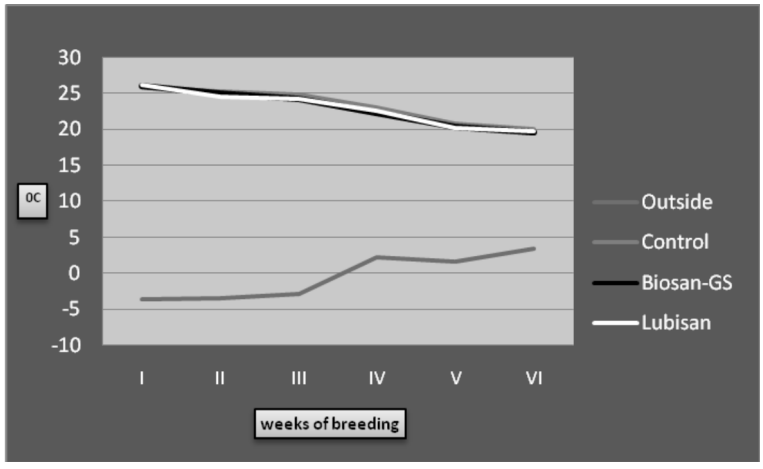

Fig. 1. Air temperature in the experimental rooms and outside

Considering the need to heat the building intensively, a problem was observed of an excessively low relative humidity of air in the building (Figure 2). It is usually recommended that the relative humidity in rooms for broiler chickens should be 70-75% during the first week of breeding and 55-70% during the rest of the period (DOBRZAŃSKI, KOŁACZ 1996), but during the first two weeks of the chicken's lives, the mean daily humidity in all the rooms was close to 30%. In subsequent weeks it rose to reach 41% (*C*) to 46% (*B*) in weeks 4 and 5. However, this caused no adverse effects in chickens. According to some zoohygienists (DOBRZAŃSKI 1992, MALONEY 1998), the currently recommended values of humidity in rooms where poultry is bred should be modified and a relative humidity of 40% should be regarded as the optimum value.

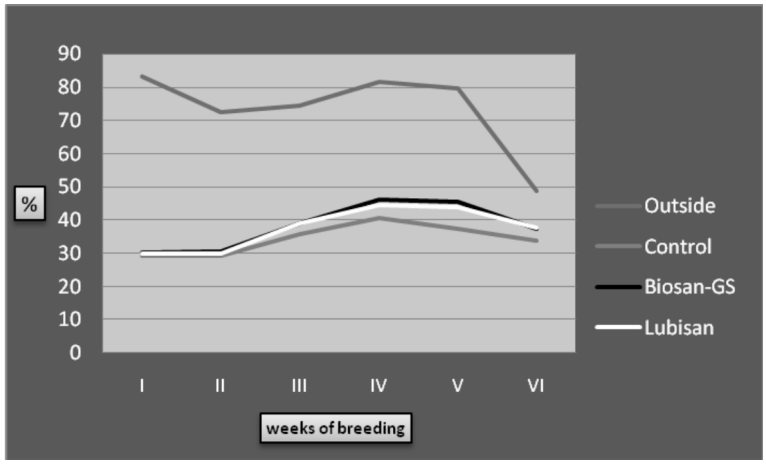

Fig. 2. Relative humidity of the air in the experimental rooms and outside

Physical and chemical properties of litter

The temperature of the internal layer of litter before the birds were put in ranged from 23.00°C in rooms of groups *C* and *B* to 22.00°C in the broiler room *L*; after the breeding was started, it dropped to 20.79°C in the control broiler room, 20.97°C in the *L* group and 21.19°C in room *B* (Table 1). In subsequent weeks of study, when the droppings accumulated and thermophilic bacteria developed, the litter temperature in all the facilities under study increased regularly, reaching similar values at the end (26.18°C – *B*, 26.27 – *C* and 26.83°C – *L*). According to DOBRZAŃSKI and MAZURKIEWICZ (1994), litter temperature should be equal to the optimum temperature of the air ±10%, whereas at the heated places it should not exceed the upper zone of heat neutrality, which for broilers equals 27°C.

The relative humidity of the inner layer of litter, as with its temperature, increased steadily in all the breeding rooms (Table 1). Before the chickens were put in, it was lower than 10%, whereas during the first week of the birds; lives it increased to reach the average value of 14.13% in room *C*, 13.94% in room *B* and 13.75% in group *L*. As early as in the second week of breeding, the relative humidity of the inner layer of litter in the control room was highly significantly higher ($p \leq 0.01$) than the litter in room *B*, and significantly higher ($p \leq 0.05$) than in room L. Similar tendencies were observed in week three and four, though the differences were not confirmed statistically. A higher relative humidity than in the control was also recorded during the last two weeks of the birds' lives, and the differences proved to be highly significant

Table 1

Physical and chemical properties of bedding by week of chicken breeding

Weeks of breeding	Statistical measure	Temperature (°C)			Relative humidity (%)						Reaction (pH)		
					inner bedding layer			outer bedding layer					
		control	Biosan--GS	Lubisan	control	Biosan--GS	Lubisan	control	Biosan--GS	Lubisan	control	Biosan--GS	Lubisan
Before putting chickens in	$\bar{x}$	23.00	23.00	22.00	8.74	8.41	8.64	> 10	> 10	> 10	6.15	6.15	6.15
1	$\bar{x}$ s	20.79 1.21	21.19 0.96	20.97 1.12	12.55 5.38	10.85 7.69	12.65 5.67	14.13 5.35	13.94 4.37	13.75 4.17	6.11 0.25	5.76 0.13	5.73 0.09
2	$\bar{x}$ s	21.00 1.37	20.88 1.27	20.98 1.11	30.06 0.86	27.36 2.12	29.75 3.51	24.00^{Aa} 6.62	18.71^{B} 3.20	20.62^{b} 4.33	6.77 1.46	5.85 0.08	5.52 0.18
3	$\bar{x}$ s	22.12 0.93	21.93 1.13	21.81 1.40	50.46 1.49	43.46 2.97	39.92 1.38	31.71 9.98	26.52 5.86	30.05 8.68	6.18 0.06	6.06 0.05	6.21 0.16
4	$\bar{x}$ s	24.61 1.25	23.95 1.65	24.16 1.46	53.61 1.36	46.57 1.59	49.23 4.76	53.10 10.75	48.24 10.96	52.05 11.75	6.01 0.04	5.95 0.16	6.44 0.82
5	$\bar{x}$ s	25.60 2.33	25.72 1.41	26.29 1.39	51.50 4.29	51.07 2.80	52.14 4.20	69.76^{A} 7.65	63.62^{B} 6.82	63.48^{B} 6.62	6.32 0.46	6.21 0.13	6.39 0.15
6	$\bar{x}$ s	26.27 1.25	26.18 1.77	26.83 1.95	48.85 5.02	44.51 6.72	43.94 5.01	72.29^{A} 5.21	63.96^{B} 7.15	62.92^{B} 5.28	7.10 0.11	6.78 0.52	6.83 0.34
1-6	$\bar{x}$ s	23.57 2.62	23.46 2.55	23.69 2.82	41.17 16.13	35.64 13.40	37.94 14.91	46.06^{A} 23.45	40.78^{B} 21.48	42.10^{B} 20.97	6.41 0.62	6.10 0.39	6.18 0.54

Commentary: A, B – $p \leq 0{,}01$; a, b – $p \leq 0.05$

($p \leq 0.01$). During the period, the analysed quantity in litter *B* and *L* ranged from 63 to 64%, whereas in room *C* it lay between 70 and 72%. In addition, the relative humidity of the inner layer of litter during the whole period of chicken breeding was very significantly higher ($p \leq 0.01$) than in the control rooms compared to the broiler rooms where drying preparations were applied, as it was 46.06% in group *C*, 40.78% in group *B* and 42.10% in group *L*. Statistically significant differences were not found to exist between the litters optimised with the examined preparations, though the discussed value was a little lower in the room where Biosan-GS was applied. It should be emphasised that these levels of relative humidity were measured at the depth of 15-20 cm inside the litter and were much higher than the relative humidity of the outer layer of litter (Table 1). However, similar tendencies were observed in both layers: in subsequent weeks of the experiment, the humidity of the outer layer of litter in the control room was the highest, while the lowest values were measured in the room where the Biosanu-GS preparation was applied, although the differences were not confirmed by statistical analysis. The levels of relative humidity have been shown to differ greatly in studies conducted by various authors. RUDZIK (1998) found the mean relative humidity of litter after 7-week-long chicken breeding to range from 13.83 to 15.27%, whereas TYMCZYNA et al. (1995) found that during eight weeks of breeding, the relative humidity of litter ranged from 27.5% to 57.1%; the same method of measurement may be the reason why these results were similar to those obtained in this study (Table 1). NAWALNY et al. (2004) examined the relative humidity of litter in broiler houses in various seasons of the year and found it to range from 14.8% to 61.9% in the winter and from 31.0% to 58.4% in the summer. The differences may be caused not only by the methods of measurement, but also by the climatic conditions in the breeding facilities, types of litter or by bird stock; however, each study has revealed a positive effect of treating the litter with optimising preparations. The favourable hygroscopic properties of natural aluminosilicates added to the litter of wood chips were examined by TYMCZYNA (1993) and TYMCZYNA et al. (1995), with the strongest drying effect observed during the last three weeks of the litter use. RUDZIK (1998) also measured a lower level of relative humidity of litter with an addition of zeolite, but not with kaolin.

This study did not find any statistically significant differences in the litter reaction, but that of the control litter was usually close to basic values (Table 1). The mean pH values of litter from the whole period of breeding was also the highest in the control room (6.41), slightly lower in the broiler room *L* (6.18), and the lowest in group *B* (6.10); the index of variability for the feature was very low (Table 1). Considering the fact that low pH values do not favour the growth of pathogens and uricolytic bacteria (IVANOV 2001), and litter at pH values below 7 emits small amounts of NH3 (AL HOMIDAN et al.

2003), the reaction of the analysed litter can be considered appropriate. Even lower pH values (with 6.33 as the highest) were measured by RUDZIK (1998), but the study found an unfavourable effect of zeolite, which raised the pH of the litter. Studies on bentonite and extract from yucca (TYMCZYNA 1993, TYMCZYNA et al. 1995, 1996) did not show any significant effect of the admixtures on the reaction of litter; however, as in this study, the pH value of the control litter was slightly higher.

An analysis of ammonia concentration in the outer layer of litter in the rooms under study reveals the effect of Biosan-GS and Lubisan (Figures 3, 4).

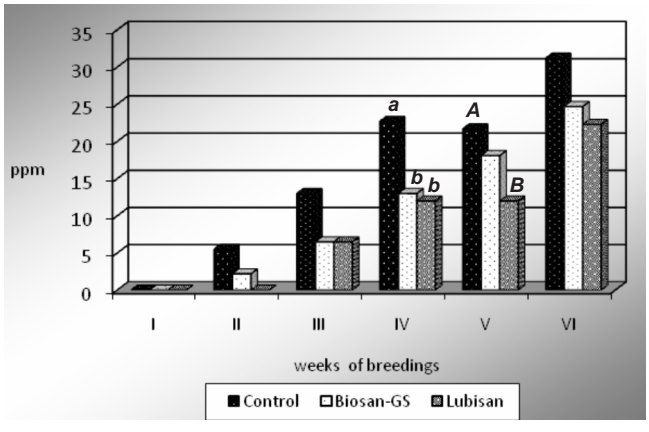

Fig. 3. Mean concentration of ammonia in the bedding: *A*, *B* – $p \leq 0.01$; *a*, *b* – $p \leq 0.05$

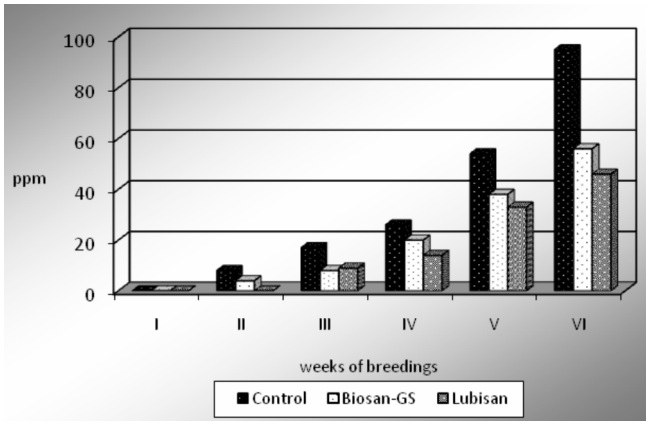

Fig. 4. Maximum concentration of ammonia in the bedding

An intensive growth of ammonia concentration in the litter in all the rooms was recorded every week; the highest growth rate was measured in the control group and the lowest was in the room where Lubisan had been applied. In week four, a significantly higher concentration of NH_3 was recorded as compared with the experimental litter, and the difference between rooms *C* and *L* in week five was highly significant (Figure 3). Throughout the period of study, the maximum ammonia concentration was the highest in the control litter (Figure 4). Very high concentrations (95 ppm) of the gas were recorded in group *C* during the last week of breeding; it was lower by 52% in group *B* and lower by 42% in group *L* (Figure 4). No standards for ammonia concentration have yet been adopted. Some zoohygienists claim that the highest acceptable concentration in the upper part of litter can be twice as high as the standard value for the air, i.e. 26 ppm for young birds and 52 for adult poultry. Considering this, the ammonia concentration in the litter of room *B* only slightly exceeded the zoohygienic recommendations, while in the control litter it was almost twice as high as the highest acceptable value. In the study into kaolin and zeolite, RUDZIK (1998) showed the reducing effect of the examined mineral admixtures on the concentration of the noxious substance in the inner litter and in the layer above the litter. Other authors have examined the effect of such admixtures as lignite (DOBRZAŃSKI et al. 1989), humus preparation called Humokarbowit (DOBRZAŃSKI et al. 1994), natural aluminosilicate – bentonite (TYMCZYNA et al. 1995), ZnSO4 (KIM, PATTERSON 2003), a microbiological preparation Cobio-litiere (DOBRZAŃSKI et al. 2000) on the poultry litter and found the ammonia concentrations to be lower than in untreated litter.

Results of chicken breeding

The percentage of deaths and rejections in all the rooms was similar (*C* – 5.83%, *B* – 6.66%, *L* – 5%). Fodder consumption was also similar and equalled 1.80 in group *C* and 1.81 in groups *B* and *L* (Table 2). Analysis of the birds; final bodyweight showed it to be significantly higher ($p \leq 0.05$) in both experimental groups as compared with the control: 2782.17 g in group *C*, 2881.59 in group *B* and 2 885.48 in group *L*. This tendency was also observed starting from week three to the end of chicken breeding, with the difference between group *L* and control in week three and four being highly significant ($p \leq 0.01$). No significant differences were observed between experimental groups, although a slightly higher bodyweight was measured for the birds bred on litter treated with Lubisan® (Table 2).

Table 2

Mean bodyweight ($\bar{x} \pm s$), fodder consumption, deaths and rejections of the chickens

Examined parameters	Age (weeks)	Group		
		control	Biosan-GS	Lubisan
Bodyweight (g)	1	198.07 ± 21.14 n = 116	198.28 ± 21.07 n = 116	200.63 ± 20.19 n = 117
	2	520.53 ± 49.96 n = 116	526.98 ± 55.55 n = 114	530.59 ± 54.66 n = 117
	3	973.89^{Bb} ± 94.73 n = 114	1003.40^{a} ± 103.48 n = 111	1018.52^{A} ± 110.74 n = 117
	4	1538.82^{Bb} ± 164.41 n = 114	1597.64^{a} ± 191.23 n = 191.23	1603.50^{A} ± 180.76 n = 115
	5	2096.54^{b} ± 235.18 n = 114	2134.97^{a} ± 281.77 n = 107	2180^{a} ± 265.32 n = 114
	6	2782.17^{b} ± 349.39 n = 113	2881.59^{a} ± 383.10 n = 107	2885.48^{a} ± 378.30 n = 114
Fodder consumption (kg/kg bodyweight)	1-6	1.80	1.81	1.81
Deaths and rejections (%)	1-6	5.83	6.66	5.00

Explanation: *A, B* – $p \leq 0{,}01$; *a, b* – $p \leq 0.05$

Conclusions

The application of the microbiological preparation Biosan-GS and a disinfectant by the name of Lubisan in chicken breeding had a favourable effect in reducing the humidity and ammonia concentration in litter, and the bodyweight growth was higher in the birds bred on the optimised litter as compared to the control group.

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ANALYSIS OF THE PERFORMANCE AND BREEDING VALUE OF HORSES REGISTERED IN THE TRAKEHNER ASSOCIATION IN POLAND CONSIDERING THEIR AFFILIATION TO FAMILIES OF MARES

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K e y w o r d s: Trakehner Horses, mare families, conformation, performance and breeding value.

A b s t r a c t

This study involved a population of Trakehner Horses and horses of Eastern-Trakehner--Prussian origin registered in the Trakehner Association in Poland. A qualitative analysis was carried out for horses belonging to 33 selected mare families. The selected families were characterized in terms of performance and breeding value of the horses belonging to them. The analysis included results of: performance test for stallions and mares, shows and breeding championships, and sports competitions. The highest quality assessment was noted for horses belonging to families of the following mares: O – Diana, E – Agresja xx, E – Balanda xx, and E – Avesta xx. In terms of gait quality, of high quality were also horses from the families: T – Chwała, E – Chiazza xx, and E – Igława xx. The analyses carried out within the study indicated a high performance and breeding value of horses belonging to the families examined.

ANALIZA WARTOŚCI UŻYTKOWEJ I HODOWLANEJ KONI ZAREJESTROWANYCH W ZWIĄZKU TRAKEŃSKIM W POLSCE Z UWZGLĘDNIENIEM ICH PRZYNALEŻNOŚCI DO RODZIN ŻEŃSKICH

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Abstrakt

Badania dotyczyły populacji koni trakeńskich i o pochodzeniu trakeńsko-wschodniopruskim, zarejestrowanych w Związku Trakeńskim w Polsce. Dokonano analizy bonitacyjnej koni należących do 33 wyodrębnionych rodzin żeńskich. Wybrane rodziny zostały scharakteryzowane pod względem wartości użytkowej i hodowlanej. Analiza ta uwzględniła wyniki: prób dzielności ogierów i klaczy, wystaw i czempionatów hodowlanych oraz zawodów sportowych. Stwierdzono, iż najwyżej bonitowanymi końmi były osobniki należące do rodziny O – Diany, E – Agresji xx, E – Balandy xx i E – Avesty xx. Pod względem jakości ruchu wysoko oceniono także konie z rodziny: T – Chwały, E – Chiazzy xx i E – Igławy xx. Na podstawie przeprowadzonych analiz stwierdzono wysoką wartość użytkową i hodowlaną koni należących do badanych rodzin.

Introduction

An analysis of conformation and motor traits of a horse constitutes a basic criterion of the selection process in its initial phase. In the later stages of selection, a significant role is attributed to an evaluation, carried out with various methods, of the performance and breeding value of animals subjected to the selection process as well as their relatives (NOWICKA-POSŁUSZNA, LISZKOWSKI 2001a, b, GERINGER, KIEŁBASIEWICZ 2004). Knowledge of the performance and breeding values of individuals belonging to sire's lines and dam families is of key significance in horse breeding in the selection process of pairs for reproduction. The success of the Polish breeding of pure bred Arabs and foreign breeds, in which practical use is made of the results of analyses of the performance and breeding value of individuals belonging to dam families and sire strains (TAVERNIER 1990, SCHWARK et al. 1993, SPRENGER et al. 1993), point to the indispensability of conducting such analyses without which the selection of individuals for mating is burdened with a higher risk of failing to achieve the assumed selection response.

Material and Methods

The experimental material were mares and stallions from Polish and foreign (Lithuania, Belarus, Ukraine) breeding stations, registered in the Trakehner Association in Poland in the years 2000-2006, in a total number of 322 and 125 individuals, respectively, as well as their relatives. The source of data on the horses were: breeding records kept by the Trakehner Association in Poland, stud books of Wielkopolska breed horses (Kwlkp) (volumes I -VI) as well as registers of sports horses by CHACHUŁA, BUCHOLC-FERENSTEIN (1981) and ŁOJEK (1995).

Based on the data collected, the following analyses were carried out in the study:

1. Pedigree analysis of horses that enabled determination of horses affiliation to mare families. According to the methodology described by SCHILKE (1965), the first mare used in the postwar breeding of Trakehner Horses in Poland was acknowledged as the foundation mare. In front of the name of the family, a letter was added that indicated the origin of mare, i.e.: T – for families established by mares of Trakehner origin, O – for the family in which the foundation mare was of East Prussia origin, E – for the family in which the foundation mare was Thoroughbred, A – for the family in which the foundation mare was Arab, and S – for the family in which the foundation mare originated from other warmblood breeds. In some cases, apart from family name, the name of the mare regarded by some hippologists as the foundation mare was provided in brackets.

2. Qualitative analysis of horses belonging to 33 selected mare families, whose population was not smaller than 5 horses. The conformation and gait of horses were evaluated based on quality classification scale used by international associations of Trakehner Horses Breeders, in which points from 1 to 10 are scored for particular traits (type, conformation, limbs, walk, trot, gallop, general impression). The data collected were elaborated statistically by calculating mean values and standard deviations. The significance of differences between mare families was examined with one-way analysis of variance and Duncan's test using Statistica software (ver. 6.0 model Anova).

3. Analysis of the performance and breeding value of horses belonging to the selected families.

This involved the characteristics of results of performance tests as well as breeding and/or the sports career of selected individuals belonging to the examined families.

Results

Based on the pedigree analysis, 33 mare families were discriminated whose populations counted from 5 to 35 horses. The results of the qualitative evaluation of horses belonging to those families were presented in Table 1.

The mean quality scores of the group of horses selected for analyses (447 animals) accounted for: type – 7.2, conformation – 7.1, limbs – 6.8, walk – 6.9, trot – 7.3, gallop – 7.0, general impression – 7,1, total 49.5 pts.

The highest total quality assessment (x > 51pts.) was noted for horses belonging to the following families: O – Diana, E – Agresja xx, E – Balanda xx, and E – Avesta xx. Horses of these families were characterized by one of the highest mean scores both for the so-called "static" traits (type, conformation and limbs) as well as motor traits, i.e. quality of walk, trot and gallop (Table 1).

Table 1

Mean quality parameters of horses belonging to selected mare families

No.		Family	N	Type		Conformation		Limbs		Walk		Trot		General impression		Gallop		Total	
			n	*x*	*s*	*x*	*s*	*x*	*s*	*x*	*s*	*x*	*s*	*x*	*s*	*x*	*s*	*x*	*s*
1	E	AGRESJA 1947	8	7.8^{a}	1.2	7.6^{a}	0.9	6.6^{b}	0.9	6.9	0.7	7.6^{AC}	0.8	7.7^{cAC}	0.8	7.4	0.7	51.6^{a}	4.1
2	O	ARA 1944 NU	22	7.2	1.0	7.1	0.7	6.7	0.7	6.7^{b}	0.5	7.2	0.6	7.1	0.7	7.1	0.6	49.0	3.2
3	O	ARIZONA 1935	5	7.6	0.5	7.1	0.4	6.9	0.4	6.8	0.8	7.0	0.8	6.4^{b}	0.4	7.0	0.6	48.8	2.4
4	E	AVESTA 1966 (JASIOŁDA XX)	15	7.2	0.7	7.3	0.8	7.0	0.7	7.1	0.8	7.5^{eA}	0.8	7.5^{eA}	0.7	7.4	0.7	51.1^{a}	4.2
5	E	BALANDA 1961	21	7.6	0.6	7.4	0.6	6.8	0.5	7.2	0.8	7.4^{g}	0.8	7.7^{gAC}	0.7	7.4	0.6	51.5^{a}	3.3
6	E	CHIAZZA 1986	7	7.0	1.0	7.1	0.4	6.7	0.7	7.2	0.8	7.1	0.9	7.4^{iA}	0.8	7.1	0.7	49.6	4.8
7	T	CHWAŁA 1944 (LORE)	16	7.0	1.1	7.3	0.7	6.5^{Bd}	0.7	6.8	0.7	7.7^{cAC}	0.7	7.4^{ai}	0.8	7.1	0.7	49.8	3.9
8	O	CYGANKA 1939	21	7.5	0.9	7.3	0.8	6.7	0.7	7.0	0.8	7.4^{a}	0.7	6.9	0.8	7.1	0.7	50.0	4.0
9	E	DAMURA 1962	5	7.2	0.6	6.8	0.6	7.4^{aA}	0.5	7.1	1.3	6.5^{bhB}	0.4	7.2	0.8	6.8	0.4	49.0	3.3
10	E	DĘBORA 1985	9	6.9	0.8	7.1	0.5	6.4^{fhBD}	0.5	6.8	0.4	7.2	0.6	7.1	0.5	7.0	0.5	48.4	2.7
11	O	DIANA 1942 NU	10	7.2	0.8	7.6^{a}	0.9	7.3^{eC}	0.5	7.4	0.8	7.7^{AC}	0.7	7.6^{ikA}	1.3	7.4	0.9	51.9^{ac}	4.7
12	O	DYREKCJA 1937 (ex DIREKTION)	11	7.1	0.7	7.1	0.7	7.0	0.9	6.8	0.9	7.2	0.8	6.9^{h}	0.8	7.2	0.7	49.4	2.9
13	T	GOLDENKAMMER 1943 (ORGEOLA)	10	6.7^{bdA}	0.8	6.9	1.2	6.7	0.8	7.1	0.9	7.4^{a}	0.7	7.0	0.9	6.9	1.1	48.6	5.2
14	O	HORTENSJA 1940	9	7.5	1.1	7.2	0.7	6.4^{dfB}	1.1	7.3	0.7	7.3^{a}	0.7	7.0	0.8	7.0	0.9	49.8	3.4
15	E	IGŁAWA 1957	13	7.5	0.6	7.5^{c}	0.8	7.2^{g}	0.7	6.8	0.5	7.3^{a}	0.7	7.3^{a}	0.8	7.4	0.7	51.0	3.0
16	O	JAGODA 1939	13	7.4	0.7	7.3	0.7	6.9	0.6	6.8	0.4	7.5^{eA}	0.7	7.2	0.5	7.4	0.5	50.5	2.7
17	E	JOLANTINA 1955	6	7.8^{eB}	1.3	7.5^{c}	1.0	6.4^{dfB}	1.2	6.8	0.9	7.1	1.2	6.8^{dh}	1.2	7.2	1.2	49.6	7.0
18	O	KONTUZJA 1942 NU	19	7.0	1.0	6.9	1.0	6.7^{b}	0.6	7.2	0.8	7.1	0.7	6.9	0.8	7.1	0.8	48.7	4.1
19	O	KORSYKA 1940	20	7.6	0.6	7.2	0.7	6.8	0.4	6.6^{b}	0.6	6.9^{d}	0.6	6.9^{h}	0.6	7.2	0.5	49.2	2.4
20	O	KRASKA 1942 NU	10	7.1	0.6	7.0	0.7	7.1	0.6	7.5^{a}	0.6	7.2	0.6	6.8^{dh}	0.6	6.9	0.5	49.4	2.7
21	O	LOŻA 1936	9	6.9	1.1	7.0	0.8	6.5^{dB}	0.4	6.7	0.7	7.4^{eA}	0.5	6.9	0.8	7.1	0.5	48.7	3.4
22	O	MALWA 1944 (AURORA)	9	7.0	0.8	6.9	0.5	6.5^{dB}	0.9	7.0	0.8	6.9^{d}	0.8	6.6^{fjD}	1.2	6.9	0.9	47.9	5.1
23	O	NAJADA 1943	8	7.5	1.1	7.4	0.8	6.8	0.8	6.6^{b}	1.2	7.2	0.6	7.2	0.6	7.1	0.7	49.7	4.6
24	T	NORMA 1943 (GAWAŃ 1955)	19	7.2	0.7	7.1	0.8	6.5^{dfB}	0.6	6.9	0.8	7.1	0.7	7.1	0.7	7.2	0.6	49.1	3.4
25	O	PANIUSIA 1941 NU	11	6.8^{bfh}	0.6	6.6^{bd}	0.5	6.5^{bd}	0.5	6.7	0.4	6.6^{fD}	0.6	6.7^{dh}	0.6	6.8	0.6	46.9^{b}	2.3

cont. table 1

No.	Family	N	Type		Conformation		Limbs		Walk		Trot		General impression		Gallop		Total	
		n	*x*	*s*	*x*	*s*	*x*	*s*	*x*	*s*	*x*	*s*	*x*	*s*	*x*	*s*	*x*	*s*
26	O PARADA 1937	24	7.4	1.0	7.4	0.7	6.9	0.7	7.1	0.6	7.5^{eA}	0.9	6.9	0.9	7.3	0.8	50.5	4.8
27	T PARTIE 1944 (PIPETKA)	21	7.2	0.7	6.7^{b}	0.8	6.5^{bD}	0.8	6.7	0.7	6.9^{d}	0.6	6.7^{dhl}	0.8	6.8	0.8	47.5^{d}	4.1
28	O RURKA 1939 NU	11	7.7^{cg}	0.7	7.2	0.8	6.8	0.6	7.0	0.8	7.6^{AC}	0.7	7.0	0.7	7.3	0.6	50.6	4.1
29	O STOKROTKA 1944 NU	38	7.0	0.7	7.1	0.7	6.9	0.6	6.9	0.8	7.2	0.7	7.0	0.7	7.0	0.7	49.2	3.4
30	O WEŁTAWA 1936 (exWELLENBRANT)	18	7.1	0.8	6.8	0.9	6.7	0.6	6.8	0.5	7.0	0.7	6.8^{dh}	0.8	6.9	0.7	48.2	3.9
31	E WENDA	7	7.7^{ej}	0.8	7.4	0.9	7.2^{e}	0.6	7.1	0.7	7.1	0.8	7.1	0.7	7.2	0.8	50.7	3.9
32	O WENUS 1940 NU	16	6.9^{f}	0.8	7.2	0.4	6.8	0.7	7.0	0.6	7.3^{a}	0.7	6.9^{h}	0.7	7.0	0.7	49.1	3.5
33	O WISIENKA 1942 NU	6	6.8^{bfhj}	0.6	7.3	0.8	6.7^{b}	0.7	6.8	0.8	7.8^{cAC}	0.8	7.0	0.9	7.1	1.0	49.3	4.6
	Total	447	7.2	0.8	7.1	0.8	6.8	0.7	6.9	0.7	7.3	0.7	7.0	0.8	7.1	0.7	49.5	3.8

Means denoted with successive letters "*ab*" are significantly different, means denoted with successive capital letters "*AB*" are highly significantly different

In terms of type, conformation and limb build, high quality scores were also given to individuals from the families: E – Wenda xx (type – 7.7, conformation – 7.4, limbs – 7.2 pts.), and E – Igława xx (type – 7.5, conformation – 7.5, limbs – 7.2 pts.). In turn, horses of the following families: O – Rurka NU (type – 7.7, conformation -7.2, limbs – 6.8 pts.) and E – Jolantina xx (type – 7.8, conformation – 7.5, limbs – 6.4 pts.), with relatively good scores for type and conformation and relatively low rates for limb build.

In the case of gait quality of the horses examined, the highest (statistically significant) mean rates for trot and gallop were reported for the above-mentioned individuals belonging to the families of mares: E – Balanda xx (trot – 7.4, gallop – 7.7 pts.), E – Agresja xx (trot – 7.6, gallop – 7.7 pts.), O – Diana (trot – 7.7, gallop – 7.6 pts.), E – Avesta xx (trot – 7.5, gallop 7.5 pts.), as well as those belonging to the families of mares: T – Chwała (trot – 7.7, gallop – 7.4 pts.) E – Chiazza xx (trot – 7.1, gallop – 7.4 pts.), and E – Igława xx (trot – 7.3, gallop – 7.3 pts.).

Good quality trot was reported for horses belonging to the families of East Prussia mares (O): Wisienka, Rurka, Parada and Jagoda. Mean rates for trot quality obtained by individuals from those families accounted for 7.8, 7.6, 7.5 and 7.5 pts., respectively. In turn, the quality of gallop and walk of these horses was evaluated at a level of the mean for the population under scrutiny.

The mean rate for walk quality of all horses (x = 6.9 pts.) was the lowest amongst the three gaits evaluated. The highest mean score was reported for horses from the family of O – Kraska (7.5 pts.), whereas the lowest was for those from the families of O – Korsyka (6.6 pts.) and O – Najada (6.6 pts.).

From the point of view of extending the period of horse exploitation, of significance is limb build. The high rates for this trait, in respect of the entire population examined (x = 6.8 pts.), were noted for horses of the following mare families: E – Damura xx (7.4 pts.), O – Diana (7.3 pts.), E – Igława xx (7.2 pts.), E – Wenda xx (7.2 pts.). Some lines were characterized by a low mean rate for limb build and relatively high rates for the quality of trot or gallop (O – Wisienka, E – Agresja xx, O – Loża, T – Chwała, O – Hortensja). Such a situation is likely to exert a negative effect on the length of exploitation of horses from these families and, thus, on their performance value.

In addition, an analysis of quality points found the worst results of the qualitative assessment for representatives of mare families: T – Partie and O – Paniusia. The horses of these families obtained low mean rates for type, conformation and limbs as well as for the quality of gaits. The low quality gaits were also observed for individuals originating from the following families: O – Korsyka, O – Malwa and to a lesser extent for those of O – Arizona (poor rates for gallop). In turn, horses from the families: O – Wenus and O – Wisienka presented an undesired type and faulty limbs.

Performance and breeding value of families

The family of T – Chwała mare foaled in 1944

A significant measure of the performance value of horses is the number of progeny with a high performance and/or breeding value. In this respect, one of the most distinguished families in the breeding of the horses examined is that of the Chwała mare. Two champions of Trakehner stallions originate from that family, i.e.: CEDRUS foaled in 1993 (by Ogar out of Cerkwica by Kondeusz) of 2004 and CZEDAR foaled in 2003 (by Ignam out of Czarelia by Akcept xx), the V-Champion of the I International Northeastern Trakehner Championship of 2006. Another stallion originating from this family – CHARLY BOY foaled in 2005, son of Ignam and Czarelia's daughter, Czarna Dama (by Aragonit), in 2006 became the V-Champion of two-year-old stallions in the Trakehner Horses Championship in Dąbrówka Mała.

In the past, many horses of this family have participated in the highest national and international equestrian competitions, including CHRENOWSKA foaled in 1958 (by Chryzolit out of Gondola by Guido), CZAPRAK foaled in 1979 (by Poprad out of Cimarosa by Surmacz xx), CZARDASZ foaled in 1978 (by Kobryń out of Czarnobrewa by Surmacz xx), CZARTER foaled in 1982 (by Poprad out of Cimarosa by Surmacz xx), CHEŁBIA foaled in 1978 (by Kobryń out of Chluba by Kosmos) – the dam of mare CHEŁMŻA foaled in 1983 (by Kerman) sold to the USA (KULISA et al. 2001) and of stallion CZERSK foaled in 1977 (by Arrigle Valley xx out of Czesanka by Colombo) – a participant in the international championship in the Three-Day Event, and the European Junior Championship in 1984 (ŁOJEK 1995). Currently, the stallion CZAR foaled in 1998 (by Lwów out of Czantoria by Aragonit) in participates in competitions and is currently used in breeding.

Family of T – Goldenkammer mare foaled in 1943 (Orgeola)

One of the horses registered in the Trakehner Association in Poland, from the mare family Goldenkammer, is the mare OPOKA (by Ignam out of Orka by Huk), a Champion of 3-Year-Old and Older Mares of the Polish Championship of Trakehner Horses in Dąbrówka Mała in 2006 and the winner of the Field Trial of Mares in Wozławki in 2006. In the same year, this mare took IV place at the I International Northeastern Trakehner Championship in Dąbrówka Mała. Worthy of notice are also the very highly evaluated mares (56.5 pts. each): ORAWKA (by Arak out of Orisawa by Aspirant) and her daughter OLIWIA LIVE (by Live and Liberty).

The horses of this family bred in the Horse Stud Liski are characterized by very good performance traits (KULISA et al. 2001), and the dam mares have a low index of foal loss as well as by a very high per cent of progeny approved to breeding (WEJER, TOMCZYŃSKI 2001). Many of them have been exported abroad. This family also includes the sport and breeding stallion OGAR (by Aspirant, out of Orgietka by Cross xx), a sire of five sons who completed a performance test in the training centre (BYSZEWSKI 1996), including stallion Cedrus (out of Cerkwica by Kondeusz) the winner of the title of Stud Champion during the Polish Championship of Trakehner Horses in Liski in 2004.

Family of O – Ara, a mare foaled in 1944

A family numerously represented in the Trakehner Association in Poland is the family of the mare O – Ara. This family includes the mare Alpeja (by Apogej out of Alga by Dzięgiel) foaled in 2005, which has twice has won the title of Champion in its category in the Polish Championship of Trakehner Horses in 2005 and 2006.

This is the family of origin to a number of valuable breeding and sports horses, including ASTRAHAŃ (by Harbin out of Arteria by Akcjonariusz) and ARETIT (by Doliniarz out of Arteria by Akcjonariusz). Both horses were very highly classified in the "Breeding ranking – reproducers and sport" issued by the World Breeding Federation for Sports Horses, respectively placing 311 out of 1400 horses in jumping and 265 in dressage (SCHILAK, SIEDLANOWSKI 2005).

Another representative of this family is ARIANIN, foaled in 1980 (by Sopran out of Arieta by Bard), a participant in the Polish Championships in Jumping and international CSIO and CSI competition of obstacles up to 150cm (ŁOJEK 1995). This stallion is the sire of such horses as: Dziurawiec (out of Dzięcielina by Karkas), Szamyl (out of Szamanka by Karkas), or the mare Gracja (out of Garsonka by Karkas), which have won medals at the Polish Show of Breeding Horses, as well as of the stallion Makart (out of Makia by Karkas) and the mare La Rochelle (out of Libora by Arak), who won the titles of, respectively, Stud Champion in 2002 and Mare V-Champion in 2004 at the Polish Championship of Trakehner Horses.

Arianin has produced a number of top-class sport horses participating in jumping shows in the competition of obstacles up to 150 cm in height inclusive, i.e. among others: Posejdon (out of Promocja by Mapnik), Szkopuł (out of Szkuna by Karkas) or Makart (out of Makia by Karkas).

The Ara family has also produced such horses as: ARLEKIN (by Szaser out of Arabeska by Deer Leap), ASZTOR (by Szewron out of Arta by Sambór), who

took second place at the Polish Championship of Young Horses in Jumping in the group of five-year-old horses in 2004, as well as ARHUS (by Gordon out of Artemida by Posesor xx) a Champion of the Polish Show of Breeding Horses in Warsaw. This is also the family of origin of ARBÓR (by Sambór out of Ardila by Deer Leap) a valuable stallion, a sire of the mare Chmura (out of Chiazma by Aragonit) who won the title of Mare Champion at the Polish Championship of Trakehner Horses in Liski in 2004 (SCHILAK, SIEDLANOWSKI 2005).

Family of O – Arizona, a mare foaled in 1935

The family of Arizona has produced 3 horses that have won the titles of V-Champions of Trakehner Horses: ARAGORN, V-Champion of One-Year--Old Stallions (Olsztyn 2005) and AGITACJA, V-Champion of Two-Year-Old Mares (Liski 2004). Both the horses are by the Hamlet Go stallion out of AGADA mare (by Agar). Another V-Champion of One-Year-Old Mares (Polish Championship of Trakehner Horses – Dąbrówka Mała 2006) was ASPERA VENTI, a daughter of Asyria (by Huk out of Agady) and Apogej, whose progeny is characterized by a desired type of good conformation (PIETRZAK et al. 2004).

This is also the family of origin of other valuable horses: ARAGONIT (by Kobryń out of Aragonia by Dzięgiel xx), for years being a breeding stallion of the Horse Stud Liski, a sire of numerous horses of great beauty, good gait and jumping style (KACZMAREK 1995), his halfbrother ARION (by Poprad) a competitor in many international jumping shows, whose coefficient of success was 6.53 (ŁOJEK 1995), and the mare AKOLADA (by Dziwisz xx out of Aragwa by Haakon) a dam of the breeding stallion Agar (by Mangan) characterized by very good gait and jumping abilities (CHOMENTOWSKA-FANGRAT 1997).

Family of O – Cyganka, a mare foaled in 1939

Mares of the Cyganka family registered in the Trakehner Association in Poland constitute valuable breeding material. Most of them have participated in horse riding competitions or originate from dams entered in jumping shows. These horses include: CYRANKA II foaled in 1986 (by Arianin out of Cyrenajka by Karkas), CYLLA foaled in 1992 (by Libellus out of Cyranka II by Arianin), CERA foaled in 1984 (by Narwik out of Cecor II by Cekin), CZARKA foaled in 1990 (by Czubaryk xx out of Cyrenajka by Karkas). These mares were mated with proven sport stallions: Ignam (by Akropol out of Inna), Elpar xx (by Parole Board xx out of Elegia xx), Gluosnis xx (by Gerodot xx out of Glorija xx), Arianin (by Sopran out of Arieta), Dłużnik (by Akropol out of Dłużyna), Radiator (by Aragonit out of Regina), Czubaryk xx (by Erotyk xx out

of Czeczma xx), Pigmalion (by Arak out of Plejada), and Arak (by Parysów xx out of Arka).

One of more valuable breeding mares belonging to the family of Cyganka is CENTURIA foaled in 1986 (by Arianin out of Cecora II by Cekin), a dam of two approved stallions: CELLO (by Liman) and CENZOR (by Pigmalion), as well as the mare CENTRA (by Elpar xx), the Champion of Three-Year-Old and Older Mares at the Polish Championship of Trakehner Horses in Olsztyn in 2005.

Family of O – Diana, a mare foaled in 1942

In the opinion of PIETRZYK, WĄSOWSKA (1974), horses of Diana family, bred in the Horse Stud Rzeczna, have been characterized by good health condition, lively temperament, proper conformation, good gait and very good predisposition to jumping.

A number of outstanding sports horses have originated from that family, including: CONDYLUS foaled in 1958 (by Szczecin xx out of Con Amore), which in 1964 when ridden by Michał Siemion took 3rd place in the Polish Dressage Championship, as well as the mare DIGESTA foaled in 1960 (by Dreibund out of Diana), which competed successfully in the years 1968-1969 in international jumping shows (CHACHUŁA, BUCHOLC-FERENSTEIN 1981). Having finished its sports career, the mare foaled, among others, the excellent stallion DIOGENES foaled in 1967 (by Elew), a winner of the following titles: V-Champion of Poland in 1972 and Champion of Poland in Dressage in 1973 (CHACHUŁA, BUCHOLC-FERENSTEIN 1981).

Another horse distinguished in sport originating from this family is DAMAZY, foaled in 1969 (by Elew out of Diagola), a participant in the Olympic Games in Moscow in 1980 and a double medalist at the Polish Championship in Dressage in the years 1978-1979 (CHACHUŁA, BUCHOLC-FERENSTEIN 1981).

Another two stallions belonging to this family are: CELEBES foaled in 1993 (by Kondor out of Cedrówka by Akropol) and CEFAL foaled in 1995 (by Gordon out of Ceduła by Akropol). The first was the champion amongst four-year-old horses in Dressage at the Championship of Young Horses held in 1997. A year later, at another championship of this rank, it repeated the success by taking 2nd place amongst five-year-old horses (TOMASZEWSKI 1999). Ridden by Joanna Włoczewska, it was highly classified in numerous national and international competitions (BEK-KACZKOWSKA 1998). In 2003, this stallion also won the title of Stallion Champion at the Polish Championship of Trakehner Horses in Sułowo. In turn, CEFAL has been a participant in multiple national championships in Jumping.

Family of O – Jagoda, a mare foaled in 1939

The family of the mare Jagoda has been developed mainly in the Horse Stud Rzeczna. In this Stud, it constituted one of the most numerous families, and according to PIETRZYK, WĄSOWSKA (1974), mares of this family are characterized by high fertility, valuable progeny and very good character.

The family of Jagoda has produced the mare DORYJKA foaled in 1958 (by Dreibund out of Doryda by Sorent), a dam of a few good sports horses exported abroad and of the breeding stallion DONBAS foaled in 1977 (by Drelink). The latter occupied the box of a breeding stallion in the maternal stud in the years 1981-1985, being a sire of a number of good breeding mares. Out of Doryjka also originates the mare DAGLEZJA foaled in 1977 (by Bard), a dam of the gelding DAMOKLES foaled in 1994 (by Kaliniec), entered successfully in the national championship in the competition of Jumping (WITKOWSKA 2005).

A daughter of DAGNA (by Akcjonariusz) and Oltis xx was DANUBIA foaled in 1981, a Champion of the Polish Show of Livestock in Olsztyn in 1984 (GĄGOROWSKI 1984). This mare was characterized by exceptional beauty, mild character, and excellent jumping predispositions. Her grand-daughter, DURRA foaled in 1994 (by Kaliniec out of Dubla by Kondor), was awarded the title of V-Champion of Wielkopolska breed mares at the XIV National Show of Livestock in Warsaw at Służewiec (CZEŚNIK 1998).

In turn, JASTARNIA, foaled in 1992 (by Makart out of Jamajka by Jen), is a dam of JOWISZ (by Aragonit), competing in Dressage and presenting outstanding gaits and a high level of training, as well as of the mares Jurata, (by Gluosnis xx) the V-Champion of Foals at the Polish Championship of Trakenher Horse in Dąbrówka Mała in 2006, and JOVITA, the winner of the Championship of Foals in Galiny in 2004 (SCHILAK, SIEDLANOWSKI 2005).

Family of O – Kontuzja, a mare foaled in 1942

One of the most numerous families in the Trakehner Association in Poland is the family of the mare Kontuzja. It is the family of origin to a number of excellent sports horses, including: the mare KOSA foaled in 1966 (by Dorwid xx out of Komosa by Ditto), that in 1973 took 3nd place at the Polish Championship in Three-Day Event (CHACHUŁA, BUCHOLC-FERENSTEIN 1981), as well as the mare KOPRA foaled in 1969 (by Eliop out of Konchita by Hidalgo), in the years of 1975-1976 entered for a Dressage competition (ridden by Wanda Wąsowska).

Worthy of mention is also the mare KLIWIA foaled in 1977 (by Bard out of Kielcza by Dyskobol), which foaled with stallion Ignam (by Akropol out of Inna

by Sobiepan) two stallions talented in jumping and used in breeding. The first of them – KALINIEC, foaled in 1988 with an impressive sports career, has left in progeny in the Rzeczna Horse Stud with jumping predispositions. The second son of Kliwia – the stallion KLEON, foaled in 1989, ridden by Anna Małkowska was 6 times a medalist of the Polish Junior Jumping Championship, and in 1999 it won the Cup of Nations in Hamburg and 4th place at the CSIO in Poznań. This pair represented Poland five times at the European Junior and Young Riders Jumping Championship (DESZCZYŃSKA 2005).

The family of Kontuzja, represented by a number of horses in the Horse Stud Rzeczna, have also produced very good mares that have been exported abroad (WITKOWSKA 2005). This is also the family of origin of the mare KLAUDYNA, foaled in 1985 (by Akropol out of Kliwia by Bard), the winner of the Champion title at the Polish Championship of Trakehner Horses in Sułowo in 2003 (CHOMENTOWSKA-FANGRAT 2004) and the mare KALIA (by Winword xx, out of Kadencja by Wiadukt), the V-Champion of Three-Year-Old and Older Mares at the Polish Championship of Trakehner Horses in Dąbrówka Mała in 2006, and in the same year the winner of 7th place at the I International Northeastern Trakehner Championship in Dąbrówka Mała.

Family of O – Korsyka, a mare foaled in 1940

In the past, the most valuable horses originating from the family of Korsyka included two stallions: KOSMOS foaled in 1961 (by Belizar out of Komonica by Celsius) and KONDEUSZ foaled in 1969 (by Colombo out of Korsarka by Dreibund) that for many years have been occupying boxes of breeding stallions and have produced a number of very good horses (CHACHUŁA, BUCHOLC-FERENSTEIN 1981).

Several mares have also had sports careers: KAPUA, foaled in 1957 (by Dreibund out of Apuila by Cyklamen), which after finishing its sports career in Poland was exported to Germany, and KODEINA, foaled in 1985 (by Dziwisz xx out of Kutyna by Poprad). The latter, apart from winning the titles of Champion and V-Champion of Poland in Jumping (ridden by Grzegorz Kubiak), also participated in the European Championship of Young Riders and in a number of international competitions (ŁOJEK 1995).

This family also included the gelding KUŁAN (by Mangan xx out of Kutykuła by Kerman), who in 1996 participated in the final of the Championship of Young Horses in Jumping held in Sielinek in the category of five-year--old horses (KRZYŻANOWSKI 1996). In turn, KORYNTIA (by Czak out of Korga by Akcept xx) won the title of V-Champion of Two-Year-Old mares at the International Northeastern Trakehner Championship in 2006.

Most of mare dams originating from the family of Korsyka, registered in the Trakehner Association in Poland, have been subjected to performance tests for mares and completed them with very good and good final scores (PIETRZAK 2001).

Family of O – Loża mare foaled in 1936

The family of Loża includes the recognized stallion LORD ODER, foaled in 2002 (by Oder out of Lebioda by Ogar), a Champion of Three-Year-Old and Older Stallions at the Polish Championship of Trakehner Horses in Olsztyn in 2005. Foaled in 1987 LAVABO (by Aragonit out of Lawenda by Cynik xx) became the V-Champion of Wielkopolska Breed Horses at the VIII National Show of Livestock in 1992 (KULISA et al. 2001).

The group of award-winning sport horses in the past originating from this family and was characterized by a high coefficient of success and presenting outstanding sports achievements (mainly in jumping) includes such horses as: stallion LAMPART (by Poprad out of Lama by Ciecieruk) – 6.98 as well as the geldings Liban (by Kobryń out of Laba by Kondeusz) – 2.06; LORD (by Kondeusz out of Lamówka by Bułat) – 2.21; and LOTAR (by Kondeusz out of Lotaryngia by Pietuszok oo) – 3.44 (Łojek 1995).

In 1994, TURBUD LWIE SERCE, a son of LWICA (by Harbin out of Lama I by Ciecieruk) and the stallion Czynel xx, took the second place at the Championship of Four-Year-Old Horses in Jumping (CZEŚNIK 1994).

Family of O – Malwa mare foaled in 1944

One of the best-known horses of the family of Malwa is MAKART, foaled in 1987 (by Arianin out of Makia by Karkas), a Champion of Three-Year-Old and Older Stallions at the Polish Championship of Trakehner Horses in Plękity in 2002. This gold medalist of the Training Centre, a breeding stallion of the Horse Stud Rzeczna and Racot, has been successful in a number of Grand Prix competitions in Jumping. Its brother MIDAS, a participant of the Championship of Young Horses in Jumping in the age category of six-year-old horses (SCHILAK, SIEDLANOWSKI 2005) was also successful in that competition.

The family of Malwa also includes the stallion MARKUS (by Perkoz xx out of Malsza by Schwertbruder), entering a number of CSI and CSIO in the Jumping competition and in a Three-Day Event (classified 36 times in places 1-4), as well as a mare competing in Jumping: MACEDONIA (by Orkisz xx out

of Mapa by Priz xx -34 times in places 1-4) and MODA (by Perkoz xx, out of Modystka by Dekander xx – classified 51 times in places 1-4) (Łojek 1995).

During their sports or breeding career, a number of horses from this family have been sold abroad, including: MALAJKA (by Sopran out of Makia by Karkas), MAGA (by Gawot out of Makia by Karkas) and two sons of MALADETTA (by Arianin): the stallion MAKLER (by Gosler) and MALERIN (by Arak) (SCHILAK, SIEDLANOWSKI 2005).

Family of O – Rurka mare foaled in 1939

The family of Rurka include two winners of the Polish Championship of Trakehner Horses. The mare R-LADY (by Ignam out of Regina by Elpar) which became a V-Championship of Two-Year-Old Mares (Liski 2004). In turn, out of this mare and by the internationally-recognized stallion Abdullah there originated a Champion of Foals – the mare RAFIA (Dąbrówka Mała 2006).

Mare RADNA (by Bard) is a dam of Ral foaled in 1992 (by Dzięgiel), that twice (in 1996 and 1997) completed the Championship of Young Horses in Jumping in the final tenth. In 2002, it took 3rd place in a Grand Prix Zbrasłowice (the All-Polish Competition). In turn, ROSANA, foaled in 1999 (by Jantar xx out of Rata I by Karkas) completed the performance test with a very good result (mean score of 7.7 pts.), and was distinguished by free jumps (9 pts.) and the result of the test of an independent rider (8 pts.).

Family of O – Stokrotka mare foaled in 1944

Horses from the family of Stokrotka registered in the Trakehner Association in Poland in most cases have been bred in the Plękity Horse Stud. Originating from this stud, the stallion SŁOWAK foaled in 1996 (by Pigmalion out of Sida by Dzięgiel) in 1999 passed the performance test of the Training Centre Kwidzyn. In 2005, the mare SAKSONIA foaled in 2004 (by Elpar xx out of Salwa by Libellus) also completed the performance test with a very good result.

Other mares originating from this family and currently used in breeding: SELIA foaled in 1992 (by Libellus out of Sewilla by Arianin); SVENA foaled in 1990 (by Arak out of Sylva by Splendor), and SIKORKA foaled in 1995 (by Pigmalion out of Sida by Dzięgiel) also participated in jumping shows in the competition of obstacles up to 130cm in height. In addition, the later is a dam of SENIORITA foaled in 2004 (by Radiator out of Sikorka by Pigmalion) the V-Champion of One-Year-Old Mares at the Polish Championship of Trakehner Horses in Olsztyn in 2005.

Family of E – Agresja xx, a mare foaled in 1947

The family of E – Agresja xx includes ASPIRACJA, foaled in 2005 (by Safran out of Alana by Cedrus) – the V-Champion of Foals at the Polish Championship of Trakehner Horses in Olsztyn 2005, a mare with a very good type and conformation as well as very good basic gaits. The mare ANARCHIA (by Kerman) is a dam of stallion Armatic foaled in 2005, the V-Champion of the Championship of Trakehner Horses in Dąbrówka Mała in 2006. A sire of the latter is the stallion Gluosnis xx – a six-time medalist of the Championship of Lithuania (including, double winner of gold medal in 2002 and 2003), Indoor Champion of Lithuania in 2004, and a participant of multiple CSI competitions (http://ogiery.romanowski.pl/gluosnis.htm).

This is also the family of origin of AGRIN (by Akcept xx out of Agenda by Aspirant), a stallion which in 1996 completed the Training Centre with an outstanding score (Byszewski 1996).

Family of E – Avesta xx, a mare foaled in 1966

Horses of the Awesta xx mare family, mostly originating from the Horse Stud Plękity, are the progeny of stallions talented in jumping, including: Ignam (by Akropol out of Inna), Arianin (by Sopran out of Arieta), Sopran (by Perkoz xx out of Sobietaka), Burgund xo (by Juriste xxoo out of Blanka xo), Karkas (by Elew out of Komosa), Hades xx (by Lincoln xx out of Harpia xx) or Elpar xx (by Parole Board xx out of Elegia xx).

The mares registered in the Trakehner Association in Poland include three champion mares in the national Trahehner Championships: ALIZE (by Ignam out of Adina by Pan Franek,) which in 2004 won the title of the V-Champion of Foals, AVRIL (by Elpar xx out of Avka by Wiec) in 2005 and 2006 awarded the title of the V-Champion of Mares, and AMANDA (by Radiator out of Avola by Arianin) which in 2005 won the title of Champion of One-Year-Old Mares. A dam of the latter, AVOLA foaled in 1985 (by Arianin out of Avra by Karkas), participating in jumping shows, and is also a dam of 5 mares approved for breeding in the Horse Stud Plękity and two stallions that completed the performance test for stallions: ASTOR (by Liman) and ALL (by Libellus).

Other horses of this family have had sports career or completed performance tests. A daughter of Arianin, ANORTA foaled in 1989 (out of AVRA by Karkas), as well as the mare ALLA foaled in 1993 (by Libellus out of Ava by Sopran) and the stallion AL PARI foaled in 1994 (by Elpar xx out of Avra by Karkas), have entered jumping shows in the competition of obstacles up to 130 cm. In turn, the stallion AHMED foaled in 1998 (by Alkierz out of Avaha

by Hades xx) in 2004 took I place at the Polish Championship of Young Horses in the Three-Day Event (SCHILAK, SIEDLANOWSKI 2005), whereas the mare AUKCJA foaled in 2002 (by ELpar xx out of Avola by Arianin) in 2006 completed the performance test with a good result.

Family of E – Balanda xx, a mare foaled in 1961

The family of Balanda xx is another family whose horses are characterized by a high utility value, as indicated by results of performance tests. This is the family of origin of the gelding SILVANT-BORSALINO 1991 (by Kerman out of Blenda by Aragonit) which in 1996, when ridden by Mieczysław Zagor, took seventh place in the final classification of 5-year-old horses of the Championship of Young Horses in Jumping in Bielinek (KRZYŻANOWSKI 1996). A very valuable mare seems to be BITWA, foaled in 1993 (by Jaguar xx, out of Blenda by Aragonit), with a qualitative score of 53.0 pts. a dam of Two-Year-Old Stallions Champion BELLO NEMO (by Burgund xo) as well as BIGNAM and the mare BELLA IKA (both horses by an outstanding sports horse Ignam). In 2004, at the Polish Championship of Trakenher Horses in Liski, these siblings became the Champion and V-Champion, respectively, in their age categories. Two years later, the stallion BIGNAM took the second place in the group of three-year-old and older stallions at the Polish Championship of Trakehner Horses and the sixth place at the International Northeastern Trakehner Championship in Dąbrówka Mała in 2006. Out of that mare there originate also highly evaluated daughters of stallion Aragonit: BELLA DONNA (57 quality pts.) and BELLA LANCASTRA (54.5 quality pts.). Both mares were subjected to a performance test and completed it with very good result.

Family of E – Igława xx, a mare foaled in 1957

A number of horses from the family of Igława xx have demonstrated high jumping predispositions. This is the family of origin of numerous sports horses. One of them was the mare IGARKA, foaled in 1970 (by Etymolog out of Igława xx by Pilade xx) which ended its sports career as one of the best sports horses entered for the Three-Day Event in Poland, and the stallion IGREK foaled in 1975 (by Colombo). It participated many times in the Polish Championship in the Three-Day Event, winning the gold medal in that competition in 1988. This pair participated in also, among others: The Olympic Games in Barcelona in 1992, The World Championship in Stockholm in 1990, and in The European Championship in the years 1987 and 1990 (ŁOJEK 1995).

A daughter of Igława xx, the mare INNA foaled in 1972 (by Sobiepan), was very successful in racing. By winning 3 x I, 4 x II, 1 x III and 1 x IV, it reached a coefficient of success of 2.80 (ŁOJEK 1995). This mare was a dam of the stallion IGNAM foaled in 1980 (by Akropol), that took high places in Grand Prix-level international championships in the years 1985-1990, and participated in the final of World Cup in Goeteborg. Its coefficient of success was as high as 10.71, whereas in the classification of horses entered for the competition of Jumping, it took the 6th place out of 2204 horses under evaluation (ŁOJEK 1995). In addition, Ignam was a sire of a number of outstanding breeding horses demonstrating high jumping abilities, including: Kaliniec, Kleon, Bignam, Czedar, or Ignac. The latter originating from the mare IGA of the Igława xx family, foaled in 1988 (by Gordon out of Ifigenia by Cerber) and sold to Lebanon. Out of the same dam originated the stallion IMPULS foaled in 1993 (by Agat), which is currently entered for the competition of Jumping.

A former sports champion, currently used in breeding, is the mare MODELKA (by Midas, out of Ilona by Gordon I) the winner of I place in the class of 11-Year-Old and Older Mares at the Polish Championship of Trakehner Horses in Olsztyn in 2005.

Summary and Conclusions

Summing up the analyses it can be concluded that:

1. The highest qualitative evaluation was reported for horses belonging to the families of the mares: O – Diana, E – Agresja xx, E – Balanda xx, and E – Avesta xx. They obtained high scores for type and conformation as well as for the quality of presented basic gaits. In terms of gait quality, high quality scores were also given to horses of the families of: T – Chwała, E – Chiazza xx, and E – Igława xx. Horses of the families of: O -Wisienka, E – Agresja xx, O – Loża, T – Chwała, and O – Hortensja obtained relatively low mean scores for limb build which, despite their motor values, may negatively affect the length of their exploitation, and thus their performance value.

2. Results of the analysis of conformation and gait of horses belonging to the families examined may constitute crucial information to breeders, enabling the proper selection of pairs for mating in order to obtain a selection response.

3. The analysis carried out based on sports results points to the high performance and breeding value of horses belonging to the examined families of mares. The population of Trakehner Horses and those of Trakehner-East Prussia origin bred in Poland constitutes valuable genetic material that may be used in improving other half-breeds.

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THE INFLUENCE OF HYDRAULIC RETENTION TIME AND SLUDGE AGE ON ACTIVATED SLUDGE BIOCENOSIS FORMATION IN SBRs TREATING LANDFILL LEACHATES

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K e y w o r d s: landfill leachate, SBR, activated sludge biocenosis, microfauna, hydraulic retention time, sludge age.

A b s t r a c t

In this study the influence of hydraulic retention time (HRT) and sludge age on organic substances removal (COD), nitrogen removal and formation of activated sludge biocenosis in sequencing batch reactors (SBRs) treating municipal landfill leachate were investigated. Two series were performed (series 1 and 2). Each of them was conducted at HRT 12, 6, 3 and 2 d. Series 1 and 2 were differed in sludge age. In series 1, sludge age was over 2-fold longer than in series 2, which was obtained by volume control of suspended solids disposed in SBR operating cycle.

The efficiency of organics removal from leachate (as COD) in series 1 decreased from 82.9% (HRT 12 d) to 70.5% (HRT 2 d) and in series 2 from 83.5% to 71.9%, respectively. Complete nitrification and the ammonia nitrogen concentration in the effluent below 1 mg dm^{-3} was noticed in series 1 at HRT of 3 d and longer.

In activated sludge, there were examined 20 taxons. Their number depended mainly on sludge age. At sludge age shorter than 16 d only 5-4 taxons were present. The number of individuals depended both on HRT and sludge age. The richest communities were observed at 12 d HRT in both series. At HRT of 6, 3 and 2 d the total number of individuals was clearly higher in series 1 than in series 2. Type 0092 was dominated among filamentous bacterium.

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WPŁYW HYDRAULICZNEGO CZASU ZATRZYMANIA I WIEKU OSADU NA KSZTAŁTOWANIE BIOCENOZY OSADU CZYNNEGO W REAKTORACH SBR OCZYSZCZAJĄCYCH ODCIEKI ZE SKŁADOWISK ODPADÓW

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S ł o w a k l u c z o w e: odcieki, SBR, biocenoza osadu czynnego, mikrofauna, hydrauliczny czas zatrzymania, wiek osadu.

A b s t r a k t

W pracy badano wpływ czasu zatrzymania (HRT) i wieku osadu na usuwanie zanieczyszczeń organicznych (ChZT) i azotu oraz kształtowanie się biocenozy osadu czynnego w reaktorach SBR oczyszczających odcieki ze składowisk odpadów komunalnych. Przeprowadzono dwie serie badawcze (seria 1. i 2.). W każdej z nich badania prowadzono dla czasu zatrzymania (HRT) równego 12, 6, 3 i 2d. Seria 1. i 2. różniły się wiekiem osadu. W 1. był on ponad 2-krotnie dłuższy niż w 2., co uzyskiwano poprzez regulację ilości odprowadzanego osadu nadmiernego w cyklu pracy SBR.

Efektywność usuwania zanieczyszczeń organicznych (ChZT) z odcieków w serii 1. malała z 82,9% (HRT 12d) do 70,5% (HRT 2d), a w serii 2. odpowiednio z 83,5% do 71,9%. Całkowitą nitryfikację i stężenie azotu amonowego w odpływie poniżej 1 mg dm^{-3} uzyskano w serii 1. dla wartości HRT równej 3d i wyższej.

W osadzie czynnym oznaczono 20 taksonów, a ich liczba zależała głównie od wieku osadu. Dla wieku osadu poniżej 16d odnotowano jedynie 4-5 taksonów. Liczebność mikrofauny zależała zarówno od HRT, jak i wieku osadu. W obu seriach najwyższą liczebność mikrofauny odnotowano dla HRT 12d. Przy krótszym HRT (6, 3 i 2 d) ogólna jej liczebność była wyższa w serii 1. niż w serii 2. Wśród bakterii nitkowatych dominował Typ 0092.

Introduction

The importance and the role of activated sludge biocenosis treating wastewater has been extensively documented (SALVADÓ, GRACIA 1993, MADONI 1994 a, b, CERECEDA et al. 1996, MADONI et al. 2000, KRHUTKOVÁ et al. 2002, LEE et al. 2004). The numerous studies allow to stay the structure of activated sludge microfauna mainly depended on sludge age, sludge loading, hydraulic retention time and dissolved oxygen concentration in aeration tank.

MADONI (1994a), on the basis of the research in 44 activated sludge plants treating municipal wastewater, found that high sludge age favours proliferation of crawling and attached ciliates (with exception of narrow peristome *Vorticella microstoma* and *Opercularia* spp.), but also shelled amoebae. The abundance of small flagellates and free-swimming ciliates increases with decreasing of sludge age.

POOLE (1984) pointed out that ciliates *Vorticella microstoma, Opercularia coarctata* and *Opercularia microdiscum* occurred more frequent in a low age sludge system and at high sludge loading, and in activated sludge with long age, low sludge loading and nitrification. There were observed *Blepharisma* spp., *Litonotus carinatus* and *Prorodon* spp., shelled amoebae (especially *Euglypha* spp.) and rotifers *Monogononta* spp. Similarly, SALVADÓ (1994) found that small flagellates, naked amoebae, free-swimming ciliates dominate at sludge age lower than 6 d, while flagellates > 20 μm, amoebae > 50 μm and attached ciliates appear abundantly in activated sludge with higher age.

From a literature view it can be concluded that among the researchers, there is an agreement that flagellates, naked amoebae and free-swimming ciliates predominate at high sludge loading and/or low sludge age. However, high microfauna diversity dominated by free-swimming ciliates and attached ciliates moderate sludge loading and its medium age. The domination of attached ciliates, rotifers and the presence of higher invertebrates (especially nematodes) correlates with low sludge loading and high sludge age.

Proven relation between microfauna composition and operational conditions became a basis for a bioindication system of activated sludge treating municipal wastewater (MADONI 1994a).

Now, the activated sludge is also commonly used to landfill leachate treatment. Leachate has always been considered as problematical wastewater from the treatment point of view, because its quality and quantity changes with time within the same landfill. Moreover, the complex leachate composition and the presence of hazardous organic and inorganic compounds (BENFENATI et al. 1996, BOZKURT et al. 1999, EL-FADEL et al. 2002) allow to presume that chemical control of leachate treatment effectiveness may be not sufficient. Several reports have shown that ciliated protozoa appear to be very useful as indicators of the functional conditions of wastewater treatment plants, especially, indicating the presence of toxic compounds (SALVADÓ et al. 2000, 2001, MADONI et al. 1996). KULIKOWSKA et al. (2005) showed that leachate added to municipal wastewater caused a decrease of the number of taxons and the total number of the microfauna cells. Furthermore, the dominance of the microfauna keygroups depended on the participation of leachate in wastewater. Correspondingly with the increase in the leachate contents, ciliates such as *Opercularia* spp. dominated however the number of attached ciliates, crawling ciliates and shelled amoebae decreased.

The aim of this work was to investigate the COD and nitrogen removal in sequencing batch reactors treating landfill leachate and the influence of hydraulic retention time and sludge age on the microfauna of activated sludge. Therefore, the microscopic analysis of microfauna may be able to deliver crucial information to establish optimal operational conditions in plant performance.

Materials and Methods

Leachate feed

The leachate was collected from municipal landfill located in Wysieka near Bartoszyce in the Warmia and Mazury Province. The landfill has been operating since January of 1996. The investigations were started 3 years after landfill exploitation.

The total surface area of landfill site is 22 ha. In the research period, waste were landfilled in the first field, sealed by geomembrane. The total area and volume of the first field is 1.5 ha and 61 628 m^3, respectively.

The total average amount of solid waste deposited in the landfill in 1996 was estimated to 7550 tons, in 1997 and 1998 – 7 970 and 9120 tons, respectively. Since 1999 it amounts to 11 000 – 12 000 tons. In the landfill, there are deposited municipal waste apart from fluid waste, faecal matter, hazardous substances, radioactive and toxic waste. Approximately 32% of disposed waste are of organic household origin (food waste), while glass, paper, plastic, metals and textiles constitute 14%, 12.2%, 4.4%, 3.07% and 2.75% of the total waste mass, respectively. The rest (31.6%) are inorganic remains like ashes and cinders.

During the research period leachate arising in the amount of average 5000 m^3/year. They were collected in a drain system and stored in retention reservoir from where they are spraying on the landfill or periodically took out to urban sewage treatment plant in Bartoszyce. Leachate samples were taken from retention reservoir to laboratory analysis.

The characteristics of landfill leachate is shown in Table 1. In leachate, there was low BOD_5/COD ratio – 0.37 (series 1) and 0.39 (series 2). However, N/COD ratio was 0.15 and 0.10 in series 1 and 2, respectively.

Table 1

Characteristics of landfill leachate used in series 1 and 2

Constituent	Values	
	series 1	series 2
pH	7.46	7.33
Biochemical oxygen demand (mg O_2 dm^{-3})	457	622
Chemical oxygen demand (mg O_2 dm^{-3})	1237	1596
Total nitrogen (mg N dm^{-3})	189	154
Ammonia nitrogen (mg N_{NH_4} dm^{-3})	141	113.3
Organic nitrogen (mg N_{org} dm^{-3})	47.2	38.8

Experimental unit

Two series were performed in this study. In both series investigations were carried out in four SBRs operated in parallel. The volume of each SBR was 6 dm^3. All reactors, made of plexiglass, were equipped with stirrers of adjustable rotation speed (36 r min^{-1}). Dissolved oxygen was supplied using porous diffusers, placed at the bottom of the reactors, producing bubbles of medium size. In both series, the amount of oxygen supplied to the reactor in the aeration phase was regulated in order to maintain the oxygen concentration at a level of 2.5 – 4.0 mg O_2 dm^{-3} at the end of the phase.

The amount of the leachate supplied within 24 h to the following reactors changed from 0.5 dm^3 (SBR 1) to 3 dm^3 (SBR 4), corresponding in appropriate hydraulic retention time (HRT) from 12 d (SBR 1) to 2 d (SBR 4) – Table 2. The system was operated at room temperature (19-21°C) for 4 months, 2 months for each series.

Table 2

The operational conditions in series 1 and 2

Operational conditions		Values			
		SBR 1	SBR 2	SBR 3	SBR 4
Volume of leachate influent in SBR operating cycle	(dm^3)	0.5	1.0	2.0	3.0
Hydraulic retention time (HRT)	(d)	12	6	3	2
Volumetric exchange rate (n)	(1 d^{-1})	0.083	0.167	0.33	0.50
Sludge loading (mg COD g^{-1} MLVSS $cycle^{-1}$):	series 1	63.6	95.4	130.5	167.6
	series 2	104.7	150.3	232.3	283.9
Sludge age (Θ) (d):	series 1	51	29	20	17
	series 2	24	16	10	8

In both series, the reactor's operating cycle consisted of five phases: filling (0.083 h), mixing (3 h), aeration (18 h), settling (2.834 h) and discharge (0.083 h). Series 1 and 2 differed in sludge age (Θ) – Table 2. The sludge age in series 1 was maintained by sludge excess in the amount of 50 cm^3 d^{-1} in SBR 1 and 2 (it corresponds to 10% and 5% of leachate influent and 100 cm^3 d^{-1} in SBR 3 and 4 (5% and 3.3% of leachate influent in the cycle, respectively). In series 2, the sludge age was nearly 2-fold lower than in series 1. It was obtained by draining some volume of sludge excess from following SBRs, i.e. 125, 225, 425, and 600 cm^3, respectively (from 25% to 20% of leachate influent in the cycle).

Process control

Biological analyses

Samples of mixed-liquor for microscopical observations were collected from SBR reactors during the aeration phase. The research on activated sludge biocenosis included the estimation of the taxonomic and quantitative composition of microfauna as well as the examination of filamentous and zoogloeal bacteria.

The composition of the microfauna was determined "in vivo" according to KAHL (1930-1935), CYRUS, SLADEČEK (1973), KUTIKOVA (1984), SLADKA, SLADEČEK (1985) and FOISSNER et al. (1991, 1992, 1994, 1995). Taxons: ciliates and rhizopods were identified at the genus or species level, and other protozoans and metazoans at higher systematic units. The abundance of flagellates were estimated along the diagonal of a Fuchs-Rosenthal chamber (MADONI 1994a). The abundance of other protozoans and metazoans was determined as an arithmetic average obtained from analysis of 4 subsamples with 0.05 cm^3 volume of mixed liquor. Finally, the number of individual taxons was accounted into 1 mg of volatile suspended solids (MLVSS).

The abundance and types of filamentous microorganisms in activated sludge were characterized according to JENKINS et al. (1993).

Zoogloeal bacteria were identified "in vivo" using the key of CYRUS, SLADEČEK (1973). For the estimation of zoogloeal colonies abundance our own 5-stage scale was used. The stages mean percentage of sludge flocs inhabited by these zooglea: 1 – indiv. (below 5%); 2 – sparse (5-25%); 3 – quite abundant (25-50%); 4 – abundant (50-75%), 5 – more abundant (75-100%).

Analysis were made througout 4 months (2 months for each series) with frequency of two times a week.

Chemical analyses

Raw leachate were determined for the following parameters: pH, BOD_5 (according to DIN EN 1899-1/EN 1899-2 official EPA method using OxiTop® made by WTW company), COD, total nitrogen and ammonia nitrogen (according to HERMANOWICZ et al. 1999).

The effluents from the SBR reactors were subjected to daily measurements for BOD_5 (according to DIN EN 1899-1/EN 1899-2 using OxiTop® made by WTW company), COD, total nitrogen, ammonia nitrogen, nitrite, nitrate, volatile suspended solids (VSS) and total suspended solids (TSS) (according to HERMANOWICZ et al. 1999).

The mixed reactor content was measured for mixed liquor volatile suspended solids (MLVSS), total suspended solids (MLTSS) and sludge volumetric index (according to HERMANOWICZ et al. 1999) and the oxygen concentration (using an oxygen controller HI 9142).

Results

The influence of sludge age and HRT of organics and nitrogen removal efficiency

In this study leachate originated from municipal landfill operated for three years. It is known that the leachate from young landfills (up to five years) contains rather biodegradable organic substances. In our research BOD_5/COD ratio were 0.37 (series 1) and 0.39 (series 2) which indicates that only about 40% of organic compounds could be really biologically oxidized. Organics removal efficiency (as COD) was showed in Table 3.

Table 3

Organics (COD) concentrations in the effluent and their removal efficiency

Series No.	SBR No.	COD	
		mg O_2 dm^{-3}	E (%)
1	SBR 1	212	82.9
	SBR 2	244	80.3
	SBR 3	291	76.5
	SBR 4	364	70.5
2	SBR 1	263	83.5
	SBR 2	345	78.4
	SBR 3	392	75.4
	SBR 4	449	71.9

It was shown that in both series the efficiency of organics removal at given HRT was comparable. Therefore, it might be stated that at given HRT the organics removal efficiency was nearly independent of sludge age.

The research results of nitrogen concentrations and forms in the effluent from SBRs in series 1 and 2 are presented in Figure 1.

From the data analysis it can be stated that in both series a significant amount of organic nitrogen remained in the effluent at high concentrations regardless of HRT and sludge age.

In series 1, at long sludge age and HRT 2 d, ammonium concentration in the effluent amounted to 46.6 mg N_{NH_4} dm^{-3}. Complete nitrification and

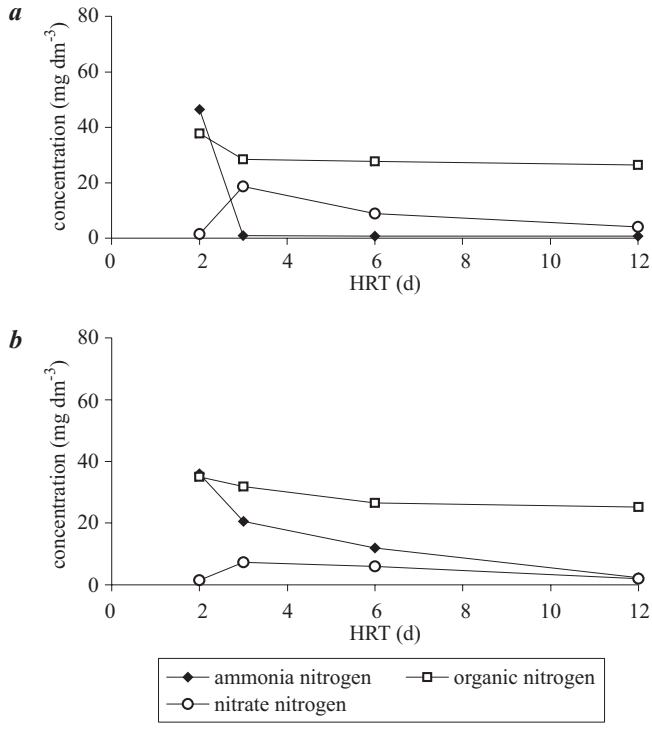

Fig. 1. Nitrogen concentration in the effluent from SBRs depending on HRT: *a* – series 1, *b* – series 2

ammonium concentration in treated leachate below 1 mg N_{NH_4} dm^{-3} were obtained in SBRs 1-3 with HRT 3-12 d (Figure 1a). It can be explained because of the lower ammonium concentration at the beginning of the cycle, which results from the extent of the capacity exchange and nitrogen losses too. The nitrate concentration in the effluent from SBRs in this series decreased from 18.7 mg N_{NO_3} dm^{-3} (SBR 3, HRT 3d) to 4.1 mg N_{NO_3} dm^{-3} (SBR 1, HRT 12 d), while in SBR 4 did not exceed 1.5 mg N_{NO_3} dm^{-3}.

In series 2 shortening sludge age caused increase of the ammonium concentration in the effluent from all reactors. The nitrate concentration was not significant and the highest value (7.3 mg N_{NO_3} dm^{-3}) was observed in SBR 3 (HRT 3d) – Figure 1b.

In SBRs, activated sludge is under transient conditions. It means that concentrations of organics and ammonium change within time. Concentration profiles in the cycle depend on the composition of leachate influent as well as leachate effluent remaining in reactor after previous cycle. The initial concentration of organics (C_0/X) in cycle was estimated as $C_0/X = (C_{inf} \cdot n)/X + (C_{eff} \cdot (1\text{-}n))/X$.

The values of C_0/X (as COD) at the cycle beginning and the amount of organics removed in SBRs were illustrated in Figure 2.

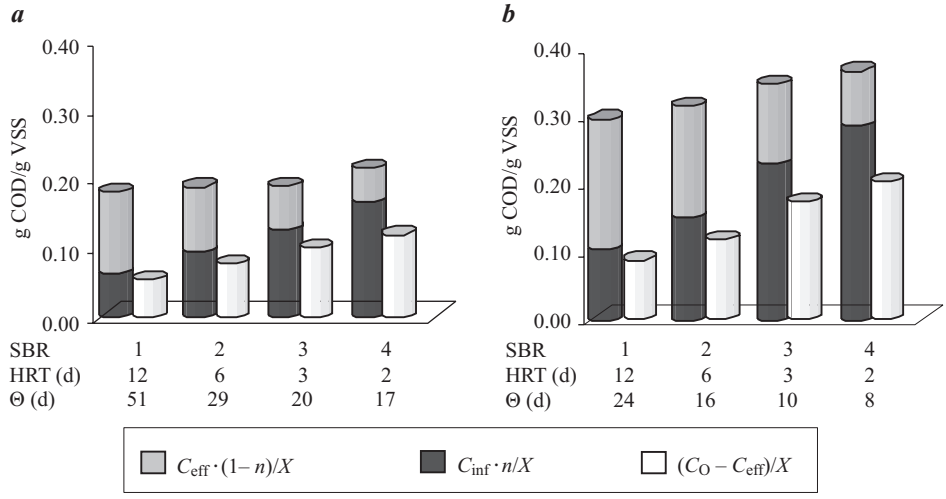

Fig. 2. Organics concentration in SBRs at different operational conditions: *a* – series 1, *b* – series 2

In both series, the C_0/X values (as COD) in the following cycles remained invariable. The fact was caused by significant participation of organics remaining in SBR reactor after previous cycle ($(C_{eff} \cdot (1\text{-}n)/X$). It concerned mainly SBR 1 and SBR 2, in which hydraulic retention time (HRT) was the longest, but the volumetric exchange rate (n) the lowest. Therefore, it can be concluded that the non-biodegradable fraction of organics remaining in reactor clearly influenced leachate composition in the cycle duration.

The influence of sludge age and HRT on microfauna composition

The data obtained in this work show that in both series increase in sludge age from 8 d to 51 d resulted in taxons number increase from 4 to 17 (Figure 3). It results that sludge age, but not HRT, influence the taxons amount in activated sludge. For example, at sludge age of 20 d and 24 d the taxons number in SBR 3 (series 1) and SBR 1 (series 2) was 11 and 10, regardless of various HRT – 3 d and 12 d, respectively. Similarly, nearly identical taxons number (7 and 8) was observed in SBR 4 (series 1) and SBR 2 (series 2). In both reactors, sludge age was comparable (17 d and 16 d), while HRT was 3-fold shorter in SBR 4 than in SBR 2.

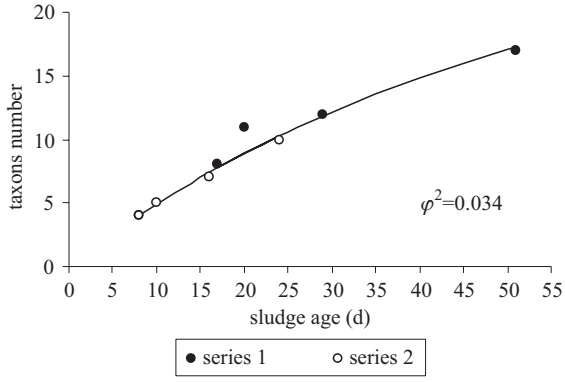

Fig. 3. Taxons number in activated sludge treating leachate at different sludge age

The relationship between sludge age and taxons number (N) proceeding according to the following equation:

$$\frac{dN}{dt} = k \cdot (N_{max} - N), \tag{1}$$

can be rearranged to form:

$$N = N_{max} \cdot (1 - e^{-k\Theta}) \tag{2}$$

where:
k – constant in the first order kinetic (d^{-1}),
N_{max} – maximum taxons number,
N – taxons number,
Θ – sludge age (d).

The constant (k) calculated from equation (2) was 0.02 d^{-1}, φ^2 equals 0.034.

The investigations indicate that the total number of microfauna without flagellates depended on sludge age and hydraulic retention time in SBRs. In series 1, the total number of microfauna decreased nearly linearly with shortening HRT from 565 to 316 ind. mg^{-1} MLVSS (Figure 4a). However, in series 2 in SBR 1 it was 514 ind. mg^{-1} MLVSS, while in SBRs 2-4 it did not exceed 200 ind./mg VSS (Figure 4b).

The composition of the microfauna and frequency of taxons were presented in Table 4. The domination structure of the microfauna were presented in Figure 5.

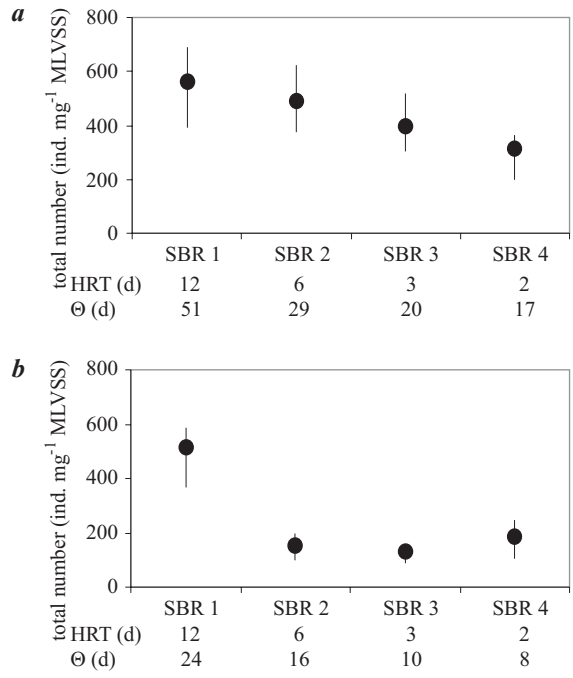

Fig. 4. Total number of microfauna in activated sludge (without flagellates): *a* – series 1, *b* – series 2

In series 1 in SBR 1 (Θ = 51 d), there was noticed rich microfauna, as regards species composition and a high total number. Ciliates and rotifers were the most numerous. The percentage of these taxons was 65% and 26%, respectively (Figure 5a). Among ciliates, *O. microdiscum* was a predominant species (41.6%). Additionally, the significant participation of wide peristome attached ciliates was noticed.

In SBR 2, an increase in ciliates percentage, especially *O. microdiscum* (69.2%) was observed (Figure 5b). Additionally, the increase in number of carnivorous ciliates, mainly *Acineria uncinata*, strong reduction in number of wide peristome attached ciliates and rotifers, and the elimination of crawling ciliates were also observed (Table 4).

In SBR 3, the community composition was characterized by 94% participation of ciliates with abundantly occurring *O. microdiscum* (81.6%) (Figure 5c). The number of flagellates was low like in SBR 1 and SBR 2 (Table 4).

Results indicate that in SBR 4 the group of ciliates showed a high participation of protozoan microfauna (98%), while naked amoebae and nematodes were in minimal proportion. Flagellates occurred numerously, over 100 individuals along the diagonal of a Fuchs-Rosenthal chamber.

Table 4

Composition of the microfauna in activated sludge in series 1 and 2

Key groups	Taxons	Series 1								Series 2							
		sludge age (d)								sludge age (d)							
		51		29		20		17		24		16		10		8	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Flagellates*	*Zooflagellates* <20 μm	10-100	100	10-100	100	10-100	100	>100	100	<10	100	>100	100	>100	100	>100	100
Naked amoebae	*Amoeba type proteus*	13	44	10	67	1	11										
	Mayorella sp.	3	11	1	22	1	11	3	33	3	33						
Shelled amoebae	*Euglypha laevis*	31	100	21	100	12	78			33	100	1	11	1	11		
Crawling ciliates	*Aspidisca cicada*	6	33			1	11			13	44						
	Chilodonella uncinata	1	22														
	Oxytricha hymenostomata	1	11														
Attached ciliates**	*Carchesium polypinum*	10	11														
	Vorticella convallaria	1	11														
	Vorticella octava									16	67						
	Vorticella sp.	44	44	4	33												
Vorticella microstoma	*Vorticella microstoma*	1	11	1	11			239	100			72	100	69	100	152	100
Opercularia spp.	*Opercularia microdiscum*	235	100	341	100	323	100	33	100	389	100	40	100	37	67	3	22
Free swimming ciliates	*Paramecium caudatum*	38	44					5	44	10	44						
	Paramecium putrinum			20	44	19	56										
	Uronema nigricans							5	56			16	22				
Carnivorous ciliates	*Podophyra fixa*	22	67	25	56	29	67	28	100	30	67	26	100	23	100	32	100
	Acineria uncinata	8	56	53	56	1	11			10	67						
Rotifers	*Rotatoria*	148	100	13	89	5	56										
Nematodes	*Nematoda*	3	22	4	33	4	67	3	67	10	67	1	11				

* – abundance of flagellates estimated along the diagonal of a Fuchs-Rosenthal chamber
** – without *V. microstoma* and *Opercularia* spp.
a – number (ind. mg^{-1} MLVSS), b – frequency (%)

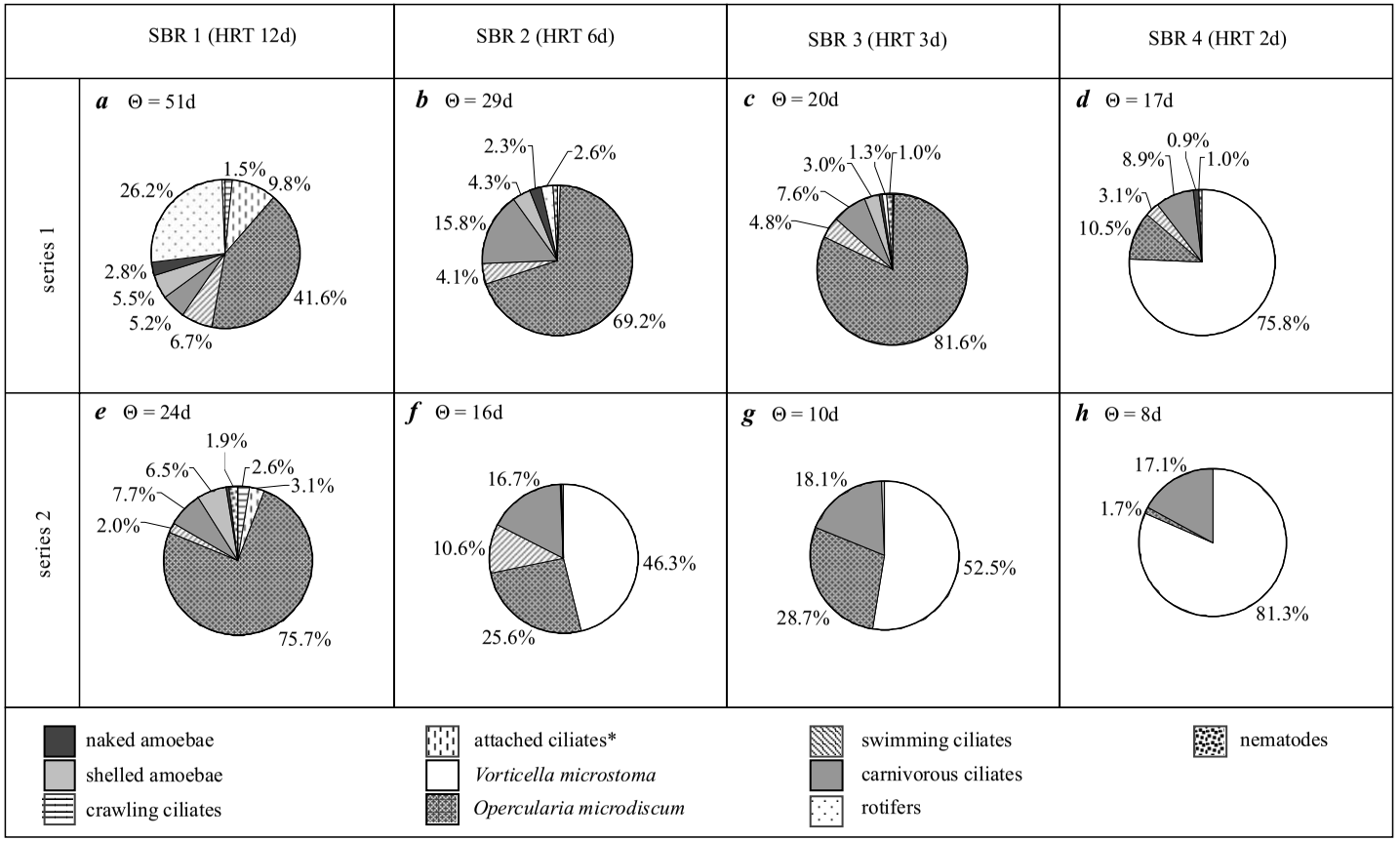

Fig. 5. Domination structure of the key groups of microfauna (without flagellates) in activated sludge treating leachate at different sludge age
* – without *Opercularia* spp. and *V. microstoma*

Among ciliates, significant abundance displayed *V. microstoma* (Table 4), while *O. microdiscum* dominating in 1-3 SBRs had percentage below 10%. *Podophyra fixa* was found as only representative species among carnivorous ciliates (Table 4).

The composition of microfauna groups in series 2 was illustrated in Table 4 and Figure 5e-h.

In series 2, the communities in SBR 1 (Θ = 33 d) were dominated by ciliates (91%), especially *O. microdiscum* (75.7%). Amoebae, mainly *Euglypha leavis* and nematodes, constituted the rest (Figure 5e). The average number of flagellates, enumerated along the diagonal of a Fuchs-Rosenthal chamber did not exceed 10 individuals (Table 4).

In SBR 2 (Θ = 16 d) the total microfauna number was 3-fold lower (156 ind. mg^{-1} MLVSS) in comparison with SBR 1. The community consisted almost 100% of ciliates, mainly *V. microstoma* (46.3%). There was also noticed a considerable percentage of *O. microdiscum* (25.6%), carnivorous and free swimming ciliates (Figure 5f) represented by *P. fixa* and *Uronema nigricans*. The flagellates abundance exceeded 100 individuals (Table 4).

In the case of SBR 3, the composition of community showed 100% percentage of ciliates with similar to SBR 2 participation of *O. microdiscum*, and carnivorous ciliates, but a higher percentage of *V. microstoma* (52.5%) – Figure 5g. The flagellates abundance exceeded 100 individuals (Table 4).

In SBR 4, the protozoans community consisted of *V. microstoma* (81.3%) and carnivorous ciliates (17.1%). It was observed that *O. microdiscum* partition decreased to 2% of the total number (Figure 5g). Similarly to SBRs 2 and 3, the abundance of flagellates was high and exceeded 100 individuals (Table 4).

Analysing preferences of more important protozoans and metazoans in relation to sludge age it can be concluded that:

– long sludge age favoured the occurrence of *Amoeba* type proteus (29 – 51 d), *E. leavis*, *Vorticella* spp, *A. uncinata* (24 – 51d), rotifers (20 – 51 d) and *Paramecium trichium* (20 – 29 d),

– *O. microdiscum* and nematodes appeared at sludge age ranged from 16 d to 51 d,

– low sludge age turned out the most appropriate for *U. nigricans* growth (16 – 17 d), *V. microstoma* and flagellates (8 – 17 d),

– *P. fixa* occurred in similar abundance almost in the whole range of sludge age, but it was the most frequent in the range of 8 – 17 d.

The number and domination of ciliates *O. microdiscum* and *V. microstoma* in activated sludge were determined by both sludge age and HRT.

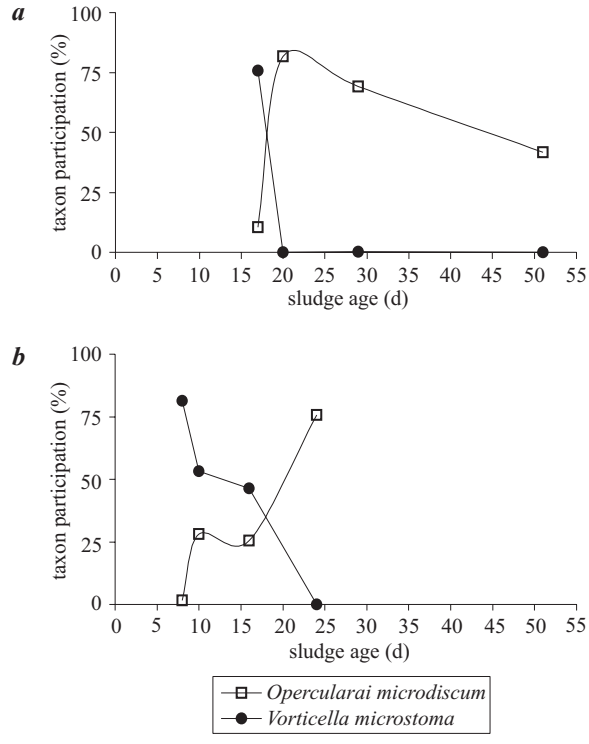

Fig. 6. Abundance of *O. microdiscum* and *V. microstoma* in activated sludge treating leachate at different sludge age: *a* – series 1, *b* – series 2

In both series *O. microdiscum* was a dominant species at sludge age higher than 20 d, whereas *V. microstoma* did not occurred or occurred seldom. However, below sludge age of 17 d the structure of domination was affected by HRT. In both series, at 2 d HRT, regardless of sludge age, *V. microstoma* showed strong domination (75.8%) (Figure 6a), what makes HRT a decisive parameter in the structure of domination.

In series 2, *O. microdiscum* and *V. microstoma* showed different abundance at almost the same sludge age (16 d), but at longer HRT (6 d), with percentages of 25.6% and 46.3%, respectively. The participation of *O. microdiscum* reduced to 1.7% and *V. microstoma* increased to 81.3%, only at 8 d sludge age and 2 d HRT (Figure 6b).

In both series, there were determined 7 types of filamentous bacteria. In series 1, the following types were present: Type 0092, Type 0041, Type 0675, *Haliscomenobacter hydrossis*, Type 1851 and *Microthrix parvicella*, whereas in series 2 only the first four types and *Thiothrix* sp.

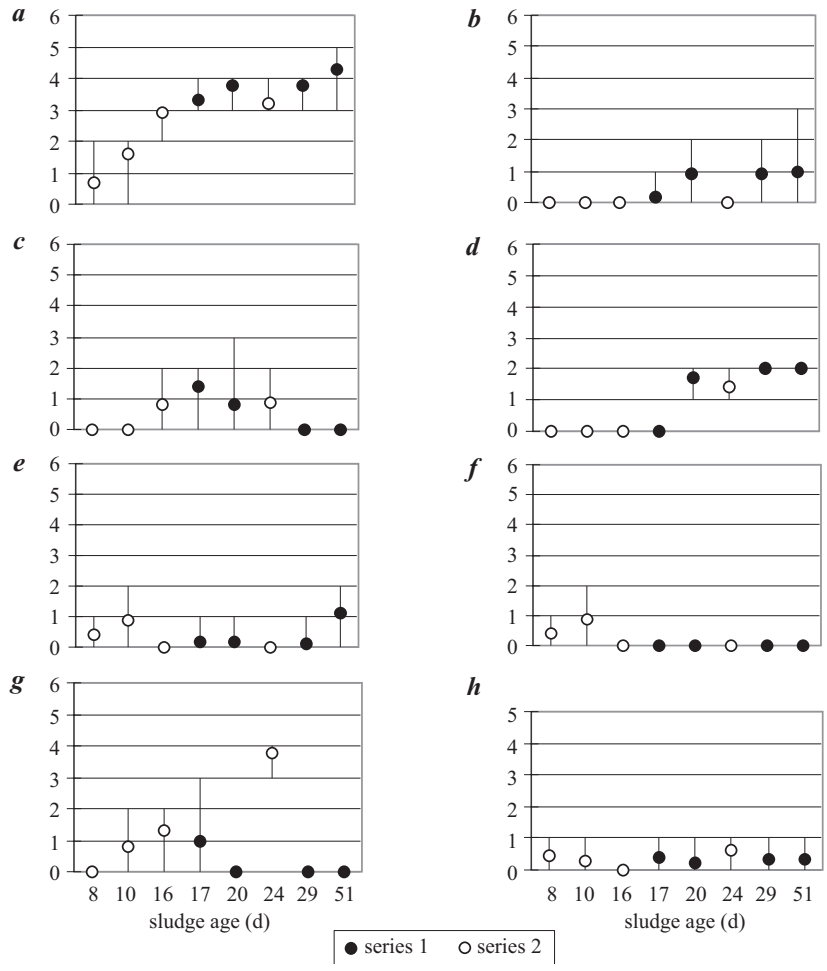

Fig. 7. Abundance of filamentous microorganisms and *Zooglea* sp. in activated sludge treating leachate at different sludge age: *a* – Type 0092, *b* – *M. parvicella, c* – Type 0041, *d* – Typ 0675, *e* – Type 1851, *f* – *Thiothtrix sp., g* – *H. hydrossis, h* – *Zooglea* sp.

In activated sludge, in series 1 the most numerously there was observed of Type 0092 (Figure 7). An average abundance of this strain increased with sludge age lengthening. Additionally, the constant presence at sludge age from 51 d to 20 d was stated for Type 0675 (Figure 7d).

The highest abundance in series 2 was achieved by *H. hydrossis* at 24 d sludge age and by filamentous bacterium of Type 0092 at the remaining range of sludge age. However, the number of dominants decreased significantly with shortening of sludge age (Figure 7).

Owing to relatively small size of filaments, dominating strains did not influenced sludge settlement conditions. Sludge volumetric index did not exceed 150 cm^3 g^{-1} VSS during experiments.

Assessing growth of filamentous bacteria in series 1 and 2, it can be concluded that experimental conditions favoured Type 0092 the most, which in general occurred in activated sludge the most abudanantly and regularly. The abundance of the filamentous bacterium decreased in proportion to sludge shortening from above 20 filaments in sludge floc at Θ = 51 d to sparse ones at Θ = 8 d.

Our results show that changes of sludge age for the range from 51 d to 8 d did not cause differences within filamentous bacterium. However, there are no unambiguous arguments on domination of *H. hydrossis* at 24 d sludge age as a consequence of its influence, especially as all identified filamentous bacteria (except for *Thiothrix*) needed long sludge age to their growth.

Zoogloeal bacteria occurred rarely in all tested range of sludge age (51-8 d) (Figure 7h).

Discussion

Our investigation showed that the effectiveness of organics removal (as COD) in both series was quite high. Almost complete ammonia removal from leachate was obtained at sludge age above 20 d and HRT > 3 d. Leachate originated from young landfill, being exploited for 3 years, which correlates well with obtained results. Many authors consider biological methods as effective during leachate treatment from young landfill sites (ROBINSON, MARIS 1983).

Our researches indicated that the composition of sludge microfauna depended, to a large extent, on operational conditions of leachate treatment. For species diversity, sludge age was the most important. The microfauna communities, treating leachate, was characterized by varying number of taxons differed between 4 and 17, in relation to sludge age increase. Moreover, the statistical relationship between sludge age and taxons number was pointed out. We observed that at constant sludge age, the number of taxons was identical even if activated sludge came from reactors at different HRT.

In present work the abudance of protozoan communities ranged from 2.99 to 9.16 ind. 10^5 dm^{-3} (130 – 565 ind. mg^{-1} MLVSS). MADONI (1991) on the basis on their own and the others researches concluded that the abundance of ciliates in properly functioning activated sludge plants, treating municipal wastewater should be 10^6 ind. dm^{-3}. When the number falls below 10^4 ind. dm^{-3}, it indicates insufficient purification of wastewater. In this case, there is

a proliferation of dispersed bacteria which makes effluent turbid and, in consequence, the worse quality.

The highest abundance and quite rich taxon diversity were stated at sludge age of 20 d and higher. At such conditions, there was obtained complete nitrification and the ammonia concentration in the effluent did not exceed 1 mg N_{NH_4} dm^{-3}. It can be presume that the high abundance and rich taxon diversity is connected, from one side, with long sludge age and from another with lack of ammonia nitrogen. However, XU et al. (2004) on the basis of measure the toxicity of ammonia and its effects on growth inhibition for the marine ciliates, *Euplotes vannus*, showed that the 2 hour median lethal concentration (LC50) was 7870.46 mg N_{NH_4} dm^{-3} on the threshold concentrations for growth inhibition was 100 mg N_{NH_4} dm^{-3}. SIMILARLY, PUIGAGUT et al. (2005) analysed the response of activated sludge microfauna in terms of abundance and diversity to evaluate both the toxic effect of ammonia nitrogen and the acclimation capacity of these microorganisms to its toxicity. The ammonia concentration tested changed from 9 to 80 mg dm^{-3}. Obtained results suggested that ammonia nitrogen in high concentrations causes a clear but reversible toxic effect on microfauna abundance. Furthermore, all microfauna groups analysed (free-swimming ciliates, crawling ciliates, attached ciliates, suctoria, gymnamoebae, metazoan, rotifers) show capacity acclimatization to the toxicity of ammonia nitrogen in terms of abundance.

In this study, the acivated sludge treating the leachate worked in low sludge loading ranges. Nevertheless, there were small abundance and frequency of ciliates such as *Chilodonella uncinata*, *Carchesium polypinum* and *Vorticella convalaria*, which are common in municipal wastewater treatment plant at low organic load (CURDS, COCKBURN 1970, KLIMOWICZ 1983, CURDS, HAWKES 1975, MADONI, GHETTI 1981, AMANN et al. 1998).

On the contrary, in sludge treating leachate there was stated relatively high abundance of *O. microdiscum*, *O. coarctata*, *O. minima, V. microstoma* and flagellates. In municipal wastewater treatment plants, such microorganisms are considered as bioidicators of sludge overloading (POOLE 1984, ESTEBAN et al. 1991, MADONI et al. 1993, MADONI 1994a).

Opercularia spp., *V. microstoma* and flagellates are associated with high feeding requirements. Thus, they can survive only in the environment enriched in dispersed bacteria in the liquid bulk. Such feeding conditions can be obtained at high sludge loading. Dispersed bacteria can also occur in sludge receiving industrial waste containing toxic substances, when bacterial cells do not aggregate and do not form flocs. Then the microorganisms may be dispersed in bulk liquid as individual cells or small clumps. In our research, leachate visual shows high turbidity what indicates good feeding conditions.

Under those circumstances, the small abundance of protozoan in activated sludge may suggest environmental toxicity.

According to SLÁDĚCEK (1973) and FOISSNER (1988) *V. microstoma* is a polysaprobic species. This ciliate shows great adaptive possibilites in relation to many various chemicals. ABRAHAM et al. (1997) observed high frequency of *V. microstoma* in activated sludge in the presence of Fe, Zn, Cu, Cr at concentrations of 2, 5, 0.06, 0.1 mg dm^{-3} and higher, respectively. However, BÉCARES et al. (1994) noticed only four taxons in activated sludge treating wastewater from the chemical-pharmacutical industry, such as *Chilodonela* sp., Drepanomonas revoluta, Opercularia coarctata and *Discophrya* sp. A decisive dominant was *O. coarctata* with participation above 87%. PUDO, ERNDT (1981) pointed out the abundant occurrence of *V. microstoma* and *Cyclidium* sp., and the lack of others ciliates in sludge treating wastewater containing Rokafenol at concentrations of 15 and 30 mg dm^{-3} and at extremely short HRT equal 2 h.

Our investigations revealed the domination of *V. microstoma* in both series which strengthened together with HRT shortening with short sludge age. Moreover, the domination of *Opercularia* sp. depended on high sludge age (above 20 d) – Table 2. On the other hand, high frequency of *V. microstoma* was determined by low sludge age below 17 d, but the strong domination of this species occurred at 2 d HRT, regardless of sludge age. SALVADÓ, GRACIA (1993) investigating the influence of sludge loading on changes in frequency and structure of protozoans observed enlarged abundance of *V. microstoma* at sludge loading above 0.2 kg BOD_5 kg^{-1} VSS d^{-1}.

In activated sludge used in leachate treatment, there were identified nine types of filamentous bacteria, among which Type 0092 was the most abundant. Its abundance in sludge was the highest at sludge age of 17 d and longer. Type 092 is commonly recognized as a microorganism of high sludge age (above 10 d) and low sludge loading (below 0.2 kg BOD_5 kg^{-1} d^{-1}) (RICHARD 1989, JENKINS 1992, WANNER 1994, JENKINS et al. 1993). Owing to the possibility of nitrate respiration, this bacterium shows resistance to the low concentration of dissolved oxygen (CASEY et al. 1992, 1994). In municipal wastewater, there are used products of hydrolysis as a carbon source, thus the bacterium is able to proliferate in the presence of slowly biodegradable substrates (JENKINS 1992). In this way, it can be suggested that long sludge age and presence of nitrates in leachate favoured proliferation of Type 0092 bacterium in this study.

We also stated that despite the filamentous bacteria presence, there were no problems with sludge settling. Additionally, the high abundance of periodically observed microorganisms with small filaments size (Type 0092, *H. hydrossis*) did not affect increase in volumetric sludge index below 100 cm^3 g^{-1}. It is highly possible that the high content of mineral suspended solids determined good sludge settling.

Conclusions

1. In both series, the organics removal efficiency (as COD) at given HRT was comparable and independent on sludge age. However, the effectiveness of ammonium removal was affected by sludge age. There was needed sludge age above 20 d to obtain the concentration in the effluent below 1 mg N_{NH_4} dm^{-3}.

2. Microscopical observations of activated sludge treating leachate from municipal landfills revealed their limiting influence on the microfauna composition. Shortening of sludge age caused lowering number of taxons, the total abundance of microfauna and changes in a percentage of single taxons participation. Crawling and attached ciliates with wide peristome turned out the most sensitive towards leachate. By contrast, flagellates and ciliates *V. microstoma* and *Opercularia* sp. were the most resistant. In series with the domination of *V. microstoma*, there were noticed the high concentration of organics (> 345 mg COD dm^{-3}) and ammonia (> 11 mg N_{NH_4} dm^{-3}) in the effluent.

3. The leachate affect microfauna communities depending on HRT. The shorter HRT in SBR reactor, the higher decrease in microfauna abundance. The higher sludge age, the milder negative effects of leachate impact on species diversity. The taxon number increases with sludge age according to first-order reaction. At sludge age 24 d and higher taxons number was 10 and more. However, at sludge age lower than 16 d only 4-5 taxons were present.

4. Filamentous microorganisms in activated sludge treatment leachate were not numerous (≤ 2 in Jenkin's scale), with exeption of Type 0092, which abundance increase with slugde age.

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EFFECT OF DYE CONCENTRATION ON ADSORPTION EFFICIENCY IN THE AIR-LIFT REACTOR

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K e y w o r d s: adsorption, chitosan, air-lift reactor, Black 8.

A b s t r a c t

The research was aimed at determining the efficiency of reactive dye Black 8 adsorption onto chitosan under dynamic conditions in the air-lift reactor. Analyses were carried out for four inlet concentrations of the dye and three flow rates. In all experimental series the concentration of adsorbent in the reactor was constant and accounted for 1 g d.m. dm^{-3}. Analyses demonstrated that technological conditions of the process affected both the maximum adsorption capacity of chitosan as well as the utilization of the maximum adsorption capacity of adsorbent at an assumed dye content in the effluent. The highest total adsorption capacity was obtained at the lowest flow rate examined, i.e. 1 V h^{-1}, irrespective of the concentration of dye inflowing to the reactor. In contrast, a significant effect was observed for the inlet concentration of dye on the utilization of adsorption capacity at an assumed dye concentration in the effluent.

WPŁYW STĘŻENIA BARWNIKA NA EFEKTYWNOŚĆ ADSORPCJI W REAKTORZE AIR-LIFT

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S ł o w a k l u c z o w e: adsorpcja, chitozan, reaktor air-lift, Black 8.

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Abstract

W pracy badano efektywność adsorpcji barwnika reaktywnego Black 8 na chitozanie w warunkach dynamicznych w reaktorze air-lift. Badania prowadzono dla czterech stężeń barwnika w dopływie do reaktora oraz trzech natężeń przepływu. We wszystkich seriach badawczych stężenie adsorbentu w reaktorze było stałe i wynosiło 1 g s.m. dm^{-3}. Badania wykazały, że warunki technologiczne procesu wpływają zarówno na maksymalną pojemność chitozanu, jak i wykorzystanie maksymalnej pojemności adsorpcyjnej adsorbentu w założonej ilości barwnika w odpływie z reaktora. Najwyższą całkowitą pojemność adsorpcyjną uzyskano w najniższym badanym przepływie 1 V h^{-1}, niezależnie od stężenia barwnika doprowadzanego do reaktora. Stwierdzono natomiast znaczący jego wpływ na wykorzystanie pojemności adsorpcyjnej adsorbentu w założonym stężeniu barwnika w odpływie.

Introduction

Treatment of wastewaters originating from the textile industry is a complex technological process requiring high financial input.

Methods of dyes decolorization differ in efficacy, degree of complexity of technological solutions and equipment used as well as costs of individual processes. Expensive yet efficient methods include the so-called advanced oxidation with the use of ozone, H_2O_2, Fenton's reagent. In a number of cases, methods of chemical oxidation are applied commonly with physical or biological processes. An efficient method of wastewater decolorization is membrane filtration. Its advantages include the possibility of high (over 99%) efficiency of dyes removal, partial removal of salt as well as thickening of solutions with a low concentration.

As compared with chemical oxidation or membrane processes, the process of adsorption does not require high financial input. Nevertheless, costs of sorbents, especially of their regeneration, are high hence searches for inexpensive and efficient sorbents are still underway.

Economic justification of propagating the adsorption process is strongly linked with the price of a sorbent and possibility of its regeneration.

In the adsorption process use is made of substances of plant origin (e.g. tannin-rich bark, marine plants, humus, peat, modified cotton and wool, chitin, chitosan, and alginate), substances of animal origin, microorganisms (activated sludge, pure bacterial cultures), and biopreparations. Usability of adsorbents in the adsorption process of particular substances is strictly connected with their structure and physicochemical properties. One of the most commonly occurring natural polysaccharides is chitin and chitosan obtained from it (DIVAKARAN, PILLAI 2001a). Chitin and chitosan readily undergo biodegradation, are non-toxic (HUANG at al. 1999), and due to a high nitrogen content of molecules constitute a very good chelating compound. Thus, they are applied as adsorbents for the removal of heavy metals, e.g.

$Hg^{2+}$and Zn^{2+} cations, from water (CHIOU, LI 2003). Chitosan belongs to the most efficient sorbents characterized by a high adsorption capacity against reactive dyes (CHIOU, LI 2003, WU et al. 2000, WU et al. 2001).

Due to their properties, chitin and chitosan have been extensively applied also in the process of wastewater treatment. Especially chitosan has been found efficient in the removal of dyes based on sulfur, alcohol or acids. Chitin in turn, as a natural polymer of acetylated or non-acetylated glucosamine, has found multiple applications in medicine, pharmacology, biotechnology, plant protection or environmental protection (JUANG, SHAO 2002, DIVAKARANET et al. 2001b).

Adsorbents, such as chitosan, can be produced, utilized and regenerated at low expenses. Hence, they constitute an alternative to costly methods of dye removal from water and sewage.

The research was thus undertaken to determine the efficiency of Black 8 removal with the adsorption method onto chitosan in the air-lift reactor. Assays involved determinations of the impact of technological conditions on dye adsorption onto chitosan in a circulating air-lift reactor, as well as the effect of dye concentration and flow rate in the air-lift reactor on the total adsorption capacity of adsorbent, working time of the reactor and utilization of the adsorption capacity of adsorbent at an assumed efficiency of dye removal.

Materials and Methods

Chitosan preparation and characteristics

In the experiment, use was made of krill chitin originating from the Sea Fisheries Institute in Gdynia. Contents of dry matter and ash in the experimental chitin reached 95.64% and 0.32%, respectively. The average size of a chitin flake used for the experiment was from 314 to 184 μm. The size of maximal flake was 756×434 μm, and minimal 62×62 μm.

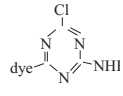
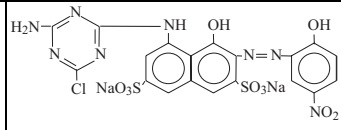
Chitin flakes was prepared following the method postulated by STANLEY et al. 1975. Preparation of chitin involved, rinsing with distilled water, rinsing with hydrochloric acid in order to wash out calcium and magnesium ions, and next cooking with 70% potassium base. The degree of acetylation of so prepared chitosan accounted for 75%.

Dye preparation

The chlorotriazine reactive dye Black 8 from "Boruta'' SA Dye Plant in Zgierz was used in the experiment. Its chemical structure and characteristics were presented in Table 1.

Table 1

Characteristics of dye

Reactive group	Class	Structural formula	Reactive Black 8	Molecular weight g mole^{-1}
chlorotriazine	azoaminochlorotriazine			656.5

Analysis of the efficiency of Black 8 adsorption onto chitosan in an air-lift reactor

The experiment was carried out in an air-lift reactor, the scheme of which is shown in Figure 1. Use was made of a reactor of a circular section made of plexiglass, with a diameter of 0.06 m, height of 0.54 m, and active volume of 0.77 dm^3. In the lower part of the reactor, there were truncated cone-shaped stub pipes allowing the inflow of air and dye solution. Inside the reactor, 0.5 m long barrier was installed centrally. Near the effluent of the reactor there was a separator.

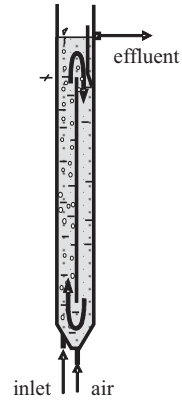


Fig. 1. Scheme of the air-lift reactor

The analyses of Black 8 adsorption onto chitosan in the air-lift reactor were carried out at different inlet flow rates and dye concentration in the reactor. The chitosan concentration was constant and reached 1 mg dm^{-3}. The pH of the solution was maintained at a level of 5.0. pH adjustment was carried out by HCl. Technological parameters of the research were presented in Table 2.

Table 2
Technological assumption of analyses of RB8 adsorption onto chitosan in the air-lift reactor

Parameter		Unit	Values
Flow rate	(q)	V/h	1, 2, 3
Chitosan concentration	(m)	g d.m. dm^{-3}	1
Inlet dye concentration	(C_0)	Mg dm^{-3}	10, 25, 50, 100

The analysis of Black 8 adsorption onto chitosan was carried out as follows: chitosan was transferred into the reactor filled with water of pH 5.0. Aeration was turned on at a pressure of 0.15 MPa (50 Ndm^3 h^{-1}), in order to force chitosan movement in the reactor. Then, a solution with dye of a specified concentration was dosed, by means of a peristaltic pump, at a rate of 0.77 dm^3 h^{-1}, 1.54 dm^3 h^{-1} or 3.1 dm^3 h^{-1}, which corresponded to the flow rate of 1 V h^{-1}, 2 V h^{-1} and 3 V h^{-1} (where V denotes reactor volume).

A total of 12 experimental series were carried out that differed in the inlet dye concentration and flow rate in the reactor.

In each series, analyses were carried out as long as the concentration of dye in the effluent was equal to its initial inlet concentration. For each initial dye concentration in the inlet and flow rate, control series were performed without chitosan. It enabled determining the real time of dye retention in the reactor and adsorption capacity of chitosan under dynamic conditions

Analytical methods

The analysis of pH was carried out with the use of a HI 123 pH-meter (Hanna Instruments), chitosan concentration as chitosan dry weight according to the methodology described by HERMANOWICZ et al. (1999).

Deacetylation degree of chitosan was evaluated on the basis of IR spectrum according to ROBERTS (1992).

Determination of dye concentration

Dye Reactive Black 8 was assigned a visual wavelength (λ= 587) at which absorbance was measured for the purpose of drafting a standardization curve and making conversion coefficient. The dye concentration was measured spectrophotometrically using a UV-VIS Spectrophotometer SP 3000 apparatus.

Determination of dye concentration in the air-lift reactor

The efficiency of the process was calculated by monitoring the decolourisation. At regular time intervals (10 min) several samples were taken, which were centrifuged (10.000 rpm) for 10 min. The residual dye concentration was measured spectrophotometrically at 587 nm.

Results and Discussion

The quantity of dye adsorbed onto chitosan Q_r under flow conditions was calculated from equation (1):

$$Q_r\,(t) = \frac{\sum_{t=1}^{n}((C_t - C_{kt}) \cdot q \cdot t)}{V \cdot m} \tag{1}$$

$Q_r(t)$ – quantity of dye adsorbed onto chitosan in time t (mg g^{-1} d.m.)
C_t – mean concentration of dye in effluent in time t in the control sample (mg dm^{-3})
C_{kt} – mean concentration of dye in effluent in time t (set up with chitosan) (mg dm^{-3})
q – flow rate of a dye solution to the reactor (dm^3 h^{-1})
t – adsorption time (h)
V – reactor volume (dm^3)
m – chitosan concentration in the reactor (g.m. dm^{-3})

Breakthrough curves obtained in 12 experimental series depicting changes in Black 8 concentration in reactor's effluent depending on time were presented in Figure 2. The experimental results obtained enabled determining the adsorption capacity of chitosan in any time t of the experiment – $C_{kt}=f(t)$ and the maximal adsorption capacity of chitosan. For each initial dye concentration in the inlet and flow rate, control series – $C_t=f(t)$ were performed without chitosan. It enabled determining the real time of dye retention in the reactor and adsorption capacity of chitosan under dynamic conditions.

Table 3 shows the total adsorption capacity of chitosan obtained at various inlet concentrations of dye in the air-lift reactor for the three flow rates examined. In all experimental series, chitosan concentration was constant and accounted for 1 g d.m. dm^{-3}.

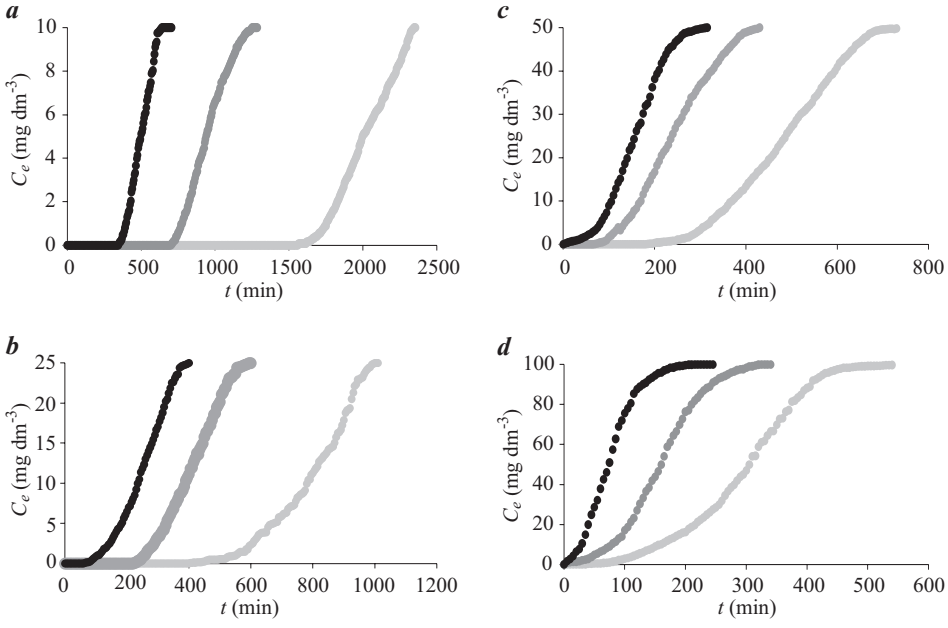


Fig.2. Breakthrough curves showing changes dye concentration in the effluent from the air-lift reactor in time at various initial concentration of dye inflowing to the reactor: *a* – C_0 = 10 mg dm^{-3}, *b* – C_0 = 25 mg dm^{-3}, *c* – C_0 = 50 mg dm^{-3}, *d* – C_0 = 100 mg dm^{-3}

Table 3
Total adsorption capacity of chitosan depending on flow rate of the dye to the reactor and inlet dye concentration in the air-lift reactor

Inlet dye concentration	Q_{max} (mg g^{-1} d.m.)		
	0.77 dm^3 h^{-1} (V h^{-1})	1.54 dm^3 h^{-1} (2 V h^{-1})	2.31 dm^3 h^{-1} (3 V h^{-1})
10 mg dm^{-3}	3977	1874	969
25 mg dm^{-3}	3871	1979	1193
50 mg dm^{-3}	4326	2174	1370
100 mg dm^{-3}	4551	2523	1421

The study demonstrated that, under dynamic conditions, the adsorption capacity of chitosan was determined to the greatest extent by the flow rate. At the inlet flow rate of 1 V h^{-1}, the adsorption capacity reached ca. 4200 mg g^{-1} d.m., irrespective of the inlet dye concentration. At the twofold higher flow rate, the quantity of adsorbed dye was lower and accounted for ca. 2100 mg g^{-1} d.m. on average. Increasing the flow rate to 3 V h^{-1} resulted in a decline in the adsorption capacity to ca. 1200 mg g^{-1} d.m. on average.

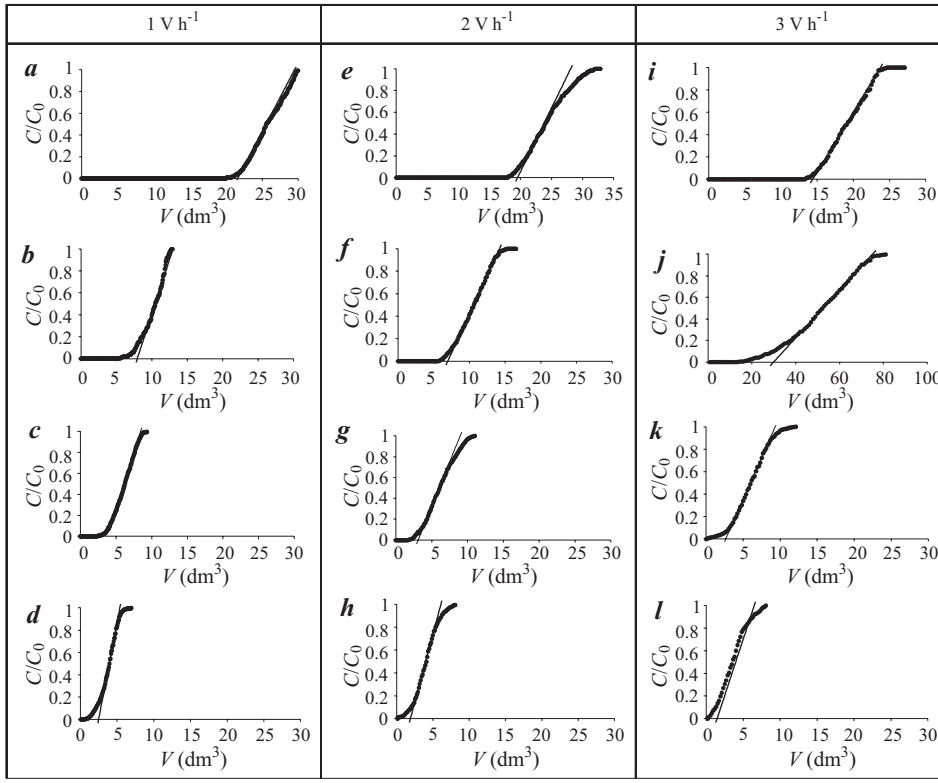


Fig. 3. Breakthrough curves and tangent of Black 8 at the flow rate of 1V h^{-1} (*a* – 10 mg dm^{-3}, *b* – 25 mg dm^{-3}, *c* – 50 mg dm^{-3}, *d* – 100 mg dm^{-3}), at the flow rate of 2 V h^{-1} (*e* – 10 mg dm^{-3}, *f* – 25 mg dm^{-3}, *g* – 50 mg dm^{-3}, *h* – 100 mg dm^{-3}), and at the flow rate of 3 V h^{-1} (*i* – 10 mg dm^{-3}, *j* – 25 mg dm^{-3}, *k* – 50 mg dm^{-3}, *l* – 100 mg dm^{-3})

In all experimental series run, it was also observed that at the constant flow rate the adsorption capacity of chitosan increased along with an increasing concentration of dye inflowing to the reactor. The increase in the total load of dye adsorbed onto chitosan was found to depend on the flow rate of the dye through the air-lift reactor. The smallest effect of inlet dye concentration on the total adsorption capacity – an increase by 14%, was recorded at the lowest flow rate examined. At the other two flow rates analyzed in the study, i.e. 2 V h^{-1} and 3 V h^{-1}, the total adsorption capacity increased by 35 and 47%, respectively, at the inlet concentration increase from 10 to 100 mg dm^{-3}. Analogous results were achieved in the analyses of Black DN adsorption under dynamic conditions onto chitin (FILIPKOWSKA 2004, 2005)

The results obtained and breakthrough curves plotted enabled stating that the selection of parameters of reactor work, including: adsorbate con-

centration and flow rate of medium through a reactor, has a substantial effect on the shape of adsorption curves. The plots of C/C_0 versus volume are given in Figure 3.

For all analytical series, tangents were plotted at $C/C_0 = {}^1/_2$. Values of tangent coefficients *a* (tangent inclination) and *b* were presented in Table 4.

Table 4

Values of tangent coefficients *a* (tangent inclination) and *b*

Inlet dye concentration	Constants					
	1 V h^{-1}		2 V h^{-1}		3 V h^{-1}	
	a	*b*	*a*	*b*	*a*	*b*
10 mg dm^{-3}	0.127	-2.744	0.117	-2.286	0.107	-1.540
25 mg dm^{-3}	0.198	-1.563	0.128	-0.960	0.115	-0.595
50 mg dm^{-3}	0.235	-0.944	0.171	-0.554	0.150	-0.399
100 mg dm^{-3}	0.345	-0.839	0.238	-0.449	0.199	-0.188

A distinct tendency was observed for tangent inclination (a) depending on the parameters of reactor work. At the constant concentration of adsorbent in the reactor the shape of a breakthrough curve obtained depends on the inlet dye concentration. The highest values of constant *a* were noted in the series with the lowest inlet flow rate of dye – 1 V h^{-1}. The analyses showed that the greatest inclination of the tangent occurred at the highest tested concentration of the dye – 100 mg dm^{-3}. These were simultaneously the series in which the highest total adsorption capacity was obtained at the constant flow rate of the dye in the reactor (Table 3).

Table 5 presents the working time of the reactor depending on the assumed dye concentration in the effluent. Four efficiencies of dye removal were assumed in the study – 100% (a lack of dye in the effluent) 99%, 95% and 95%.

Table 5

Working time of the reactor depending on the assumed dye concentration in the effluent

Inlet dye concentration	Working time of the reactor (min)											
	0.77 dm^3 h^{-1} (V h^{-1})				1.54 dm^3 h^{-1} (2 V h^{-1})				2.31 dm^3 h^{-1} (3 V h^{-1})			
	100%	99%	95%	90%	100%	99%	95%	90%	100%	99%	95%	90%
10 mg dm^{-3}	1560	1620	1705	1755	700	710	750	780	340	355	385	400
25 mg dm^{-3}	400	450	560	605	220	235	255	280	60	80	140	175
50 mg dm^{-3}	185	225	290	325	65	85	110	205	0	15	60	85
100 mg dm^{-3}	30	75	120	160	0	5	55	80	0	0	15	30

The longest working time of the reactor – from 1560 to 1755 min – was noted for experimental series with the lowest flow rate (1 V h^{-1}) and the lowest inlet dye concentration (10 mg dm^{-3}). An increase in the inlet dye concentration appeared to have a substantial effect on the shortening of the working time of the reactor.

Table 6 collates the Q/Q_{max} values depending on the adopted technological conditions. The Q value indicates the quantity of dye adsorbed onto chitosan, at a corresponding assumed efficiency of dye removal reaching 100%, 99%, 95% and 90%, whereas the Q_{max} value indicates the total quantity of dye adsorbed onto chitosan when $C = C_0$.

Table 6
Utilization of the adsorption capacity of chitosan depending on the assumed dye concentration in the effluent

Inlet dye concentration	Q/Q_{max} (%)											
	0.77 dm^3 h^{-1} (V h^{-1})				1.54 dm^3 h^{-1} (2 V h^{-1})				2.31 dm^3 h^{-1} (3 V h^{-1})			
	100%	99%	95%	90%	100%	99%	95%	90%	100%	99%	95%	90%
10 mg dm^{-3}	77.2	80.2	84.4	86.7	73.5	74.6	78.7	81.6	68.9	71.0	77.0	79.8
25 mg dm^{-3}	48.9	55.3	69.1	74.5	53.0	56.8	61.6	67.5	22.0	30.7	53.5	68.1
50 mg dm^{-3}	31.9	41.1	55.7	63.1	20.2	29.4	40.6	73.7	0.0	3.2	30.9	47.7
100 mg dm^{-3}	2.1	12.6	27.3	41.3	0.0	0.5	21.9	37.8	0.0	0.0	4.9	16.6

The results obtained indicated that at the constant concentration of adsorbent in the reactor, the utilization of the total adsorption capacity of chitosan depended to a greater extent on the inlet dye concentration than on the flow rate, as it was shown for the total adsorption capacity. At the lowest tested concentration of dye (10 mg dm^{-3}) – even at the flow rate of 3 V h^{-1}, the utilization of adsorbent was found to be very high, from 68.9 to 79.8%, as affected by the assumed efficiency of dye removal. The quantity of dye adsorbed onto chitosan, at the assumed 100% efficiency of dye removal from the solution, ranged from 77.2% (at the lowest flow rate examined – 1 V h^{-1}) to 68.9% (at the flow rate of 3 V h^{-1}). Increasing the inlet dye concentration was observed to negatively affect the quantity of dye adsorbed and adsorbent utilization. Corresponding results were obtained during adsorption in an air-lift reactor onto chitin (FILIPKOWSKA 2004),

This can be explained by the fact that at a lower flow rate the time of contact between molecules of a dye and a sorbent is longer than at high flow rates. Thus it increases the possibility of dye retention onto the sorbent and, consequently, increases the load of removed adsorbate.

In addition, the results obtained indicate that in the case of a series where C_0 = 100 mg dm^{-3}, the highest increase in the utilization of the total adsorption

capacity was observed along with the assumed efficiency of dye removal. At $\eta=100\%$ (a lack of dye in the effluent), the utilization of the total adsorption capacity reached 2.1%, whereas at the assumed $\eta=90\%$ (10 mg dm^{-3} of dye in the effluent), it increased 20-fold to reach 41.3%. Decreasing the inlet concentration of the dye was accompanied by smaller differences in the utilization of the total adsorption capacity depending on the assumed efficiency of dye removal. In the case of the 10 mg dm^{-3} concentration, the difference was as little as 10%. An analogous tendency was observed for the other flow rates of the dye to the reactor.

Conclusions

The research addressed the efficiency of Black 8 removal from aqueous solutions at pH 5.0 with the adsorption method onto chitosan, under dynamic conditions. Experiments carried out within the study enabled determining the effect of inlet dye concentration and flow rate on the course of the adsorption process as well as optimal parameters and factors inhibiting the work of a circulating air-lift reactor. The results obtained indicate that:

1. at the assumed efficiency of dye removal, the adsorption capacity of chitosan is determined to the greatest extent by the flow rate. The study showed also the impact of inlet dye concentration on the total adsorption capacity of chitosan. With an increasing flow rate, the total adsorption capacity of chitosan was observed to decrease. The greatest load of adsorbed Black 8 was obtained at the lowest flow rate (1V h^{-1}) and the highest dye concentration tested (100 mg dm^{-3}). Increasing the flow rate to 2V h^{-1} (1.54 dm^3 h^{-1}) evoked a decrease in the total load of Black 8 adsorbed by 49%, on average, as compared to the flow rate of 1V h^{-1} (0.77 dm^3 h^{-1}). The subsequent elevation of the flow rate to 3V h^{-1} (2.31 dm^3 h^{-1}) decreased the total load of Black 8 adsorbed by another 42% as compared to the load achieved at the flow rate of 2V h^{-1} and by 70% in respect of the flow rate of 1V h^{-1}.

2. the flow rate was found to affect the working time of the reactor. With the increasing flow rate, the time need for the completion of the adsorption process ($C = C_0$) was observed to shorten. The shortest working time of the reactor was obtained at the highest flow rate. Also the inlet dye concentration appeared to affect the working time of the reactor. With the increasing concentration of dye in the solution inflowing to the reactor, the time after which $C = C_0$ was observed to shorten. The longest working time of the reactor was recorded once C_0 = 10 mg dm^{-3} and the flow rate reached 1 V h^{-1}.

3. investigations demonstrated a significant effect the concentration of dye on the utilization of the adsorption capacity at the assumed efficiency of dye

removal. The higher the inlet dye concentration (at the constant chitosan concentration), the lower the load of dye adsorbed at the assumed concentration of dye in the effluent. An increase in the flow rate also resulted in diminished utilization of adsorbent at the assumed efficiency of the process.

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QUANTITATIVE FLUCTUATIONS IN THE FUNGAL MICROFLORA OF ATMOSPHERIC AIR IN THE TOWN OF OLSZTYN

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K e y w o r d s: filamentous fungi, yeast, yeast-like forms, atmospheric air.

A b s t r a c t

The article presents mycological studies on atmospheric air sampled by the sedimentation and impaction methods in the centre and in a recreational area of the town of Olsztyn. The results of our two-year-long study showed variable quantities of fungal microflora in atmospheric air at the sites selected for the research. The fluctuations depended on the location of a sampling site, season of the year, air temperature and atmospheric pressure.

ILOŚCIOWE ZMIANY MIKROFLORY GRZYBICZEJ W POWIETRZU ATMOSFERYCZNYM OLSZTYNA

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S ł o w a k l u c z o w e: grzyby pleśniowe, drożdże, formy drożdżopodobne, powietrze atmosferyczne.

A b s t r a k t

Praca dotyczyła badań mikologicznych powietrza atmosferycznego oznaczanego metodą sedymentacyjną i zderzeniową w centrum i miejscach rekreacyjnych Olsztyna. Wyniki dwuletnich badań wykazały zróżnicowanie ilościowe mikroflory grzybiczej w powietrzu atmosferycznym na badanych stanowiskach. Zmiany te zależały od ich usytuowania, sezonu oraz temperatury i ciśnienia atmosferycznego.

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Introduction

Most of fungi present in air are saprophytes, although some belong to pathogenic microflora. What is characteristic of all fungi, both saprophytic and pathogenic, is that they occur at nearly all geographical latitudes. It is so because of their capability to develop within a very wide range of temperatures, pH or humidity as well as the ability to decompose nearly all organic substances. Most species of fungi whose spores are present in atmospheric or indoor air develop in soil, on organic waste or else function as parasites of plants, animals or humans (KRAJEWSKA-KUŁAK et al. 2002). Earlier studies suggest that a household environment can contain a number of filamentous fungi, of which *Cladosporium*, *Penicillium*, *Alternaria*, *Aspergillus*, *Eurotium*, *Mucor* and *Wallemia* are the most widespread ones (PASTUSZKA 2001, KRYSIŃSKA-TRACZYK et al. 2003, MACURA, GNIADEK 2003). These fungi can release large amounts of spores, which are then carried by air streams and contaminate new environments. When breathing, people can inhale fungal spores into bronchia and lungs, thus fungal spores can be an immediate cause of many diseases and allergies (MĘDRELA-KUDER 1999, SHELTON et al. 2002).

The purpose of the present study was to determine the mycological contamination of atmospheric air in the centre and in an recreational area of the town of Olsztyn. The study was based on quantitative determinations of fungal microflora carried out from October 2002 to September 2004. Samples were collected by the sedimentation and impaction methods.

Material and Methods

Sample collection. Samples of atmospheric air for mycological determinations were collected at several research sites at monthly intervals from October 2002 to September 2004. Each time sampling consisted of three series, using two methods: the sedimentation method according to recommendation of the Polish Norms (*Ochrona czystości powietrza...* PN-89 Z-04111/01, *Ochrona czystości powietrza...* PN-89 Z-04111/02, *Ochrona czystości powietrza...* PN-89 Z-04111/03, *Ochrona czystości powietrza...* PN-89 Z-O4008/08) and the impaction method, using a Mas 100 EcoTM microbiological air sampler manufactured by Merck (ZMYSŁOWSKA, JACKOWSKA 2005).

Research sites. Three research sites were set up in the centre of Olsztyn (Figure 1): site 1 – near the office building of the SANEPID Sanitary and Epidemiological Station, site 2 – near the Fire Station, and site 3 – on a bridge over the Łyna River near the Old Town. Three other sites were localised in Kortowo, an area on the outskirts of Olsztyn (Figure 2): site 4 – in the

university park near Kortowskie Lake, site 5 – at the yacht marina on Kortowskie Lake, site 6 – at a housing estate in Kortowo.

Fig. 1. Location of the sampling sites in the centre of Olsztyn

Mycological studies. Quantitative determinations comprised:

– counts of fungi, including filamentous fungi, yeast and yeast-like forms, cultured on Sabouraud medium (Biomed) after 6-day incubation at 26°C.

The results obtained with either sample collection method, the sedimentation or the impaction one, were converted into colony forming units per 1 m^3 (cfu m^{-3}). Air sample collection was accompanied by meteorological

Fig. 2. Location of the sampling sites in some recreational areas in Kortowo, part of Olsztyn

measurements. On each day when air samples were taken, the following parameters were measured: air temperature, atmospheric air relative humidity, wind direction and speed and cloud cover.

Statistical processing of the data. The results of the mycological assays were subjected to statistical analysis. In order to demonstrate statistically significant differences between the counts of fungi and location of the sampling sites in the town centre and its outskirts, as well as the differences between seasons of the year, Duncan's test was applied in one-factor analysis of variance. Multiple regression assay was used to test the simultaneous effect of temperature, air relative humidity and wind speed on the number of fungi. The significance level assumed for all the statistical analyses was $p \leq 0.05$.

Results

Presence of fungi in 1 m^3 of atmospheric air in Olsztyn sampled with the sedimentation method was not detected in December 2002 (sites 3 and 5), in February 2003 (sites 2, 5 and 6), in January 2004 (sites 2, 4 and 6) and in February and April 2004 (site 6). Maximum counts of fungi (13 565 cfu m^{-3}) were determined in July 2003 (site 6) – Table 1. The analysed assemblages of fungi were dominated by filamentous fungi, which made up between 30% (in March 2003) to 100% (in February the same year) of the whole population. The proportion of yeast-like forms and yeasts in the total number of fungi was small, not exceeding 20%. On three occasions, in December 2002, March 2003 and January 2004, their percentage was higher (Figure 3).

Table 1

Counts of fungi in 1 m^3 of air sampled by the sedimentation method at the sites located in the centre (1-3) and in a recreational part (4-6) of Olsztyn, in 2002-2004

Months	Sites						Mean and standard deviations
	Centre			Recreational areas			
	1	2	3	4	5	6	
1	2	3	4	5	6	7	8
2002							
X	708	747	1180	983	1730	1651	1166±441
XI	275	432	1730	354	236	197	537±590
XII	39	197	0	983	0	39	210±386
Mean and standard deviations	341±339	459±276	970±884	773±363	655±938	629±888	–
2003							
I	590	786	157	79	432	236	380±273
II	79	0	39	39	0	0	26±32
III	1494	983	432	197	197	1101	734±537
IV	275	550	197	315	79	354	295±158
V	1101	1494	2556	3263	1730	3224	2228±919
VI	2320	2674	4050	5740	3853	2831	3578±1259
VII	2634	4050	3971	7195	10616	13565	7005±4316
VIII	1691	1062	1415	3853	2556	3774	2392±1207
IX	1455	1102	661	1278	1102	1278	1146±272
X	132	441	132	926	264	309	367±297
XI	431	1180	865	629	1219	590	819±326
XII	236	79	79	78	79	354	151±118
Mean and standard deviations	1036±881	1200±1141	1213±1493	1966±2465	1844±3004	2301±3774	–

cont. table 1

1	2	3	4	5	6	7	8
2004							
I	79	0	236	0	79	0	66±92
II	39	157	550	472	118	0	223±231
III	341	2621	236	419	157	996	795±942
IV	577	1075	813	577	970	0	669±385
V	708	2018	1730	5452	1520	1861	2215±1651
VI	2045	2962	1756	2228	2333	2621	2324±425
VII	2700	4115	6029	6710	2438	2674	4111±1859
VIII	1546	2071	4168	7261	2464	4168	3613±2092
IX	2176	2123	865	1180	655	970	1328±658
Mean and standard deviations	1134±998	1905±1322	1820±1994	2700±2936	1193±1023	1477±1465	–

The total count of fungi in air samples collected with the impaction method ranged between 6 cfu m^{-3} in December 2002 (site 1), February 2003 (sites 2 and 4) up to 25 525 cfu m^{-3} in July 2003 (site 5), (Table 2). The analysed group of microorganisms was dominated by filamentous fungi fungi, which made up between ca 90% (in September 2003) to 100% (in October 2002, February 2003, and in March and July 2004) of the whole population. Yeast-like forms and yeasts were not detected in October 2002, February 2003 and in March and July 2004, although their percentage determined in September 2003 was ca 10% (Figure 4).

Table 2

Counts of fungi in 1 m^3 of air sampled by the impaction method at the sites located in the centre (1-3) and in a recreational part (4-6) of Olsztyn, in 2002-2004

Months	Sites						Mean and standard deviations
	Centre			Recreational areas			
	1	2	3	4	5	6	
1	2	3	4	5	6	7	8
2002							
X	933	783	1417	808	825	850	936±241
XI	338	425	188	608	92	183	306±190
XII	6	25	95	10	20	55	35±34
Mean and standard deviations	426±470	411±379	567±738	475±415	312±445	363±427	–

cont. table 2

1	2	3	4	5	6	7	8
2003							
I	133	300	200	112	94	88	154±82
II	15	6	10	6	40	25	17±14
III	265	145	160	140	80	130	153±61
IV	117	158	192	131	62	400	177±118
V	1212	1150	1325	3300	1358	2500	1807±886
VI	*	*	*	*	*	*	*
VII	5692	6808	5500	7194	25525	22862	12263±9301
VIII	2150	2288	2150	3167	2233	5875	2977±1472
IX	808	658	533	800	369	544	619±171
X	*	*	*	*	*	*	*
XI	667	625	767	631	975	681	724±133
XII	150	267	167	1750	217	225	463±632
Mean and standard deviations	1121±1737	1240±2070	1100±1684	1723±2288	3095±7914	3333±7097	–
2004							
I	*	*	*	*	*	*	*
II	175	133	33	100	108	83	105±48
III	350	425	258	842	358	1292	587±401
IV	*	*	*	*	*	*	*
V	733	800	908	758	1192	1567	993±327
VI	2511	3794	1144	6872	1739	3394	3242±2035
VII	6133	4483	5300	5000	3633	5067	4936±835
VIII	3650	5550	4317	1667	2000	2817	3333±1470
IX	683	1108	850	1475	2000	875	1165±492
Mean and standard deviations	2033±2215	2327±2215	1830±2090	2388±2535	1576±1183	2156±1707	–

* samples not collected

The highest average contamination of atmospheric air with fungal microflora was determined at site 4 in 2004 (2700 cfu m^{-3}) and site 6 in 2003 (3333 cfu m^{-3}). These counts were determined in air sampled with the sedimentation and impaction methods, respectively (Table 1, 2).

The assays conducted in consecutive seasons of the year during the years 2002-2004 showed the highest mean counts of fungi in the air collected in summer: about 4000 cfu m^{-3} (sedimentation method) and 5000 cfu m^{-3} (impaction method). The lowest counts of fungi were discovered in winter, irrespective of the sampling method (Figure 5).

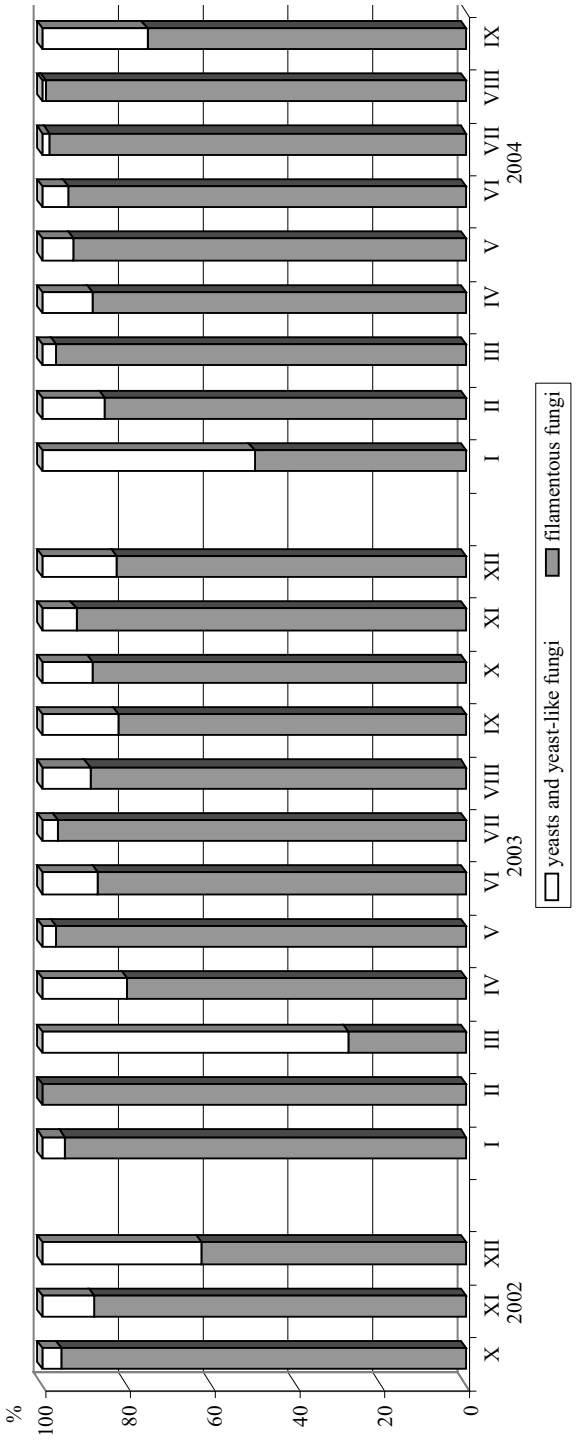

Fig. 3. Percentage of filamentous fungi as well as yeasts and yeast-like forms in 1 m^3 of atmospheric air sampled by the sedimentation method in Olsztyn, as determined in particular months of the study from 2002 to 2004

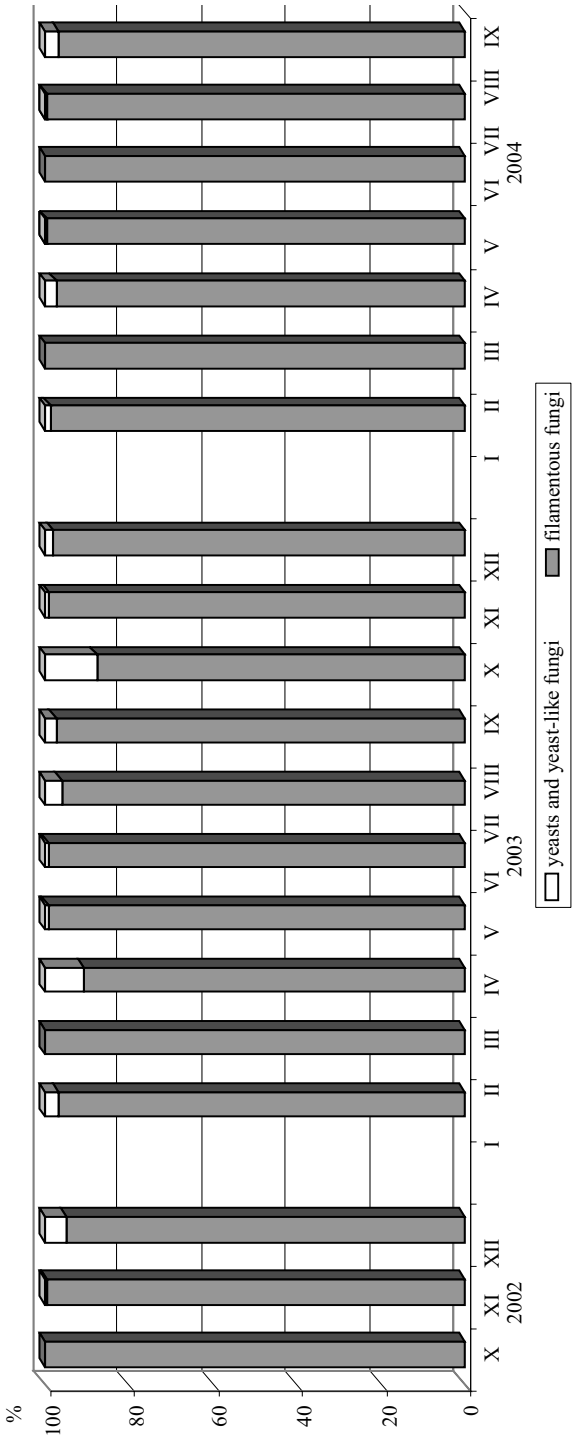

Fig. 4. Percentage of filamentous fungi as well as yeasts and yeast-like forms in 1 m^3 of atmospheric air sampled by the impaction method in Olsztyn, as determined in particular months of the study from 2002 to 2004

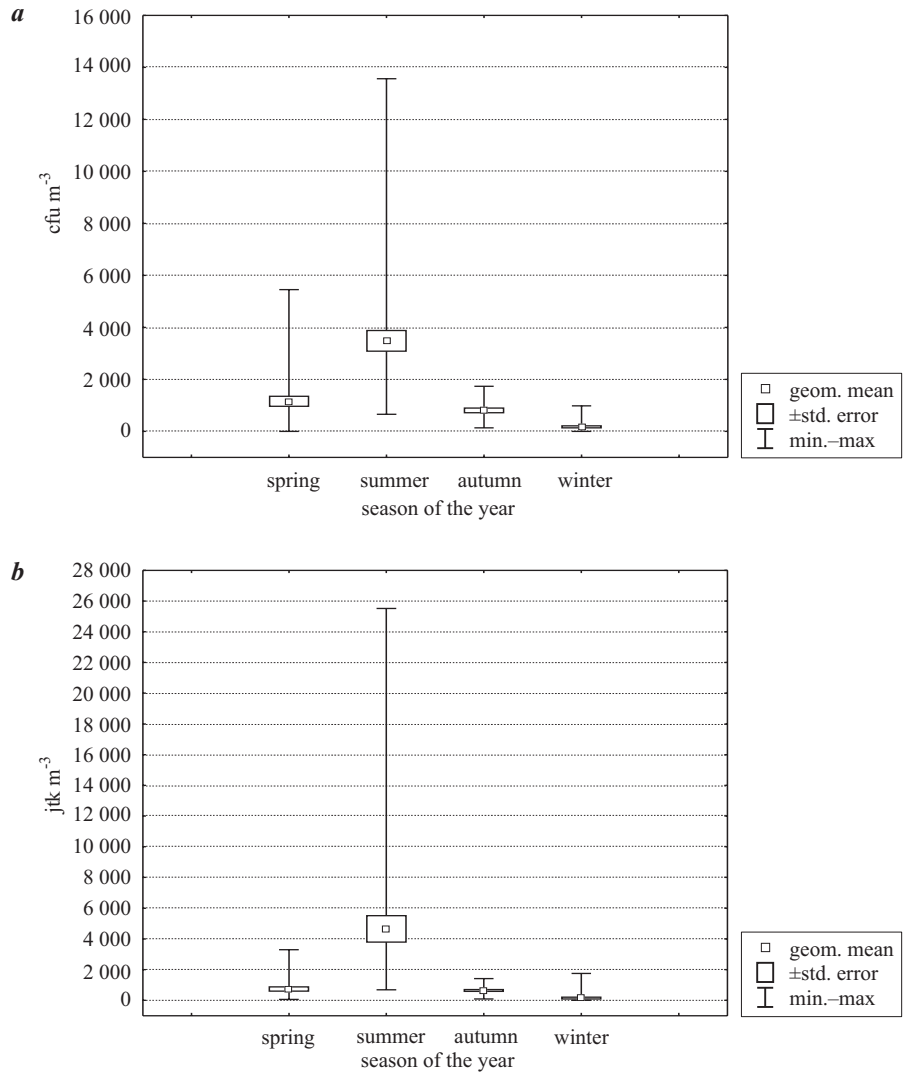

Fig. 5. Mean counts and count ranges of fungi in 1 m^3 of atmospheric air in Olsztyn collected in particular research periods between 2002 and 2004 by: *a* – the sedimentation method, *b* – the impaction method

The mycological measurements in Olsztyn were carried out during the day, between 7.30 a.m. and 1.30 p.m. The meteorological observations completed while collecting air samples proved that the weather conditions prevailing on the sampling days were suitable for proper collection of air samples for mycological determinations. It was only in December 2002, February 2003 and in January, February and May 2004 that on the day of collecting air samples

there was some intermittent drizzle, snow or rain. On the days preceding the sampling no rainfall or snow was recorded.

The elaboration of the results obtained from mycological analyses of atmospheric air which employed one-factor analysis of variance (Duncan's test) demonstrated some statistically significant differences between mean counts of fungi and location of the research sites. Higher amounts of fungi were determined at the sites located in the recreational area (sites 4, 5, 6) than in the centre of the town (sites 1, 2, 3), both in the samples collected by the sedimentation and impaction methods (Table 3). Statistically significant differences were also observed between the mean counts of fungi and season of the year. Generally, higher counts of fungi were determined in the spring and summer, in the air samples collected by either of the methods (Table 4).

Table 3

Results of the one-factor analysis of variance (Duncan's test) which assessed the significance of the effect of a site location (in the town centre and in Kortowo, a recreational part of Olsztyn) on counts of fungi, in air samples collected by the sedimentation and impaction methods, in 2002-2004

Sites	Methods	
	Sedimentation	Impaction
Centre	17.16^{b}	59.12^{b}
Recreational area	23.35^{a}	123.31^{a}

a, b – mean values in the same columns designated with different letters are significantly different

Table 4

Results of the one-factor analysis of variance (Duncan's test) which assessed the significance of the effect of seasons of the year on counts of fungi, in air samples collected by the sedimentation and impaction methods, in 2002-2004

Season	Methods	
	Sedimentation	Impaction
Spring	14.88^{bcd}	34.87^{b}
Summer	42.44^{ad}	210.95^{acd}
Autumn	10.01^{bd}	40.64^{b}
Winter	2.23^{abc}	10.01^{b}

a, b, c, d – mean values in the same columns designated with different letters are significantly different

Based on the analysis of multiple regression, which assessed the simultaneous effect of meteorological factors, i.e. temperature, relative air humidity, atmospheric pressure and wind speed, on the counts of fungi in the air samples gathered by the sedimentation and impaction methods, two climatic factors: temperature and atmospheric pressure were found to have some influence (Table 5).

Table 5

Results of the multiple regression analysis dealing with the effect of meteorological factors on counts of fungi, in air sampled by the sedimentation and impaction methods, in 2002-2004. The table contains only those coefficients which proved to have significant influence and statistics of the multiple regression analysis (corrected R^2 and F)

Groups of microorganisms	Meteorological factors						
	temperature	relative humidity	atmospheric pressure	wind speed	free term	corrected R^2	F
Method							
Fungi	Sedimentation						
	1.93		-0.376		361.51	0.389	31.357
	Impaction						
	10.268		-6.559		6541	0.187	14.696

Discussion

The literature dealing with mycology contains an increasing number reports on the presence of fungal spores in atmospheric air, which is due to the fact that some filamentous fungi produce very toxic secondary metabolites, known as mycotoxins (HENDRY, COLE 1993, HORNER et al. 1995). Long-term contact with fungi which produce harmful mycotoxins, such as alphatoxins produced by *Aspergillus flavus* and ochratoxins produced by *Aspergillus ochraceus*, may lead to various illnesses (NAYAK et al. 1998, DUTKIEIWCZ, GÓRNY 2002). The distribution of fungal microflora in the air we analysed shows some seasonal regularity. During the spring and summer the amount of fungal spores significantly increased compared to the other seasons of the year. The highest amounts of fungal spores were recorded in July 2003 at the sites located in the recreational part of Olsztyn: 13 565 cfu m^{-3} in samples collected by the sedimentation method and 25 525 cfu m^{-3} in the samples obtained by the impaction method. The season of the year as well as the climatic conditions prevailing at that time (higher temperature and relative humidity of the air) are of importance (ROSAS et al. 1993, DI GIORGIO et al. 1996, FERNANDEZ et al. 1998, MOLINA et al. 1998, NAYAK et al. 1998, GRINN-GOFROŃ 2005). Similar seasonal fluctuations in the occurrence of fungi was recorded by MĘDRELA-KUDER (1983, 1992, 1999, 2003 a, b), who carried out mycological tests of air at some locations in Kraków. MĘDRELA-KUDER found out that summer (July and August) was the time when the fungal spores occurred at the maximum number, which clearly declined in the other seasons of the year. This was also connected with a seasonal occurrence of some fungi of the genera *Cladosporium* and *Alternaria* (MĘDRELA-KUDER 1992). SHELTON et al. (2002), who investigated air in

some locations across the USA, determined the highest fungal contamination of air in summer. In the present study, the highest average contamination of air with fungal microflora, whichever sampling method was applied, was determined in the recreational area of Olsztyn. This was most likely connected with the presence of plants, which most certainly was more abundant in the recreational area than in the town centre. Compared to the Polish Norms (*Ochrona czystości powietrza...* PN-89 Z-04111/03), just 3 and 3.5% (the sedimentation and impaction method, respectively) of the air sampled in the recreational area could be classified as contaminated to the extent that might adversely affect the natural environment of man (Table 6).

Table 6

Atmospheric contamination range (in %) at the sites located in the centre and in an recreational area of Olsztyn, determined in air sampled by the sedimentation and impaction methods, in 2002-2004

Group of microorganisms		Atmospheric air contamination range		
		moderately clean atmospheric air, especially in late spring and early autumn	contamination which may affect adversely man's natural environment	contamination which affect adversely man's natural environment
Fungi	Method			
	Sedimentation			
	*	99%	1%	0%
	**	90%	7%	3%
	Impaction			
	*	90%	10%	0%
	**	88.5%	8%	3.5%

* – centre
** – recreational area

Conclusions

1. It was determined that counts of fungi were generally higher in the recreational area of Olsztyn than in the town centre, both in the air samples collected by the sedimentation method and those obtained by the impaction method.

2. Seasonal fluctuations in the counts of fungi were demonstrated, with the highest amounts of fungi in the atmospheric air in Olsztyn being recorded in the spring and summer months.

3. Significant statistical relationships were determined between the location of a sampling site, season of the year and meteorological conditions versus number of fungi in air.

4. The air in Olsztyn assayed in the years 2002-2004, both in the town centre and in the recreational area, was mostly uncontaminated with fungi.

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EFFECT OF ELECTRIC CURRENT ON THE OXYGENATION CAPACITY OF ROTATING BIOLOGICAL CONTACTOR

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K e y w o r d s: rotating biological contactor, oxygenation capacity, electric current.

A b s t r a c t

Investigations were carried out in a laboratory scale in a rotating biological contactor in which cathode was made of stainless steel disks with immobilized biological membrane, whereas electrode made of stainless steel or steel sheet or aluminium one were used as anode. In the first stage of the experiment, the oxygenation capacity of the rotating biological contactor was investigated under conventional conditions – without the flow of electric current; whereas in the second stage of the study – under the flow of electric current with density range from 0.2 A m^{-2} to 1.5 A m^{-2}. The highest values of oxygenation capacity (OC) were obtained in the system in which electrode made of stainless steel served as anode, and the lowest ones – in the conventional system.

WPŁYW PRĄDU ELEKTRYCZNEGO NA ZDOLNOŚĆ NATLENIANIA ZŁOŻA BIOLOGICZNEGO

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S ł o w a k l u c z o w e: biologiczne złoże tarczowe, zdolność natleniania, prąd elektryczny.

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Abstrakt

Badania prowadzono w skali laboratoryjnej na elektrobiologicznym złożu tarczowym, w którym katodą były tarcze ze stali nierdzewnej z unieruchomioną błoną biologiczną, anodą – jedna z elektrod – elektroda ze stali nierdzewnej, z blachy stalowej lub aluminiowa. W pierwszym etapie badano zdolność złoża biologicznego do natleniania w warunkach konwencjonalnych – gdy nie przepływał prąd elektryczny, w drugim – w warunkach przepływu prądu elektrycznego o gęstości z zakresu od 0,2 A m^{-2} do 1,5 A m^{-2}. Najwyższe wartości OC (zdolności natleniania) uzyskano w układzie, w którym anodą była elektroda ze stali nierdzewnej, a najniższe w układzie konwencjonalnym.

Introduction

RBC units are widely used in the treatment of waste water because it is possible to obtain high performance of the removal of dissolved pollutants at the expense of less energy than by using activated sludge systems. A typical trait of RBC units is the fact that bacteria colonize the surface of their filling and form a biofilm.

The biofilm is a complex system composed of microbial cells and colonies deposited in a polymeric carrier and its composition is a function of its age and environmental conditions. The main constituents of the biofilm are bacterial cells and exopolymers (LAZAROVA et al. 1995). ZHANG et al. (1994) demonstrated that the bottom layers of biofilms were 5 to 10 times thicker than the upper ones, and that the ratio of viable cells to total biomass decreased from 72-91% in the upper layers to 31-39% in the bottom ones, as well as that porosity of biofilms was changing from to 84-93% to 58-67%, respectively. Biofilm thickness increases the rate of reactions in biological membrane but, simultaneously, restricts diffusion of a substrate due to a decreased coefficient of efficient diffusion. An increase in biofilm thickness results in an increasing bacterial count followed by increased rate of substrate consumption. Due to restricted diffusion, however, the increase in biofilm thickness is accompanied by the development of a layer of inactive microorganisms participating in the process of substrate removal (ŞEKER et al. 1995).

Waste water purification according to oxidative biological processes requires maintenance of a distinct concentration of dissolved oxygen in the water to be treated. The degree of purification declines considerably when the amount of oxygen drops below 2 mg dm^{-3}. The ability of the rotating biological contactor to maintain a certain oxygen concentration in the trough depends among other things on the oxygenation capacity (OC). This is the capacity of the installation to supply oxygen to the water to be purified:

$$OC = K_L \cdot A \cdot C_s$$

where:
K_L – is the oxygen transfer coefficient to water m s^{-1},
A – wetted area m^2,
C_s – saturation concentration of oxygen in water kg m^{-3} (BINTANJA et al. 1975).

Two types of oxygen transfer take place inside the biofilm:

– through the air-liquid interface in the reactor tank due to the turbulence caused by disk rotation,

– through the liquid film adhering to the disks during the aeration phase. This liquid film, whose thickness depends on the disks rotational speed and diameter, in the absence of biomass becomes saturated with oxygen during the aeration phase and is then remixed with the liquid on re-entering into reactor (DI PALMA et al. 2003).

Studies on the physical transport of oxygen with disks free of biomass have shown that the mechanism of oxygenation through the liquid film is the one that predominates at high rotational speed (higher than about 10 rpm). At low rotational speed, the two contributions are the same order of magnitude (SHIEH 1982).

The presence of attached biomass on disk surfaces causes a significant increase in the amount of transport due to the film mechanism. This is due both to the considerable increase in the surface area exposed and to the consumption of oxygen, which prevents saturation of the film in the aeration phase. As a result of the presence of biomass, at least 90% of the oxygen is dissolved through the film mechanism (SPENGEL, DZOMBAK 1992). FU (1994) investigated changes in oxygen concentration inside the biofilm and noticed that efficient diffusivity of oxygen is linked with biofilm thickness. It decreases down to the bottom of biofilm from 90% at the surface to 25% in the bottom layer. Absorption rate of oxygen inside the biofilm is a function of substrate concentration. Once substrates, nutrients and organic compounds were removed from the solution, the rate of respiration was limited only to endogenous respiration.

STOODLEY et al. (1997) investigated the possibility of applying electric current to improve the efficiency of antibiotics incorporation into biological membranes and assumed that electric impulses would increase the transport of antibiotic mass into biofilm, acting based on the mechanism of a pump. Their investigations were carried out on biofilm immobilized on a platinic, rod-like electrode that reached thickness of ca. 50 μm after 3 days. The application of electric current evoked significant changes in the structure of biofilm. Its thickness increased by 4% (from 44.7 μm to 46.4 μm) when the rod served as cathode and was reduced by 26% (to 33.2 μm) once the rod served

as anode. The structural changes in the biofilm can be elucidated by electrostatic interaction between charged groups of the biofilm and charges on the rod. In contact with negatively-charged cathode, the negatively-charged groups of extracellular polymeric substances, forming the base of cellular membrane, and often negatively-charged cell walls will repulse one another, thus evoking development and increasing the thickness of biofilm, whereas their contact with anode will result in shrinkage of the biofilm. The above-presented results of studies enable assuming that the application of electric current in processes of waste water treatment will affect an increase in the efficiency of oxygen and pollutants introduction to biofilms and their faster neutralization.

Aim and range of research

The research is aimed at determining the effect of electric current flow on the oxygenation capacity of rotating biological contactor.

Material and Methods

Investigations involved:

– monitoring of changes in oxygen concentration in time,

– determination of the effect of various densities of electric current of oxygenation capacity,

– selection of the type of anode based on OC values computed.

The investigations were carried out on a conventional biofilm in two stages: without the flow of electric current, and under the flow of electric current, following the experimental design presented in Figure 1.

The first stage of the study was carried out on conventional biofilm, without the flow of electric current.

The second stage was run under the flow of electric current with density range of 0.2 A m^{-2} to 1.5 A m^{-2} in three variants:

– cathode – disks made of stainless steel with immobilized biofilm; anode – aluminium electrode,

– cathode – disks made of stainless steel with immobilized biofilm; anode – electrode made of steel sheet,

– cathode – disks made of stainless steel with immobilized biofilm; anode – electrode made of stainless steel.

Oxygenation Capacity (OC) of the rotating biological contactor was determined according to the methodology described by CYWIŃSKI et al. (1972).

The rotating electrobiological contactor (REBC) consisted of four sections

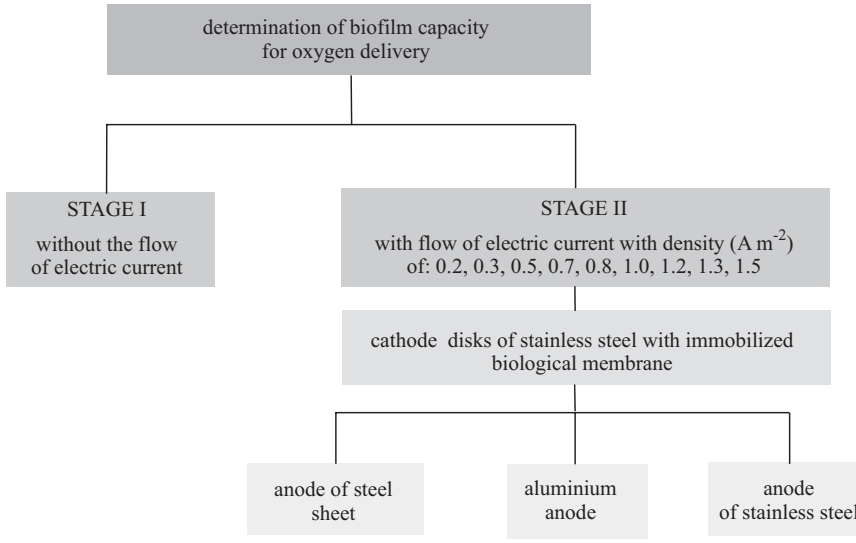

Fig. 1. Design of an experiment aimed at determining the oxygenation capacity of rotating biological contactor

(Figure 2). Each section was made of sets of discs mounted concentrically on a horizontal shaft, 0.42-m long. Each of four disc-sections consisted of 8 discs with 0.22-m diameter. Discs in the sections were made of stainless steel. The total active surface of the contactor equaled to 0.6 m^2. Each section was placed in a half-round tank with 2 dm^3 volume. The discs rotating with a speed of 60 rpm were moved by an electric motor. In the flow-tanks, electrodes made of stainless steel were mounted and connected with insulated wires to a rectifier providing the required current intensity.

In order to systematize the results obtained, the statistical analysis was begun from descriptive statistics. The minimum, maximum, mean value and standard deviation were determined for each variable, which was additionally analyzed for normal distribution. The latter was analyzed with the Shapiro--Wilk W-test with the following null hypothesis – H_o: distribution of the analyzed variable is normal. Rejection of the null hypothesis means that a given variable does not have normal distribution. Determination whether a given variable has normal distribution or not is highly important in the selection of the type of test used in the analysis of variance.

In order to verify whether n independent samples originate from the same population, use was made of the Kruskal-Wallis test being a non-parametric equivalent of one-way analysis of variance. In the tests, the level of significance was stipulated at $\alpha=0.05$.

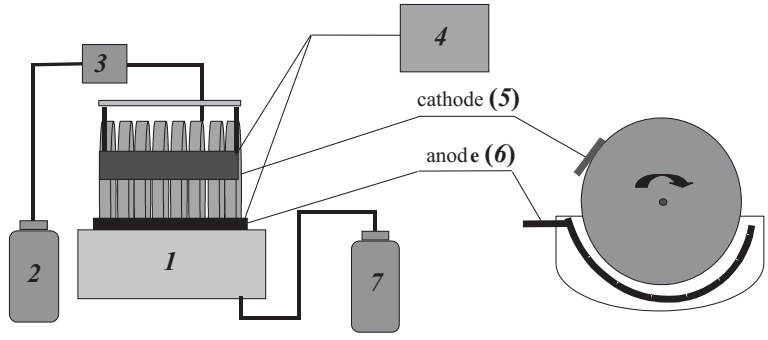

Fig. 2. The scheme of an experimental post: *1* – rotating biological contactor, *2* – tank with untreated waste water, *3* – peristaltic pump, *4* – electric current source, *5* – cathode, *6* – anode, *7* – tank with treated waste water

Results and Discussion

At the first stage of the experiment, the oxygenation capacity of the conventional rotating biological contactor was analyzed under no flow of electric current, whereas at the second stage – under the flow of electric current with densities of: 0.2, 0.3, 0.5, 0.7, 0.8, 1.0, 1.2, 1.3 and 1.5 (A m^{-2}). In the study, use was made of 3 types of anode: aluminum anode, anode made of steel sheet and anode made of stainless steel. The highest OC value reaching 418.4 (mg O_2 dm^{-3} h^{-1}) – being a mean of all results obtained – was observed in the system in which electrode made of stainless steel served as anode. The lowest OC value, i.e. 254.7 (mg O_2 dm^{-3} h^{-1}), was reported in the treatment system with no flow of electric current (Figure 3).

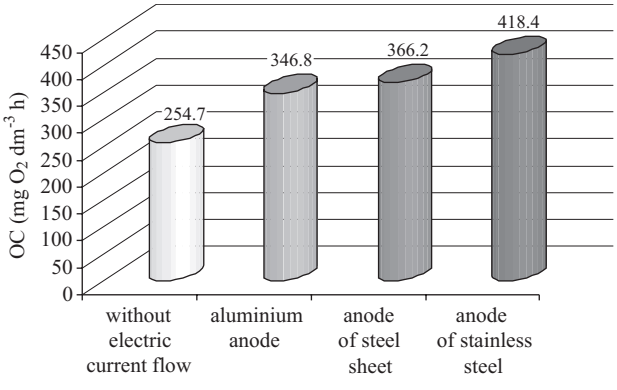

Fig. 3. Oxygenation capacity of rotating biological contactor

Results of the descriptive statistical analysis of the oxygenation capacity of the rotating biological contactor indicate that the lowest variability, expressed by the value of standard deviation (s=26.89), was observed for the conventional system without the flow of electric current. In contrast, the highest variability was reported in the system with aluminum anode and that with anode made of stainless steel – the value of standard deviation reached 59.83 and 56.55, respectively (Table 1).

Table 1
Results of descriptive statistical analysis of the oxygenation capacity of rotating biological contactor

OC value (mg O_2 dm^{-3} h^{-1})	Medium	Minima	Maximal	Standard deviation
Conventional system	254.7	196.92	286.74	26.89
System with aluminium anode	346.8	318.9	383.0	59.83
System with anode of steel sheet	366.2	313.62	438.8	46.47
System with anode of stainless steel	418.4	338.43	491.25	56.55

Based on the results of the Kruskal-Wallis test, at a significance level of 0.000, it can be stated that the type of anode applied has a statistically significant effect on biofilm capacity for oxygenation (Table 2).

Table 2
Results of the rank sum test of Kruskal-Wallis for the oxygenation capacity of rotating biological contactor

Parameter	Results of rank sum test of Kruskal-Wallis		
	n	*H*	*p*
Oxygenation capacity of biofilm	290	109.4184	0.000

In the experiment, the OC values were observed to increase along with increasing density of the electric current. The highest OC values were reported in the system with electrode of stainless steel used as anode (Figure 4). In that system, OC obtained under the flow of electric current with a density of 1.5 A m^{-2} reached 491.4 (mg O_2 dm^{-3} h^{-1}), whereas under the same conditions in the system with aluminum anode the OC value accounted for 318.9 (mg O_2 dm^{-3} h^{-1}) and in that with anode made of steel sheet – for 438.2 (mg O_2 dm^{-3} h^{-1}).

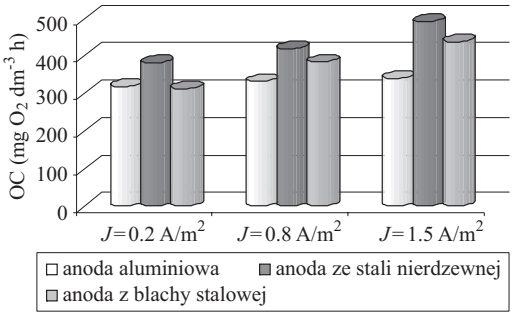

Fig. 4. Effect of electric current density on the oxygenation capacity of biofilm

Next, the OC values obtained under conditions of the flow of electric current with densities of: 0.2, 0.8 and 1.5 (A m^{-2}) in the systems with three different anodes were subjected to a statistical analysis.

Under the flow of electric current with a density of 0.2 A m^{-2} the lowest variability of the OC values obtained was observed in the system with anode of steel sweet – standard deviation equal to 26.71. Slightly higher diversity was obtained in the system with anode made of stainless steel – s=43.48. In turn, the highest variability was reported for the values obtained in the system with aluminum anode – the highest value of standard deviation – 70.34 (Table 3).

Table 3

Results of descriptive statistical analysis of the oxygenation capacity of rotating biological contactor depending on electric current density

Electric current density	0.2 (A m^{-2})				0.8 (A m^{-2})				1.5 (A m^{-2})			
OC value (mg O2 $dm^{-3} \cdot h^{-1}$)	mean	min.	max.	s	mean	min.	max.	s	mean	min.	max.	s
System with aluminium anode	318.9	175.31	442.76	70.34	332.2	177.05	385.26	43.95	341.0	258.34	408.35	18.54
System with anode of stainless steel	381.6	244.57	427.83	43.48	419.8	362.65	466.58	34.65	491.4	447.36	536.78	30.81
System with anode of steel sheet	313.6	271.01	340.54	26.71	384.14	368.18	403.29	12.81	438.8	399.32	493.03	36.62

s – standard deviation

Similar distribution of variability was observed under the flow of electric current with a density of 08 A m^{-2}. The lowest diversity was reported for the OC values obtained in the system with anode made of steel sheet – standard

deviation equal to 12.81, and slightly higher one in the system with anode made of stainless steel – s=34.65. The highest variability was recorded in the system with aluminum anode – s=43.95.

The lowest variability of the OC values, under conditions of the flow of electric current with a density of 1.5 A m^{-2}, was found in the system with aluminum anode – standard deviation equal to 18.54. Twice as much diversity was observed in the system with anode made of steel sheet – s=36.62 and a slightly lower one – in the system with anode made of stainless steel – s=30.81.

Results of the Kruskal-Wallis test indicate that the type of anode applied has a statistically significant effect on the oxygenation capacity of the rotating biological contactor, and that under conditions of electric current flow with a density of 0.2 A m^{-2} the null hypothesis can be rejected at a significance level of 0.000, whereas at the flow of electric current with density of 0.8 A m^{-2} and 1.5 A m^{-2} it can be rejected at a significance level of 0.009 and 0.0002, respectively (Table 4).

Table 4

Results of the rank sum test of Kruskal-Wallis for the oxygenation capacity of rotating biological contactor depending on electric current density

Parameter	Results of rank sum test of Kruskal-Wallis		
	n	H	p
0.2 A m^{-2}	30	19.41840	0.000
0.8 A m^{-2}	30	14.03077	0.0009
1.5 A m^{-2}	30	17.30954	0.0002

The principal aim of aeration is delivery of oxygen to sewage and mixing the contents of the reactor. In the aeration process the rate of gases exchange between air bubbles and sewage is determined by diffusion exchange of mass. HARRIS et al. (1996) investigated the effect of changeable flow rate of air and changeable volumetric loading of a reactor with organic pollutants on the oxygen transfer efficiency in the biological aerated filters. They observed the increase in the oxygen transfer efficiency at the low air flows. Besides the improvements in the oxygen transfer efficiency with rising volumetric loading rate was observed at the three lower loadings, but ceased upon the imposition of a very high load (2.7 kg BOD m^{-3} d^{-1}). This may be explained by the findings of Lee and Stensel (1986) which were that the oxygen transfer efficiency was raised during periods of high organic load and raised oxygen limitation. As the load increased, so the microbial activity within the columns was raised, improving the efficiency by increasing the demand for oxygen, and so the driving force.

One of the main parameters that determine the efficiency of nitrogen compounds removal is oxygen concentration in the chamber. It has been confirmed in a study by JANG et al. (2002) who proved, that the bacterial distributions were significantly changed according to the dissolved oxygen (DO) condition. In the case of 2 mg DO dm^{-3}, the organic oxidizing bacteria, heterotrophs dominated in the whole depth of the biofilm. When DO was kept at 10 mg dm^{-3}, on the other hand, the numbers of nitrifying bacteria were significantly increased. In addition the distributions changed according to the relative depth of biofilm indicating that the microorganisms with slow growth-rate in a mixed autotrophic/heterotrophic biofilm are typically more abundant in deeper sections of the biofilm where they can compete for oxygen. Especially slow-growing autotrophic organisms, *Nitrobacter* exist abundantly near the biofilm surface. It is understood that nitrifying bacteria can effectively compete with heterotrophs under high dissolved oxygen conditions. DANGOONG et al. (2000), while investigating the effect of nitrogen administration on the nitrification process carried out in an SBR, demonstrated that oxidation of ammonia nitrogen and nitrates III occurred simultaneously under controlled inflow of oxygen (oxygen concentration in the reaction ranged from 2 to 3 mg dm^{-3}). In contrast, the application of uncontrolled administration of nitrogen (oxygen concentration in the reactor was changing) resulted in a complete inhibition of oxidation of nitrates III at low concentrations of oxygen.

The results achieved by POLLICE et al. (2002) indicate dissolved oxygen as an alternative parameter for controlling nitrification to nitrite. The possibility of converting ammonium into nitrite by modifying the aeration patterns instead of the sludge retention time is attractive considering potential energy savings and looking at the operational practices. Air supply is probably an easier parameter to control in SBRs with respect to the sludge age, and allows better flexibility to plant operations. Moreover, the adoption of this parameter would decrease the risks of biomass washout, which may be relevant when operating at very short SRTs. Finally, alternated aeration as a strategy to limit the oxidation of ammonia to nitrite may also favour the processes of autotrophic simultaneous ammonia and nitrite removal. Examinations at different DO concentrations (HELMER et al. 2001) make apparent that in dependence on the oxygen supply there are not only different reactions rate, but obviously also different reactions courses. In the nitrifying reactor 1, a nitrifying population with the capability to effect nitritation was found, which reacted to an improvement of the oxygen supply with a considerable increase in efficiency. In reactors 2 and 3, there was found – apart from the capability to effect nitritation – a clear potential to deamonification, that Is ammonium could to a high degree be eliminated without the emergence of either nitrite or nitrate.

In contrast to the nitritation, the deammonification reacted sensitively to increased DO concentrations: at a DO concentration of 0.7 mg dm^{-3}, ammonium was eliminated almost completely at 98.9%, whereas with higher DO concentrations the production of nitrite and nitrate from ammonium increased.

Besides Wilen and BALMÉR (1999) showed, that low DO concentrations produced activated sludge with poor settling and thickening properties, mainly due to excessive growth of filamentous bacteria and the formation of porous flocs. Also they proven, that the turbidity of the supernatant was mostly higher at low than at high DO concentrations.

An appropriate concentration of nitrogen is also a significant parameter in the removal of some pollutants. MELO et al. (2005) investigated the efficiency of the phenol biodegradation in a batch reactor system and a rotating biological contactor (RBC). They proved, that the rate of phenol degradation increased with increase in oxygen supply. At a biomass concentration close to 1000 mg dm^{-3} MLSS, the phenol degradation rate almost doubled when the volume of air supplied to the system was increased from 2 to 4 dm^3 h^{-1} in the batch reactor system. They investigated the effect of process variable such as speed of rotation on phenol removal and on level of dissolved oxygen in a different flow rates and phenol concentrations. Improvement in phenol degradation was observed with increase in the speed of rotation.

Conclusions

The research demonstrated the effect of electric current and type of anode applied on the oxygenation capacity of the rotating biological contactor. The highest OC values were obtained in the system in which electrode made of stainless steel served as anode. In addition, increased OC values were reported along with increasing density of electric current. The statistical analysis of the results obtained confirms that the type of electrode applied has a statistically significant impact on biofilm capacity for oxygenation.

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ENTEROBACTERIACEAE FAMILY BACTERIA 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: enteric bacteria, water pollution, cormorant, lake.

A b s t r a c t

Counts of *Enterobacteriaceae* family bacteria were assayed in the water of Długie Wigierskie Lake, which are situated in Wigry National Park and is settled by a colony of about 1000 black cormorants. The studied were conducted at monthly intervals in spring, summer and autumn of 2000-2001. Water samples were collected at 14 sites. Counts of *Enterobacteriaceae* family bacteria in the water of Długie Wigierskie Lake were very small in studied capacity water samples. The highest numbers of these bacteria not exceed 910 in 1 cm^3; simetimes, no presence of these bacteria was determined. *Enterobacteriaceae* family bacteria was determined in higher numbers from July to September, especially in the water sampled at the sites located in the areas inhabited by cormorants. Among the bacteria which belong to the *Enterobacteriaceae* family bacteria *Enterobacter gergoviae* (15.5%), *Klebsiella oxytoca* (9.8%), *Serratia odorifera* (9.8%), *Citrobacter freundii* (9.2%) and *Enterobacter cloacae* (7.6%) were most numerous, whereas pathogenic *Salmonella* and *Shigella* genus were not detected in water samples.

BAKTERIE Z RODZINY *ENTEROBACTERIACEAE* W WODZIE JEZIORA MEZOTROFICZNEGO (JEZIORO DŁUGIE WIGIERSKIE) W WARUNKACH BYTOWANIA KORMORANA CZARNEGO (*PHALOCROCORAX CARBO*)

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S ł o w a k l u c z o w e: bakterie kałowe, zanieczyszczenie wody, kormorany, jezioro.

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A b s t r a k t

Praca dotyczy badań liczebności i składu jakościowego bakterii z rodziny *Enterobacteriaceae* w Jeziorze Długim Wigierskim na obszarze Wigierskiego Parku Narodowego, w związku z bytowaniem tam licznej (ok. 1000 osobników) kolonii kormorana czarnego. Badania przeprowadzono wiosną, latem i jesienią 2000-2001, w odstępach 1-miesięcznych na wodzie pobieranej z 14 stanowisk. Bakterie z rodziny *Enterobacteriaceae* w wodzie badanego zbiornika występowały nielicznie. Maksymalna ich liczebność nie przekraczała 910 jtk cm^{-3}, a często nie odnotowywano ich w ogóle. Z reguły więcej tych mikroorganizmów stwierdzano w okolicach dopływu z Jeziora Mulicznego i w rejonie bytowania kormorana czarnego. Najwyższe liczebności bakterii z rodziny *Enterobacteriaceae* odnotowywano od lipca do września. Dominowały gatunki: *Enterobacter gergoviae* (15,5%), *Klebsiella oxytoca* (9,8%), *Serratia odorifera* (9,8%), *Citrobacter freundii* (9,2%) i *Enterobacter cloacae* (7,6%). Nie stwierdzono natomiast chorobotwórczych dla ludzi bakterii z rodzaju *Salmonella* i *Shigella*.

Introduction

Contamination of surface waters can be compounded by birds feeding on a given water body and nesting away from it, or birds which inhabit a given reservoir but feed somewhere else. Birds' excrements are a potential source of biogenic elements, responsible for eutrophication of water bodies (GWIAZDA 1996), as well as pathogenic microorganisms (BUCK 1990, FRICKER 1984, KAPPERUD, ROSEF 1983, KUHN et al. 2002, PALMGREN et al. 1997, SEYMOUR et al. 1994). The amount of such microorganisms introduced to a water reservoir by birds is a function of environmental pollution and the feeding behaviour of birds, and those factors are not necessarily constant throughout a year (LÉVESQUE et al. 2000). As birds, especially migratory ones, travel over long distances, dispersal of pathogenic bacteria over large areas is not unlikely (BUCK 1990). Birds can carry pathogenic microbes through faecal contamination of soil an surface water (ALDERISIO, DELUCA 1999). On the other hand, pathogens spread among groups of birds via coprophagy or through contaminated air and water (BROMAN et al. 2000). The effect of waterfowl on contamination of surface waters depends on the number of birds in question, their species, daytime, season, rate of defecation and duration of stay on a given water reservoir (ALDERISIO, DELUCA 1999). The size of birds of a species and their population size are also important (HUSSONG et al. 1979). As a result of the microbiological contamination of surface waters, water quality deteriorates and humans or animals in contact with such water risk higher incidence of illnesses (HAGEDORN et al. 1999, MEDEMA et al. 1997). Regarding Długie Wigierskie Lake, a colony of about 1000 black cormorants (*Phalocrocorax carbo*) living on and around the lake is claimed to affect the quality of the lake water. During storms, excrements of those birds, as well as bird pellets and expectorated fish can be washed away into the lake, where, due to microbiological processes (WIŚNIEWSKA et al. 2006), the volume of biogenic

substances responsible for the lake's eutrophication increases. In addition, this type of contaminants can be a source of enteropathogenic bacteria. Whether or not this is the case of Długie Wigierskie Lake, and if so – to what degree the bird-dependent pollution affecting that lake causes contamination with enteropathogenic bacteria – has been the purpose of our study. The research involved the quantity and quality composition of the family *Enterobacteriaceae* bacteria, their spatial distribution (particularly focusing on the sites where black cormorants tended to dwell) and seasonal variations in two consecutive years (2000 and 2001).

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

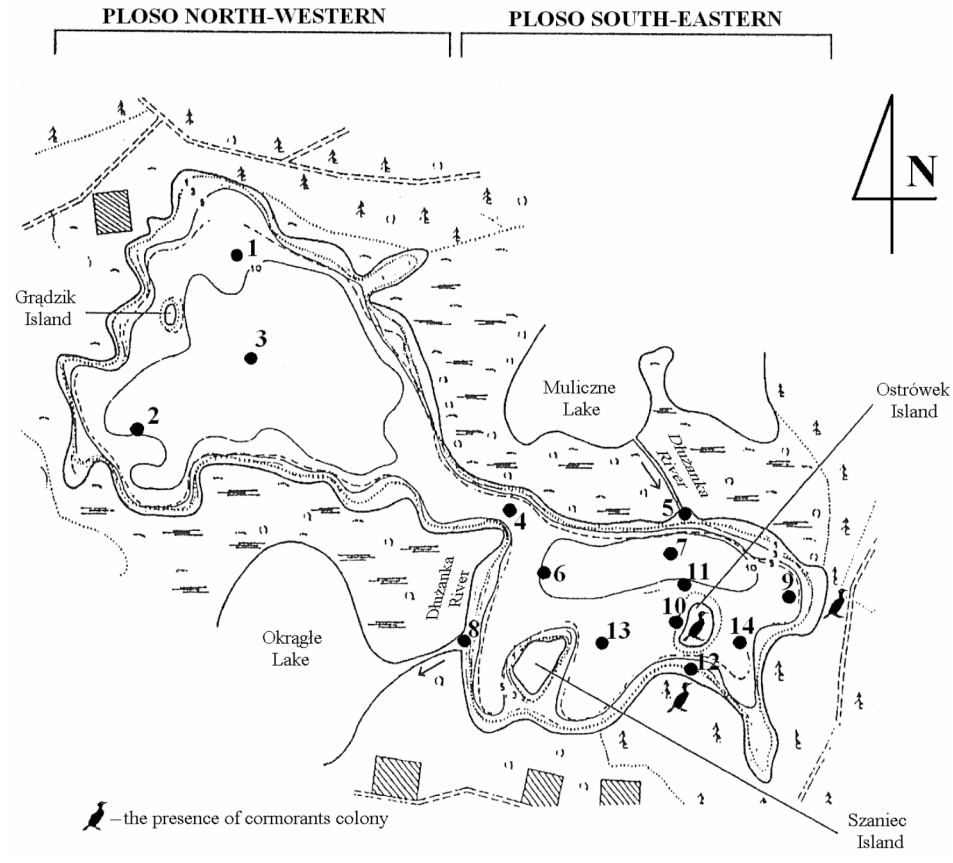

Fig. 1. Location sketch of the Długie Wigierskie Lake. 1, 2,..., 14 – water sampling sites

2000-2001. All the sampling sites were situated at some characteristic parts of the lake, with special attention given to the areas settled by cormorants (Table 1). The north-western part of the lake held sites 1-4, while 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 cm^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, 350 water samples were collected and analyzed. Parallel to water sampling, the following measurements were made: water temperature, Secchi disc visibility, water pH and additionally, water for chemical analyses was collected (WIŚNIEWSKA et al. 2006). 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.

Table 1

Location of water sampling sites at the Długie Wigierskie Lake

Location	Site	Number of samples	Depth (m)
Ploso north-west	1 → north part of ploso	46	0.30; 8.20
	2 → south part of ploso	44	0.30; 9.70
	3 → the deepest site	69	0.30; 7.00; 14.50
	4 → narrowness between ploso	46	0.30; 8.20
Ploso south-east	6 → between Szaniec Island and north waterside	30	0.30; 9.20
	7 → the deepest site	69	0.30; 5.00; 9.70
	9 → at east waterside (the presence of cormorants colony site)	45	0.30; 7.70
	10 → near Ostrówek Island (the presence of cormorants colony site)	23	0.30
	11 → between Ostrówek Island and the deepest site	30	0.30; 8.70
	12 → near south waterside (the presence of cormorants colony site)	23	0.30
	13 → near Szaniec Island	29	0.30; 7.70
	14 → between Ostrówek Island and south-east waterside	30	0.30; 7.70
Dłużanka' inflow	5 → inflow of Muliczne Lake	23	0.30
Dłużanka' outflow	8 → outflow of Okrągłe Lake	23	0.30

Microbiological analyses

Microbiological analyses involved determination of family *Enterobacteriaceae* bacteria count in 1 cm^3 of water on Endo medium after 24 hours incubation at 37°C (APHA 1992). Each measurement was done in three simultaneous repetitions with the same sample of water. After incubation typical colonies were counted and then inoculated onto the agar-bullion

medium with 2% glucose and 5% sheep blood added in order to multiply the bacteria and detection of haemolysins. Additionally, bacteria of the family *Enterobacteriaceae* were analysed for the production of β-D-glucuronidase on Fluorocult medium (VRB agar – Merck), catalase (using 3% hydrogen peroxide solution) and cytochrome oxidase (using 1% tetramethylo-p-phenyldiamine solution). All the determinations were preceeded by determination of motility of bacteria and their response to staining by the Gram's method. They were finally identified with API 20E tests (bioMerieux).

Statistical evaluation

The results of the microbiological, chemical and meteorological examinations were subjected to statistical evaluation by determining the correlation (estimation by Spearman' correlation) between a given set of parameters with simple correlation coefficients. In order to obtain information concerning potential differences between bacteria numbers (dependent variables) for various time and place of samples collecting (independent variable), 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).

Results

The counts of bacteria belonging to the family of *Enterobacteriaceae* in the water of Długie Wigierskie Lake during the time period covered by our study ranged from 0 cfu cm^{-3} at various sites to 910 cfu cm^{-3} in the surface water sampled at site 4. The single factor analysis of variance indicated the highest influence of time (months) of water samples collecting for numbers of the analyzed group of bacteria ($p = 0.0000$). The mean numerousness of the tested bacteria in water sampled from Lake Długie Wigierskie was the highest in water collected during summer' months. Bacterial counts were the smallest in April, whereas the highest counts were recorded from July to September (Figure 2). The location of water samples collecting sites didn't have any statistically significant influence for *Enterobacteriaceae* numbers (Figure 3). The highest mean counts of these bacteria were determined respectively at site

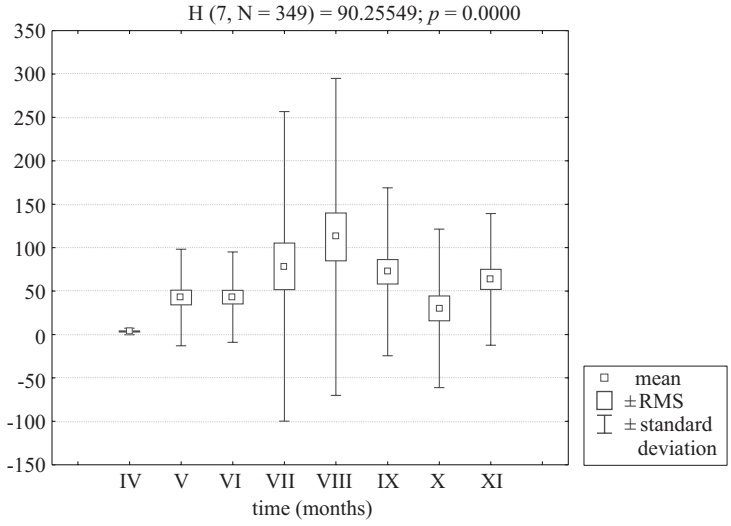

Fig. 2. Averages numbers (± standard deviation and ± random mean square-RMS) of bacteria from family *Enterobacteriaceae* (cfu cm^{-3}) in the water of Długie Wigierskie Lake sampled in 1999-2001 years. Independent variable (assembling): time-months. ANOVA test of Kruskal-Wallis, ranges

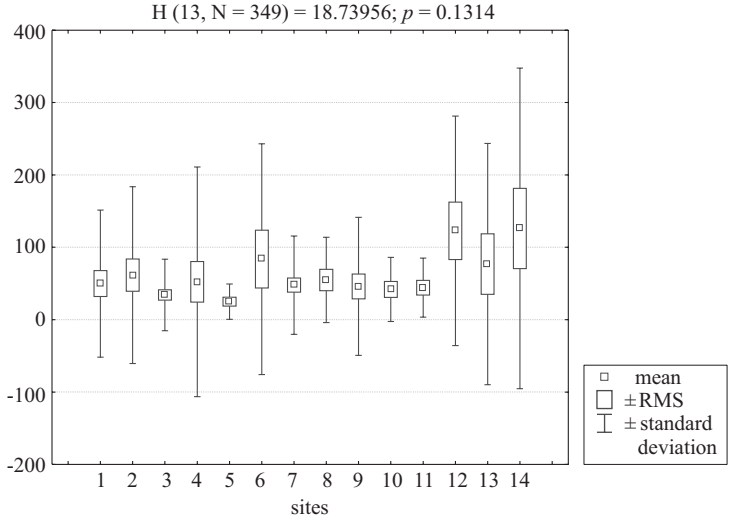

Fig. 3. Averages numbers (± standard deviation and ± random mean square-RMS) of bacteria from family *Enterobacteriaceae* (cfu cm^{-3}) in the water of Długie Wigierskie Lake sampled in 1999-2001 years. Independent variable (assembling): sites. ANOVA test of Kruskall-Wallis' ranges

12, close to a place colonised by the largest group of cormorants. Regarding the composition of *Enterobacteriaceae* isolated in the water samples, the following species dominated: *Enterobacter gergoviae* (15.5%), *Klebsiella oxytoca* (9.8%), *Serratia odorifera* (9.8%), *Citrobacter freundii* (9.2%) and *Enterobacter cloacae* (7.6%). A large contribution to the population of bacteria of the family *Enterobacteriaceae* determined in the lake water was made by *Rahnella aquatilis* (5.8%), a species which occurs naturally in aqueous environments. No cells of pathogenic bacteria of the genera *Salmonella* or *Shigella* were determined. *Escherichia coli*, a species typical of the human and animal digestive tract, constituted just 0.3% of the total count of all isolated strains of *Enterobacteriaceae* (Figure 4).

According statistic estimation by Spearman; correlation the phosphorus total, phosphate contents and temperature of water were correlated positively while oxygen and nitrate contents were correlated negative (statistically significant) with number of *Enterobacteriaceae* bacteria (Table 2).

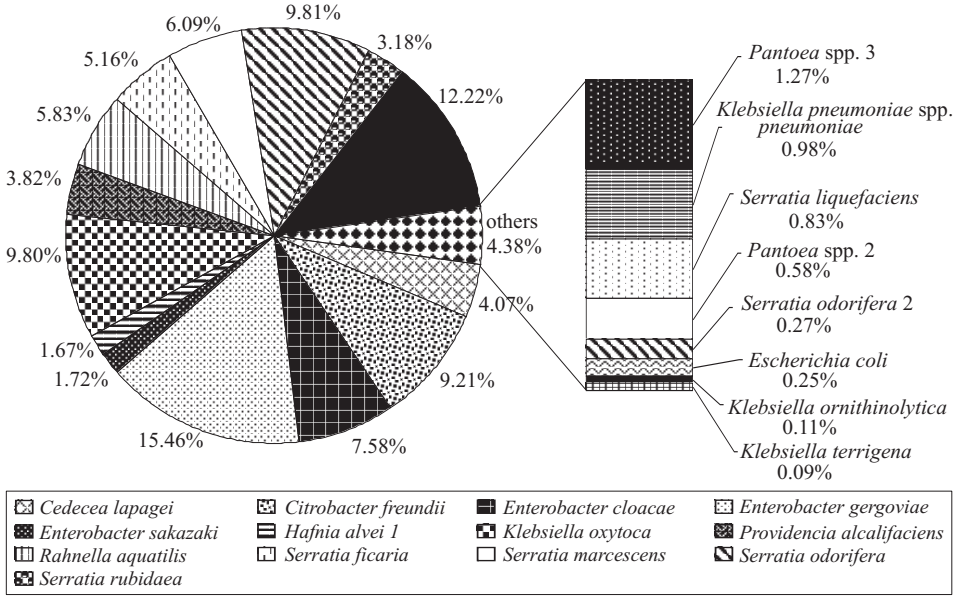

Fig. 4. Percentage of different species of bacteria from Enterobacteriaceae family in the water of Lake Długie Wigierskie collected in the years 2000-2001

Table 2

Statistic estimation by Spearman' correlation between numbers (cfu cm^{-3}) of *Enterobacteriaceae* bacteria received during whole time of studies and some chemical and physical compounds in water of Długie Wigierskie Lake. BD eliminated in couple. Important correlations ($p < 0.05000$) marked

Variable	*Enterobacteriaceae*
$N\text{-}NO_3$ (mg dm^{-3})	**-0.256706**
$N\text{-}NO_2$ (mg dm^{-3})	0.048974
$N\text{-}NH_4$ (mg dm^{-3})	0.196824
N_{org} (mg dm^{-3})	-0.150442
P_{tot}(mg dm^{-3})	**0.474348**
$P\text{-}PO_4$ (mg dm^{-3})	**0.273708**
Temperature of water (°C)	**0.260634**
O_2 (mg dm^{-3})	**-0.247640**
pH	-0.302672
Chlorophyl *a* (μg dm^{-3})	-0.053295
Seston (mg dm^{-3})	0.286910
Secchi disc (m)	-0.247067
Air temperature on water sampling days (°C)	0.034339
Mean of monthly air temperature (°C)	**0.133107**
Weekly precipitation (mm)	0.032747
Monthly precipitation (mm)	**0.232033**
Wind (m s^{-1})	**-0.396260**

Discussion

Bacteria of the family *Enterobacteriaceae* are widespread in nature. Most of them (except a few species) are constantly present in human and animal digestive tracts as the comensal microflora. Some of them inhabit soil and water, others are known as pathogens of plants (MAHON, MANUSELIS 2000), animals and people (EWING et al. 1985). Their number in water or soil depends on the sanitary state of a given environment. The water of Długie Wigierskie Lake contained 10- to 100-fold fewer *Enterobacteriaceae* than corresponding counts determined in bathing waters, sailing marinas and fishing harbours of nearby Wigry Lake during the years 1995-1999 (KORZENIEWSKA et al. 2001, KORZENIEWSKA 2005). Those differences become understandable when considering different character and trophic type of both lakes. Wigry Lake is a eutrophic water body, used for fishing and recreational purposes; it also receives treated wastewater from a sewage and wastewater treatment plant in Suwałki, which is discharged near Hańczańska Bay (NIEWOLAK 2001). Długie Wigierskie Lake is a mesotrophic lake, protected as a strict nature reserve, which includes ban on swimming and bathing.

Any contamination of this lake is due to wild animals and birds rather than humans. Długie Wigierskie Lake lies in the Augustowska Forests, with a rich variety of wild living animals, such as wolves (*Canis lupus*), foxes (*Vulpes vulpes*), racoon dogs (*Nyctereutes procyinoides*), otters (*Lutra lutra*), muskrats (*Ondathra zibethica*), beavers (*Castor fiber*), American minks (*Mustela vison*), elk deer (*Alces alces*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), brown hares (*Lepud eupopaeus*) and other animals (BOGUSŁAWSKI 1999). CRABILL et al. (1999), who investigated the water of the Oak Creek in Colorado (United States), determined an unfavourable effect on the bacteriological state of such water reservoirs caused by numerous mousses, deer and cattle living in that area. Water and mud birds, which populate Wigry National Park, make up a large proportion of the whole avifauna (from total 204 species). Cormorants, whose increasing numbers settle on the local lakes (also along the shoreline of Długie Wigierskie Lake), are likely to play an important role. They are seasonal occupants of the lake, and the exact time of their arrival at the lake depends on the thawing of the lake ice cover, which usually takes place in March. Spring returns of birds to their nesting sites in Wigry National Park last for three months, from mid-February to the end of May. By early June nearly all birds nesting in Wigry National Park have already arrived. Beside the birds which nest here, there are also other migratory birds, flying over the park in flocks composed of a few dozens to hundreds of birds, such as bean geese, white-fronted geese and greylag geese (ZAWADZKA, ZAWADZKI 1999). All those birds; species, both nesting and migratory, can affect counts of the family *Enterobacteriaceae* bacteria and other groups of microorganisms in the waters of Długie Wigierskie Lake.

According to GELDREICH (1970), 1 g of human excrements can contain $13 \cdot 10^6$ coli bacteria; the corresponding counts in animal excrements are $16 \cdot 10^6$ (sheep), $33 \cdot 10^6$ (duck), $1.3 \cdot 10^6$ (chicken). Delaware seagull (*Larus delawarensis*) excrements contained $4.3 \cdot 10^6$-$1.1 \cdot 10^{10}$ coli bacteria per 1 g of faeces. Excrements of those birds can also contain pathogenic bacteria of the genus *Salmonella* (LÉVESQUE et al. 1993, PALMGREN et al. 1997). Nevertheless, HUSSONG et al. (1979) did not determine coli bacteria in samples of faeces collected from Canada geese (*Branta canadensis*) and whistling swans (*Cygnus columbianus columbianus*). In general, a more numerous presence of the family *Enterobacteriaceae* bacteria in the water samples near settling grounds of black cormorants (*Phalocrocorax carbo*) can be associated with the leaching of bird faeces and droppings during rains; this effect was particularly visible at sites 9 to 12. Higher counts of those bacteria in samples of water collected near the channel which carries water from Muliczne Lake (site 5) could be linked to the fact that the lake bottom was disturbed by wild living animals, which came

there to a watering place. Bottom sediments of rivers and lakes contain manifold higher amounts of bacteria of the family *Enterobacteriaceae* than the layers of water higher above the bottom (NIEWOLAK 1998). Differences in the counts of those bacteria found between particular seasons during our investigations can be attributed to the activity of birds and atmospheric conditions. Many authors (BOURROUET et al. 2001, DAVIES, EVISON 1991) point to the fact that sunrays have antimicrobial effect on *Enterobacteriaceae*. Thus, other important factors for growth and survival of bacteria are periodic blooms and death of phytoplankton (e.g. blue-green or green algae), which not infrequently have antimicrobial properties, as well as occurrence of protozoans, which feed on bacteria (BOUALAM et al. 2002, WCISŁO, CHRÓST 2000). The fact that counts of *Enterobacteriaceae* tended to gradually increase from spring to summer may have been due to the thawing of the ice cover on the lake and the runoff of melting snow and ice from the lake's catchment. It was also affected by the transport of bacteria deposited in the lake's basin by wild animals during the winter season. Some genera and species of bacteria which belonged to the family of *Enterobacteriaceae*, determined in the water of Długie Wigierskie Lake, were also identified in other water bodies (NIEMELA, NIEMI 1989, KORZENIEWSKA et al. 2001), although the percentages of particular species found in Długie Wigierskie Lake differed from those cited by other authors. Typical faecal coli bacteria (*Escherichia coli*) constituted barely 0.3% of the total amount of *Enterobacteriaceae* in Długie Wigierskie Lake, whereas in the bathing waters of Wigry Lake they made up between 3.5 and 18.4% of the total count of enterobacteria. In addition, the contribution of *Enterobacter cloacae* to the total volume of *Enterobacteriaceae* was three-fold lower than in Wigry Lake (KORZENIEWSKA et al. 2001) and two-fold lower than in small lakes in Finland (NIEMELA, NIEMI 1989). Several species identified in bathing waters of Wigry Lake (*Serratia odorifera, Citrobacter freundii*), frequented by people, were not found in the water of Długie Wigierskie Lake. In contrast, other bacteria were determined in much higher proportions than in bathing waters of Wigry Lake (KORZENIEWSKA et al. 2001). Those differences are understandable when one considers different contamination degrees of both lakes. The presence of *Escherichia coli* in water is strongly associated with human and animal activity. A nearly negligible contribution of those bacteria to the total composition of *Enterobacteriaceae* in Długie Wigierskie Lake can be attributed to the strict nature reserve protection, which covers this lake along with Muliczne and Okrągłe Lakes, to which it is connected. Among the species of the family of *Enterobacteriaceae* bacteria, pathogenic species which belonged to *Salmonella* or *Shigella* genera were not identified; this may indicate the fact that the population of black cormorant on Długie Wigierskie Lake either is not a carrier of those bacteria or the bacteria in question cannot survive in the waters of the lake.

Conclusions

1. Generally, larger counts of bacteria of the family *Enterobacteriaceae* in the south-eastern part of Długie Wigierskie Lake, particularly near the settling sites of black cormorants, could be associated with the activity of wild animals (otters, muskrats, beavers, wild boar, elk deer and red deer), waterfowl and mud birds as well as cormorants.

2. Seasonal variations in the counts of *Enterobacteriaceae* bacteria, with peaks in summer, can be related to higher temperature of the lake water which can also play some role as it does not favour the survival of those bacteria.

3. Higher counts of that group of bacteria in water samples collected near the settling sites of black cormorants in spring could be associated with spring migration of birds and their returns to nesting sites (migratory activities last for 3 months – from February to end of May) on and around the lakes in Wigierski National Park.

4. Among the bacteria which belong to the family of *Enterobacteriaceae*, there were no pathogenic bacteria of the genera *Salmonella* or *Shigella*, which suggests that the cormorants in the colony which settles on and near Długie Wigierskie Lake do not carry those bacteria, or else *Salmonella* and *Shigella* cells do not survive in the lake water.

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THE INFLUENCE OF CONSTANT MAGNETIC FIELD ON OZONOLYSIS OF DETERGENT ROKAFENOL N8

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K e y w o r d s: detergent degradation, magnetic field, ozonolysis, oxidation.

A b s t r a c t

The aim of the study was to determine the impact of constant magnetic field (SPM) on the effectiveness of ozonolysis process of detergent Rokafenol N8 (R N8) in water solutions. The experiment was performed in two stages at the laboratory scale. The application of SPM, as an improved factor of ozonolysis process of detergent R N8, gave positive technological results. The higher doses of oxidizing agent, the higher impact of constant magnetic field on the effectiveness of detergent oxidation was observed. When ozone doses was 3.0 g O_3 dm^{-3} h^{-1}, additional application of physical factor let obtain several times lower R N8 concentration in contrary to the results of first experimental stage.

WPŁYW STAŁEGO POLA MAGNETYCZNEGO NA PROCES OZONOLIZY DETERGENTU ROKAFENOL N8

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S ł o w a k l u c z o w e: degradacja detergentu, pole magnetyczne, proces ozonolizy, utlenianie.

A b s t r a k t

Celem badań było określenie wpływu stałego pola magnetycznego (SPM) na sprawność procesu ozonolizy detergentu Rokafenol N8 (R N8) w roztworach wodnych. Eksperyment przeprowadzono w skali laboratoryjnej, wykorzystując 2 odmienne systemy technologiczne. Skoncentrowano się na określeniu wpływu dawki ozonu, stężenia detergentu w roztworze, czasu zatrzymania w układzie

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technologicznym oraz znaczenia stałego pola magnetycznego (SPM) na uzyskane efekty degradacji detergentu. Stwierdzono, iż wprowadzenie do układu SPM wpłynęło bezpośrednio na poprawienie efektów, uzyskiwanych w procesie ozonolizy detergentu R N8. Najwyraźniej wpływ czynnika fizycznego zaobserwowano podczas stosowania najwyższej testowanej w eksperymencie dawki utleniacza wynoszacej 3,0 g O_3 dm^{-3} h^{-1}. W tym przypadku zastosowanie SPM pozwoliło na uzyskanie wielokrotnie wyższych efektów końcowych w stosunku do stosowania jedynie procesu ozonolizy.

Introduction

Problem of high detergent concentration in wastewater treatment effluent is commonly known and current. This fact is directly connected with general and mass using of surface-active substances in households but also in the industries (SCOTT, JONES 2000). At the beginning of the XXIth century the annual detergent production in USA, West Europe and Japan exceeded 6 10^6 tons and it still grows up (MODLER, ISHIKAWA 2001, KOJO, FORSTER 2003).

Increasingly, there is a high concentration of surface-active substances (SPC) in surface water (SZYMANSKI et al. 2001, PASTEWSKI, MĘDRZYCKA 2003). Its elimination from wastewater is important for the sake of the fact that the concentration above 1.0 mg dm^{-3} caused the disturbances of the natural balance in the environment (RENNER 1997, SONNENSCHEIN, SOTO 1998, SHURIN, DODSON 1997). Detergents limit oxygen accessibility, cause oxygen shortage, reduce natural processes of self-purification of water and deteriorate organoleptic properties of water (PAPADOPOULOS et. al. 1997, SCOTT, JONES 2000).

Thus, there is a real necessity of modernization of the technological systems operating nowadays and creation of high-tech solutions determining effective degradation of SPC that are significantly nuisance for the natural environment. The optional techniques can be used successfully at water and wastewater treatment in contrary to commonly used methods. Methods based on intensive pollutants oxidation improved by the physical factor are often mentioned in the literature (SCHEMER et al. 2006, SCHRANK et. al. 2005, AGUSTINA et. al 2005, PANIZZA et. al 2005).

One of the methods of advanced oxidation is system with ozone. Ozone can react with pollutants as a result of directive oxidation or after initial ionization on free radicals (BARREDO-DAMAS et. al 2005, FONTANIER et. al 2006, SELCUK 2005).

Currently, the studies focus on the intensification of the final results using low doses of chemical reagents what directly relates to the decrease of treatment costs. To achieve lower costs physical factors are applied into the technological systems (MONTEAGUDO et. al 2005, SHU, CHANG 2005). In presented experiment it was assumed that constant magnetic field (SPM) may be

the factor that could fulfil that condition. Usefulness of the physical factor was previously proved in case of intensification of Fenton reaction (KRZEMIENIEWSKI et. al 2003, KRZEMIENIEWSKI et. al 2004).

The aim of the study was to determined the impact of constant magnetic field (SPM) on the effectiveness of ozonolysis process of detergent Rokafenol N8 (R N8) in water solutions. The investigations focused on the significance of ozone dosage, detergent concentration in water solution, length of the retention time in technological system on the obtained results.

Matherials and Methods

The experiments were carried out in two various technological systems, operated in the laboratory, at the ambient temperature in the range of 20±1°C. In the first stage the influence of ozone on R N8 degradation was investigated. In the second stage relevance of SPM on the final technological result of detergent removal in ozonolysis process was determined.

In our study anionic detergent – R N8 (ether of nonylphenylpolixethylenoglycol), with the following a chemical formula: $C_9H_{19}C_6H_4O(CH_2CH_2O)nH$, n ~ 9. This is common component of liquid detergents used in industry, sanitary area and at homes. R N8 is used as detergent, damper, dispersing agent and a component of dyes. Graphic formula of this detergent is shown on the Figure 1, and it characteristic is presented in Table 1.

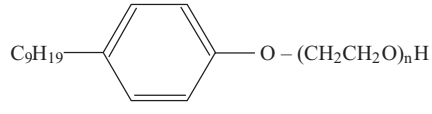

Fig. 1. Structural formula of R N8

Table 1

Physicochemical properties of R N8

Parameter		Value
Molecular mass	(mol)	616
Density	(g cm^{-3})	1.06
Freezing temperature	(°C)	0
Ignition temperature	(°C)	200
The main component content	(%)	99
Absolute viscosity	(kg m s)	340
Hydrophile-lipophile balance		12.5

Each of the experimental stage involved two research series varying in R N8 concentration in water solution. Depending on the series the following solutions were used: 5.0 mg R N8 dm^{-3} and 10.0 mg R N8 dm^{-3}. During the experiment three dosage of ozone were tested: 1.0 g O_3 dm^{-3} h^{-1}; 2.0 g O_3 dm^{-3} h^{-1}; 3.0 g O_3 dm^{-3} h^{-1}. Scheme of the organization of the experiment is presented in Table 2.

Table 2

Organisation of the experiment

Stage	I		II	
	Ozonolysis		Ozonolysis + SPM	
Series	I	II	I	II
	5.0 mg R N8 dm^{-3}	10.0 mg R N8 dm^{-3}	5.0 mg R N8 dm^{-3}	10.0 mg R N8 dm^{-3}
Variant	1.0 g O_3 dm^{-3} h^{-1}	1.0 g O_3 dm^{-3} h^{-1}	1.0 g O_3 dm^{-3} h^{-1}	1.0 g O_3 dm^{-3} h^{-1}
	2.0 g O_3 dm^{-3} h^{-1}	2.0 g O_3 dm^{-3} h^{-1}	2.0 g O_3 dm^{-3} h^{-1}	2.0 g O_3 dm^{-3} h^{-1}
	3.0 g O_3 dm^{-3} h^{-1}	3.0 g O_3 dm-3 h^{-1}	3.0 g O_3 dm^{-3} h^{-1}	3.0 g O_3 dm^{-3} h^{-1}

The investigations of the first stage were performed in model, tightly-closed laboratory rectors at the working volume of 1.0 dm^3. The reactors were equipped with magnetic stirrers. Retention time of water solution of R N8 in technological system was 1 h. The content of the reactors was mixed with the capacity 300 r. $min.^{-1}$ in order to obtain the same concentration of ozone in all parts of the solution. Ozone was generated by the OZOMATIC LAB 802 by WEDECO. Scheme of the firs stage of the experiment is show in Figure 2.

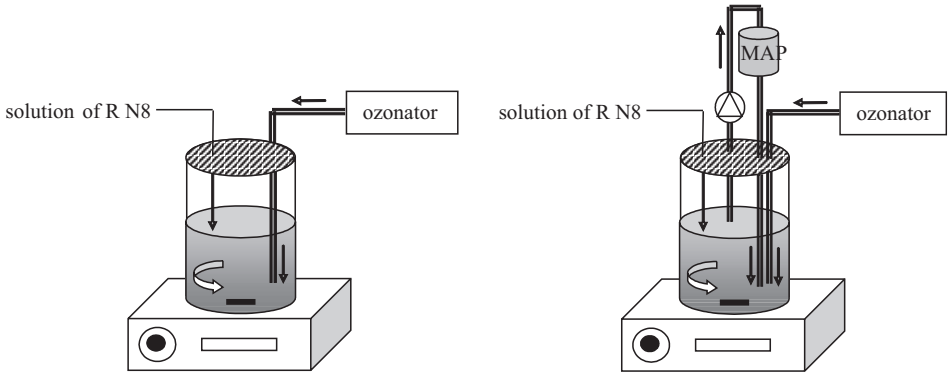

Fig. 2. Scheme of the research stations used in the experiment

The second stage of the experiment concerned the determination of SPM as the element influencing the effectiveness of R N8 ozonolysis. Magnetic liquid activator (MAP) made by MAGNETIZER company (technical parameters of MAP are presented in Table 3) was applied in the technological system used in the first stage of the experiment.

Table 3

Technical date of MAP

Parameter		Value
Width of ring (B)	mm	65
Height of single ceramic magnet (H)	mm	45
Mass of single ring (M)	kg	1.25
Range of gauge diameters	mm	90-110
Range of gauge diameters of pipeline	mm	75-95
Intensity of induced constant magnetic field	T	0.6

MAP is build of two parts composing a ring at the particular diameter. The elements emitting SPM, in case of this kind of device, are ceramic magnetize sinters (Figure 3). In order to ensure the contact of detergent R N8 with SPM, water solution with R N8 was pumped through the region directly exposed on physical factor interaction (Figure 2). The effectiveness of membrane pump was 0.5 dm^3 $min.^{-1}$ It indicates that the whole content of the reactor was brought through the region of SPM 30 times per hour of the retention of R N8 solution in the reactor.

Retention time of R N8 solution in technological system was 1 hour in all parts of the experiment. Samples were collected, using peristaltic pump, installed in the system, at the beginning of cycle and afterwards at 10 min. intervals.

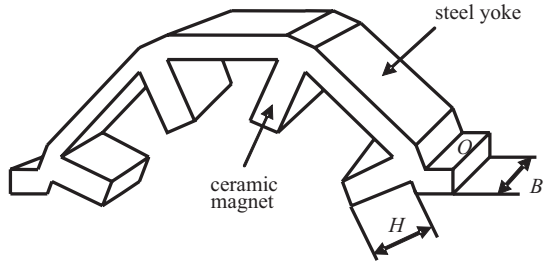

Fig. 3. The schema of half-ring of MAP used in the experiment

R N8 content was determined with spectrophotometer UV-VIS SP-3000 by OPTIMA. In order to mach wavelength characteristic for R N8 concentration standard solutions of detergent at the concentrations: 0.5 mg dm^{-3}; 1.0 mg dm^{-3}; 2.0 mg dm^{-3}; 5.0 mg dm^{-3}; 10.0 mg dm^{-3}; 25.0 mg dm^{-3}; 50.0 mg dm^{-3}; 75.0 mg dm^{-3}; 100.0 mg dm^{-3}; 200.0 mg dm^{-3} were prepared. The highest value of absorbance was chosen and standard curve was drafted. Wavelength used for the determination of detergent concentration was 224.5 nm, and the conversion coefficient was 77.76 to present R N8 content in mg dm^{-3}.

Statistical analysis of the obtained results was done on the basis of STATISTICA 7.1 PL. Verification of hypothesis concerning distribution of each variable was determined using W Szapiro-Wilk test. In order to show the significance of the difference between variables – single factor variance analysis (ANOVA) was used. Variance homogeneity inside the groups was verified on the basis of Leveney's test. In order to determined significance of the difference between analysing variables RIR Tukey test was used. The assumed accuracy level was $\alpha = 0.05$.

Results

In series I of presented experiment, with the initial R N8 concentration on the level of 5.0 mg R N8 dm^{-3}, independently on the technological variant, the lowest concentration was obtained after 10 min. of R N8 retention in the system. Longer retention time caused an increase in extinction for analyzing solution. The highest effectiveness of R N8 degradation was observed in second stage at ozone dosage 3.0 g O_3 dm^{-3} h^{-1}. In this technological variant the effluent R N8 concentration was 0.1 mg R N8 dm^{-3} (Figure 4). The application of constant magnetic field significantly influenced on R N8 oxidation in the range of ozone dosage from 2.0 g O_3 dm^{-3} h^{-1} to 3.0 g O_3 dm^{-3} h^{-1}, irrespective of the retention time in the technological system. The highest dose of the oxidant and using of physical factor resulted in much higher degradation efficiency of R N8 than obtained in I stage of the experiment (Figure 4). The highest differences in the concentrations were observed between 20 min. and 50 min. of the retention time of the detergent solution in the reactors. Ozonolysis only, let obtain final R N8 concentration in the range from 2.6 mg R N8 dm^{-3} to 4.5 mg R N8 dm^{-3}. Whereas system worked with constant magnetic field the concentrations of the detergent was ranging from 0.1 mg R N8 dm^{-3} to 0.7 mg R N8 dm^{-3} (Figure 4). There was no significant effect of SPM on the final results of the detergent oxidation in case of the lowest doses of ozone – 1.0 g O_3 dm^{-3} h^{-1}. In this technological variant relevant impact of physical factor was proved merely after 10 min. and 20 min. of the

retention time of the detergent solution in the reactor. Longer retention time resulted in the similar final R N8 concentrations, independently on the stage of the experiment (Figure 4).

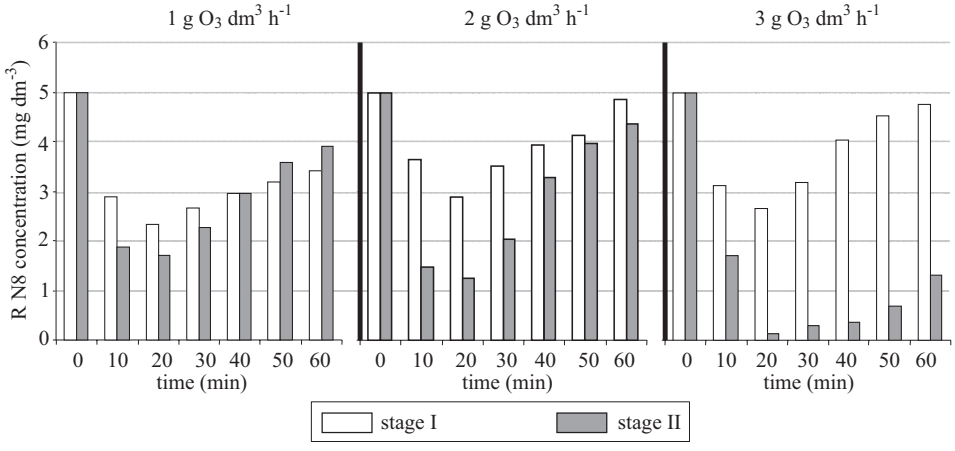

Fig. 4. Variations in R N8 concentration depending on the technological system in series I

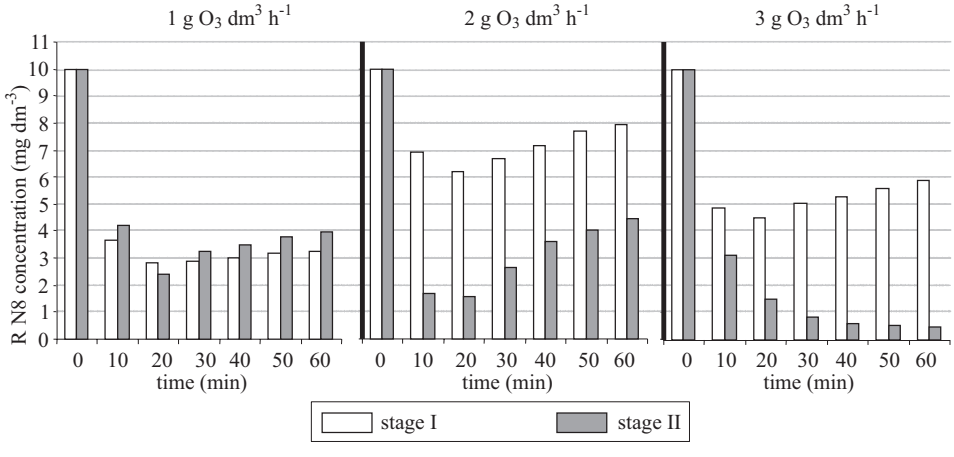

Fig. 5. Variations in R N8 concentration depending on the technological system in series II

Analogical dependences were obtained in series II with the initial R N8 concentration 10 mg dm^{-3}. The highest technological results was at ozone dosage on the level 3.0 g O^{33} dm^{-3} h^{-1}. The lowest detergent concentration was achieved after 20 min. of the retention time of R N8 solution in the reactors. Longer reaction time did not influence significantly on detergent concentration in the effluent (variant I) or caused an increase in extinction (variant II)

(Figure 5). The application of ozone dosage – 1.0 g O_3 dm^{-3} h^{-1} let obtain final R N8 concentration in the range from 2.8 mg R N8 dm^{-3} to 3.6 mg R N8 dm^{-3} in the I stage of the experiment and from 2.4 mg R N8 dm^{-3} to 4.2 mg R N8 dm^{-3} in the II stage with SPM as an improving of oxidizing process (Figure 5).

It was proved that ozone dosage on the level 2.0 g O_3 dm^{-3} h^{-1} deteriorated final results especially in I stage of the study. The application of ozone as the only factor influencing of detergent degradation let achieve the effluent R N8 concentrations ranging from 6.2 mg R N8 dm^{-3} to 7.9 mg R N8 dm^{-3}, depending on the retention time in the technological system. In II stage R N8 concentration was significantly lower from 1.5 mg R N8 dm^{-3} to 4.4 mg R N8 dm^{-3} (Figure 5). The best final results in this series of the experiment was noted in II stage, in variant with 3.0 g O_3 dm^{-3} h^{-1}. After ozonolysis, improved by constant magnetic field, after 60 min. of the detergent retention time in the reactor, final concentration was on the level 0.4 mg R N8 dm^{-3}. An increase in extinction was not observed in case of longer retention time in the technological system. In this technological variant the highest statistical differences between the results from I and II stage were obtained.

Discussion

Surface active substances, commonly used nowadays, possess linear structure what determines its biodegradability (SCOTT, JONES 2000). Despite the progress and development in detergent production, and the ways of its removal from wastewater, some problems with detergent elimination from the wastewater treatment plant influent and water leading to the receivers still exist. This situation is caused by the increasing level of detergent using as well as in households and industry (MODLER, ISHIKAWA 2001, KOJO, FORSTER 2003).

The surface active substance, produced currently, are biodegradable, and wastewater including these kinds of compounds can be treated in conventional systems with activated sludge or in anaerobic reactors (BRUNNER et al. 1988). It was proved that aerobic and anaerobic wastewater treatment was efficient in case of low concentrations of surface active substances in wastewater (PAINTERS, ZABEL 1989). The studies confirmed that at SPC concentration higher than 50 ppm there is limiting influent on microorganisms (KOJO, FORSTER 2003). Literature date shoved that detergent with linear structure was effectively degraded under aerobic condition (PAINTER, ZABEL 1989). Wastewater containing SPC concentration in the range from 20 mg dm^{-3} to 50 mg dm^{-3} limited biogas production in anaerobic reactors (KHALIL et al. 1988). It may be assumed that conventional technological methods, in case of high detergent concentration in treated solutions, would be inefficient.

For that reason, there is a higher importance of the methods based on advanced pollutants oxidation in case of slowly-biodegradable and toxic, for activated sludge, substances. Among these methods there is ozonolysis process improved by constant magnetic filed. This process is applied for degradation of surface active substances (KOZYREVA et al. 2005, SANZ et al. 2003).

The studies by SANZ et al. (2003) shoved that anion surface active substances with linear structure of alkylbenzenesulfonate was degraded by the system with UV/H_2O_2. An application of advanced oxidation caused peroxidation of analysing substance to 50% during the time shorter than 1 hour. The initial concentration of alkylbenzenesulfonate in the solution was 2500 mg dm^{-3}.

In other experiment Fenton reaction was tested for degradation of anion surface active substance of alkylbenzenesulfonate. In those study authors focused on the determination of dosages of chemical reagents, and the reaction pH on the final results. It turned out that the application of 90 mg $FeSO_4/dm^3$ and 60 mg H_2O_2/dm^3 was the most effective technological variant. Retention time was 50 min., and pH was 3. It was proved that effectiveness of tested detergent degradation was about 95% (LIN et al. 1999).

In presented experiment standard ozonolysis with constant magnetic field, as a factor improving oxidation process, was used by the authors for degradation of surface active substances. Earliest studies by KRZEMIENIEWSKI et al. (2003, 2004), proved to improve of Fenton reaction and hence the application of the physical factor in our investigation.

High technological effects were obtained for various kinds of wastewater treatment using Fenton reaction and constant magnetic field. Advanced oxidation improved by SPM caused three times lower doses of reagents at the same effectiveness of degradation of pollutants in diary wastewater. Slightly lower efficiency was achieved in case of synthetic wastewater made of powder whey. It should be pointed out that process proceeded fast, and the impact of SPM revealed after several minutes of wastewater exposition (KRZEMIENIEWSKI et al. 2004).

Positive impact of SPM, observed in presented experiment, on the effectiveness of detergent removal using ozone, could be the result of favourable influence on generation and indestructibility of free radicals (JAJTE et al. 2002). It was shown that the direct oxidation or generation of strongly reactive free hydroxyl radicals could result from the reaction of ozone and organic compounds. The appearance of free radicals starts chain reaction when products of oxidation become the catalysts of the successive radicals' generation (HOIGNE', BADER 1994, SHEMER et al. 2006).

Improvement of detergent R N8 removal, observed in our study, caused by the application of physical factor into the technological system, might

the result of some physical or chemical mechanisms. With a high probability it could be said that constant magnetic field directly influenced on higher $OH^{\bullet}$ generation. Some studies revealed SPM affect free radicals generation (JAJTE et al. 2002). It was found that free radicals have one or more non-paired electrons the spin quantum number of spin is -1/2 or +1/2. During the reaction of two free radicals, unpaired electrons can have the same spin what is defined as a "triplet configuration" or the reverse spin with "singlet configuration". Free radicals possessing a triplet configuration do not create bonds. Through the intersystem crossing, the triplet configuration can be transformed to the singlet configuration that makes the creation of the bonds between free radicals possible. Intersystem crossing may be limited by poor influence of SPM what may leads to a decrease in the amount of radicals that are transformed to singlet configuration with simultaneous maintenance of on the same level or even increase in free radicals total quantity. This is the reasons that this physical factor is thought to be the factor responsible for generation of hydroxyl radicals $OH^{\bullet}$ (MCLAUGHLAN, STEINER 1991).

In presented experiment in most cases there was a decrease in detergent concentration during the initial 20 min. of the reaction, and next theoretical increase was observed. Theoretical increase, after previous decrease, was probably caused by the product of half-break-up of R N8 generation that caused an increase in the extinction value. Simultaneously, it may indicate that commonly used methodology of detergent determination in the experiments in the investigations under sorption process is not appropriate, especially when advanced oxidation is used.

Literature date and the results achieved by the authors give the basis backgrounds to continue the research under the optimisation of physic--chemical methods of detergents degradation. Moreover, other factors improving this technology should be determined. The priority is saving the amount of chemical reagents what limits it using without any impact on the final technological results.

Conclusions

The application of constant magnetic field as the improving factor on ozonolysis of detergent R N8 did not influence on the obtained technological results. It was proved that higher effectiveness of detergent oxidation was achieved along with higher doses of the oxidant. Ozone dosage on the level 3.0 g O_3 dm^{-3} h^{-1}, and application of physical factor into the technological system resulted in several times lower R N8 concentrations in contrary to the results obtained in I stage of the experiment.

Independently on the initial concentration of the detergent in the solution there was no correlation between ozone dosages and technological results in the system without SPM. In series I of this part of the study analogous final effects was achieved irrespective of ozone dosage, in series II the lowest R N8 concentration was noted at ozone dose – 1.0 g O_3 dm^{-3} h^{-1}.

In the experimental stage with the application of constant magnetic field as the factor improving ozonolysis, it was proved that the effectiveness of R N8 degradation was significantly the highest in case of ozone dosage on the level 3.0 g O_3 dm^{-3} h^{-1}. There was no relevant differences in variants testing ozone dosages in the range from 1.0 g O_3 dm^{-3} h^{-1} to 2.0 g O_3 dm^{-3} h^{-1}.

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LONG-TERM CHANGES IN THE BIOMASS AND COMPOSITION OF PHYTOPLANKTON IN A SHALLOW, FLOW-THROUGH LAKE KIRSAJTY (MASURIAN LAKELAND, POLAND)

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K e y w o r d s: phytoplankton, biomass, chlorophyll, Lake Kirsajty.

A b s t r a c t

The objective of this study was to analyse of the taxonomic composition and biomass of phytoplankton also concentration of chlorophyll in water of the lake Kirsajty, and to assess the changes the ecological state of the lake. Phytoplankton abundance and structure were analyzed in Lake Kirsajty during the years 1986-1988 and 2000-2001 (from March to November), and chlorophyll concentrations were determined over the 1986-2001 (August) period.

During the years 1986-1988 total phytoplankton biomass in this lake was relatively low (0.6-2.2 mg dm^{-3}), while over the years 2000-2001 the biomass maximum was approximately twofold higher (5.0 mg dm^{-3}). The concentrations of chlorophyll *a* and pheophytins measured in August over the 1986-2001 period remained within the 2.2-13.1 mg m^{-3} range, reaching a maximum in 1996. During the years 1986-1987 the phytoplankton community of Lake Kirsajty was dominated by cryptophytes of the genera *Cryptomonas*, *Chroomonas* and *Rhodomonas* and chrysophytes – *Dinobryon divergens* and *D. sociale,* whereas in the summer 1988 blue-green algae (including *Aphanizomenon gracile* and *Leptolyngbya thermalis*) accounted for about 50% of total biomass. In the spring 2000-2001 the diatom community (mostly *Fragilaria ulna*, *Stephanodiscus neoastrea* and *Cyclotella* sp.) expanded to the greatest degree. During late summer the phytoplankton composition was again dominated by blue-greens, with a high contribution of *Limnothrix redekei* and *Microcystis aeruginosa*. Long-term studies on phytoplankton abundance revealed undesirable changes in the trophic state of the shallow, polymictic Lake Kirsajty.

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WIELOLETNIE ZMIANY BIOMASY I STRUKTURY FITOPLANKTONU W PŁYTKIM, PRZEPŁYWOWYM JEZIORZE KIRSAJTY (POJEZIERZE MAZURSKIE, POLSKA)

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S ł o w a k l u c z o w e: fitoplankton, biomasa, chlorofil, jezioro Kirsajty.

A b s t r a k t

Celem badań była analiza składu taksonomicznego i biomasy fitoplanktonu oraz koncentracji chlorofilu w wodzie jeziora Kirsajty, a także oszacowanie zmian stanu ekologicznego tego zbiornika. Wykonano analizę ilościową planktonu roślinnego z prób pobieranych od marca do listopada w latach 1986-1988 oraz 2000-2001. Oznaczono także zawartość chlorofilu w wodzie jeziora w sierpniu w okresie 1986-2001 r.

W latach 1986-1988 notowano dość małą biomasę ogólną fitoplanktonu (0,6-2,2 mg dm^{-3}), natomiast w okresie 2000-2001 maksimum było ok. dwukrotnie większe (5,0 mg dm^{-3}). Zawartość chlorofilu *a* i feofityn w sierpniu 1986-2001 r. mieściła się w granicach od 2,2 do 13,1 mg m^{-3}, z maksimum w 1996 roku. W latach 1986-1987 w fitoplanktonie dominowały głównie kryptofity z rodzajów *Cryptomonas*, *Chroomonas* i *Rhodomonas* oraz złotowiciowce *Dinobryon divergens* i *D. sociale*. Już latem 1988 r. około 50% biomasy ogólnej tworzyły sinice m.in. *Aphanizomenon gracile* i *Leptolyngbya thermalis*. Wiosną w latach 2000-2001 najobficiej rozwijały się okrzemki (głównie *Fragilaria ulna*, *Stephanodiscus neoastrea* i *Cyclotella* sp.). Późnym latem w fitoplanktonie dominowały znowu sinice, z dużym udziałem *Limnothrix redekei* i *Microcystis aeruginosa*. Wieloletnie badania obfitości fitoplanktonu wskazywały na niekorzystne zmiany stanu troficznego płytkiego i polimiktycznego jeziora Kirsajty.

Introduction

The shallow, polymictic, flow-through Lake Kirsajty supplies water to one of the most attractive natural water bodies in the Country of Great Masurian Lakes, i.e. to Lake Mamry Północne (Northern Mamry). Lake Kirsajty is extremely prone to degradation. According to CYDZIK et al. (1995), this lake belongs to a group of degradation-susceptible water bodies (category 3 of natural lake resistance to degradation). Already in the years 1958-1961 Lake Kirsajty was considered the most polluted water body in the Mamry Lake Complex (ZACHWIEJA 1975). In the summer 1976 low phytoplankton biomass and a high contribution of blue-green algae and dinoflagellates to total phytoplankton biomass confirmed a low trophic state of all lakes of this complex (SPODNIEWSKA 1979). Until the end of the 20th century the lakes of the Mamry Lake Complex maintained the most stable water cleanliness state among all bodies of water included in the System of Great Masurian Lakes.

However, water purity conditions deteriorated even in this group of lakes (CYDZIK et al. 1995, WRÓBLEWSKA 2002). Water transparency decreased in all lakes forming the Mamry Lake Complex, and total phosphorus concentration exceeded the limit values for water purity class III, even in the cleanest and deepest, stratified Lake Mamry Północne (WRÓBLEWSKA 2002). This trend was also confirmed by phytoplankton studies conducted on Lake Mamry Północne during the years 1986-2001, which showed that total phytoplankton biomass increased over this period, while phytoplankton structure changed considerably. In late summer 2000 and 2001 blue-green algae dominated in this lake, and *Gloeotrichia echinulata* J. S. Smith ex Richt formed surface blooms (NAPIÓRKOWSKA-KRZEBIETKE, HUTOROWICZ 2005).

The objective of this study was to analyse of the taxonomic composition and biomass of phytoplankton also concentration of chlorophyll in water of the lake Kirsajty, and to assess the changes the ecological state of the lake.

Material and Methods

Phytoplankton samples were taken at the deepest place of the lake (7 m) (Figure 1), from April to November over the years 1986-1988 and 2000-2001. Water samples were collected with a sampler, every meter from the surface to the bottom, and mixed thoroughly. Then a final sample (approx. 200 ml) was taken and preserved with buffered Lugol's solution (UTERMÖHL 1958) and next with formalin and glycerin. 42 samples were collected in total.

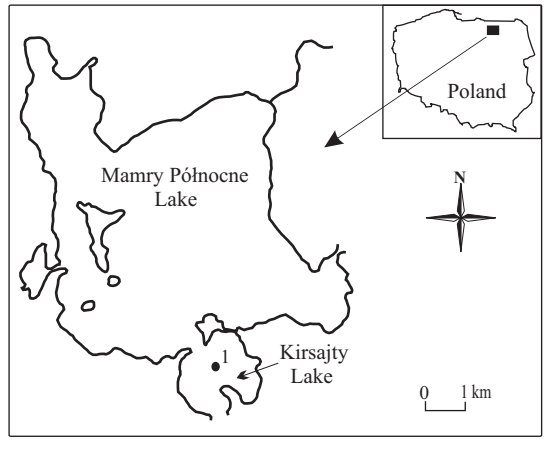

Fig. 1. Location of the sampling site in Lake Kirsajty

A quantitative analysis of phytoplankton was performed using a reverse microscope imaging system, as described by UTERMÖHL method (1958). 10 ml water samples were placed in sedimentation chambers. Single cells, cenobia, colonies and filaments were counted. Phytoplankton biomass was estimated by cell volume measurement (PLIŃSKI et al. 1984, KAWECKA, ELORANTA 1994).

Water samples for the determination of chlorophyll *a* and pheophytins were collected in August, over the 1986-2001 period. Pigment content was determined spectrophotometrically by the alcohol method of NUSCH (1980). Data on chlorophyll concentrations obtained in 1987, partly published by ZDANOWSKI et al. (1993) and ZDANOWSKI, HUTOROWICZ (1994), were used in this study.

Results

Phytoplankton biomass

During the years 1986-1988 phytoplankton biomass in Lake Kirsajty ranged from 0.6 to 2.2 mg dm^{-3} (Figure 2). In 1986 a peak was recorded in June, and in the other months algal biomass was always lower than 0.6 mg dm^{-3}. In 1987, apart from the maximum in May, high biomass was also noted in July, at the end of September and October (approx. 1.3 mg dm^{-3}). Also in the other months total phytoplankton biomass was always higher than in 1986. In 1988 the greatest algal biomass was recorded in July, as well as in May and August. A similar trend in biomass changes over the growing season was also observed in the year 2000. The spring maximum of phytoplankton biomass occurred towards the end of April, but biomass value was by over twofold higher (5.0 mg dm^{-3}) than the maximum recorded in the second half of the 1980s. Phytoplankton biomass higher than in the 1980s was also noted at the beginning of April and in June. The minima observed at the end of May and at the end of November (0.2 mg dm^{-3}) were comparable to the minimal values recorded in 1986. The dynamics of changes in total phytoplankton biomass in 2001 was similar to that observed during the years 1987-1988, although a distinct maximum was noted as late as at the beginning of September.

Phytoplankton composition

In the spring (April) 1986-1987 the phytoplankton community of Lake Kirsajty was dominated by cryptophytes (Figure 3), primarily of the genus *Cryptomonas* (Table 1), and the contribution of diatoms did not exceed 30%. In May over the 1986-1987 period, as well as in June and July, an important

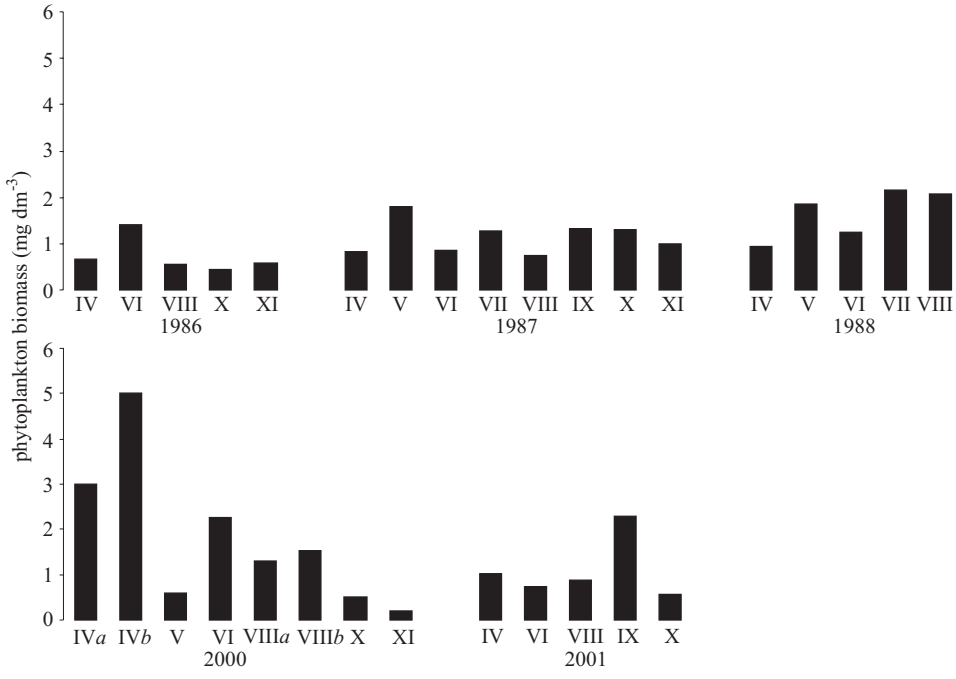

Fig. 2. Phytoplankton biomass (mg dm^{-3}) during the growth season in Lake Kirsajty in the years 1986-1988 and 2000-2001 (*a* – the beginning of the month, *b* – the end of the month)

role was played by the *Chrysophyceae* (Figure 3), mostly *Dinobryon divergens* Imh. and *D. sociale* Ehr. In August 1986 the phytoplankton composition was dominated by cryptophytes, whereas in August 1987 cryptophytes were accompanied by diatoms. In the fall 1986-1987 the phytoplankton community was almost entirely dominated by cryptophytes. The proportion of diatoms was 11 to 22%.

A slightly different structure of the phytoplankton community was observed in 1988. In the spring, similarly as during the years 1986-1987, a distinct domination of cryptophytes was noted, but in early summer (June) the proportion of diatoms in total biomass was still relatively high. At first a relatively high contribution of *Chrysophyceae* (13-38%) was observed – numerous species of the genus *Dinobryon* were recorded during the years 1986-1987. Diatoms and cryptophytes co-dominated in May, accompanied by blue-greens in June. Algae, mainly *Aphanizomenon gracile* (Lemm.) Lemm. and *Leptolyngbya thermalis* Anagn., dominated in July and August, with cryptophytes of the genus *Cryptomonas* as co-dominants.

In 2000 and 2001 the pattern of changes in phytoplankton biomass structure was different than in 1986-1988. In the year 2000, from April until

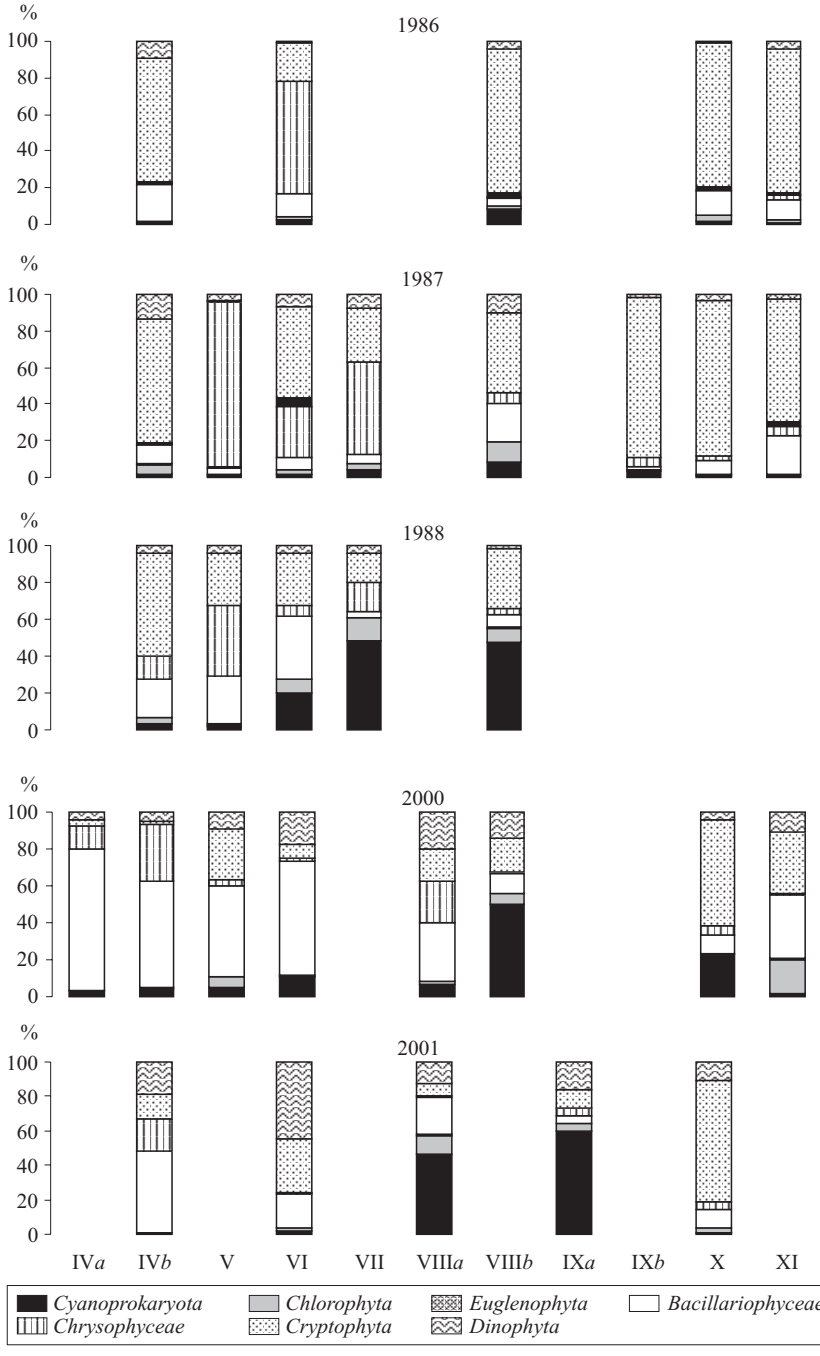

Fig. 3. Percentage contribution of particular groups of phytoplankton to the total biomass in Lake Kirsajty in the years 1986-1988 and 2000-2001 (*a* – the beginning of the month, *b* – the end of the month)

Table 1

Dominat species (≥ 10% total biomass) of phytoplankton in Lake Kirsajty during the growth season in the years 1986-1988

Date		1986			1987						1988		
Species	Depth (m)	22.04	24.06	26.08	22.10	19.11	23.04	19.05	23.06	28.07	25.08	30.09	27.10	23.11	26.04	24.05	21.06	26.07	23.08
Cryptomonas sp. div. (59%)	0-5																		
Dinobryon divergens Imh. + *D. sociale* Ehr. (61%) *Cryptomonas* sp. div. (14%)	0-4																		
Cryptomonas sp. div. (45%) *Chroomonas acuta* Uterm. + *Rhodomonas* sp. (36%)	0-5																		
Cryptomonas sp. div. (73%)	0-4																		
Cryptomonas sp. div. (76%)	0-4																		
Cryptomonas sp. div. (53%) *Chroomonas acuta* Uterm. + *Rhodomonas* sp. (16%) *Peridinium* sp. div. (12%)	0-4																		
Dinobryon divergens Imh. + *D. sociale* Ehr. (91%)	0-4																		
Cryptomonas sp. div. (47%) *Dinobryon divergens* Imh. + *D. sociale* Ehr. (28%)	0-4																		
Dinobryon divergens Imh. + *D. sociale* Ehr. (50%) *Cryptomonas* sp. div. (25%)	0-5																		
Cryptomonas sp. div. (36%)	0-4																		
Cryptomonas sp. div. (81%)	0-4,5																		
Cryptomonas sp. div. (83%)	0-4,5																		
Cryptomonas sp. div. (68%) *Aulacoseira* sp. div. (18%)	0-4																		
Cryptomonas sp. div. (48%) *Dinobryon divergens* Imh. + *D. sociale* Ehr. (12%)	0-4																		
Dinobryon divergens Imh. + *D. sociale* Ehr. (38%) *Cryptomonas sp. div.* (27%) *Asterionella formosa* Hass. (17%)	0-4																		
Cryptomonas sp. div. (24%)	0-5																		
Aphanizomenon gracile (Lemm.) Lemm. (23%) *Leptolyngbya thermalis* Anagn. (17%) *Dinobryon divergens* Imh. + *D. sociale* Ehr. (15%) *Cryptomonas* sp. div. (14%) *Mougeotia* sp. (10%)	0-4																		
Leptolyngbya thermalis Anagn. (28%) *Cryptomonas* sp. div. (25%) *Aphanizomenon gracile* (Lemm.) Lemm. (12%)	0-4																		

Table 2

Dominat species (≥10% total biomass) of phytoplankton in Lake Kirsajty during the growth season in the years 2000-2001

Species	Date	2000						2001			
	Depth (m)	04.04	26.04	31.05	28.06	02.08	31.08	03.10	06.11	24.04	27.06	01.08	05.09	29.10
		0-4	0-5	0-5	0-4	0-5	0-4	0-5	0-5	0-5	0-4,5	0-4	0-4	0-4
Fragilaria ulna (Nitzsch) Lange-Bert. (30%) *Stephanodiscus neoastrea* Hâk. et Hick. (21%) *Dinobryon divergens* Imh. + *D. sociale* Ehr. (13%)														
Fragilaria ulna (Nitzsch) Lange-Bert. (41%) *Dinobryon divergens* Imh. + *D. sociale* Ehr. (11%)														
Cyclotella sp. (30%) *Chroomonas acuta* Uterm. + *Rhodomonas* sp. (14%) *Cryptomonas* sp. div (13%)														
Ceratium hirundinella (O. F. Müll.) Bergh (18%) *Cyclostephanos dubius* (Fricke) Round (16%) *Stephanodiscus neoastrea* Hâk. et Hick. (16%) *Stephanodiscus hantzschi* Grun. (15%)														
Dinobryon divergens Imh. + *D. sociale* Ehr. (22%) *Ceratium hirundinella* (O. F. Müll.) Bergh (17%) *Cryptomonas* sp. div. (15%) *Fragilaria crotonensis* Kitt. (11%)														
Cryptomonas sp. div (17%) *Limnothrix redekei* (Van Goor) Meff. (16%) *Aphanizomenon gracile* (Lemm.) Lemm. (11%) *Peridinium* sp. div. (11%) *Leptolyngbya thermalis* Anagn. (10%)														
Cryptomonas sp. div. (48%) *Aphanizomenon gracile* (Lemm.) Lemm. (17%)														
Cryptomonas sp. div. (31%) *Stephanodiscus* sp. div. (24%) *Spirogyra* sp. (16%) *Gymnodinium* sp. (11%)														
Dinobryon divergens Imh. + *D. sociale* Ehr. (19%) *Gymnodinium* sp. (15%) *Cyclotella* sp. (12%) *Cryptomonas* sp. div. (11%)														
Ceratium hirundinella (O. F. Müll.) Bergh (44%) *Cryptomonas* sp. div. (21%) *Chroomonas acuta* Uterm. + *Rhodomonas* sp. (11%)														
Microcystis aeruginosa (Kütz.) Kütz. (33%) *Merismopedia tenuissima* Lemm. (10%)														
Leptolyngbya thermalis Anagn. (34%) *Microcystis aeruginosa* (Kutz.) Kutz. (14%) *Peridinium* sp. div. (11%)														
Cryptomonas sp. div. (64%)														

the end of June, diatoms (mainly *Fragilaria ulna* (Nitzsch) Lange-Bert. and *Stephanodiscus neoastrea* Håk. et Hick.) were characterized by the fastest growth rate (Table 2). The chrysophytes were numerous already in April, but their contribution (13-31%) was much lower than over the years 1986-1988. At the beginning of August as many as four taxonomic groups co-dominated in the phytoplankton community: diatoms, chrysophytes, dinoflagellates and cryptophytes. Each of these groups accounted for about 23% of total phytoplankton biomass. However, at the end of August the community was dominated by blue-green algae, among which *Limnothrix redekei* (Van Goor) Meffert, *A. gracile* and *L. thermalis* were the most abundant. The proportions of cryptophytes and dinoflagellates of the genus *Peridinium* in total phytoplankton biomass were also high. In the fall (October) the phytoplankton composition was dominated by cryptophytes. Blue-greens, mainly *A. gracile*, were also quite numerous. At that time diatoms made up only 9% of total phytoplankton biomass. Green algae and dinophytes were also quite abundant, accounting for 19% and 11% of total phytoplankton biomass, respectively. In November diatoms, mostly of the genus *Stephanodiscus*, and cryptophytes of the genus *Cryptomonas* co-dominated in the phytoplankton (Table 2).

In 2001 the pattern of changes in the phytoplankton community was quite similar, but in April diatoms accounted for only 47% of total biomass, and the other co-dominants were cryptophytes, chrysophytes and dinoflagellates. Dinoflagellates *Ceratium hirundinella* (O. F. Müll.) Bergh. dominated in June. At that time their mass (0.4 mg dm^{-3}) was similar as in 1988, and they made up 44% of total phytoplankton biomass. Other large groups were cryptophytes and diatoms (Figure 3; Table 2). In August phytoplankton biomass was composed primarily of *Cyanoprokaryota*, mainly *Microcystis aeruginosa* (Kütz.) Kütz. and in September of *M. aeruginosa* and *L. thermalis*. The biomass of blue-greens was almost twofold higher than in 2000. In the fall, similarly as in the year 2000, cryptophytes again had the highest proportion in phytoplankton biomass.

Chlorophyll content

The concentrations of chlorophyll *a* and pheophytins measured in the summer (August) over the 1986-2001 period remained within the 2.2-13.1 mg m^{-3} range (Figure 4). The lowest chlorophyll concentration was recorded in 1986. The values noted from 1988 to 1995 (5.7-8.8 mg m^{-3}) were three- to fourfold higher than in 1986. The maximum concentration of these pigments was observed in 1996, and in the subsequent years (6.7-11.7 mg m^{-3}) it was usually higher than before 1996.

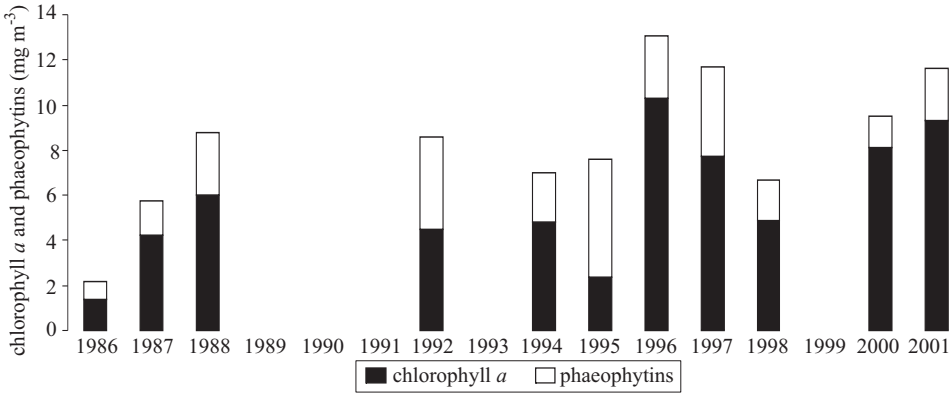

Fig. 4. Chlorophyll *a* and phaeophityns concentration (mg m^{-3}) in Lake Kirsajty in August between 1986 and 2001

Discussion

The structure and function of the phytoplankton community in lakes may be considered as the product of various environmental factors, including light conditions, temperature, nutrient availability, chemical environment and biocenotic interactions (OLEKSOWICZ 1988, KAWECKA, ELORANTA 1994). In deep, stratified lakes the development of algal communities is dependent primarily on nutrient availability and water column stability (REYNOLDS 1984). Phytoplankton biomass is usually higher in shallow aquatic ecosystems than in dimictic lakes, at a similar degree of eutrophication (KAJAK 1983, SPODNIEWSKA 1983). This is a consequence of a higher mean total phosphorus concentration recorded in the summer in the epilimnion of polymictic and dimictic lakes (KAJAK 1983, ZDANOWSKI 1983). Most probably, this is also caused by more efficient biogenic compounds utilization by living organisms in polymictic bodies of water, where remineralization plays a key role in the functioning of the whole ecosystem (HERRERA-SILVEIRA et al. 2002). A crucial element modifying these relationships is the flowability of water bodies (VOLLENVEIDER 1976, HILLBRICHT-IŁKOWSKA 1994).

Lake Kirsajty is a small, shallow, polymictic and flow-through body of water, considerably different than the neighboring large, deep, dimictic lakes Mamry Północne and Dargin. The geomorphological and morphometric distinctness of Lake Kirsajty, and the specific character of its catchment area, are reflected in the dynamics of changes in phytoplankton biomass as well as in the proportions of particular taxonomic groups over the growing season, despite the fact that the taxonomic composition of phytoplankton observed in Lake

Mamry Północne during the years 1986-2001 was very similar to that of Lake Kirsajty (NAPIÓRKOWSKA-KRZEBIETKE, HUTOROWICZ 2005). The main factors responsible for differences in the total biomass and structure of phytoplankton between both lakes could be temperature, water mixing and water flow, since these parameters usually decide about differences between lakes (SOSNOWSKA 1988). The stimulating influence of temperature is probably most noticeable in the spring, when the water in Lake Kirsajty warms up earlier and faster than the water in Lake Mamry Północne (NAPIÓRKOWSKA-KRZEBIETKE 2004).

In the 1980s algal abundance was still comparable to that noted in 1976 (SPODNIEWSKA 1979). However, there was quite a strong tendency towards an increase in phytoplankton biomass during the summer maximum. A similar trend was also observed in Lake Mamry Północne, although phytoplankton biomass in this water body was in most cases lower (NAPIÓRKOWSKA--KRZEBIETKE, HUTOROWICZ 2005). During the years 1986-1987 the phytoplankton community in Lake Kirsajty was dominated by small cryptophytes, easily adapting to changing environmental conditions (SZYSZKA 1990). Numerous chrysophytes *Dinobryon divergens* and *D. sociale* contributed to the summer maximum of phytoplankton biomass. A different phytoplankton structure recorded in 1988, with diatoms *Asterionella formosa* and filamentous blue--greens *Aphanizomenon gracile* and *Leptolyngbya thermalis* as co-dominants, was most probably a consequence of higher water temperatures (NAPIÓRKOWSKA-KRZEBIETKE 2004). Over the 1986-1988 period the relatively low abundance of phytoplankton biomass in Lake Kirsajty was accompanied by rather low concentrations of chlorophyll *a* and pheophytins, as well as quite good Secchi disk visibility (ZDANOWSKI et al. 1984, 1993, ZDANOWSKI, HUTOROWICZ 1994). An analysis of water physicochemical parameters, performed at that time, enabled to classify Lake Kirsajty into water purity class I (CYDZIK et al. 1995).

Disadvantageous changes in the trophic state of Lake Kirsajty were initiated by an increase in the concentrations of chlorophyll *a* and pheophytins, observed in the summer 1996. Such undesirable changes were recorded in the large, deep Lake Mamry Północne, located in the close vicinity of Lake Kirsajty, as late as in the year 2000 (NAPIÓRKOWSKA-KRZEBIETKE, HUTOROWICZ 2005).

The intensive growth of phytoplankton, whose abundance at the end of April 2000 was over twofold higher than the maximal values noted during the years 1986-1988, was most probably caused by very high water temperatures (NAPIÓRKOWSKA-KRZEBIETKE 2004). Although in the other months over the 2000-2001 period total algal biomass was comparable to that recorded in the second half of the 1980s, a different domination structure in the phytoplankton community indicated negative changes in the trophic state of Lake

Kirsajty. Filamentous and chroococcal blue-greens of the genera *Leptolyngbya* and *Microcystis*, forming water blooms, dominated at the end of summer, similarly as in Lake Mamry Północne (NAPIÓRKOWSKA-KRZEBIETKE, HUTOROWICZ 2005).

The most significant consequence of the disadvantageous processes occurring in Lake Kirsajty was a decrease in water purity, from class I during the years 1993-1998 (DOROCHOWICZ 1994, CYDZIK et al. 1995, WRÓBLEWSKA 2002) to class II during the years 1999-2001 (WRÓBLEWSKA 2002). This could result from increasing tourist pressure as well as from fluctuations in water levels in the Mamry Lake Complex in the 1980s and 1990s (BAJKIEWICZ-GRABOWSKA 1991, NOWICKI, GLIŃSKA 2000, DĄBROWSKI 2002), being a potential source of uncontrolled pollution (HESSION 2000), which in shallow, non-stratifies lakes may considerably affect the biomass of diatoms and blue-green algae (FRISK et al. 1999).

Conclusions

The undesirable changes in the trophic state of the shallow, polymictic Lake Kirsajty, recorded during the years 2000-2001, were reflected in a different domination structure of the phytoplankton community and twofold higher total maximum biomass, compared to the 1986-1988 period. The increase in pigment concentrations observed in this lake in the summer in the second half of the 1990s resulted from a response of the phytoplankton community to increasing tourist pressure, prompter than in Lake Mamry Północne.

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EFFECT OF GRILL TYPE ON SENSORY QUALITY OF MEAT STEAKS

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Key words: grilled meat steaks, grill type, sensory quality, quantitative descriptive analysis.

Abstract

The objective of the study was to compare the sensory characteristics of pork and chicken steaks obtained on three different types of grills. Grills such as charcoal (*A*), gas (*B*) and electric (*C*) were used in the study. After grilling (final internal temperature 72°C) the steaks were subjected to sensory evaluation using quantitative descriptive analysis (QDA) and hedonic tests In QDA a trained panel (n = 12) rated the steaks for odour, taste and texture. In the affective tests the panelists evaluated the samples for overall quality. The results proved that both the grill type and the meat type had significant effects on the sensory quality of the steaks. Gas grilled pork and chicken steaks obtained the highest scores of overall quality (6.1 units and 6.6 units respectively). However, pork steak prepared on electric grill and chicken steak obtained from charcoal grill indicated the least palatable effect (5.0 units and 4.7 units respectively). The QDA found significant differences ($p < 0.05$) between the steaks grilled for the following attributes: darkness, "fatty" odour and taste, "herbal/spicy" odour and taste, "smoky" odour, bitter taste and juiciness. The principal component analysis (PCA) indicated that the first (PC1) and the second (PC2) component together explained 87.56% of the variation of sensory quality of samples.

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WPŁYW TYPU GRILLA NA JAKOŚĆ SENSORYCZNĄ STEKÓW MIĘSNYCH

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Słowa kluczowe: grillowane steki mięsne, ogólna ocena sensoryczna, ilościowa analiza opisowa, analiza składowych głównych (PCA).

Abstrakt

Celem pracy było porównanie jakości sensorycznej steków mięsnych, uzyskanych z grilla węglowego (*A*), gazowego (*B*) i elektrycznego (*C*). Do przygotowania standardowych steków wykorzystano dostępną na rynku karkówkę wieprzową i filet drobiowy. Proces grillowania prowadzono do uzyskania wewnątrz steków temperatury 72°C. Badania jakości sensorycznej prób przeprowadzono metodą profilowania sensorycznego barwy, zapachu, smakowitości i tekstury oraz w kategoriach jakości konsumenckiej. Oceny sensoryczne wykonał wyszkolony 12-osobowy zespół. Stwierdzono zróżnicowanie jakości sensorycznej badanych prób steków, na co miał wpływ zarówno typ urządzenia grillującego, jak i gatunek mięsa. Steki przygotowane na grillu gazowym spełniały w największym stopniu oczekiwania jakościowe konsumentów. Najmniej pożądany był stek z karkówki przygotowany na grillu elektrycznym i stek drobiowy z grilla węglowego. Wyróżnikami statystycznie istotnie różnicującymi ($p < 0,05$) profile sensoryczne steków były: barwa, zapach i smak „tłuszczowy", „ziołowy", „dymowy", gorzki oraz soczystość. Analiza składowych głównych (PCA) wykazała, że pierwsza (PC1) i druga składowa (PC2) opisały łącznie 85,58% całkowitej zmienności jakości sensorycznej badanych prób.

Introduction

Nowadays, grilled foods have gained popularity in Poland both in restaurant and at home. It is commonly believed that fat free grilling makes it possible to obtain low caloric meat products. People are interested in consuming foodstuffs with reduced fat and energy as part of weight-reducing diet. It is well known that obesity causes severe health problems, including adult type diabetes, coronary artery disease and breast and colon cancer (RIPPE et al. 1998). In order to fulfil the expectations of consumers in this field the industry produces various types of grills such as charcoal, gas and electric. Despite the growing interest in the health aspects of meat good sensory properties remain a key priority as a consumer choice criterion (TOURAILLE 1992, CHAMBERS, BOWERS 1993, ISSANCHOU 1996, VERBEKE et al. 1999). Many studies have reported the impact of cooking on the sensory quality of meat (VASANTHI et al. 2007, BEJERHOLM, AASLYNG 2003, BERTRAM et al. 2005, ZHANG et al. 2004). However, information about the relation between the sensory quality

of grilled meat and the type of grill is scant. Thus the aim of this work was to evaluate the effect of commercially available type grills on the sensory quality of pork and chicken steaks using a trained sensory panel.

Materials and Methods

Characterization of grills

The three types of grills such as: charcoal (Gwarancja, model 017), gas propane/butane (Broil King, model Porta Chef) and electric (Silex, model P3) abbreviated as *A, B* and *C* were used in the study.

Preparation of steaks

Pork loin and chicken breast purchased from a local supermarket were used in the study. Steaks weighing approximately 165 g were marinated with a solution of salt and spices – recipe from Raichlen 2001 (salt, juice from lemon, olive oil, thyme and pepper) and stored overnight at approximately 4°C until processed. The samples were grilled to a final internal temperature of 72°C, checked using a calibrated hand-held digital thermometer. After grilling the steaks were placed in separated thermoses and immediately transported to the sensory laboratory. Before the sensory evaluation they were cut (into 1 cm x 1 cm x 2.5 cm) and placed in transparent plastic boxes covered with a lid. Three digest random numbers were used to code the samples.

Sensory panel

The sensory assessments of the samples were carried out by a panel consisting of 12 members selected and trained according to ISO guidelines (ISO 8586-1:1993). All assessors have passed the basic taste test, the odour test and the colour vision test. Prior to their participation in the experiments, the subjects were trained on sensory descriptors for grilled pork and chicken.

Sensory methods and evaluation conditions

Quantitative descriptive analysis (QDA) was used to determine the differences in the sensory characteristics of the samples (*Sensory analisis.*

ISO/DIS 13299:1998, STONE, SIDEL 1993, LAWLESS, HEYMANN 1999). Prior to the analysis, the vocabulary of the sensory attributes was developed by the panel in round-table session, using standardised procedure 14 attributes related to the appearance (darkness), odour (roasted meat, fatty, herbal/spicy, smoky) taste (roasted meat, smoke meat, fatty, herbal/spicy, bitter) and texture (toughness, juiciness, hardness, chewiness) of steaks were selected and thoroughly defined for profiling. Panellist rated the sensory attributes on continuous unstructured line scale from low intensity (0) to high intensity (10). Evaluation of overall quality of steaks was conducted using the same type of scale as above. The results were expressed as "liking" the sample, within the range: unlinking (0) – extremely linking (10). The samples were served in random order to panelists and mineral water and unsalted crackers were offered between samples. The assessments were carried out at the sensory laboratory room, which fulfils the requirements of the ISO standard (*Sensory analisis...* ISO 8589:1998). Scores were recorded and collected using a computerised system (BARYŁKO-PIKIELNA 1992).

Statistical analysis

ANOVA was used to test statistical differences in sensory attributes between the steaks. Treatment means were compared using Fisher's protected least significant difference (LSD) test. Statistical significance was considered at $p < 0.05$. Principal component analysis (PCA) was performed in order to describe the variance among the whole sensory data obtained. Statistical analysis was performed using software package (StatSoft Inc., v. 7.1, Tulsa, OK, USA).

Results and Discussion

To find attributes which influenced the sensory quality of samples quantitative descriptive analysis (QDA) was used. Descriptive analysis are the most sophisticated tools in of sensory evaluation and involve the discrimination and description of both the qualitative and quantitative sensory attributes of product by trained panels (LAWLESS, HEYMANN 1999, MURRAY et al. 2001). The mean sensory ratings for the samples and the analysis of variance are presented in Table 1. ANOVA showed that there were significant ($p < 0.05$) differences in the intensity of attributes such as: darkness, "herbal/spicy" odour and taste, "smoky" odour, "roasted meat" taste, bitter taste and juiciness caused the type grill. The average overall quality of scores for the

steaks ranged from 4.7 to 6.6 on a 0 – 10 non-structured scale (Figure 1). Gas grilled pork and chicken steaks obtained the highest scores of overall quality (6.1 units and 6.6 units respectively). However, pork steak prepared on electric grill and chicken steak obtained from charcoal grill indicated the least palatable effect (5.0 units and 4.7 units respectively). In order to observe the above differences in the analysed samples more clearly, the sensory profiles of chicken steak with the highest scores of overall quality (gas grill – *B*) and chicken steak with the lowest scores of overall quality (charcoal grill – *A*) were displayed as the spider diagrams in Figure 2. It can be seen that the sensory profiles of these samples were significantly different ($p < 0.05$) in the intensity of attributes for appearance (darkness), odour ("herbal/spicy" "smoky"), taste ("smoke meat", "herbal/spicy", bitter) and texture (juiciness). In the profile of chicken steak with the lowest scores of overall quality the dominating attributes were as follows: darkness, "smoky" odour and "smoke meat" taste. These notes except "smoke meat" taste were also dominating in the pork steak obtained from charcoal grill (Table 1). However, it should be stressed that they did not affect the overall quality.

Table 1

Mean values of the intensity of sensory attributes in meat steaks

Sensory attributes[c]	*A*		*B*		*C*	
	pork	chicken	pork	chicken	pork	chicken
c. darkness	7.4^{bB}	4.9^{aA}	3.0^{aA}	3.5^{aA}	3.4^{bA}	0.9^{aB}
o. roasted meat	5.2^{aA}	4.0^{aA}	4.5^{aA}	5.5^{aA}	5.9^{aA}	4.5^{aA}
o. fatty	2.0^{bA}	0.0^{a}	2.5^{bA}	0.0^{a}	3.3^{bA}	0.0^{a}
o. herbal/spicy	2.0^{aA}	2.1^{aA}	1.8^{aA}	2.9^{aA}	5.5^{aB}	6.5^{aB}
o. smoky	6.4^{aA}	6.0^{aC}	5.6^{aA}	3.5^{aB}	0.3^{aB}	0.6^{aA}
t. roasted meat	6.9^{aA}	5.2^{aA}	6.2^{aA}	6.0^{aA}	5.4^{aA}	4.4^{aA}
t. smoke meat	0.0^{a}	4.6^{bA}	0.0^{a}	3.0^{bA}	0.0^{a}	0.7^{bB}
t. fatty	2.6^{bA}	0.0^{a}	3.1^{bA}	0.0^{a}	3.9^{bA}	0.0^{a}
t. herbal/spicy	3.0^{aA}	3.0^{aA}	3.1^{aA}	4.6^{aAB}	5.3^{aB}	5.7^{aB}
t. bitter	2.3^{aB}	2.4^{aB}	0.5^{aA}	1.2^{aAB}	0.2^{aA}	0.4^{aA}
toughness	4.7^{aA}	4.8^{aA}	4.6^{aA}	4.1^{aA}	4.1^{aA}	4.0^{aA}
juiciness	4.9^{bA}	2.4^{aB}	6.1^{aA}	4.6^{aAB}	5.5^{aA}	4.8^{a}
Ahardness	3.9^{aA}	3.8^{aA}	3.6^{aA}	2.6^{aA}	2.9^{aA}	2.1^{a}
Achewiness	4.3^{aA}	4.2^{aA}	3.9^{aA}	3.2^{aA}	3.3^{aA}	2.5^{a}
Aoverall quality	5.9^{aA}	4.7^{aA}	6.1^{aA}	6.6^{aA}	5.0^{aA}	6.4^{aA}

a – Mean descriptive analysis ratings of steaks (0-10 units)
b – means marked in each row with the same letters do not have significant differences (LSD test, $p < 0.05$) small letters describes comparison between pork and chicken steaks in each grillbig letters describes comparison between kind of grills in each variety of steaks
c – colour, o – odour, t – taste
A – charcoal grill, *B* – gas grill, *C* – electric grill

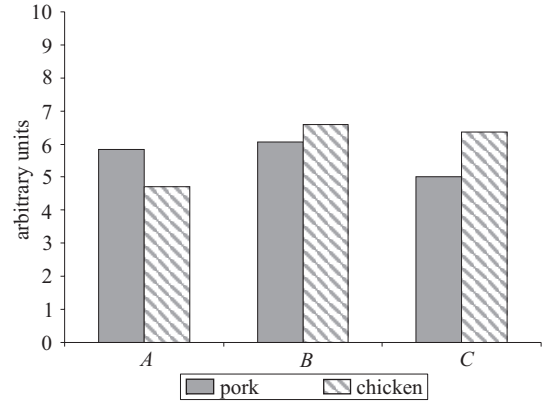

Fig. 1. Overall sensory quality of pork and chicken steaks processed in three grill types: charcoal (*A*), gas (*B*), electric (*C*)

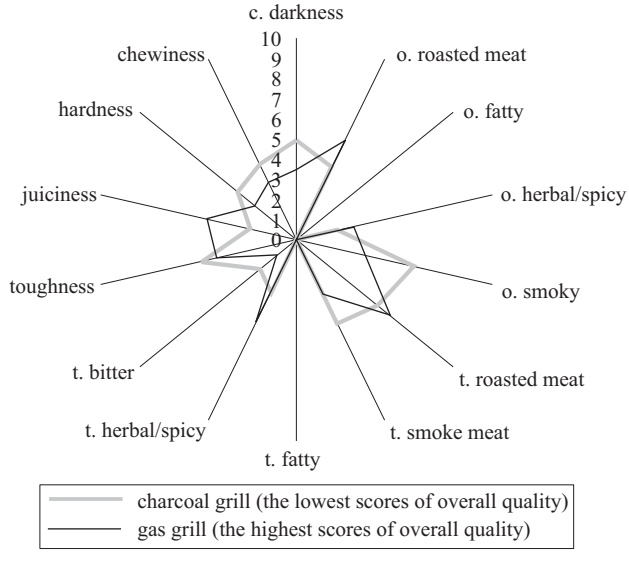

Fig. 2. Sensory profiles of chicken steaks of the lowest and highest overall quality

The data obtained from the profile analysis (QDA) were subjected to PCA employing statistical software (Figure 3). This method allows us to see a graphic representation of the data so that the variations between the samples can be more easily interpreted. PCA was performed on the covariance matrix of the samples with no rotation. The first four principal components (PC) had eigen values greater then one and accounted for 97.68% of the total variance. Eigen values accounted for by each principal component (PC1 – PC4) were

18.146; 9.862; 2.189 and 1.050 respectively (not shown here). The first two principal components which together explained 87.56% of the variation were plotted in Figure 3. It can be see that PCA technique differentiated of samples by type of grill and type of meat. Table 2 shown the loadings of the first two PC. This indicated that the first PC was differentiated by darkness, "herbal/spicy" odour and taste, "smoky" odour, "roasted meat" taste and bitter taste as well as texture: toughness, hardness, chewiness. They were negatively related to the first PC except "herbal/spicy" taste. It is a well-known that the first PC contains the most important information and includes the more important attributes. Therefore these attributes are the most significant for differentiating the samples. The second PC was composed of sensory attributes such as "fatty" odour and taste, "smoke meat" taste and juiciness. They were also negatively correlated to the PC except "smoke meat" taste.

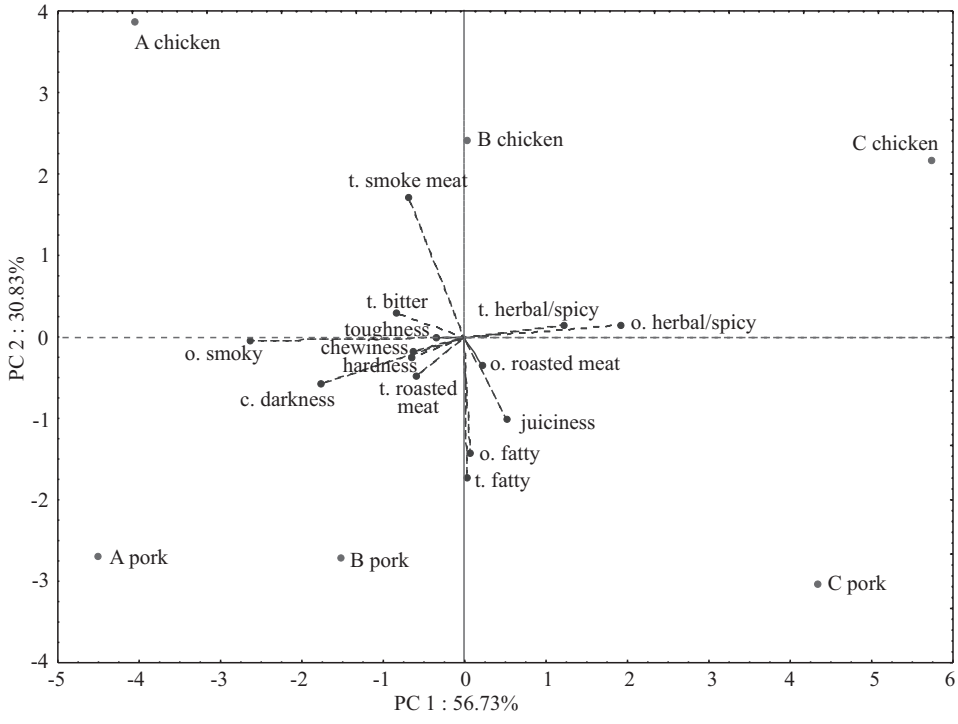

Fig. 3. PCA biplot of pork and chicken steak samples and loadings of score of steak samples and loadings of sensory attributes on the first and secondą principal component

Table 2
Principal component factor loadings from PCA of sensory scores (QDA) from meat steaks

Attributes	Principal component PC	
	1	2
c. darkness	-0.824056	-0.265219
o. roasted meat	0.303218	-0.501963
o. fatty	0.040115	-0.955420
o. herbal/spicy	0.952186	0.069248
o. smoky	-0.977567	-0.014164
t. roasted meat	-0.694007	-0.560188
t. smoke meat	-0.347932	0.864486
t. fatty	0.013242	-0.963619
t. herbal/spicy	0.962779	0.114266
t. bitter	-0.863196	0.307224
toughness	-0.915681	-0.042119
juiciness	0.402123	-0.784114
hardness	-0.885868	-0.344752
chewiness	-0.932496	-0.272016

Conclusions

In conclusion we found that sensory quality of the meat steaks was affected by both the grill type and the meat type. The results of the overall quality evaluation of pork steaks were as follows: gas grill (*B*) > charcoal grill (*A*) > electric grill (*C*) whereas for the chicken steaks the order was the following: gas grill (*B*) > electric grill (*C*) charcoal grill (*A*). On this basis it can be stated that the gas grill was successful tool for preparing the products with the highest scores of overall quality both for the pork and the chicken steaks.

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UTILITY OF ACUTE PHASE PROTEIN DETERMINATION IN WEANED PIGLETS REARED IN CONVENTIONAL FARMS

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K e y w o r d s: APP, diarrhoea, combining stress, piglets.

A b s t r a c t

Social stress and diarrhoea are major threats to piglets during weaning. This study compared serum levels of acute phase proteins (APP) during diarrhoea, silent infection, under stress and in healthy weaned piglets from a conventional herd.

Diarrhoea and silent infection influenced the APP level to a moderate degree. It was a weak reaction compared with alteration of these indices in cases of respiratory infections and the possible reasons and mechanisms for this are discussed. It is considered that stress events may not only influence neuroendocrine mechanisms, but also the immune system. The study supports this theory as it obtained elevated serum levels of positive (as well as lower) levels of negative APP in piglets subjected to combining stress. All groups were compared and it was found that stress increased APP reaction, but not as dramatically as infection did.

PRZYDATNOŚĆ OZNACZANIA BIAŁEK OSTREJ FAZY (BOF) U PROSIĄT PO ODSADZENIU POCHODZĄCYCH Z FERMY PRZEMYSŁOWEJ

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Abstrakt

Stres odsadzania i biegunki są największymi zagrożeniami dla zdrowia prosiąt w okresie odsadzania. W artykule oceniono przydatność oznaczania białek ostrej fazy (BOF) u prosiąt z biegunką, zakażonych bezobjawowo i poddanych stresowi łączenia miotów w porównaniu ze zwierzętami zdrowymi pochodzącymi z fermy przemysłowej. Uważa się, że czynniki stresowe wpływają nie tylko na układ neuroendokrynowy, ale także na odpornościowy. Opisywane doświadczenie potwierdza tę teorię jako że, u grup prosiąt poddanych stresowi związanemu z łączeniem miotów, uzyskano zarówno podniesione poziomy pozytywnych, jak i obniżone negatywnych białek ostrej fazy. Zmiana stężeń BOF nie była jednak tak wyraźnie zaznaczona jak w przypadku stanów zapalnych.

Introduction

It is known that some serum proteins change their levels during disturbances, such as infections, inflammations, stress etc. The proteins are not, or not always, exclusive immunological particles, synthesised and released only during systemic reaction to disorders, but they exist and play various roles in normal conditions. During immunological challenges, their properties are essential (or less important) for systemic defence reactions, and changes in the body result in an increase or decrease in their production and blood plasma level. Their levels are able to peak or rapidly drop within hours of the onset of a challenge and, due to these properties, they are called acute phase proteins (APP). Traditionally, it has been thought that the release of pro-inflammatory cytokines (which are involved in APP release) and so-called Acute Phase Response (APR) triggering only occurs when the immune system is activated by a viable pathogen. There is now evidence that a variety of stressors alone can induce pro-inflammatory cytokine secretion. The body utilizes the same pathways and the same signal molecules – showing cross-sensitisation between the neuroendocrine and immune systems.

Social stress and diarrhoea are the most common threats to piglets during weaning. During weaning, piglets experience several stressful events such as sudden diet change, mother-young link disruption, mixing with unfamiliar piglets and a move to a new housing environment. These conditions induce behavioural, endocrine and immune alterations (MERLOT et al. 2004).

Although there are many reports on APR in pigs suffering from respiratory diseases, there is a lack of studies concerning diarrhoea and the clinical significance of APPs in such a state. Furthermore, most of the research in this field was completed under laboratory conditions, or at least in pathogen-free (SPF) herds and without determination of the clinical utility of APP tests. This paper is an attempt at such a study, conducted on a conventional farm during a normal farming program. Previously, the authors reported a slight reaction of APP during diarrhoea in piglets (RYCHLIK et al. 2001). In the present study,

APR caused by diarrhoea and combining stress was studied. These two states were compared in the study.

Materials and Methods

34 Large Polish White weaned piglets of both sexes, from a large conventional farm in northeast Poland were used in this study. Of these, 10 piglets aged 35 days showed acute diarrhoea symptoms during 3 days (the consistency and colour of faeces resembled mayonnaise), 24 had no such clinical signs. 8 piglets experienced combining stress (2 equal litters from 2 sows placed together in one pen). 6 animals aged 35 days without diarrhoea after microbiological examinations were included in another group – sub-clinically infected animals; 10 piglets, 7 days after weaning served as a control group.

Four groups were established as follows: group *D* – diarrhoeal piglets; group *C* – exposed to combining stress; group *S* – sub-clinically infected; group *H* – healthy control.

Clinical, biochemical and microbiological tests were performed 7 days after weaning (groups *D, S* and *H*) or 2 days after weaning in the animals of combining stress group (group *C*). Blood samples were collected from the jugular vain and prepared by centrifugation. Analyses were conducted immediately, without storage or freezing. Biochemical examinations included protein electrophoretic separation on agarose using a Cormay Gel Protein 100 kit. C-reactive protein was determined by employing the immunoturbidimetric method, using a Backman Array 360 System. Comments included in papers by HEEGAARD et al. (1998) and SCHRÖDL et al. (1998) were taken into consideration. The fibrinogen level was determined by the Clauss method using a Multibibren U kit (Dade Behring).

All animals in the study were subjected to microbiological examination*. Analyses were performed by applying standard methods. The scope and targets of the bacteriological and virological examinations are presented below.

1. Corona virus gastroenteritis (*Coronaviridae*) – testing the presence of antibodies in the blood serum for TGE/PRCV; ELISA test.

2. Rotavirus piglet diarrhoea (*Rotaviridae*) – faeces analysis aimed at detecting rotavirus infections; ELISA test;

3. Pig dysentery (*Brachyspira hyodysenteriae*) – bacteriological analysis of faeces samples on TSB, TSA and blood agar under anaerobic conditions; differentiation with *Brachyspira pilosicoli* (PCR method).

* The examination was carried out at the Epizoootiology Unit, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn and National Veterinary Research Institute in Puławy.

4. Salmonellosis (*Salmonella sp.*) – material proliferation on SF; SS culture and passage from SF to the MacConkey and Sołtys medium. Confirming or excluding the presence of Salmonella rods on additional differentiating series.

5. Yersiniosis (*Yersinia enterocolitica*) – material proliferation on ITC; passage to CIN; biochemical identification of the colonies.

6. Colibacteriosis (*Escherichia coli*) – isolation of colonies from the Levin medium, aimed at detecting *Escherichia coli*, and their biochemical identification.

7. Necrotic enteritis in newborn piglets (*Clostridium perfringens* type *C*) – material inoculation into the Wrzosek medium, and then into the Wilson--Blair medium.

The results of biochemical examinations were subjected to a statistical analysis employing the ANOVA, and Spjotvoll and Stoline (Tukey) post hoc test.

Results

In the diarrhoeal piglets (group *D*), the average level of CRP was 21.2 mg/l, which was significantly higher than that determined in the control (group *H*) – 10.33 mg/l (Table 1). An increased value of this protein was observed two days after combining, and it amounted to 17.4 mg/l on average (group *C*). The highest level of CRP was noted in the subclinical infected piglets, assigned to group *S* (28.4 mg/l) (Figure 1) which was associated with the lowest of all groups in the study albumins level (1.91mg/l) – Table 2. In the control group (*H*), the average level of albumins was 2.59 g/dl, whereas in the combining (*C*) and diarrhoeal group (*D*), it amounted to 2.106 and 2.55 g/dl respectively. The lowest fibrinogen level was observed in the diarrhoea (*D*) and subclinical (*S*) groups (256.30 and 276.33 mg/dl, respectively) and was significantly higher in the two remaining groups: combining (*C*) – 395.71 and control (*H*) – 329.44 mg/dl.

Bacteriological examination of faeces analysis aimed at detecting *Rotaviridae* in all the animals with diarrhoea symptoms (group *D*) and in those included in the subclinical ill group (*S*). The faeces samples analysed to detect

Table 1

Mean values of indices in the study

	Group *D*	Group *C*	Group *S*	Group *H*
CRP (mg/l)	21.20	17.40	28.40	10.32
Albumin (g/dl)	2.55	2.11	1.91	2.59
Fibrinogen (mg/dl)	256.30	395.71	276.33	329.44

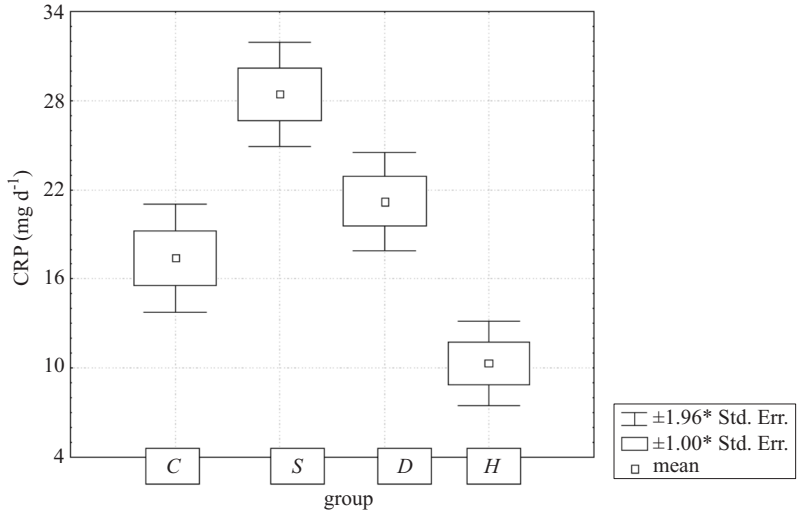

Fig. 1. Plot for CRP in the study

Table 2

Means and variability factors of indices in the study

Variable	Statistical figures	Group			
		D	*C*	*S*	*H*
CRP	x	21.2^A	17.4^B	28.4^A	10.03^B
	v	0.25	0.3	1.15	0.42
Albumin	x	2.55^A	2.1^B	1.91^B	2.59^A
	v	0.17	0.2	0.13	0.06

A, B – differences statistically significant ($p < 0.01$)
x – mean
v – variability factor

Brachyspira hyodysenteriae yielded negative results in all the animals. Neither *Salmonella spp*. nor *Yersinia enterocolitica* rods were found. *Clostridium perfringens* type C was isolated from jejunum specimens in all the diarrhoeal (*D*) and 3 of 6 subclinical infected piglets (*S*). All the diarrhoeal animals were infected with *E. coli* with fimbrial antigens F18. In the case of subclinical infected animals (*S*), 3 of them were infected with F4 rods and they were the same individuals that were infected with *Clostridium perfringens*. The remaining piglets were infected with the K88 strain, 3 of which were carrying F18 fimbrial antigens.

Discussion and Summary

As a control, piglets 7 days after weaning were used because in younger pigs the level of APP is still not stable and increases from birth up to 35 days of age (RYCHLIK et al. 2001, KOSTRO et al. 2004); simultaneously, 7 days after weaning, the stress connected with this event passes and the APP level seems to stabilise (LECHOWSKI et al. 1998, MERLOT et al. 2004).

The level of CRP in piglets suffering from diarrhoea (*D*) corresponded with that found by BÜRGER et al. (1992). According to his papers, a CRP serum level below 15 mg/l should be considered as normal (physiological). He has also noted that in diarrhoeal piglets the increase in CRP level is rather slight – only 50% of ill individuals showed an increase over 20 mg/l. Interestingly, others point to the value of 30 mg/l (LECHOWSKI et al. 1998) and even 43 mg/l (HEEGAARD et al. 1998) as the upper normal range in healthy pigs. In the present study, the most intensive growth of this factor was observed in the subclinically infected animals (group *S*) and amounted to 28.4 mg/l (Figure 1), but it is still too of a weak reaction (especially with a variance factor 1.15) to be of clinical importance. Albumin reaction, as a negative APP, was also ambiguous: quite significant in silent infection – 1.91 versus 2.59 g/dl in the control group ($p < 0.01$) – but rather weak (2.55 g/dl) in the diarrhoeal piglets (Table 2). Fibrinogen was designated by The European Commission Directorate General Research Concerted Action Group, as an acute phase protein in pigs, but its reaction found in the present study was unpredictable and varied between 329.44 and 395.71 in the control (*H*) and combining (*C*) group, to 276.33 and 256.33 in the subclinically infected (*S*) and diarrhoeal piglets (*D*), respectively.

It should be noted that immune reactions differ slightly in conventionally reared pigs and in animals from SPF herds. Healthy conventional pigs often have elevated immune indices and show moderate reaction to immune challenge (PETERSEN et al. 2000). 27% of 100 healthy pigs conventionally reared had elevated plasma IL-6 level (16.2 ± 8.0 U ml^{-1}) in the study conducted by FOSSUM et al. (1998).

A weak immune reaction in conventional pigs was previously reported by HALL et al. (1992). According to his paper, SPF pigs had lower mean haptoglobin concentration values than conventional pigs at the onset of the study, furthermore, conventional pigs showed a less significant immune reaction after a challenge of *Actionbacillus pleuropneumoniae*. CARROLL et al. (2004) noted that even after an oral *E. coli* K88 challenge, serum haptoglobin and TNF-α concentrations are poor indicators of the acute phase response (APR) prior to 24 h post infection, and CRP levels in piglets varied significantly even before the challenge, as was noted in the present study (the variance factor in H group amounted to 0.42). Furthermore, the experimental model of porcine

post-weaning colibacteriosis presented by MADEC et al. (2000) yielded interesting results, suggesting that diarrhoea after an *E. coli* K88 (F4) challenge occurs only in 50% of cases, develops rapidly (sometimes within half a day), and is usually transient (last 1.7 days on average). These facts are worth remembering during discussion on the current findings, especially those obtained from group *S*. Despite a lack of symptoms of illness, the piglets in this group had lower albumins and the highest CRP level (Table 2). It is possible that these piglets did not develop diarrhoea symptoms after a natural encounter with the microorganism or had passed through it earlier; nevertheless, they developed successful immune defences against the invader. It is of great importance that silent infections produce APP level alterations, even to a moderate degree.

It is also interesting that a respiratory pathogen challenge usually produces a rapid and solid alteration in plasma interleukins (FOSSUM et al. 1998) and APP level (FRANCISKO et al. 1996, AGERSØ et al. 1998, SKINER 2001); for example, *Actionbacillus pleuropneumoniae* – 3.3 to 7 times pre-challenge CRP level or haptoglobin even 40 times the pre-challenge mean (HALL et al. 1992, AGERSØ, FRIIS 1998, HEEGAARD et al. 1998) and, although fields results are not so clear (PETERSEN et al. 2000), they are considered valid markers of respiratory infections in pigs.

The question is: Why does the systemic response vary with respiratory and diarrhoeal diseases? Mammals focus their defence mechanisms on the sites of most frequent contacts with pathogens. The most obvious example of such a surface is the skin but, in fact, the surfaces of the mucosa of the respiratory system and digestive tract are about 200 times larger. These large surfaces are defended by highly specialised systems: bronchus-associated lymphoid tissue (BALT) and gut-associated lymphoid tissue (GALT), which are autonomic in a significant degree. As primed B cells, they may migrate among gut, respiratory tissue, lacrimal and milk glands and are considered to be a part of the common mucosal immune system (CMIS), which additionally includes active sites in the reproductive system and salivary glands (TIZARD 2000).

BALT is organised to cope with single particles, mostly not viable, which are successfully trapped by macrophages easily releasing proinflammatory cytokines during activation. On the contrary, GALT is sufficient to neutralise thousands of antigens daily (TIZARD 2000). Simultaneously, there is no need to destroy all of the microorganisms, it is enough to allow invaders to pass through the alimentary tract without interfering with the mucosa. Pathogen exclusion in the gut is performed mainly by antibodies. Although the first immunoglobulin of a newborn is IgM, enriched later by IgE and IgG, the most important in adult life is IgA (secretory IgA). IgA activates complement only by an alternate pathway and its main activity is the prevention of microorganism mucosal surface adhering, which is called immune exclusion. If the bacteria

cannot adhere to the intestinal mucosa, it simply passes along the gut and is expelled without doing any harm. This is why, in the present study, dramatically elevated APP levels were not found despite the presence of *E. coli* and diarrhoea symptoms. Interestingly, gut translocation by bacteria, meant as the passage of viable microorganisms across the intact mucosa of the gastrointestinal tract, is more common in surgical patients with obstructive bowel disease (FLORENCE 1997). It is possible that the diarrhoea is not present at that time, but the state may lead to an increased immune response – as in group *S* in the current study. If invading microorganisms get away immune exclusion, they must be trapped by another mechanism – IgE – but this involves rapid degranulation of mast cells and the release of vasoactive factors. These factors increase the permeability of blood capillaries, which eventually develop acute inflammation. The present study supports the hypothesis that diarrhoea without general disorders yields only moderated alterations in APP levels. It is highly probable that resistance to pathogens is more connected with the sIgA level on the mucosa surface than levels of immunoglobulins circulating in blood serum (de ARRIBA et al. 2002, KELLY, COUTTS 2000). In summary: GALT and its mechanisms are efficient enough to protect the body against pathogens without engaging systemic defences, as long as microorganisms break down the barrier causing the systemic response. These facts suggest that the weak reaction of APPs found in the present study might be connected with a properly functioning GALT and its defences.

In the present study, two days after the combining event, piglets showed (as predicted earlier) a moderate reaction of CRP (Figure 1). This corresponds with findings obtained by LECHOWSKI et al. (1998) concerning stress conditions and APR, although the mean values differed from results found in the present study (43.16 mg/l and 17.4 mg/l respectively). HICKS et al. (1998) examined pig reactions for acute stressors such as heat, cold and shipping. The animals were subjected to their assigned stressor (heat, cold or shipping) for 4 hours. Pigs were stressed until they showed increased respiration rate and the cold animals began to huddle and shiver. The reaction of haptoglobin was poor, 1.1 mg/ml, 1.02 mg/ml and 0.89 mg/ml in cold, heat and control group, respectively. After shipping, the serum level of haptoglobin was even lower than that found in the control group – 0.80 mg/ml. Similarly, the blood level of fibrinogen in the shipping group was lower than that observed in the control group, and in the heat and cold groups failed to show any significant reaction. Although fibrinogen is considered to be an acute protein in pigs, it was not confirmed that fibrinogen is an APP in pigs in stress conditions, as well.

Some preliminary findings suggested that combining two groups of weaned pigs did not evoke global haptoglobin change. The results might have been caused by the pigs being subjected to insufficiently short stress events. It is

possible that the three-hour stress challenge used in that study was not potent enough to raise cytokine levels enough to cause hepatic conversion to APP production. Even a dose of 100 μg/kg LPS endotoxin *E. coli* injected intraperitoneally in weaned pigs only once was insufficient to induce an IL-6 reaction and a significant reaction of APP (haptoglobin) (WRIGHT et al. 2000), whereas repeated LPS administration and a *Actinobacillus pleuropneumoniae* challenge did manage to increase IL-6, which is responsible for haptoglobin and albumin expression in pigs (GONZÁLEZ-RAMÓN et al. 2000). GONZHLEZ--RAMÓN et al. (2000) showed that releasing haptoglobin and expression of its DNA was time- and dose-dependent during an in vitro experiment. They reported that haptoglobin concentration in a culture medium of porcine hepatocytes increased 1.5 fold after a 24h incubation with recombinant human IL-6 and about 2 fold after a 48 hour incubation period. In the present study, the piglets were subjected to stress continuously for 48 hours and this might have exerted a difference on the results.

Stress response may be adaptive in the short-term but it can become highly maladaptive in the long-term (BARTOLOMUCCI et al. 2005). Combining stress does not produce cortisol and cytokine level alterations in a prolonged manner and – what is even more important – in all individuals in the group equally. A lack in immune reactivity and higher disease susceptibility was described in lower ranking pigs compared with dominants (MERLOT et al. 2004, TUCHSCHERER et al. 1998). A stable hierarchy among young piglets in the natural environment develops within a few hours without harmful consequences. Increased immune reactions may be expected mainly in dominant animals – residents (RD) and invaders (ID) – and submissive invaders (MERLOT et al. 2004). Furthermore, during combining, the immune reactions are not maladaptive and do not evoke critical responses. This theory was supported in the present study, where the reactions of APP were significant, but not as strong as during inflammation. In the present study, the animals were not assigned to dominant or submissive group because the aim of the experiment was to estimate if combining stress influences the global level of APP in a pen. The findings indicate that if elevated levels of APPs appear in young piglets, this cannot be caused by combining stress. It is not very likely that diarrhoea without general disorders is the cause of increasing plasma APP alterations. It should be noted that elevated levels of positive APPs during weaning are not evoked by diarrhoea or combining stress and, if they appear, they must have been triggered by other factors – which should be identified.

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THE INFLUENCE OF THE OZONE AND VITAMIN C ON STEROIDOGENIC ACTIVITY OF RAT TESTES

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K e y w o r d s: ozone, testes, steroidogenesis, vitamin C.

A b s t r a c t

The study was performed on sexually mature male rats divided randomly into six groups: I – control rats; II and III – ozonized rats and receiving im injections of vitamin C at doses, respectively, 20 and 40 mg/rat, and IV and V – rats did not expose to ozone, treated with mentioned above doses of vitamin C, VI – animals only ozonized, without vitamin injections.

On the ground obtained results can ascertain that oxidation stress caused by ozone disturbs process of steroidogenesis in testes leading to a decrease in the content of enzymes – $P450_{scc}$ and 3β-HSD, and consequently the concentrations of testosterone (T) and estradiol-17β (E_2) in gonadal tissue (in spite the elevated content $P450_{arom}$) Vitamin C distinctly increases the 3β-HSD content in testes restoring the physiological T and E_2 concentrations.

WPŁYW OZONU I WITAMINY C NA AKTYWNOŚĆ STEROIDOGENICZNĄ JĄDER SZCZURA

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S ł o w a k l u c z o w e: ozon, jadra, steroidogeneza, witamina C.

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Abstrakt

Doświadczenie przeprowadzono na dojrzałych płciowo samcach szczurów podzielonych na 6 grup: I – zwierzęta kontrolne; II i III – zwierzęta ozonowane i otrzymujące domięśniowe iniekcje witaminy C w dawkach odpowiednio 20 i 40 mg/szczura oraz IV i V – zwierzęta nieozonowane otrzymujące w/w dawki witaminy C, VI – szczury ozonowane bez iniekcji witamin.

Na podstawie uzyskanych wyników można stwierdzić, że stres oksydacyjny powodowany przez ozon zaburza proces steroidogenezy w jądrach, obniżając zawartość enzymów $P450_{scc}$ i 3β-HSD, a w konsekwencji koncentrację testosteronu (T) i 17β-estradiolu (E_2) w tkance gonad (pomimo podwyższonej zawartości $P450_{arom}$). Witamina C wyraźnie zwiększa zawartość dehydrogenazy 3β-HSD, przywracając fizjologiczny poziom T i E_2.

Introduction

Reactive oxygen species (ROS) serve an important role in regulating numerous cell functions. Besides transferring intercellular signals, they modulate gene expression and depending on their needs activate cell trascription, proliferation and apoptosis (LAHANCE et al. 2001). Low amounts of ROS, which are controlled by the antioxidation system, play a vital role in testis physiology as well as the processes of gamete production and steroidogenesis.

The whole steriod synthesis process is accompanied by ROS production, beginning with regulatory mechanisms with the help of luteinizing hormones (LH). By itself physiological LH action on rats Leydig cells (LC) is connected with lipid peroxidation and maintaining the high activity of peroxide-metabolizing enzymes in interstitial tissue (PELTOLA et al. 1996). The next stages of steriod production are controlled by cytochrome P-450 enzymes, which are mixed-function oxidases and steroid dehydrogenases. ROS are mainly generated in stages regulated by P-450 enzymes. The steroidogenesis and the amount of reactive species produced determines which antioxidation systems present in the cell are activated, ensuring their proper operation (HANUKOGLU 2006).

Exposing rats to ozone upsets the balance between ROS production and neutralization, leading to oxidative stress. Our earlier experiments showed that ozonized animals had a lower testosterone (T) concentration in blood plasma and lower 17β-hydroxysteroid dehydrogenase (17β-HSD) activity in testicular tissue (JEDLIŃSKA-KRAKOWSKA 1998). A damaged seminiferous epithelium was also noticed (JEDLIŃSKA-KRAKOWSKA et al. 2006). Administering vitamin C (ascorbic acid – AA), known for its antioxidative properties, does not always prevent oxidative stress, and instead sometimes increases its intensity (JEDLIŃSKA-KRAKOWSKA et al. 2006, JEDLIŃSKA-KRAKOWSKA 2006).

The study had two aims: to detemine how and to what degree ozone disturbs the steroidogenesis in testes, as well as to check if high doses

of vitamin C reduce oxidative stress brought on by ozone and also influence steriod biosynthesis.

Materials and Methods

Animals and experimental procedure

Mature male Wistar Hannover rats aged 12 months, weighing 420 ± 10 g and clinically healthy were used in this experiment. The animals were kept in the room under natural lighting, at a temperature of 21-22°C, and with gravitational ventilation, and were fed *ad libitum* Murigran standard pellets for rodens (Motycz n/Lublin, Poland) with free access to water. The principles of animal care (NIH publication No. 86-23, revised 1985) as well as the specific national law on the protection of animals were followed.

All animals were randomly divided into 6 groups (6 rats each): I (C) – control; II (20C+Oz) and III (40C+Oz) – rats exposed to Oz and receiving intramuscularly vitamin C (Vitaminum C, Pliva Kraków, Poland) at a dose 20 and 40 mg/rat, respectively; every 5 days, during 35 days; IV (20C) and V (40C) – rats treated with vitamin C at a dose 20 and 40 mg/rat, respectively, every 5 days, during 35 days; and VI (Oz) – rats exposed to Oz. All rats from the II, III and VI groups were exposed to 0.5 ppm Oz during 5 h, thought 35 days.

Ozone was generated from compressed air in an IMPOZ-4 ozonizer (Institute of Precise Mechanics, Warsaw, Poland) and transferred through a polyethylene conduit to an exposure chamber (a room chemically-sealed with a chemically neutral and biologically friendly polyethylene foil) with a capacity of 42.6 m^3. In the chamber, ozone was spontaneously mixed with air, and its concentration was monitored using an iodometric method (SALTZMAN et al. 1959). The air in the chamber was exchanged at the midpoint during exposure to avoid excessive accumulation of CO_2, and the ozone concentration was returned to the initial level. All the rats, the control and experimental rats, were kept in cages (4 rats per cage). During exposure to ozone, the animals had free access to water, while the pellets were removed due to the oxidizing effect of ozone. Except during the 5 h of exposure to ozone, all rats were kept under the same conditions with reference to air composition, temperature, and manner of feeding.

After 35 days, all rats under halothane anesthesia (Narcotan, Leciva, Czech Republic) were sacrificed by cardiac puncture and the testes were immediately dissected out. Samples of the gonads were collected and kept at -70°C until estimation of the content of steroid hormones was completed. Fragments of the testes were also shock-frozen in liquid nitrogen and then stored at -70°C in order to estimate the content of enzymes studied (see below).

Hormone analyses

Extraction of progesterone (P_4), T, estrone (E_1) and estradiol-17β (E_2) from the testes was performed by method described by TSANG et al. (1990). Briefly, 300 mg of tissue was homogenized in 4 ml of 0.05 M EDTA, 0.2 ml of homogenate was mixed with 2 ml of oil-ether and then sample was frozen. On the next day, after evaporating of etheric fraction, 1 ml of 0.1 M phosphate buffer was added into dry fraction and this probe was used for analysis. The contents of these hormones in extracts prepared from testes were estimated by the radioimmunoassay procedures, published for P_4 and E_2 by HOTCHKISS et al. (1971), for A_4 and E_1 by DZIADKOWIEC et al. (1982) and for T by KOTWICA et al. (1982). Characteristics of antibodies (obtained from the Institute of Animal Physiology, University of Warmia and Mazury in Olsztyn, Poland) have previously been provided for P_4 by DZIADKOWIEC et al. (1982) and for the remaining steroids by SZAFRAŃSKA et al. (2002). The sensitivity of the assay for P_4, T, E_1 and E_2 were 15, 2.5, 2.5 and 5 pg/ml, respectively. Intra- and interassay coefficients of variation for P_4, T, E_1 and E_2 were 5.2 and 10.6%, 7.6 and 12.8%, 6.7 and 11.3%, 7.1 and 12.2%, respectively.

Western blotting analysis

The content of cholesterol side-chain cleavage cytochrome P450 ($P450_{scc}$), 3β-HSD and aromatase cytochrome P450 ($P450_{arom}$) in the testes were estimated with the method described by STĘPIEŃ et al. (1999), with following modifications: 20 μg of protein was administered into each well; the non-specific binding sites were blocked by 5% skim-milk, overnight at 4°C, with constant shaking; visualization of the immune complex was done by incubation with freshly prepared mixture of 3.3'-diaminobenzidine tetrahydrochloride and H_2O_2 in Tris-buffered saline (pH 7.2), for 2-3 min. Primary antibodies including rabbit anti-rat $P450_{scc}$ polyclonal antibody (diluted 1:1000; Chemicon, Temecula, CA, USA), rabbit anti-mouse 3β-HSD polyclonal antibody (diluted 1:5000; a gift from Dr. N. Rahman, University of Helsinki, Finland) and rabbit anti-human placental $P450_{arom}$ polyclonal antibody (diluted 1:2000; provided by Hauptman-Woodward Medical Research Institute, Inc., Buffalo, USA) were used. All immunoblotts were then quantitated by scanning on KODAK 1D Image Analysis Software (USA). The intensity of bands detected by on-dimensional image analyses is reported in arbitrary units.

Statistical analysis

The mean (± SEM) contents of steroids in testes as well as the intensity of bands staining (arbitrary units) were calculated for each group/each treatment and compared by Bonferroni test (ANOVA, InStat Graph Pad, San Diego, CA).

Results

Content of steroids in the testes

Progesterone

The content of P_4 in the testes of rats from all groups was insignificantly changed (Figure 1a).

Testosterone

In compared to the control group, the content of T in the testes of rats receiving vitamin C at a dose 20 and 40 mg was enhanced ($P < 0.05$) whereas in the ozonized animals was decreased ($P < 0.01$). Amounts of this steroid in the gonads of rats exposed to ozone and treated with 20 and 40 mg of vitamin C parallel enhanced ($P < 0.05$ and $P < 0.01$, respectively) in relation to rats only ozonized. After administration of 20 and 40 mg of vitamin C the content of T was higher than that determined in the rats treated with 20 and 40 mg of vitamin C and ozonized together ($P < 0.01$ and $P < 0.05$, respectively; Figure 1b).

Estrogens

The content of E_1 in the testes of all studied rats was similar (Figure 1c). While exposition of the rats to ozone lead to a decrease ($P < 0.05$) in the amount of E_2 in the testes as compared to the control group (Figure 1d).

Content of enzymes

Expression of $P450_{scc}$, 3β-HSD and $P450_{arom}$ proteins was observed, at clearly detectable levels, in the testes of all studied rats.

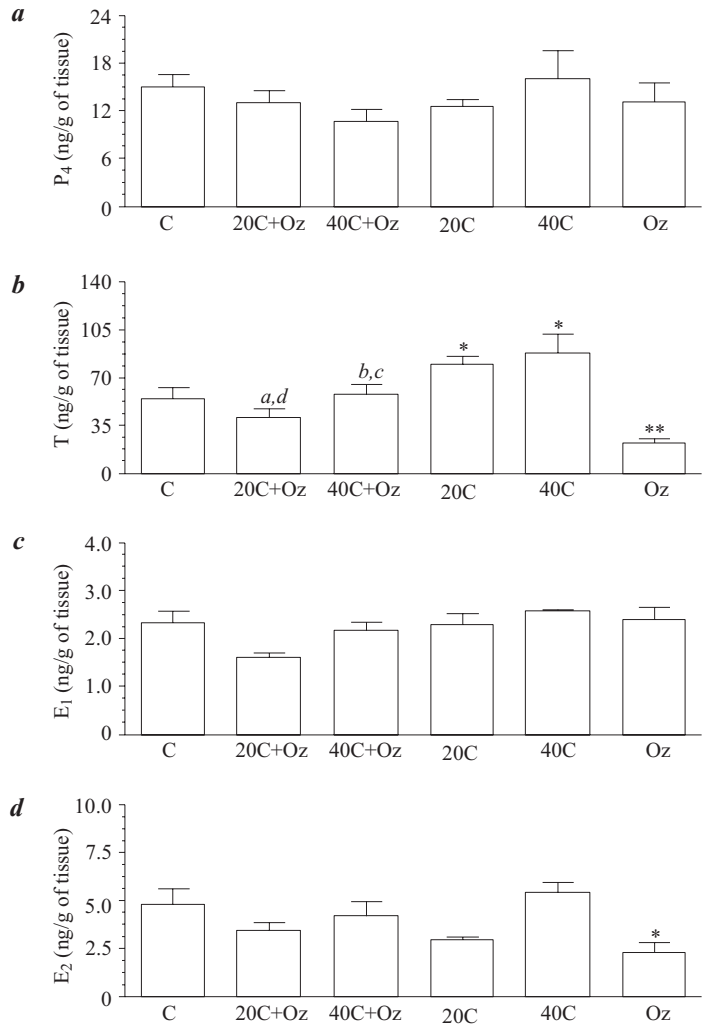

Fig. 1. Mean (± SEM) content of P4 (a), T (b), E1 (c) and E_2 (d) in the testes of the rats: control (C), receiving vitamin C at a dose 20 mg/rat and exposed to Oz (20C+Oz), receiving vitamin C at a dose 40 mg/rat and exposed to Oz (40C+Oz), treated with vitamin C at a dose 20 mg/rat (20C), treated with vitamin C at a dose (40C), and exposed to Oz (Oz). *$P < 0.05$; **$P < 0.01$ indicate significant differences between the control and experimental groups. [a]$P < 0.05$; [b]$P < 0.01$ – indicate significant differences between 20C+Oz or 40C+Oz and Oz groups; [c]$P < 0.05$ – indicates significant differences between 40C+Oz and 40C groups, [d]$P < 0.01$ indicates significant differences between 20C+Oz and 20C groups

The content of $P450_{scc}$ in the testes of the animals treated with 20 and 40 mg of vitamin C and exposed to ozone simultaneously ($P < 0.01$, 27.4 and 70.2%, respectively), receiving vitamin C at a dose 20 ($P < 0.01$, 33.5%)

and 40 ($P < 0.05$, 14.2%) mg as well as exposed only to ozone ($P < 0.01$, 55.8%) was lower as compared to the control group. In the gonads rats treated with 20 mg of vitamin C and ozonized together the content of this enzyme was higher ($P < 0.01$, 64.3%) than that found in the ozonized animals. While administration of vitamin C at a dose 40 mg and ozonization conducted to a decrease ($P < 0.05$, 65.3%) in the content of $P450_{scc}$ in testes as compared to rats receiving only vitamin C at a dose 40 mg (Figure 2a).

The immunoexpression of 3β-HSD in the testes of rats after administration of vitamin C at a dose 20 and 40 mg and ozonization together ($P < 0.01$, 23.4 and 34.3%, respectively), injections 20 and 40 mg of vitamin C ($P < 0.05$, 16.8 and 26.8%, respectively) and ozonization alone ($P < 0.01$, 59.5%) was reduced in relation to the control group. In the animals injected with vitamin C at a dose 20 and 40 mg and ozonized simultaneously the content of this enzyme was higher ($P < 0.01$) as compared to the rats only ozonized (89.1 and 62.2%, respectively; Figure 2b).

Injections of vitamin C at a dose 20 and 40 mg and exposition to ozone together ($P < 0.01$, 176.2 and 197.5%, respectively), injections of vitamin C at a dose 20 and 40 mg ($P < 0.05$, 139.4 and 197.8%, respectively) and ozone alone ($P < 0.05$, 328.1%) lead to an increase in the content of $P450_{arom}$ protein in the testes when compared to the values found in the gonads testes of the control animals. In turn, immunoexpression of $P450_{arom}$ in the testes of rats treated with vitamin C at a dose 20 mg and ozonized together was lower ($P < 0.05$, 35.5%) than that estimated in the rats ozonized only (Figure 2c).

Discussion

Based on the obtained data we can state that oxidative stress, caused by ozone, disturbes steroidogenesis in rat Leydig cells. A direct cause of these changes is increased ROS production parallel to a drop in the output of antioxidative systems. In animal testes exposed to oxidative stress, there were a drop in the activity of superoxide dismutase, catalase and glutathione--peroxidase; lower levels of vitamin C and E as well as growth in malondialdehyde content (JEDLIŃSKA-KRAKOWSKA 2006, SAMANTA et al. 2006).

The main site of ROS-suppression of steriod synthesis are depend on LH, and occured in mitochodria processes the provide and transport cholesterol. This may be related to the decrease in expression of StAR protein (steroidogenic acute regulatory protein), which is necessary for cholesterol to reach the mitochondrial membrane (DIEMER et al. 2003) or interuptions in regulating this process, when oxidative stress significantly lowers the LH level (SAMANTA et al. 2006, MANNA et al. 2003). The first step of steroidogenesis (the conversion

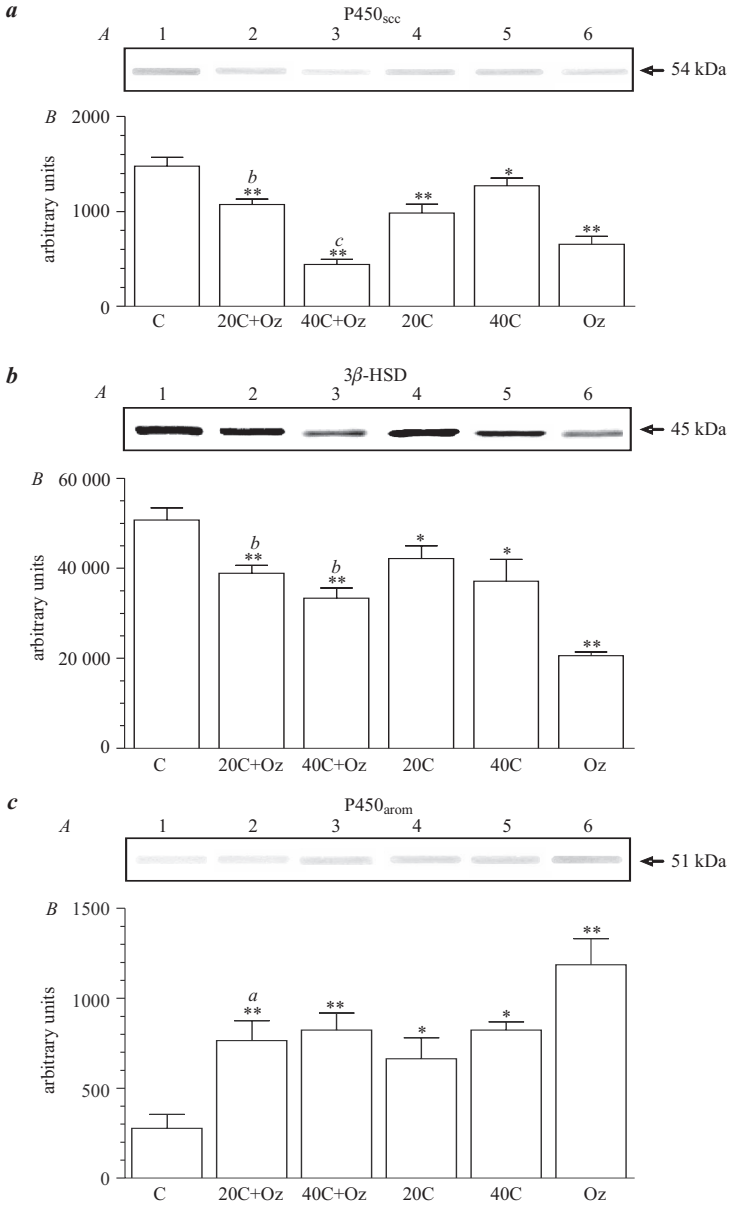

Fig. 2. Western blot of $P450_{scc}$ (A), 3β*-HSD (B) and $P450_{arom}$ (C) in the testes of rats: a – control (C; line 1), receiving vitamin C at a dose 20 mg/rat and exposed to Oz (20C+Oz; line 2), receiving vitamin C at a dose 40 mg/rat and exposed to Oz (40C+Oz; line 3), treated with vitamin C at a dose 20 mg/rat (20C; line 4), treated with vitamin C at a dose (40C; line 5), and exposed to Oz (Oz; line 6), b – densitometric analysis of enzymes in arbitrary units. Data presented here are representative of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$ indicate significant differences between the C and experimental groups; $^{a}P < 0.05$; $^{b}P < 0.01$ – indicate significant differences between 20C+Oz and/or 40C+Oz and Oz groups; $^{c}P < 0.05$ – indicates significant differences between 40C+Oz and 40C groups

of cholesterol to pregnenolon) is controlled by the cytochrom-$P450_{scc}$ enzyme complex, whose activty was lower in all the experiment groups. The most significant decrease in the content of this cytochrome in ozonized animals and receiving large doses of vitamin C can be explained by the fact that an insufficient supply of substrate can result in electrons "escaping" while being transferered from NADPH via FAD adrenodoxin reductase and adrenodoxin (HANUKOGLU 2006, PELTOLA et al. 1996) and thus increasing ROS production. In addition, ozone alone initiates the chain reaction which may result in generating ROS, and furthermore large doses of vitamin C, even more so in connection with ozone, do not always antioxidative activity (JEDLIŃSKA-KRAKOWSKA 2006, CARR et al. 1999). On the other hand, administering C or along with ozone, but in lower doses, clearly increased reduced $P450_{scc}$ content. Availabe literature lacks data concerning ozone's influence on the process of steriodogenesis in testes, but it is known that during oxidative stress there is a drop in the activity of both 3β- and 17β-HSD (SAMANTA et al. 2006, MANNA et al. 2003). A reduced amount of 3β-HSD was found in all the experimental groups. It grew significantly after vitamin C administration compared to ozone-only animals, however the amount did not reach the values of the control group. Despite the drop in 3β-HSD content, reduced P4 concentration was not noticed, even though the ozonized groups exhibited a downward trend. However this enzyme is not only present in the endoplasmic reticulum but also in the LC mitochondria, which enables the synthesis of this hormone directly from cholesterol, ommitting pregnenolon (BILIŃSKA 1997). Vitamin C in turn plays a vital role in coregulating StAR gene expression (GUPTA et al. 2004) and furthermore participates in steroidogenesis, mainly in hydroxylation reactions (LUCK et al. 1995).

The highest, more than double, decrease in the T level was observed in the group of animals ozonized without vitamin protection. Administering AA to ozone-exposed rats increased concentration of this steroid to value comparable to that of the control group. In non-ozonized animals, the level even surpassed that of the control group. Our earlier experiments demonstrated that ozone significantly lowers 17β-HSD activity, the enzyme directly responsible for T synthesis (JEDLIŃSKA-KRAKOWSKA 1998). In addition a high correlation exists between the activity of this enzyme and catalase and peroxidase with anitoxidative properties (MANNA et al. 2003). By removing ROS and inhibiting the peroxidative processes, Vitamin C increases the activity of both dehydrogenase at the protein level and the transcription process, regulating the T level in this way (SAMANTA et al. 2006, MANEESH et al. 2005, GUPTA et al. 2004).

The activity and localization of $P450_{arom}$ in rats depends on age. In adult males, they are mainly round and elongated spermatides as well as LC, and

sporadically Sertoli cells (CARPINO et al. 2001). The increased content of $P450_{arom.}$ in all the experimental groups at unchanged estrogen levels (with the exception of the decrease of E_2 in group VI Oz) could be a consequence of increased gene expression of this enzyme, which was seen earlier in porcine testes (GOLDRING et al. 1987). Despite large amounts of $P450_{arom.}$, ozonized animals wihtout vitamin protection displayed a drop in the E_2 concentration, which was not observed in ozonized rats receiving vitamin C. This decrease may have been caused by a lack of sufficient T, a substrate of the aromatization process. However, E1 whose level did not change, could be produced independently through the aromatization of androstenedion (ISHIKAWA et al. 2006), which at a high content of $P450_{arom.}$ probably stabilizes the level of this hormone.

Based on the obtained results, we declare that oxidative stress caused by ozone exposure disturbs the steroidogeneis in rat testes lowering $P450_{scc}$ and 3β-HSD content, and consequently T and E_2 levels. Vitamin C clearly increases 3β-HSD content restoring the physiological levels of both these hormones.

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