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TABLE OF CONTENTS

Agriculture

Animal Breeding and Husbandry

Biology

Environmental Protection

Food and Nutrition Sciences

SPIS TREŚCI

Rolnictwo

Chów i Hodowla Zwierząt

Biologia

Ochrona Środowiska

Nauka o Żywności i Żywieniu

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EFFECT OF REPRODUCTION PLACE AND WAY OF HARVESTING FRUITS ON QUALITY OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) SEEDS

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K e y w o r d s: tomato, seed production, seed quality, *Lycopersicon esculentum*.

A b s t r a c t

In the years 2000–2002, a possibility was investigated of the seed production of a tomato cultivar „Etna F_1". The experiments were carried out in the southern and middle Poland. Fruits were collected one, two and many times. The seeds from the collected fruits were extracted directly after harvest or one week after their maturing. It was proved that in the conditions of the middle and southern Poland, it is possible to produce tomato seeds of the cultivar „Etna F_1" in the field conditions. Despite a big variability in the seed yield per plant amongst the years, the received seed yield was satisfied. The production place affected seed yield, 1000 seeds weight and seed germination capacity of the cultivar. The seeds produced in the middle Poland germinated better (89.0%) than the ones from the southern Poland (85.8%). The fruit harvesting method in the middle Poland conditions did not affect the seed yield, 1000 seeds weight and their germination. When producing seeds in the southern Poland, it is better to harvest fruits from the field successively (many times). The seeds received from the fruits of the tested cultivar, when maturing for 7 days, germinated worse than the ones extracted from fruits directly after their harvesting. The way of extracting seeds did not affect their seed yield per plant and 1000 seeds weight.

WPŁYW REJONU REPRODUKCJI I SPOSOBU ZBIORU OWOCÓW NA JAKOŚĆ NASION POMIDORA (*LYCOPERSICON ESCULENTUM* MILL.)

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S ł o w a k l u c z o w e: pomidor, produkcja nasion, jakość nasion, *Lycopersicon esculentum*.

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Abstrakt

W latach 2000–2002 zbadano możliwość produkcji nasion pomidora odmiany Etna F_1. Badania przeprowadzono w Polsce południowej i środkowej. Owoce zbierano jedno-, dwu- i wielokrotnie. Nasiona pozyskiwano natychmiast po zbiorze owoców lub po tygodniu ich leżakowania. Wykazano, że w warunkach Polski środkowej i południowej możliwa jest polowa produkcja nasienna pomidora odmiany Etna F_1. Pomimo stwierdzonej dużej zmienności między latami, uzyskany plon nasion był zadowalający. Rejon produkcji miał wpływ na plon nasion z rośliny, masę 1000 nasion i zdolność kiełkowania badanej odmiany. Nasiona wyprodukowane w Polsce środkowej kiełkowały lepiej (89,0%) niż uzyskane z Polski południowej (85,8%). Metoda zbioru owoców w warunkach Polski środkowej nie miała wpływu na plon nasion, masę 1000 nasion i ich zdolność kiełkowania. Produkując nasiona w Polsce południowej, lepiej jest natomiast prowadzić sukcesywny (wielokrotny) zbiór owoców z pola. Nasiona uzyskane z owoców badanej odmiany po leżakowaniu przez 7 dni kiełkowały gorzej niż uzyskane z owoców bezpośrednio po zbiorze. Sposób ich pozyskania nie wpłynął na plon nasion z rośliny i masę 1000 nasion.

Introduction

Tomato has been the most important vegetable species in the world. Its area and production have been constantly increasing (*FAO* 2006). The market has been dominated by hybrid cultivars despite their high seeds costs due to their expensive production. Introduction of the cultivars changed the calculation of transplants costs. The field tomato growers started to limit amount of seeds used for sowing. They also started to look for seeds with the highest quality, enhanced (HILL et al. 1989) and preferred cultivars which could be grown through sowing seeds directly in the field. Tomato seed producers, on the other hand, maximally simplified production lowering its costs and used systems of promotion and discounts (BRALEWSKI, HOŁUBOWICZ 2006). For this reason, most of field tomato seeds is nowadays produced in China and India (BRALEWSKI, HOŁUBOWICZ 2005).

However, still one faces problems when producing seeds of this species. Some authors referred to store tomato fruits after harvesting, whereas others recommended to process them immediately after collecting. Also contradictory reports confirm the way of harvesting fruits: completely in one time or as practiced by many growers, gradually in 2–3 times. The time of fruit ripening depends on the cultivar but can be also affected by the place of reproduction and weather: higher temperature speeds up the ripening of tomato fruit (NAIK, SRINIVAS 1989, VALDES, GRAY 1998, ADAMS et al. 2001).

The main purpose of this experiment was to find out how seed plantation's location and the way of fruit harvesting affect tomato seed quality.

Material and Methods

The used in the experiments cultivar „Etna F_1" was developed in Plant Breeding and Seed Production Co. „PlantiCo – Świętosław, Ltd." in Poland. It was developed by using two lines: female (R_m) – PH 1102 SWE with functional male sterility received from the Polish Vegetable Institute in Skierniewice and male (R_o) – SWO 1. The cultivar was registered in Poland in 1994. It is a field early, determinate cultivar with red, round fruit of 70–100 g. It is resistant to both chill and drought. It is suitable for direct seeds sowing technology. In the 90s, the cultivar had been widely used in Poland with the commercial production area of about 3.000 ha per year. Moreover, it had started a series of breeding sister lines followed then by new cultivars with the use of male sterile (MS) lines.

The experiment was carried out in the years 2000–2002 in two different locations: Kutno (middle Poland) and Krzeszowice near Cracow (southern Poland). The seeds of parent lines used in the experiment came from the seed company Plant Breeding and Seed Production Co. „PlantiCo – Gołębiew, Ltd.". The two factors of the experiments included 3 ways of fruit harvesting: many times during fruit ripening, twice or once when the majority of fruits was ripen, and two ways of getting seeds from fruits: directly after harvesting them and 7 days after stocking them. The transplants were planted in the 50 cm and 80 cm spacing on the field of fertile, heavy glay soils with routine fertilizing. Each treatment included 8 plants. The experiment was run in 3 replications. The pollen from R_o was collected mechanically. The flowers of R_m were hand--emasculated and artificially pollinated. Then, the fruits were collected, extracted, fermentated, rinsed with water and dried. Seed quality was evaluated using routine ISTA methods. The received data was statistically processed. The variance was calculated, significant differences were counted using the Duncun's test for $\alpha = 0.05$.

The weather conditions in the year 2000 were medium good for tomato seed production in Poland. After cold May, the weather in June was suitable (dry and hot) for hand pollination of flowers in the field. The spring of 2001 was colder than usually with night frosts. High precipitation in June and July created problems with successful flower pollination in the field. However, hot August helped good ripening of fruits and resulted in high seed production. The year 2002, although hotter than usually, was difficult for seed production. Heavy rains in June and July made problems in emasculating and hand--pollinating of flowers in the field. Still, eventually the year was good due to high fruit yields.

Results and Discussion

The carried out experiment proved, that in the climatic conditions of the middle and southern Poland, it was possible to produce in the field tomato seeds of the hybrid cultivar „Etna F_1". The received seeds germinated between 85.8 and 89.0% (Table 1). The production place affected seed yield, 1000 seeds weight and seed germination capacity (Table 1). The fruit collected in the middle Poland gave bigger seed yield per plant, their seeds were heavier and germinated better than the fruits and seeds collected in the southern Poland. This was received despite different weather conditions, sometimes not so favourable for seed production of hybrid seeds in the field.

Table 1
Effect of production location of the field tomato hybrid cultivar „Etna F_1" grown for seeds on their seed yield and quality

Production location	Seed yield (g plant^{-1})	1000 seeds weight (g)	Germination capacity (%)
Middle Poland	3.07^{b}*	4.39^{b}	89.0^{b}
Southern Poland	1.89^{a}	4.06^{a}	85.8^{a}

* means in a column followed by the same letter are not significantly different according to the Duncan's test for $\alpha = 0.05$

The fruit harvesting way affected their seed yield per plant, 1000 seeds weight but did not affect their germination capacity (Table 2). In the middle Poland conditions, the seed yield per plant was higher if fruits were harvested many times than with a single harvest, however their 1000 seed weight was bigger with a single harvest than with a multiple one (Table 2). Thus, the way of fruits harvesting did not affect their seeds quality. In the southern Poland though, the way of fruit harvesting did not affect the first two parameters, i.e. seed yield per plant and 1000 seeds weight. It did affect, however, their germination capacity. If the fruits were harvested gradually (many times), their seeds germinated in 88.1%, whereas if in two times or one time, they germinated in 84.7% and 85.0%, respectively (Table 2). The way of processing fruits after their harvesting affected germination capacity of the seeds extracted from them (Table 3). When extracted just after harvesting, they germinated in 91.2%, whereas if the fruits were stocked for 7 days, their seeds germinated only in 83.5% (Table 3). Neither seed yield per plant nor 1000 seed weights were affected by the way of processing the fruits (Table 3).

Table 2
Effect of production location and way of fruits harvesting of the field tomato hybrid cultivar „Etna F_1" grown for seeds on their seed yield and quality

Production location	Way of fruit harvesting	Seed yield (g plant^{-1})	1000 seeds weight (g)	Germination capacity (%)
Middle Poland	many times	3.53^{b}*	4.34^{a}	88.3^{a}
	two times	2.83^{ab}	4.40^{ab}	88.0^{a}
	one time	2.55^{a}	4.44^{b}	91.1^{a}
Southern Poland	many times	2.04^{a}	4.07^{a}	88.1^{b}
	two times	1.84^{a}	4.06^{a}	84.7^{a}
	one time	1.81^{a}	4.05^{a}	85.0^{ab}
Mean for the production location	many times	2.79^{a}	4.21^{a}	88.2^{a}
	two times	2.34^{a}	4.23^{a}	86.3^{a}
	one time	2.18^{a}	4.25^{a}	88.1^{a}

* means in a column for a given location and character followed by the same letter are not significantly different according to the Duncan's test for $\alpha = 0.05$

Table 3
Effect of production location and way of fruit processing of the field tomato hybrid cultivar „Etna F_1" grown for seeds on their seed yield and quality

Production location	Way of fruit harvesting	Seed yield (g plant^{-1})	1000 seeds weight (g)	Germination capacity (%)
Middle Poland	just after harvesting	3.24^{a}*	4.43^{a}	91.0^{b}
	after stocking	2.89^{a}	4.36^{a}	86.8^{a}
Southern Poland	just after harvesting	1.90^{a}	4.09^{a}	91.3^{b}
	after stocking	1.89^{a}	4.03^{a}	80.2^{a}
Mean for the production location	just after harvesting	2.57^{a}	4.26^{a}	91.2^{b}
	after stocking	2.39^{a}	4.20^{a}	83.5^{a}

* means in a column for a given location and character followed by the same letter are not significantly different according to the Duncan's test for $\alpha = 0.05$

In terms of the world's production area, tomato is today the third most important (after lemon-like species and grapes) horticultural crop in the world. The tomato commercial production area worldwide has been constantly growing for the last 10 years (*FAO* 2006). No wonder that at the moment, tomato is a leading species in breeding works in many seed companies and there has been a need to lowering its seeds production costs. One of the

possible ways to solve this problem is to place the hybrid greenhouse cultivars seed production in the countries with the climatic conditions for such production close to optimal, e.g. in China. With the field tomato though, the seed price has always been much lower and other ways of solving this problem should be found. The carried out experiments proved, that in the conditions of Poland, it is possible to produce high quality tomato seeds of hybrid field cultivars in the field. Moreover, hand-carried emasculation and artificial pollination of flowers in the field resulted in satisfactory but variable seed yield per plant, what is in an agreement with earlier findings of ATANASSOVA, GEORGIEV (1986), POTACZEK (1995, 1999) and ATANASSOVA (1999). The crucial point here was the weather during the period of flowers hand-emasculation and pollination. The dominating effect of weather in tomato seed production refers not only to high production of viable pollen but could make difficult or even impossible hand flower emasculation and pollination. A tomato pollen grain in order to germinate and pollinate needs not only a proper humidity and temperature but firstly set of conditions which will enable it to stay on a pistil's stigma. These observations were confirmed by FERNANDEZ-MUNOZ et al. (1995, 2002a,b), PEET, BARTHOLEMEW (1996), JANKULOVSKI et al. (1997), GROOT (2002). The weather factor in the field production of hybrid tomato cultivars should always be taken under consideration and undoubtedly in seed production of tomatoes it creates a certain risk. Hand emasculation and pollination of tomato flowers is the most labour-consuming part in its hybrid seeds production. For this reason, its profitability may be related not only to quantity of quality of the received seed yield, but also the cost of work of people employed to pollinate (JANKULOVSKI et al. 1997). In terms of labour costs, the best conditions at present for hybrid tomato seed production are in China. However, to measure and compare this another study is needed.

Direct extraction of seeds from fruits was found to be better than keeping them for 7 days. This is due to the fact that in fruit seeds may lose their germinability. In practice, it is also easier to eliminate small, ungerminated seeds from a seed lot than already germinated and dried back seeds from stocked fruits too long kept (HILL et al. 1989).

Conclusions

1. In the field conditions of the middle and southern Poland one can get satisfactory seeds yield of tomato hybrid cultivar „Etna F_1".

2. The place of production of the seeds of the cultivar affected their quality. The seeds produced in the area of middle Poland germinated better than the ones produced in the southern Poland.

3. When producing seeds of the cultivar in the middle Poland, the method of fruits collection did not affect the germination capacity of the seeds extracted from them.

4. When producing seeds of the cultivar in southern Poland, the best is to harvest fruits many times from the field.

5. Seeds extracted from fruits of the cultivar after stocking the fruits germinated worse then when they were extracted directly after their harvest.

Translated by ROMAN HOŁUBOWICZ

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REAL INSOLATION DEFICIENCY DURING THE COOL HALF-YEAR IN POLAND

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K e y w o r d s: insolation trends, deficiency index, discomfort periods, zones.

A b s t r a c t

Decadal sums of real insolation in October–March of the years 1976–2000 at 46 meteorological stations operated by the Institute of Meteorology and Water Management (IMWM) were used to identify temporal and spatial characteristics of real insolation deficiency (i.e., daily insolation of less than 4 h). Three periods of real insolation deficiency-induced discomfort were identified in the cool half-year: period of high discomfort, from the first decade of October to the third decade of November; period of medium discomfort, from the first decade of December to the first decade of January; and period of low discomfort, from the second decade of January to the third decade of March. The duration of periods with daily real insolation less than 4 h in was found to vary spatially in Poland, the difference exceeding one month (170–200 days on the average). The duration of insolation deficiency was found to decrease southwards, except for a zone extending from Racibórz to Tarnów where, because of the excessive air pollution and cloudiness, the deficiency period is by about 20 days longer than in the adjacent areas. The northern part of the country features frequent (more than 40%) long-lasting deficiency periods with the mean daily insolation not longer than 1 h. Under the climatic regime of Poland, the frequency of years with real insolation <1 h stretching for ≥ 6 decades in the cool half-year was found to range from 4 to 16%, the frequency in the area of Suwałki being as high as 20%. No such long-lasting periods of real insolation < 1 h are experienced in the mountainous areas. Using the real insolation deficiency index W_{nu}, the area of Poland was divided into 4 insolation deficiency zones, the deficiency ranging from low to very high.

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NIEDOBORY USŁONECZNIENIA RZECZYWISTEGO W PÓŁROCZU CHŁODNYM W POLSCE

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S ł o w a k l u c z o w e: trendy usłonecznienia, wskaźnik niedoborów, okresy dyskomfortu, strefy.

A b s t r a k t

Wykorzystując dekadowe sumy usłonecznienia rzeczywistego z 46 stacji meteorologicznych IMGW od października do marca, za lata 1976–2000, opracowano czasową i przestrzenną charakterystykę jego niedoborów, trwających poniżej 4 godz. dziennie. W chłodnej porze roku wydzielono 3 okresy dyskomfortu usłonecznienia rzeczywistego: od 1. dekady października do 3. dekady listopada – duży dyskomfort, od 1. dekady grudnia do 1. dekady stycznia – średni dyskomfort, od 2. dekady stycznia do 3. dekady marca – mały dyskomfort. Przestrzenne zróżnicowanie długości okresu z usłonecznieniem rzeczywistym poniżej 4 godz. dziennie wynosi w Polsce ponad miesiąc (średnio 170–200 dni), zmniejszając się ku południowi, za wyjątkiem strefy rozciągającej się od Raciborza po Tarnów, gdzie z powodu nadmiernego zanieczyszczenia powietrza i zachmurzenia okres ten jest dłuższy o ok. 20 dni w porównaniu z sąsiednimi terenami. Na północy kraju często występują (ponad 40%) długotrwałe okresy ze średnim dziennym usłonecznieniem nie przekraczającym 1 godz. W klimatycznych warunkach Polski, w półroczu chłodnym, częstość lat z ciągami trwającymi ≥ 6 dekad z usłonecznieniem rzeczywistym < 1 godz. wynosi od 4 do 16%, a jedynie w rejonie Suwałk – 20%. Na obszarze gór nie notuje się tak długich okresów z usłonecznieniem. Uwzględniając wielkość wskaźnika niedoboru usłonecznienia rzeczywistego W_{nu} wydzielono w Polsce 4 strefy niedoborów – od małych do bardzo dużych.

Introduction

In central and northern Europe, winter is characterised by a high insolation deficiency, relative to the insolation level necessary for human well-being and health (KOZŁOWSKA-SZCZĘSNA et al. 2004, KOŹMIŃSKI, MARTYN 2004). Moreover, the heating season induces a considerable increase in concentration of air pollutants and in the number of days with precipitation; that season is also accompanied by wide variations in atmospheric pressure as well as by high prevalence of fog and strong wind. All those factors, combined with the low real insolation, create bioclimatic conditions which are highly adverse for human life, work, and recreational activities (BŁAŻEJCZYK 2004, CZARNECKA, KOŹMIŃSKI 2006). The consequences of such conditions include increased rate of diseases and elevated job absenteeism; in extreme circumstances, the conditions described contribute to increased incidence of depression and raised

suicide rate (KUCHCIK, BŁAŻEJCZYK 2001, TREPIŃSKA et al. 2006). Particularly uncomfortable are the months featuring days with complete cloud cover or with insolation lasting less then 2 h, which is frequently the case in November, December, and January in Poland. During those months, the mean daily relative insolation ranges from 12 to 22% (KOŹMIŃSKI, MICHALSKA 2005a, b). The physiological and biometeorological standards set the minimum real insolation level at 4 h a day (BŁAŻEJCZYK 2004, PAPIERNIK 2004).

Assuming the daily real insolation shorter than 4 h is an important factor adversely affecting human performance and comfort, this work was aimed at assessing temporal and spatial variability of the real insolation deficiency in Poland. Knowledge of the insolation regime in the cool season of the year is important not only for climatological reasons, but also for tourism and recreastion, particularly with respect to planning of and investing in the development of tourism (FREITAS 2003).

Materials and Methods

The paper analyses data on decadal sum of real insolation in the cool half-year (October–March) of 1976–2000, collected at 46 meteorological stations operated by the Institute of Meteorology and Water Management (IMWM) (Biuletyny IMGW). The data allowed to extract temporal trends (in the decadal system) as well as dates of the onset and termination of periods of daily real insolation shorter than 4 h and the duration of those periods. Average daily real sunshine was determined by dividing a ten day period total with sunshine by the number of days in a given ten day period. The probability that the daily real insolation would be shorter than 1, 2, 3, and 4 h in consecutive decades within October–March was calculated for some stations representing different climatic regions of Poland. Next, per cent differences in real insolation between the bioclimatological standard of 4 h minimum daily human requirement and the actual insolation occurring during the cool season were calculated. It was assumed that the longer the period with real insolation deficiency (daily insolation < 4 h) and the higher the deficiency, the worse the biometeorological conditions at the station concerned, compared to the country-wide average. Spatial variability of insolation during the cool season was described using the real insolation deficiency index (W_{nu}), calculated as in the formula below:

$$W_{nu} = \frac{D_o \cdot \Sigma N_u}{\bar{D}_o \cdot \Sigma \bar{N}_u}$$

where:

D_o – duration of period with daily real insolation < 4 h at a station;

ΣN_u – sum of differences between daily insolation of 4 h a and actual insolation at a station;

$\bar{D}_o$ – country-wide mean duration of the deficiency period;

$\Sigma \bar{N}_u$ – country-wide mean sum of insolation deficiency.

Results and Discussion

The cool half-year (October–March) in Poland may be divided into 3 characteristic periods differing in the daily real insolation (Figure 1). The first, from the first decade of October to the third decade of November, is characterised by a very distinct decrease of the number of hours of sunshine a day, averaging

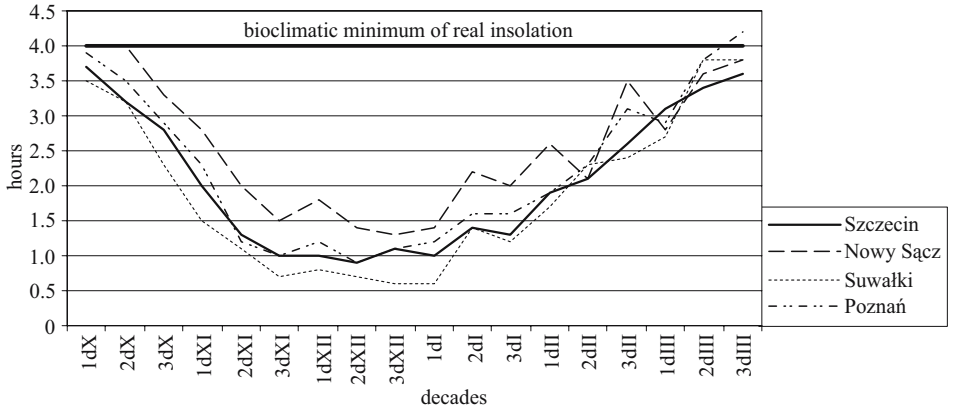

Fig. 1. Mean daily real insolation (hours) by decades at selected stations in 1976–2000

from about 4 to about 1 h. This is a result of the days becoming shorter, and of extensive cloud cover associated with the increasing frequency of cyclonic weather types over Poland, from 42.4% in October to 52.9% in November, accompanied by a marked decrease of anti-cyclonic types, from 54.2 to 43.2%, respectively (OSUCHOWSKA-KLEIN 1991). The second period, from the first decade of December to the first decade of January, shows a relatively stable insolation period of short duration, from about 0.5 to about 1.5 h a day, depending on the station. This is a result of the highest overall frequency of cyclonic weather types in December (55.4%) and the lowest frequency of anti-cyclonic types (42.4%). The third period, from the second decade of January to the third decade of March, features a slow and non-uniform

increase in the number of hours of real insolation during the day, from about 1 to about 4 h. The increase is associated with, i.a., a reduction of frequency of cyclonic weather types, from 50.3% in January to 48.2% in March. During the third period, duration of daily real insolation distinctly drops in the second decade of February and in the first decade of March, which is related to an increased intensity of westerly advections (DEGIRMENDŹIĆ 2004). In autumn, the reduction in the duration of daily real insolation is accompanied by a substantial decadal variability of that factor, from about 40% in early October to 60–70% in November and December (and even to above 100% in the north-western part of the country). In March, the variability in the duration of insolation drops again to about 40% (Figure 2).

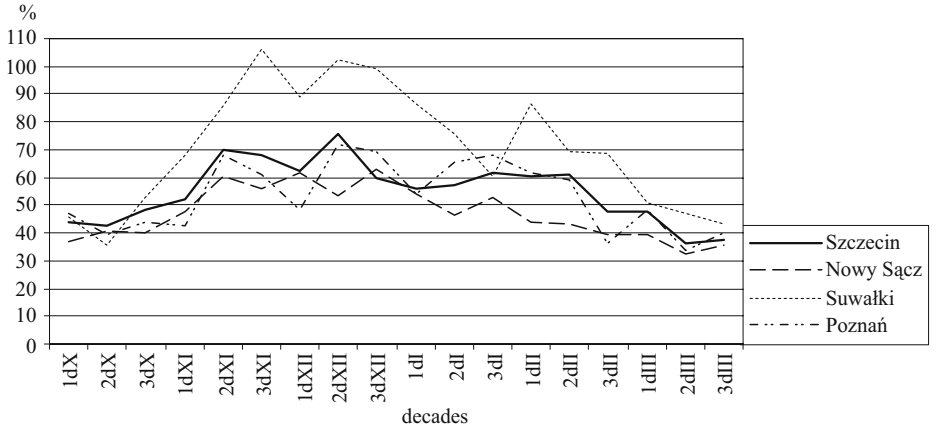

Fig. 2. Coefficient of variation (%) of real insolation by decades at selected stations in 1976–2000

The first period of insolation deficiency identified during the cool half-year is, from the bioclimatic standpoint, the most adverse. The rapid decline in the duration of daily real insolation in autumn, coupled with wide variations in the atmospheric pressure, frequent fogs, strong winds (including the foen in the mountains), and an increased level of air pollution due to house heating, exerts a considerable stress on the human performance (TREPIŃSKA et al. 2006). As reported by Markham and Markham (2005), extreme biometeorological factors such as temperature, precipitation, atmospheric pressure, and snowfall significantly increased the plant-wide worker absenteeism rate. From the standpoint of daily life requirements, it is important to know not only the mean daily duration of insolation, but also the magnitude of changes in the extent of insolation deficiency in consecutive decades of the cool half-season. Regression coefficients illustrating positive and negative changes in the daily real

insolation deficiency in consecutive decades, relative to the bioclimatological standard of 4 h, were calculated for selected stations representing different regions of Poland (Figure 3). A decrease in the deficiency is particularly distinct in the central (Koło) and south-western (Opole) parts of the country, particularly from the third decade of December to the third decade of January. Noteworthy is the worsening of the daily insolation regime in the second and third decades of February in the eastern part of Poland (Białystok, Terespol). During the 25-yr period examined, the highest reduction in the daily real insolation deficiency occurs in Poland in the third decade of January (Figure 4), particularly in the central-western part where the trend calculated proved highly significant ($\alpha = 0.01$). The least distinct reduction of insolation deficiency during that decade was visible in the south-western part of Poland.

To illustrate the particularly adverse insolation regime in the cool season of the year, diagrams showing frequencies of years with daily real insolation < 2 h stretching for at least three decades and frequencies of years with daily real insolation < 1 h extending for at least two decades were plotted (Figure 5). The ten day periods with sunshine values below an accepted threshold, for example below 1.0, 2.0, 3.0 and 4.0 hours daily, were recognized as the sequences involving 2, 3 and more successive ten day periods. Such decadal series occurred most often from late November to early January. The highest frequencies (above 80%) of the minimum 3 decade-long series were detected in the northern part of Poland, the lowest frequencies (below 60%) being observed in the mountain areas. Particularly adverse for the human health and performance are those periods when daily insolation does not exceed 1 h, which are particularly frequent in the north-eastern part of the country (years with such periods showed frequency of above 60%) (Figure 5). There were years during which daily real insolation did not exceed 1 h for 7 or 8 consecutive decades; this was the case in Suwałki during winters of 1976/77, 1982/83, 1983/84, 1985/86, and 1993/94. As shown in Figure 6, the minimum 6 decade-long series of days with real insolation below 1 h did not occur at all in the Sudety Mountains and in the Carpathians; on the other hand, the frequencies of years with such temporal insolation series were about 8, 16, and even above 20% in the central part of the country, in uplands of the Pomeranian and Masurian Lake Districts, and in the vicinity of Suwałki, respectively.

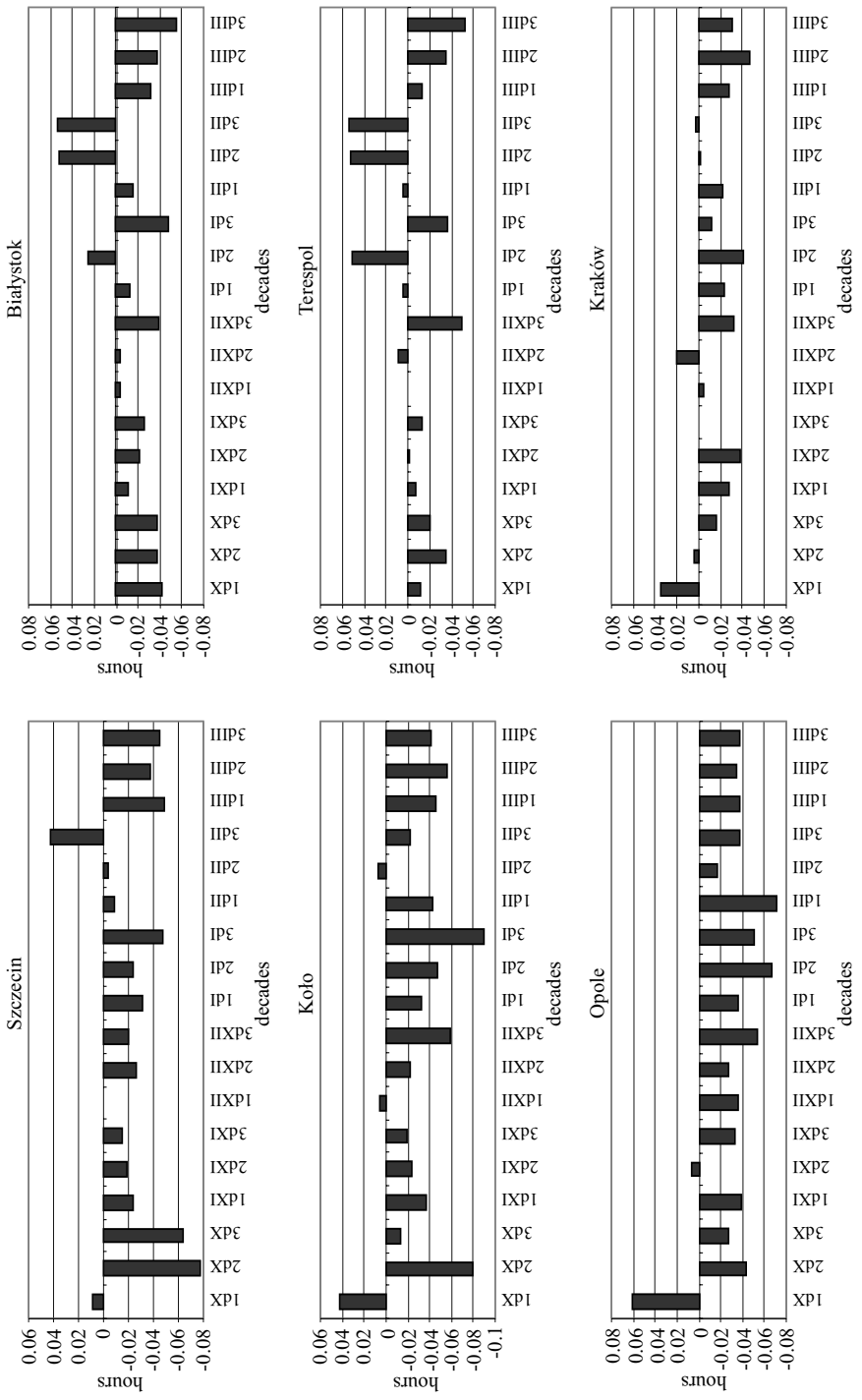

Fig. 3. Mean annual variability in insolation deficiency (h) in 1976–2000 relative to the minimum required daily real insolation of 4 h

Fig. 4. Correlation coefficients of negative linear trend of real insolation deficiency in the third decade of January in 1976–2000

Important for evaluation of Poland's bioclimatic conditions in terms of daily real insolation is the probability that the daily real insolation during the cool half-year decades would be shorter than a certain number of hours (Table 1). As shown by the data in Table 1, the factor analysed varies extensively from decade to decade, particularly in the second half of November and in the second half of February. The probability that the sun will shine for less than 4 h a day may be even 100% for the period from the first decade of November to the third decade of January, particularly in the Pomeranian and Masurian Lake Districts. Moreover, the probability that the daily duration of sunshine will be even shorter than 1 h ranges, in the period mentioned, from 50 to 60% in northern part of Poland and from 30 to 50% in the southern and central parts of the country.

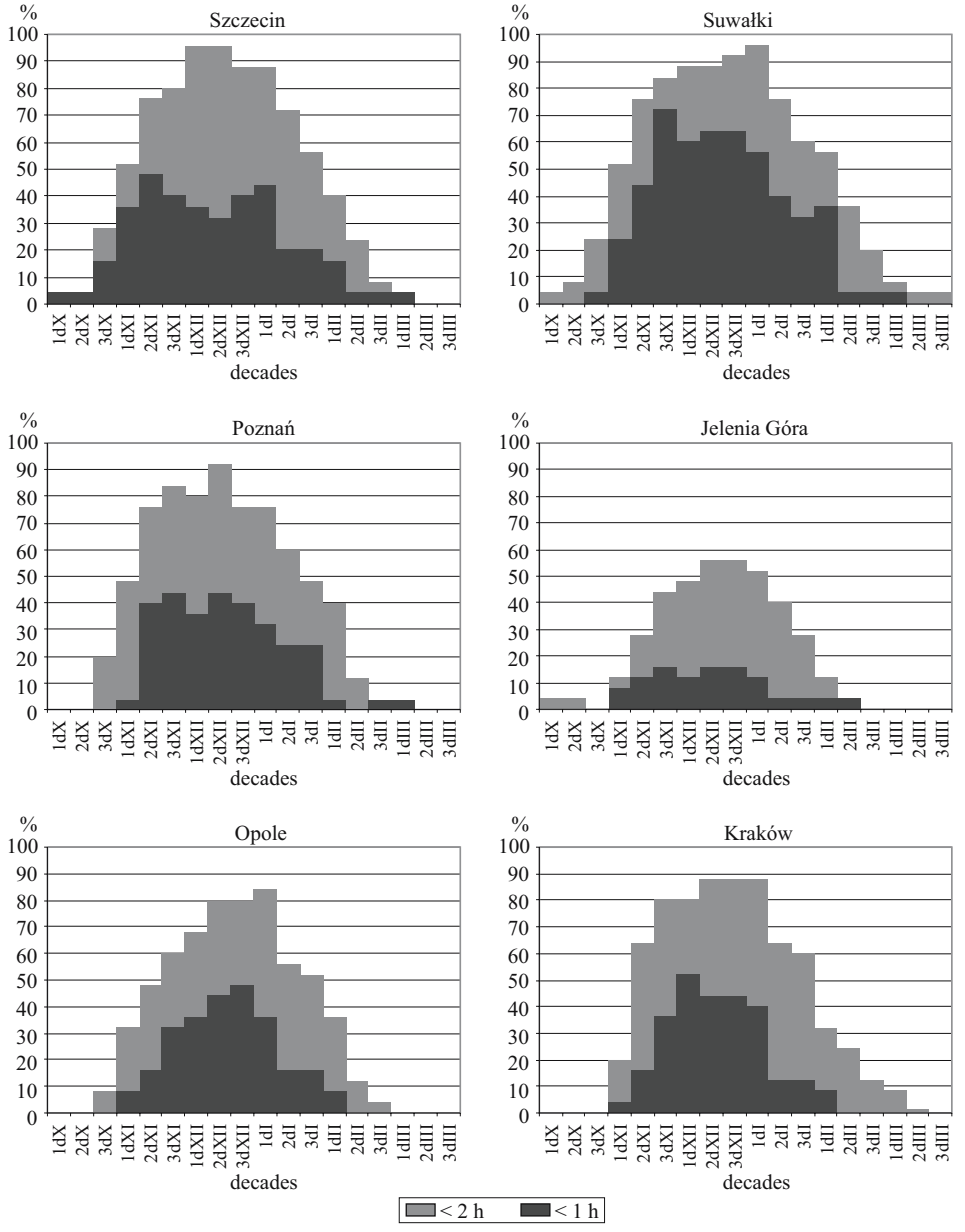

Fig. 5. Frequency (%) of years with daily real insolation shorter than 2 h in minimum 3 decade-long series, and shorter than 1 h in minimum 2 decade-long series

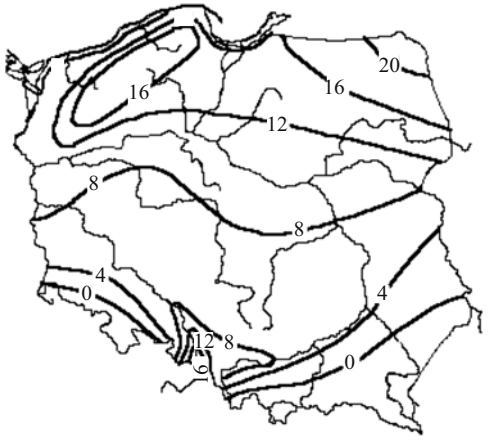

Fig. 6. Frequency (%) of years with ≥6 decade-long series of daily real insolation <1 h in 1976–2000

Table 1

Probability (%) that the daily real insolation would be: shorter than 1, 2, 3, and 4 h in consecutive decades of the cool half-year in minimum 3-decades long series, and shorter than 1 h minimum 2-decades long series

Station	h	October			November			December			January			February			March		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Koszalin	1	3	2	7	18	37	55	50	50	50	66	26	37	28	20	12	7	6	3
	2	12	12	25	50	78	92	98	92	84	99	61	78	60	47	35	24	17	11
	3	30	39	57	82	97	100	100	100	98	100	88	97	86	76	65	53	36	27
	4	55	74	84	97	100	100	100	100	100	100	98	100	97	93	88	80	59	50
Suwałki	1	8	3	14	34	46	60	61	62	58	67	32	39	31	25	19	10	7	5
	2	20	16	43	73	84	93	96	97	88	97	68	87	58	48	38	27	16	15
	3	39	46	77	95	98	100	100	100	99	100	91	99	82	71	62	53	31	31
	4	61	79	95	100	100	100	100	100	100	100	99	100	95	88	81	77	50	52
Szczecinek	1	4	4	10	29	50	63	66	63	61	77	38	44	31	20	16	13	6	4
	2	13	18	36	71	92	98	99	98	96	100	76	90	69	50	37	32	19	13
	3	32	47	71	95	100	100	100	100	100	100	96	100	93	80	63	58	42	31
	4	58	77	93	100	100	100	100	100	100	100	100	100	99	95	84	81	68	55
Poznań	1	5	5	8	12	40	50	37	50	41	43	27	27	21	16	6	8	3	2
	2	15	18	27	42	84	95	91	95	81	93	66	66	54	41	22	24	11	8
	3	31	42	56	79	99	100	100	100	98	100	91	91	84	70	50	50	30	22
	4	52	69	82	96	100	100	100	100	100	100	99	99	97	90	78	76	55	43
Terespol	1	9	5	10	18	26	45	39	50	44	39	18	20	20	16	11	8	3	3
	2	12	16	30	50	61	87	87	89	73	87	47	72	44	41	26	27	11	10
	3	28	57	59	81	88	99	99	99	92	99	77	98	72	70	48	56	27	24
	4	50	63	84	97	98	100	100	100	98	100	94	100	90	90	70	82	50	45
Wrocław	1	6	2	5	9	18	27	25	40	33	33	20	21	14	11	7	7	3	2
	2	15	10	18	32	54	73	67	84	75	84	50	58	38	34	21	21	13	8
	3	30	28	44	68	86	97	94	99	96	99	80	88	68	66	45	45	33	23
	4	50	56	73	91	98	100	100	100	100	100	95	99	89	89	70	70	61	47

cont. table 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Kielce	1	7	3	5	14	24	45	35	44	43	33	12	23	16	18	8	6	2	3
	2	17	12	20	40	62	87	81	90	77	84	39	57	41	44	21	19	10	12
	3	35	30	47	72	90	99	98	100	95	99	74	86	70	73	41	42	28	29
	4	57	55	76	92	99	100	100	100	99	100	94	98	90	92	64	68	56	52
Kłodzko	1	5	1	4	10	30	37	29	37	29	25	20	16	12	12	4	8	3	2
	2	14	8	16	68	71	91	71	91	71	84	47	75	37	39	16	24	12	9
	3	33	28	41	76	95	100	95	100	95	100	76	99	69	74	39	50	35	25
	4	57	60	70	96	100	100	100	100	100	100	93	100	91	94	67	76	65	50
Kraków	1	4	4	4	14	29	50	44	37	50	42	23	28	20	16	10	10	5	5
	2	16	16	21	43	64	92	90	91	92	96	69	80	50	54	27	30	20	14
	3	39	41	58	77	90	100	100	100	100	100	96	100	80	89	53	59	47	33
	4	67	70	88	95	99	100	100	100	100	100	100	100	95	99	77	84	76	57
Rzeszów	1	6	5	7	16	21	39	40	40	46	31	14	19	18	16	12	8	4	5
	2	17	18	24	43	54	87	84	84	84	88	43	65	47	43	29	27	14	15
	3	38	39	53	75	84	99	99	99	98	100	77	95	77	75	52	56	36	31
	4	62	66	80	93	97	100	100	100	100	100	95	100	94	93	75	82	64	52

The high insolation deficiency in the northern part of Poland during the cool half-year is illustrated also by Figure 7 which shows that the mean number of decades with daily insolation of at least 1 h is 4–5, 1–3 decades being typical of other areas except the mountains where periods of such short insolation were not recorded. This is thus a confirmation of the winter insolation regime being more favourable in the piedmont and mountainous areas compared to that in lowlands.

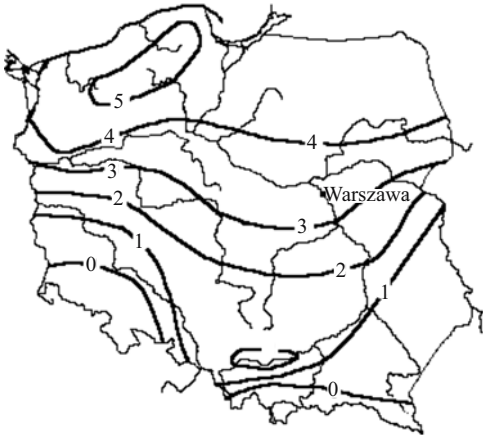

Fig. 7. Number of decades with mean daily real insolation ≤ 1 h in October–March in 1976–2000

The period of days with real insolation shorter than 4 h is a measure of bioclimatic conditions in an area. The earliest onset of such period in Poland (before 15 September) is recorded in higher-lying areas of the Pomeranian Lake District, the latest onset (after 5 October, and locally even after 10 October) being typical of the eastern part of the country and of the Silesian Lowland. The period terminates before 25 March in areas extending along the central part of the River Bug valley, in the Sandomierz Valley, and in the Wielkopolska Lake District, the latest termination (after 10 April) being recorded in the Sudety Mountains and in the Carpathians (KOŹMIŃSKI, MICHALSKA 2006).

As seen in Figure 8, the shortest (less than 170 days) periods of days with insolation shorter than 4 h prevailed in the central part of the Silesian Lowland and in the region of Polesie Podlaskie, followed by the central, lowland part of Poland (less than 180 days). The longest (more than 200 days) duration of the periods with insolation deficiency was typical of a narrow band extending from Racibórz to Tarnów, mainly due to the extensive cloud cover and high level of and air pollution originating from the agglomerations of Ostrava, Rybnik, Katowice, and Cracow (CZARNECKA, KOŹMIŃSKI 2006). The net result of those factors is an earlier onset and later termination of the period with daily real insolation shorter than 4 h.

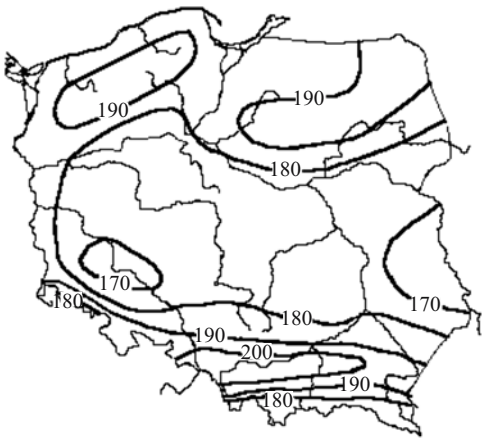

Fig. 8. Mean duration of periods with daily real insolation shorter than 4 h in cool seasons of 1976–2000

Another characteristics used in the analysis of cold season insolation is the per cent deficiency, i.e., a difference, expressed as a percentage, between the bioclimatic daily real insolation minimum of 4 hours and actual insolation in a locality (Figure 9). The per cent deficiency showed a south-north increase

Fig. 9. Per cent difference between minimum standard daily insolation of 4 h and real insolation in 1976–2000

in Poland, from less than 34% in the mountains to more than 49% in higher-lying areas of the Pomeranian Lake District. The isarythms show a latitudinal pattern resulting from, i.a., a southward increase in the day length in the cool half-year.

The real insolation deficiency index W_{nu} calculated for individual stations throughout Poland allowed to identify 4 zones of real insolation deficiency in cool season (Figure 10). Low deficiencies (**zone 1**) prevail in the mountains and in the central part of the Silesian Lowland, where the period of daily real insolation less than 4 h is at its shortest (less than 170 days) and the mean daily insolation is high (2.4–2.6 h), compared to the remaining parts of the country. **Zone 2**, with medium insolation deficiency, covers the largest, central part of the country characterised by an intermediate duration of the deficiency period (170–180 days) and medium-long (about 2.2 h) daily real insolation. Zone 2 includes the Carpathian Piedmont where the deficiency period is longer (about 190 days), but the mean daily insolation is higher (about 2.5 h). **Zone 3**, characterised by a high insolation deficiency, covers the Pomeranian Lake District (except for its higher parts) in the north and a small area in the south, from Racibórz to Przemyśl, except for the vicinity of Cracow. **Zone 4**, with very high real insolation deficiencies in the cool season compared to the bioclimatic standard, encompasses higher elevations of the Pomeranian Lake District as well as the Suwałki Lakeland. The adverse insolation regime prevalent in zone 4 is a net result of a long-lasting (more than 190 days) period of days with daily insolation shorter than 4 h and the shortest duration of real insolation (about 2 h). Zone 4 includes also the vicinity of Cracow, with its longest

(more than 200 h) period of insolation deficiency and the relatively high (about 2.4 h) daily duration of insolation.

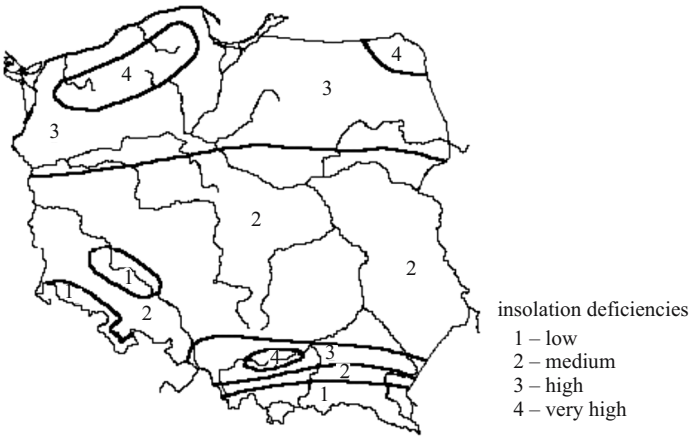

Fig. 10. Zones of real insolation deficiencies in the cool season in Poland

Conclusions

1. During the 25-yr-long period examined, a significant reduction in the real insolation deficiency was observed to extend from the third decade of December to the third decade of January, with respect to the bioclimatological daily insolation minimum of 4 h.

2. Under the climatic regime of Poland, the cool season shows three characteristics periods of real insolation deficiency-induced discomfort: from the first decade of October to the third decade of November: high discomfort; from the first decade of December to the first decade of January: intermediate discomfort; from the second decade of January to the third decade of March: low discomfort.

3. The duration of periods with daily real insolation shorter than 4 h differs by more than one month (170–200 days on the average) between various areas in Poland; the difference decreases southward, except for a zone extending from Racibórz to Tarnów where, due to excessive air pollution and high cloudiness, the period is by about 20 days longer than in the neighbouring areas.

4. Long-lasting periods with mean daily insolation not longer than 1 h are frequently (with frequencies exceeding 40%) experienced in the northern part of the country.

5. Calculations of the real insolation deficiency index, W_{nu}, for various areas of Poland allowed to identify 4 deficiency zones, the insolation deficiency changing from low to very high.

Translated by ANDRZEJ KAŁUSZYŃSKI

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EFFECTS OF GROWTH REGULATORS, APPLIED ALONE OR IN COMBINATION WITH MAGNESIUM SULFATE, ON OAT YIELD

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K e y w o r d s: oat, yield, growth regulators.

A b s t r a c t

The effects of growth regulators, applied alone or in combination with magnesium sulfate, on oat yield components 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 (GA_3) were applied to leaves, alone or in combination with a 5% aqueous solution of magnesium sulfate, twice during the growing season of oat – at the panicle emergence stage and before flowering.

It was found that the total aboveground biomass of oat increased significantly following the application of BAP and GA_3, while decreased as the plants were sprayed with IBA, TRIA and NAA. As regards the combinations of growth regulators with magnesium sulfate, only IBA+Mg caused a significant increase in aboveground biomass, whereas the other combinations had the opposite effect. The applied growth regulators (except for IBA) increased the proportion of grain in the total aboveground biomass of oat. This influence was more noticeable when magnesium sulfate was added to the growth regulators. The obtained results indicate an ambiguous, often contradictory impact of growth regulators, applied alone or combined with magnesium sulfate, on the morphometric characters of oat plants, their mass and the share of the analyzed organs in total aboveground biomass.

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WPŁYW REGULATORÓW WZROSTU ORAZ ICH MIESZANEK Z SIARCZANEM(VI) MAGNEZU NA PLONOWANIE OWSA

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S ł o w a k l u c z o w e: owies, plonowanie, 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 składowe plonu owsa. Regulatory: benzyloaminopurynę (BAP), kwas α-naftylooctowy (NAA), kwas indolilo-3-masłowy (IBA), triacontanol (TRIA) oraz kwas giberelinowy (GA_3) stosowano dolistnie, samodzielnie lub łącznie z 5-procentowym wodnym roztworem siarczanu magnezu, dwukrotnie w okresie wegetacyjnym owsa – w fazie wysuwania wiech oraz przed kwitnieniem.

Stwierdzono istotny przyrost całkowitej masy nadziemnej owsa pod wpływem BAP i GA_3, natomiast ograniczenie jej przyrostu po oprysku IBA, TRIA i NAA. Spośród zastosowanych mieszanek regulatorów wzrostu z siarczanem(VI) magnezu jedynie IBA+Mg spowodował istotne zwiększenie masy nadziemnej, pozostałe ją zmniejszały. Aplikowane preparaty wzrostu (oprócz IBA) zwiększały udział ziarna w całkowitej masie nadziemnej owsa, a dodatek siarczanu magnezu do nich powodował na ogół wzrost tego udziału. Uzyskane wyniki wskazują na niejednoznaczny, często sprzeczny, wpływ regulatorów wzrostu, stosowanych samodzielnie lub łącznie z siarczanem magnezu, na cechy morfometryczne owsa, jego masę oraz udział badanych organów w całkowitej masie nadziemnej.

Introduction

One of the key yield-forming factors is adequate cultivation regime, including balanced fertilization. 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, in particular natural phytohormones. Research results show that growth regulatory can significantly modify crop yield (AUFHAMMER, FEDEROLF 1992, CZAPLA et al. 2005, HARMS, NOWAK 1990).

Growth regulators, applied at adequate concentrations and growth stages of spring wheat, contribute to root system development, which in turn increases nutrient uptake from the soil (NOWAK, WIERZBOWSKA 1991). Other authors (PATEL 1993, RAKESH et al. 1995) demonstrated that triacontanol intensifies the growth and increases the yield of cereals. It also affects some basic metabolic processes, including photosynthesis, nutrient assimilation and enzymatic activity. According to WOJCIESKA (1992), gibberellic acid (GA_3) and indole-3-acetic acid (IAA) have an insignificant effect on the number

of spikelets in oat panicles and – usually – a negative impact on their productivity. However, their influence on the weight of a single kernel was positive and stronger when the above hormones were applied at an early growth stage.

The aim of this study was to determine the effects of selected growth regulators, applied alone or in combination with magnesium sulfate, on changes in oat yield.

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. Oat cv. Borowiak 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.

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

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).U 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 oat to five growth regulators: benzylaminopurine (BAP), α-naphthylacetic acid (NAA), 3-indolebutyric acid (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 oat – at the panicle emergence 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 oat requirements.

Oat plants, 20 of each plot, were collected at the full maturity stage. They were divided into organs and weighed, and biometric measurements were taken.

The results were processed 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 $p = 0.05$.

Results and Discussion

The data in Table 2 show that growth regulators and their mixtures with magnesium sulfate, used in a three-year field experiment, had no significant

Table 2

Some morphological traits of oats plants

Treatment	1999		2000		2001		Mean	
	lenght culm (cm)	number of grains in panicle	lenght culm (cm)	number of grains in panicle	lenght culm (cm)	number of grains in panicle	lenght culm (cm)	number of grains in panicle
Control	92,0	49.2	75.9	57.8	112.6	99.8	93.5	68.9
IBA	92.7	51.1	78,0	53.6	104,0	100.1	91.6	68.3
IBA+Mg	87.3	46.1	72.6	53.8	113.5	112.7	91.1	70.9
TRIA	93,0	50.1	73.4	51.3	112.3	103.1	92.9	68.2
TRIA+Mg	88.5	44,0	76.2	56.9	113,0	94.1	92.6	65.0
BAP	92.2	52.1	74.9	59.3	111.9	104.9	93.0	72.1
BAP+Mg	90.1	50.1	74.1	57.6	108.3	94.7	90.8	67.5
NAA	89.3	49.9	77.6	58.9	109.3	92.7	92.1	67.2
NAA+Mg	87.3	45,0	75.1	55.2	112.1	91.5	91.5	63.9
GA_3	93.1	50.7	80.2	55.5	112.3	113,0	95.2	73.1
GA_3+Mg	89.8	47.1	81.5	58.9	110.6	100.1	94.0	68.7
Growth regulators	92.1	50.8	76.8	55.7	110,0	102.8	92.9	69.8
Mixtures	88.6	46.5	75.9	56.5	111.5	98.6	92,0	67.2
$LSD_{0.05}$								
a	n.s.	n.s.	3.1	n.s.	n.s.	n.s.	n.s.	n.s.
b	1.8	2.8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
$a \cdot b$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Legend: *a* – growth regulator, *b* – mixture growth regulators with magnesium sulphate, $a \cdot b$ – interaction, n.s. – non-significant

effect on the morphometric characters of oat plants in the majority of cases. In the first year of the study (1999) growth regulators applied with magnesium sulfate significantly reduced length increment of oat culms, compared to the control treatment and plots sprayed with pure solutions of growth regulations. Culm length decreased by around 3.5 cm on average. A significant decrease in the number of grains per panicle was also recorded following the application of growth regulators mixed with magnesium sulfate, except for the BAP+Mg combination. This decrease reached 8.5% on average, compared to the treatments where growth regulators were applied alone. In the second year (2000) gibberellic acid (GA_3) caused significant culm elongation, in comparison with the control plots and plots sprayed with triacontanol (TRIA) and benzylaminopurine. Similar results were obtained in previous studies involving barley and triticale (NOGALSKA, CZAPLA 2002, 2005). Also other authors (KUBICKA, DEC 1998, WOODWARD, MARSCHALL 1988) observed an increment in culm length resulting from the application of GA_3. CZAPLA and NOGALSKA (2000) found that oat plants sprayed with triacontanol were characterized by the longest culms, while CZAPLA et al. (2000) noted a significant decrease in oat culm length under the influence of GA_3 and NAA.

Mean values obtained during a three-year experiment indicate that the morphological characters of oat were significantly affected neither by pure solutions of growth regulators nor by their mixtures with magnesium sulfate. Similar results were obtained when growth regulators and their mixtures with magnesium sulfate were applied to spring triticale under field conditions (CZAPLA et al. 2005).

In the present study (Table 3) the greatest changes in the mass of particular oat organs were observed in the first year (1999). Compared to the control treatment, growth regulators applied alone (except for α-naphthylacetic acid NAA) caused an increase in grain weight, which reached the highest (though statistically non-significant) level of 5.6% in the case of triacontanol. Growth regulators combined with magnesium sulfate (except for BAP+Mg) significantly reduced grain weight, culm weight and total aboveground biomass, compared with plants collected from the control plots and plots sprayed with pure growth regulators. The average decrease in the weight of grain and culms, in comparison with the control treatment, was 11.5%. In a previous experiment conducted by NOGALSKA and CZAPLA (2002), growth regulators increased grain weight in barley, but decreased in oat and triticale (CZAPLA, NOGALSKA 2000, NOGALSKA, CZAPLA 2005). Other authors (CHAPLOT et al. 1992, MEHRA, KAMAL 1995) demonstrated that α-naphthylacetic acid contributed to a higher seed yield.

Table 3

Weight of above-ground organs of oats, in g per productive culm

Treatment	1999			2000			2001			Mean		
	culm	grain	total above-ground weight	culm	grain	total above-ground weight	culm	grain	total above-ground weight	culm	grain	total above-ground weight
Control	0.80	1.78	3.23	0.71	2.00	3.30	1.77	3.53	6.67	1.09	2.44	4.40
IBA	0.80	1.82	3.33	0.71	1.92	3.17	1.66	3.30	6.20	1.06	2.35	4.23
IBA+Mg	0.72	1.62	2.94	0.64	1.93	3.11	1.93	3.97	7.26	1.10	2.51	4.44
TRIA	0.79	1.88	3.35	0.66	1.78	2.98	1.63	3.46	6.29	1.03	2.37	4.21
TRIA+Mg	0.64	1.44	2.62	0.69	1.98	3.25	1.79	3.38	6.35	1.04	2.27	4.07
BAP	0.79	1.86	3.30	0.72	2.11	3.44	1.84	3.51	6.79	1.12	2.49	4.51
BAP+Mg	0.74	1.77	3.13	0.68	2.02	3.28	1.62	3.21	6.00	1.01	2.33	4.14
NAA	0.74	1.76	3.15	0.73	2.09	3.41	1.57	3.27	6.03	1.01	2.37	4.20
NAA+Mg	0.64	1.60	2.82	0.71	1.92	3.18	1.65	3.22	6.20	1.00	2.25	4.07
GA_3	0.77	1.81	3.20	0.74	2.01	3.32	1.85	3.72	6.87	1.12	2.51	4.46
GA_3+Mg	0.72	1.67	2.95	0.80	2.13	3.48	1.71	3.47	6.38	1.08	2.42	4.27
Growth regulators	0.78	1.83	3.27	0.71	1.98	3.26	1.71	3.45	6.44	1.07	2.42	4.32
Mixtures	0.69	1.62	2.89	0.70	2.00	3.26	1.74	3.45	6.44	1.05	2.36	4.20
$LSD_{0.05}$												
a	n.s.	n.s.	n.s.	n.s.	n.s.	0.04	n.s.	n.s.	n.s.	n.s.	n.s.	0.04
b	0.04	0.10	0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.02
$a \cdot b$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.14	n.s.	n.s.	n.s.

Explantations as in Table 2

In the second and third year of the study (2000 and 2001) growth regulators and their mixtures with magnesium sulfate significantly affected only the total aboveground biomass of oat. In 2000 benzyloaminopurine and α-naphthylacetic acid caused a statistically significant increase (by 4.2 and 3.3% respectively) in total aboveground biomass, compared to the control treatment. On the other hand, 3-indolebutyric acid (IBA) and triacontanol reduced total aboveground biomass by 4 and 10% respectively. In 2001 the interaction between growth regulators and magnesium sulfate resulted in a significant increase in the total mass of oat organs in the IBA+Mg and NAA+Mg treatments (by 17.1 and 2.8% respectively), as well as a significant decrease in this parameter in the BAP+Mg and GA_3+Mg treatments (by 11.6 and 7.1% respectively).

Mean values recorded over a three-year period point to a significant increment in total oat mass under the influence of BAP and GA_3, in comparison with the control treatment, as well as to a significant decline in total oat mass after the application of the other growth regulators, i.e. IBA, TRIA and NAA. Growth regulators combined with magnesium sulfate caused a significant decrease in the aboveground biomass of oat. The only exception was the IBA+Mg treatment, where aboveground biomass increased by 5%.

Figure 1 shows that changes in the percentages of particular organs in the total aboveground biomass of oat plants were slight. Compared to the control plots, the proportion of grain in oat biomass increased under the influence of TRIA, BAP, NAA and GA_3. The effect of growth regulators (IBA, BAP and

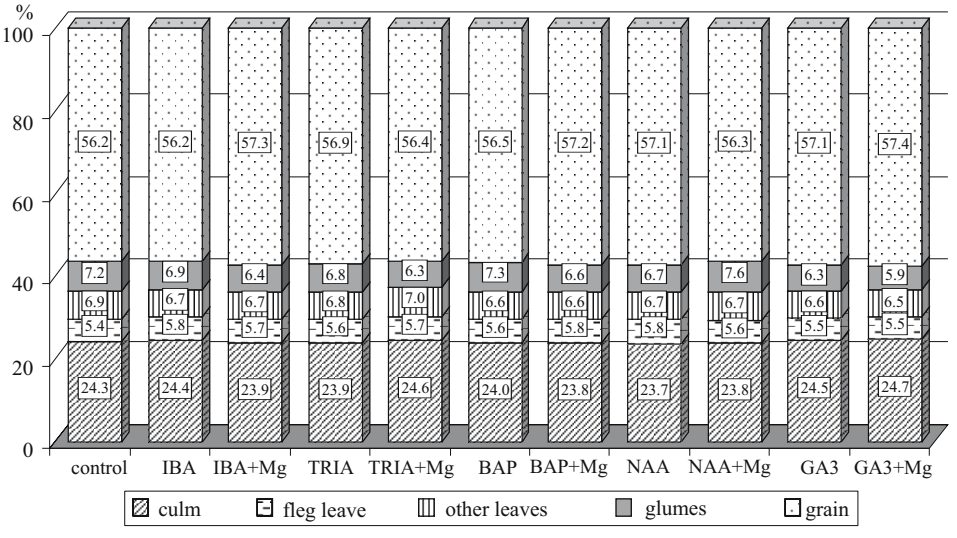

Fig. 1. Perticipation of individual organs in biomass of oats (1999–2001)

GA_3) applied together with magnesium sulfate was found to be more beneficial. Similar results were obtained in a field experiment with spring triticale (CZAPLA et al. 2005). Also SIVAKUMAR et al. (2001) reported a substantial increase in the number of grains per spike following the application of BA, compared to control plants. The opposite trend was noted during a pot experiment (NOGALSKA, CZAPLA 2005), where growth regulars reduced the proportion of grain in the total aboveground biomass of spring triticale, particularly if mixed with magnesium sulfate.

Conclusions

1. The total aboveground biomass of oat increased significantly following the application of BAP and GA_3, while decreased as the plants were sprayed with IBA, TRIA and NAA. As regards the combinations of growth regulators with magnesium sulfate, only IBA+Mg caused a significant increase in aboveground biomass, whereas the other combinations had the opposite effect.

2. The applied growth regulators (except for IBA) increased the proportion of grain in the total aboveground biomass of oat. This influence was more noticeable when magnesium sulfate was added to the growth regulators.

3. The obtained results indicate an ambiguous, often contradictory impact of growth regulators, applied alone or combined with magnesium sulfate, on the morphometric characters of oat plants, their mass and the share of the analyzed organs in total aboveground biomass.

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RELATIONSHIPS BETWEEN THE DENSITY AND COMPOSITION OF COLOSTRUM AND THE PERFORMANCE OF CALVES

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K e y w o r d s: cows, colostrum composition, colostrum density, calf rearing.

A b s t r a c t

The aim of this study was to determine relationships between the density of first-milking colostrum (collected within the first hour after calving), the composition of colostrum from subsequent milkings on the first day after birth and the performance of calves until 120 days of age. Cows that delivered bull-calves in the fall (October, November) were divided into three groups, depending on the specific gravity of first-milking colostrum: group I – below 1.047 g/cm^3 (6 head), group II – 1.048 g/cm^3 to 1.054 g/cm^3 (6 head), group III – above 1.054 g/cm^3 (7 head). Higher density of first-milking colostrum (cows of groups II and III) was correlated with higher concentrations of total whey protein and immunoglobulins. This correlation was observed until the second milking (6 to 9 hours after calving). Bull-calves fed colostrum of higher specific gravity were characterized by a faster growth rate, higher daily gains and greater body measurements, compared with those fed colostrum of lower density.

ZALEŻNOŚCI MIĘDZY GĘSTOŚCIĄ SIARY A JEJ SKŁADEM I EFEKTAMI ODCHOWU CIELĄT

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S ł o w a k l u c z o w e: krowy, skład siary, gęstość siary, odchów cieląt.

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Abstrakt

Analizowano zależności między gęstością siary z doju wykonanego w pierwszej godzinie po wycieleniu a jej składem w kolejnych dojach w pierwszej dobie oraz efektami odchowu cieląt do 120 dnia życia. Krowy, które urodziły buhajki jesienią (w październiku, listopadzie) podzielono na trzy grupy w zależności od ciężaru właściwego siary w pierwszym doju: I grupa – do 1,047 g/cm^3 (6 szt.), II grupa 1,048 g/cm^3 – 1,054 g/cm^3 (6 szt.), III grupa powyżej 1,054 g/cm^3 (7 szt.). U krów, których siara miała większą gęstość w pierwszym doju (grupa II i III) stwierdzono wyższą ilość białka serwatkowego i immunoglobulin. Zależność ta utrzymywała się do drugiego doju (6-9 godz. po porodzie). Buhajki odpajane siarą o większym ciężarze właściwym intensywniej przyrastały i uzyskały większe wymiary ciała w porównaniu z odpajanymi siarą o mniejszej gęstości.

Introduction

Colostrum produced by cows during the first few days after calving differs significantly from milk with respect to composition – it is yellow, alkaline, thick and sticky. Colostrum contains more proteins and has a higher concentration of lipids than milk. Other distinguishing features of colostrum include a fat content of around 7%, a very high content of fat-soluble vitamins, vitamin B12 and iron (SZULC, ZACHWIEJA 1998). Due to the presence of large amounts of other bioactive components, colostrum has a positive effect on calves not only on the first day postpartum, but over the entire period of its secretion (SKRZYPEK 2000). The activity of compounds released in the process of digestion of major milk proteins may be higher than that of the precursor protein (BERNATOWICZ, REKLEWSKA 2003).

The rate of immunoglobulin absorption by calves is directly proportional to their concentration in colostrum – at first it is very fast, but then gradually slows down as their serum content increases. The time of absorption of immunoglobulins of particular classes is different, ranging from 16 to about 27 hours postpartum (BAREJ 1986). The possibility of colostral immunoglobulin transfer to calf's blood reduces within successive hours after calving. The period of time between birth and receiving colostrum is crucial to meeting the immunological requirements of calves (BIELECKA 1987a, b, MC COY et al. 1984, NOCEK et al. 1984, STOTT et al. 1979 a, b, c, SZULC, ZACHWIEJA 1998).

First- and second-milking colostrum is of the greatest significance, because the ability to absorb unmodified colostral components by the intestinal epithelium of newborn calves decreases rapidly after birth and is practically non-existent at 16 to 36 hours. After that time even large quantities of high-quality colostrum cannot provide the newborn calf with the necessary antibodies for early immune protection (BIELECKA 1987 a, b, SZULC, ZACHWIEJA 1998). It is estimated that a calf requires around 12 g of immunoglobulins in 100 cm^3 of colostral whey to achieve adequate immunity (BALBIERZ et al. 1983). A positive correlation was also observed between colostral immunity

and daily body weight gains of calves aged 6 to 7 months (JARMUŻ et al. 2001, ROBINSON et al. 1988).

The aim of this study was to determine relationships between the density of first-milking colostrum, the composition of colostrum from three subsequent milkings and the performance of calves until 120 days of age.

Materials and Methods

The study was conducted on a dairy farm where the average stocking density was 220 Polish Holstein-Friesian cows. The animals were housed in free stalls and fed a partly mixed ration (PMR). Cows that delivered bull-calves in the fall (October, November) were divided into three groups, depending on the specific gravity of first-milking colostrum: group I – below 1.047 g/cm^3 (6 head), group II – 1.048 g/cm^3 to 1.054 g/cm^3 (6 head), group III – above 1.054 g/cm^3 (7 head).

On the first day postpartum the amount of colostrum obtained from cows was determined by weighing it to an accuracy of 0.1 kg, and samples were taken for analysis. Colostrum density was determined with a lactodensimeter graduated for 18°C, and active acidity was measured with the use of a Piccolo Plus pH-meter with a HF-1295 electrode. Next the samples were cold-stored for around 4 hours (+ 4°C). The collected colostrum samples were marked for thermostability – by titration with 96% ethanol, and coagulability – by measuring the time of casein curd formation following the application of a 1% rennet solution (PIJANOWSKI 1984). Whey was assayed for protein content by the biuret method of Weichselbaum (ANGIELSKI 1980). Whey proteins were divided into fractions by agarose gel electrophoresis (Biosciences Europe-SAS-Mx Serum Proteinum Kit). The percentages of immunoglobulins, β-lactoglobulins and α-lactoalbumins were determined with a Marcel Mini densitometer. The content of total protein, fat, lactose and dry matter in colostrum was measured with the use of a Combi Foss 6200 apparatus.

Newborn calves were separated from their mothers and placed in disinfected pens, on litter. The first four milkings and colostrum feedings were performed as follows: first colostrum feeding – one hour postpartum, second colostrum feeding – 6 to 9 hours postpartum, third colostrum feeding – 14 to 17 hours postpartum, fourth colostrum feeding – 22 to 25 hours postpartum. Starting from the second day after calving, calves received colostrum twice daily, and starting from the fourth day they were fed a milk replacer from nipple buckets. In the second week, in addition to the milk replacer, calves had free access to good-quality meadow hay and concentrated feed. All calves were weighed using a livestock scale, to an accuracy of 0.5 kg, at birth and at four months of age. Zoometric measurements were taken on day 120.

The results were processed statistically with the use of STATISTICA 6.0 software, by a one-factorial analysis of variance in a non-orthogonal design. The significance of differences was estimated with the use of Duncan's test.

Results and Discussion

The density of bovine colostrum is closely related to its composition and provides information on its overall quality. FLEENOR and STOTT (1980) demonstrated that due to a high correlation coefficient, density measurement permits accurate determination of the immunological value of colostrum. The specific gravity of colostrum above 1.047 g/cm^3 is indicative of its high quality, between 1.037 g/cm^3 and 1.045 g/cm^3 – of average quality, and below 1.035 g/cm^3 – of poor quality. None of the examined cows had colostrum with specific gravity below 1.036 g/cm^3. Higher specific gravity of colostrum was correlated with a lower colostrum yield as well as with higher concentrations of fat, protein and dry matter (Table 1). An increase in colostrum density was accompanied by a decrease in lactose content and coagulation rate. No relationships were observed between the other analyzed parameters and colostrum density. The basic composition of colostrum, determined in the present study, was comparable to that reported by other authors (PODKÓWKA 2002, SZULC et al. 1990, ZACHWIEJA 1995 a,b).

In group I (density of first-milking colostrum below 1.047 g/cm^3) the density of second-milking colostrum decreased to 1.032 g/cm^3, and it was statistically significantly lower than the values recorded in the remaining two groups. The density of third-milking colostrum was also the lowest in group I. The average density of colostrum obtained within eight hours after calving (the first two milking runs) was as follows: group I – 1.036 g/cm^3, group II – 1.048 g/cm^3, group III – 1.055 g/cm^3. The respective mean values for four milking runs performed on the first day postpartum were 1.035, 1.042 and 1.045 g/cm^3. It was found, based on density measurement, that in group I colostrum from the second and subsequent milkings was marked by poor quality, whereas in groups II and III good-quality colostrum was obtained during the first three milking runs.

A decrease in the concentrations of fat, protein and dry matter, as well as an increase in lactose content, were noted at subsequent milkings in all groups of cows. These changes were related to colostrum density. No correlations were found between colostrum density and acidity, thermostability and coagulability. Higher density of first-milking colostrum was correlated with higher concentrations of total whey protein and immunoglobulins (Table 2). This correlation was observed until the second milking (6 to 9 hours after calving). The highest whey protein content of first-milking colostrum was recorded

Table 1

Composition and "technological properties" of colostrum depending on its density at first milking

Specification		Successive milking runs											
		1			2			3			4		
		group			group			group			group		
		I	II	III	I	II	III	I	II	III	I	II	III
Density (g/cm^3)	$\bar{x}$	1.040^A	1.051^B	1.064^B	1.032^a	1.045^b	1.045^b	1.034	1.040	1.038	1.033	1.035	1.033
	v	0.68	0.17	0.42	0.86	0.80	1.13	0.59	0.71	0.32	0.15	0.44	0.07
Yield (kg)	$\bar{x}$	7.07	6.36	6.30	6.42	3.93	4.59	6.73	6.20	6.44	9.22	7.17	7.34
	v	55.82	70.60	59.43	60.28	89.42	52.24	51.68	69.34	39.93	2056	51.07	37.48
Content (%):													
fat	$\bar{x}$	4.99	5.31	5.50	5.94	5.42	4.88	4.66	4.08	4.55	4.99	4.56	4.27
	v	25.91	21.67	42.37	25.35	33.06	31.52	24.10	20.63	24.88	19.00	36.18	23.38
Total protein	$\bar{x}$	15.21	16.72	17.40	9.22^a	12.17	13.41^b	6.37	9.17	7.86	6.39	6.31	5.95
	v	10.89	17.95	14.87	34.30	23.32	25.25	30.52	24.84	19.12	45.17	20.34	9.45
Lactose	$\bar{x}$	3.88	3.49	3.46	4.04	3.69	3.70	4.53	5.49	4.15	5.19	4.62	4.71
	v	9.28	12.93	7.26	19.69	26.43	13.37	14.93	41.98	21.86	31.46	2.26	7.52
Dry matter	$\bar{x}$	25.19^a	27.79	28.87^b	22.24	23.09	24.38	17.06	17.27	18.00	16.85	16.85	16.38
	v	11.18	7.05	13.34	13.02	15.95	18.52	2.83	10.31	11.23	7.47	4.64	7.49
Acidity (pH)	$\bar{x}$	6.46	6.49	6.36	6.38	6.44	5.69	6.46	6.51^a	6.39^b	6.45	6.41^a	6.51^b
	v	2.84	1.81	2.03	1.55	0.69	32.45	1.54	0.69	1.37	1.44	1.60	0.80
Thermostability (cm^3)	$\bar{x}$	2.13	2.15	1.93	2.02	1.93	2.21	2.18	1.92	2.11	2.33	2.28	2.22
	v	15.59	14.34	16.86	7.92	25.27	21.09	15.98	32.74	16.02	12.62	19.27	7.07
Coagulation time (min)	$\bar{x}$	7.05	4.75	3.93	2.63	3.66	5.09	2.38	4.34	3.30	2.53	3.19	5.31
	v	88.16	62.23	29.48	43.19	53.14	59.41	46.93	102.37	59.93	47.69	74.32	110.58

within a row, mean values with differing superscript letter are significantly different; capital letters – $P \leq 0.01$; small letters – $P \leq 0.05$

Table 2

Proportions of particular whey protein fractions in colostrum from successive milkings depending on its density at first milking

Specification		Successive milking runs											
		1			2			3			4		
		group			group			group			group		
		I	II	III	I	II	III	I	II	III	I	II	III
Total whey protein g/100 cm^3	$\bar{x}$	19.19	24.23	26.33	10.02	13.00	13.09	9.12	9.99	7.81	4.67	5.15	4.75
	v	36.77	62.81	52.71	36.85	51.85	53.85	34.53	37.45	58.13	17.76	31.97	51.73
Immunoglobulins (%)	$\bar{x}$	71.27	74.87	75.20	65.22	66.42	69.57	66.39	70.24	61.43	57.09	62.29	53.17
	v	10.13	4.00	5.07	11.59	19.56	7.67	9.83	6.17	17.54	16.51	14.91	28.98
β-lactoglobulins (%)	$\bar{x}$	18.99	14.77	17.42	19.52	15.55	18.63	22.14	18.14^a	24.18	26.50	23.95	30.57
	v	28.68	22.11	12.90	26.94	16.96	12.60	21.55	16.46	24.37	19.67	25.73	37.50
α-lactoalbumins (%)	$\bar{x}$	6.89	7.86^a	4.25^b	11.74	14.77	8.40	8.70	9.07	10.13	13.46	11.14	12.42
	v	44.73	35.09	41.38	50.16	99.33	44.88	32.41	28.88	58.22	28.88	31.04	41.92

within a row, mean values with differing superscript letter are significantly different; small letters – $P \leq 0.05$

in group III, while the lowest – in group I. A substantially lower whey protein content of first-milking colostrum was reported by SZULC et al. (1989) and RZĘDZICKI, MIKUCKI (1983). According to BAREJ (1986), the initial protein content of colostrum is highly dependent on the diet of in-calf-cows, especially in the second half of gestation. The content of both whey proteins and immunoglobulins in colostrum decreased at subsequent milkings. Whey protein concentration in second-milking colostrum was nearly twofold lower than in first-milking colostrum. Immunoglobulin concentration decreased from around 6 percentage points (group III) to over 8 percentage points (group II). A further (although slighter) reduction in the content of protein and immunoglobulins in colostral whey was observed during successive milking runs. The whey protein content of fourth-milking colostrum was over fourfold (group I) to over fivefold (group III) lower, compared to first-milking colostrum. During successive milking runs, the concentrations of whey protein and immunoglobulins decreased faster in colostrum marked by higher density at first milking than in colostrum characterized by lower density at first milking. SZULC, ZACHWIEJA (1998) cited the results obtained by other authors which indicate that the absorption capacity of the calf with respect to particular immunoglobulin classes falls from 80–90% within the first two hours postpartum to less than 15% at 24 hours. The absorption period may be somewhat longer provided that nutrient concentrations in colostrum are lower.

At the beginning of calf's life colostrum is the only source of immunoglobulins. The blood levels of immunoglobulins from colostrum considerably affect the health status and survival rates of calves within the first weeks of their life. Immunoglobulins are absorbed from colostrum, in biologically active form, only on the first day after birth. The calf's ability to absorb colostral immunoglobulins decreases gradually within 5 to 6 hours postpartum. It follows that colostrum timing and quantity on the first day are the main factors affecting the blood concentrations of immunoglobulins in newborn calves.

An analysis of the body measurements and weight revealed that the quality of colostrum fed to calves on the first day of their life had a significant effect on their performance traits (Table 3). Calves that received colostrum with the highest density had the highest body weights on day 120. In this group average daily gains for 120 days reached 0.72 kg, and were by approximately 0.1 kg higher than in the group of calves fed colostrum with the lowest density. At 120 days of age, the body weights and daily gains of calves were significantly higher ($P \leq 0.05$) in group III, in comparison with the other two groups. Other authors reported similar daily gains of calves during the first four months of their life (CZAJA et al. 2002, GRADOMSKA et al. 2002, SZEWCZUK, KAMIENIECKI 2003). The fact that the birth weights of calves increased threefold over the first 120 days of their life

in almost all groups testifies to a successful calf raising program (PODKÓWKA 2002). An increase in the density of colostrum fed to calves on the first day after parturition was followed by an increase in their body measurements.

Table 3
Body weights, daily gains and body measurements of calves depending on its density at first milking

Specification		Groups of calves		
		I	II	III
Birth weight (kg)	$\bar{x}$	39.8	38.8	40.9
	v	10.55	19.38	14.78
Body weight on day 120 (kg)	$\bar{x}$	114.4^{a}	116.6^{a}	127.7^{b}
	v	12.10	11.16	7.23
Daily gains over a period from birth to day 120 (kg)	$\bar{x}$	0.622^{a}	0.647^{a}	0.721^{b}
	v	17.66	13.40	10.66
Body measurements on day 120 (cm):				
– height at withers	$\bar{x}$	92.3	94.3	95.7
	v	4.20	5.56	2.94
– height at sacrum	$\bar{x}$	96.7	97.5	99.1
	v	3.38	3.16	4.34
– shoulder width	$\bar{x}$	22.8	22.3	24.0
	v	14.24	4.62	6.80
– depth of forechest	$\bar{x}$	38.2	41.5	43.3
	v	23.52	3.65	2.2
– rump length	$\bar{x}$	32.0	32.2	33.3
	v	5.23	4.98	4.15
– oblique length of trunk	$\bar{x}$	94.7	94.3	98.7
	v	5.07	6.06	5.72
– chest girth	$\bar{x}$	109.3^{a}	112.8	114.9^{b}
	v	4.35	3.52	3.60
– oblique thigh circumference	$\bar{x}$	98.8	100.0	102.4
	v	4.12	4.90	3.38
– spiral thigh circumference	$\bar{x}$	105.0	105.7	108.1
	v	4.93	4.58	4.12

within a row, mean values with differing superscript letter are significantly different; small letters – $P \leq 0.05$

As demonstrated by SZULC et al. (1991), 70 to 80% of all diseases occur within the first 14 days of calf's life. According to SZAREK et al. (1994), 90% of all deaths are recorded in calves aged up to 3 months. Since no cases of illness or culling were reported over the experimental period, it seems that the serum concentrations of antibodies were high enough to protect the calves against infection.

It may be concluded that higher density of colostrum obtained within the

first hour after calving was correlated with higher concentrations of whey protein and immunoglobulins as well as with a shorter time required for curd formation. A considerable decrease in colostrum density, observed during subsequent milking runs in all groups, was accompanied by a reduction in the content of total protein, whey protein and immunoglobulins. Differences in colostrum density and composition noted at third milking (approx. 16 hours postpartum) were not linked to the density of first-milking colostrum. A significant correlation was found between the quality of colostrum from the first two milkings (1 and 8 hours after birth) and the growth rate of calves. The specific gravity of colostrum obtained within the first hour after parturition is a good indicator of its quality.

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THE EFFECT OF FLOW-THROUGH RESERVOIRS ON ZOOPLANKTON OF THE PŁONIA RIVER

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K e y w o r d s: zooplankton, river outflow, flow-through reservoirs, freshwater.

A b s t r a c t

In the years 2002–2003 the zooplankton collected by the Płonia river waters from the standing freshwater reservoirs it passed through was analysed. The reservoirs represented different trophic, biological and morphological conditions. The species composition and densities of zooplankton and changes taking place in these parameters with increasing distance from the reservoir were determined. Significant differences in the qualitative and quantitative composition of the zooplankton were noted at different sites of observation. In the samples collected at the majority of sites the Cladocera were represented by the greatest number of taxa, while Rotifera were represented by the largest number of individuals.

WPŁYW ZBIORNIKÓW PRZEPŁYWOWYCH NA ZOOPLANKTON RZEKI PŁONI

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S ł o w a k l u c z o w e: zooplankton, odpływ rzeki, zbiorniki przepływowe, zbiorniki śródlądowe.

A b s t r a k t

W latach 2002–2003 badano zooplankton wynoszony przez rzekę Płonię ze zbiorników o różnych warunkach troficznych, biologicznych i morfologicznych. Określono jego skład jakościowy i ilościowy, jak również zmiany w nim zachodzące w miarę oddalania się od zbiornika stojącego. Zaobserwowano istotne różnice w składzie jakościowym i ilościowym zooplanktonu na poszczególnych stanowiskach. Na większości z nich pod względem taksonomicznym dominowały *Cladocera*, natomiast ilościowo zdecydowanie przeważały *Rotatoria*.

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Introduction

Outflows of natural freshwater reservoirs carry out large amounts of zooplankton (SZLAUER 1977, EJSMONT-KARABIN, WĘGLEŃSKA 1996, CZERNIAWSKI 2004). This phenomenon can be directly confirmed by the observations of fry, feeding on zooplankton, accumulating at the outflows. According to the above-mentioned authors the higher the level of trophy in the water of a given reservoir the greater densities of zooplankton in its outflows.

Lake zooplankton is characterised by relatively high species diversity. The number of species living in lakes is much greater than that noted in small water reservoirs, which is a consequence of the diversity of available ecological niches and the intensity of intra- and interspecies competition (MŁOCICKI, GÓRNIAK 2003). Depending on the trophy, the numbers can vary from a few to a few thousand individuals in dm^3 (SELIN, HAKKARI 1982, KARABIN 1985, WĘGLEŃSKA, RYBAK 1998, ROMANOWICZ 1998, LINK, KEEN 1999). Regarding the numbers and biomass of zooplankton in natural and artificial water reservoirs, is probable of its proportional representation in the outflows from the reservoirs. According to EJSMONT-KARABIN, WĘGLEŃSKA (1996), the qualitative and quantitative structure of river plankton is mainly determined by the lakes through which a given river flows. In particular, high similarity of zooplankton in the river and in the reservoir is observed in the river zone near the outflow from the reservoir.

The aim of study

The study was undertaken to establish the effect of the freshwater reservoirs through which the river flows on the river zooplankton and to make a comprehensive analysis of the phenomenon of the carrying out of the zooplankton from the lakes and a shallow reolimnic reservoir by the river waters. In particular, our aim was to determine the species composition of zooplankton carried out from the reservoirs, its amount, the seasonal and distance changes in its amount and composition, and to compare the zooplankton carried out from the reservoirs characterised by different degrees of trophy.

The study area

The study was performed on the Płonia river (NW Poland). The river flows out of Lake Barlineckie and through the few lakes in the north-western Poland: Lake Płoń, Lake Miedwie and Lake Żelewko, then it joins the Odra

river through Lake Dąbie. The catchment area of the river Płonia is mostly covered by meadows and arable land. According to SZYPER, GOŁDYN (1990), the Płonia river waters in the section between Lake Płoń and Lake Miedwie in 1986 represented the second class of purity. Zooplankton was sampled at four study sites.

Site 1 (St. 1) was localised near the village Lubiatowo, at nearly 100 m from the river outflow from the Lake Płoń (covering 790.7 ha), at the bridge over the narrowed fragment of the river course. The river Płonia flows out of the eutrophic Lake Płoń (FILIPIAK, RACZYŃSKI 2000) through an artificial channel of soft bottom, sporadically grown with submerged plants.

Site 2 (St. 2) was localised near the village Żelewo, at about 300 m from the river outflow from the mesotrophic Lake Miedwie (covering the area of 3500 ha) (FILIPIAK, RACZYŃSKI 2000), also at a bridge over the narrowed section of the river. Above this site there is a dam.

Site 3 (St. 3) was localised at the bridge in the village Jezierzyce, below the reolimnic reservoir densely grown with submerged and emergent plants. The reservoir covers an area of about 3 ha and its mean depth is close to 1m. In March 2003 above this site a stone dam was made.

Site 4 (St. 4) was localised in Szczecin Dąbie, below a bridge near the railway track, at about 6 km from site 3. Throughout the year 2003 road work was conducted above this site and directly along the river. The measurements at the site provided data on the qualitative and quantitative structure of the river zooplankton at the end section of the river Płonia, directly before its inflow into Lake Dąbie.

The watercourse of the Płonia river and the localisation of the measuring sites are presented in Figure 1.

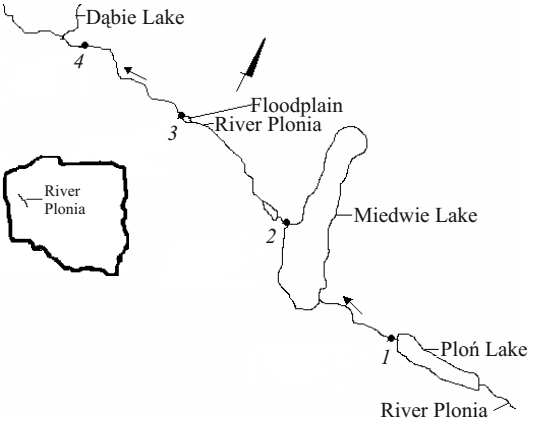

Fig. 1. Location of sites of zooplankton sampling on the River Płonia: *1* – Lubiatowo (site 1), *2* – Żelewo (site 2), *3* – Jezierzyce (site 3), *4* – Dąbie (site 4)

Material and Methods

Samples were collected in the years 2002–2003 every month (except February 2002) at all four sites. At each site two samples were collected: one for a quantitative study – 100 dm^3 of filtered water and another one for qualitative study. The former sample was collected on the bolting-cloth of 50 μm mesh size kept vertically in the river for 10 minutes. The number of individuals representing systematic groups of the crustaceans, the species of the rotifers and their numbers were established. In each sample the lengths of at least 30 individuals at each species were measured to be able to estimate the zooplankton biomass. The conversion of the animals; lengths into their mass was made using the tables proposed by MORDUCHAJ-BOŁTOWSKA (1954), STARMACH (1955). The qualitative analysis of the zooplankton was made using the keys for identification of the species prepared by WAGLER (1937), KUTIKOVA (1970), KIEFER, FRYER (1978), RYBAK (1996, 2000). The taxonomic similarity between the sites was calculated using the formula derived by MARCZEWSKI, STEINHAUS (1959):

$$S = {}^{W}/_{A+B} - W$$

where:
S – the statistical similarity of the two sites compared,
A – the number of elements of set A,
B – the number of elements of set B,
W – the number of elements shared by sets A and B.

The results, that were the numbers of individuals and the biomass of species, were subjected to statistical analysis by the programs Excel and Statistica. The statistical significance of the differences was estimated by the Scheffe test ($p < 0.05$) and variance analysis (W) (for many samples) (STANISZ 1998). In order to illustrate the differences in selected features between particular groups of the zooplankton the hierarchic agglomeration method (agglomeration analysis) was applied, based on the Euclidean distance calculated according to the formula:

$$(x, y) = [\Sigma_1 (x_i - y_i)^2]^{1/2}$$

where:
x, y – the objects whose distance is analysed; the value of this parameter is the geometrical distance between the objects analysed in a multidimensional space.

Results

The character of the lakes and the reolimnic reservoir through which the river flew influenced the taxonomic composition of the zooplankton carried by the river, the pattern of the zooplankton dominants and its amount. Over the Płonia river section between Lake Płoń and the site in Szczecin Dąbie, the zooplankton samples collected contained representatives of 62 taxa, of which 16 represented Copepoda, 25 – Cladocera and 21 – Rotifera (Table 1). The most important zooplankton representatives showing seasonal variations were: *Acanthocyclops robustus, Eucyclops serrulatus*, *Mesocyclops leuckarti, Thermocyclops crassus, Thermocyclops oithonoides, Ceriodaphnia quadrangula, Brachionus angularis, Brachionus variabilis* appearing in greater amounts in the warm season and *Cyclops strenuus, Cyclops vicinus, Notholca acuminata* appearing in greater amounts in the cool season.

Table 1

Taxonomic composition and frequency* of zooplankton in examined sites

Taxons	Sites			
	River Płonia			
	Site 1	Site 2	Site 3	Site 4
1	2	3	4	5
Copepoda				
Nauplii Cyclopoida	+++++	+++++	+++++	++++
Copepodites Cyclopoida	+++++	+++++	+++++	++++
Nauplii Calanoida	++	+	+	
Copepodites Calanoida	+++	++	+	+
Acanthocyclops robustus (Sars)	+	++		
Cyclops strenuus Fischer	+	+		
Cyclops vicinus Uljanin	++	+		
Diacyclops bicuspidatus (Claus)	+	+		
Eucyclops macrurus (Sars)	+			
Eucyclops serrulatus (Fischer)	++	++		+
Mesocyclops leuckarti (Claus)	++	+		
Thermocyclops oithonoides (Sars)	+	++	+	
Thermocyclops crassus (Fischer)	+	+		
Eudiaptomus gracilis (Sars)	++	+		
Eudiaptomus graciloides (Lilljeborg)	+	+	+	
Harpacticoida			+	+
Cladocera				
Acroperus harpae (Baird)			+	
Alona affinis (Leydig)	+	+	+	

cont. table 1

1	2	3	4	5
Alona quadrangularis (O. F. Müller)			+	+
Alona rectangula Sars		++	+	
Alona costata Sars	+	+	+	+
Alona guttata Sars		+	+	+
Alonella nana (Baird)		++	+	+
Bosmina coregoni Baird	++++	++++	+++	++
Bosmina longirostris (O. F. Müller)	++	+++	+++	++
Ceriodaphnia quadrangula (O. F. Müller)	+	+	+	+
Chydorus sphaericus (O. F. Müller)	++++	++	++++	+
Chydorus gibbus (Lilljeborg)	+	+	+	
Daphnia cucullata Sars	+++	++	+	
Daphnia longispina O. F. Müller	++	+	+	
Daphnia magna Straus		+	+	
Graptoleberis testudinaria (Fischer)			+	+
Leptodora kindtii (Focke)		+		
Monospilus dispar Sars				+
Pleuroxus aduncus (Jurine)		+	+	
Pleuroxus striatus Schoedler		+		
Pleuroxus trigonellus O. F. Müller	+	+	+	
Scapholeberis mucronata (O. F. Müller)			+	
Simocephalus sp.	+			
Peracantha truncata O. F. Müller			+	
Rhynchotalona rostrata (Koch)		+	+	+
Rotifera				
Asplanchna sp.	+++	++++	++++	++
Brachionus angularis Gosse	+	+	+	++
Brachionus budapestinensis Daday			+	
Brachionus calyciflorus Pallas	++	+++	++++	+++
Brachionus quadridentatus Hermann			+	
Brachionus rubens Ehrenberg			+	
Brachionus urceus (Linnaeus)		+		
Brachionus variabilis Hempel		+	+	+
Brachionus sp.		+		
Filinia longiseta (Ehrenberg)	++	+++	++	+++
Kellicottia longispina (Kellicott)	++++	++++	+++	+
Keratella cochlearis (Gosse)	+++++	+++++	+++++	+++++
Keratella irregularis (Lauterborn)	+			
Keratella quadrata (Müller)	+++++	++++	++++	+++
Notholca acuminata (Ehrenberg)			+	+

cont. table 1

1	2	3	4	5
Notholca foliacea (Ehrenberg)	+	+	+	+
Notholca squamula (Müller)	+	+	+	+
Notholca labis			+	+
Polyarthra sp.	+++++	+++++	+++++	+++++
Synchaeta sp.	++	+++	+++	+++
Rotatoria non det.	+++++	+++++	+++++	+++++

*Attendance:
+++++ 80–100%
++++ 80–60%
+++ 60–40%
++ 40–20%

The most frequent taxa in the river water were: nauplii of Cyclopoida, copepodites of Cyclopoida, *Eucyclops serrulatus*, *Daphnia cucullata, Daphnia longispina, Bosmina longirostris, Bosmina coregoni, Chydorus sphaericus, Asplanchna, Brachionus calyciflorus, Kellicottia longispina, Keratella cochlearis, Keratella quadrata, Polyarthra* sp.

The number of species found in the samples was higher in summer months. The highest taxonomic similarity index (MARCZEWSKI, STEINHAUS 1959) value of 0.73 was found between sites 1 and 2. The sites shared 40 taxa per 49 identified ones. The similarity index between the zooplankton communities at sites 3 and 4 was 0.63, the samples shared 29 taxa per 49 identified ones. This result points to a significant influence of the character of the reservoirs on the zooplankton carried by the river, observed even over a distance of a few km below the outflow. The lowest similarity index was found for the zooplankton communities at sites 1 and 4, so at the most distant ones. The samples of zooplankton collected there shared 21 taxa per 48 identified ones (Table 2).

Table 2

Taxonomic similarity between compared sites in the river Płonia

	Site 1	Site 2	Site 3	Site 4
Site 1	x	x	x	x
Site 2	0.73	x	x	x
Site 3	0.53	0.6	x	x
Site 4	0.49	0.52	0.63	x

The mean numbers and biomass of the zooplankton collected at the sites studied are presented in Table 3. The mean number of plankton animals in the Płonia river water was 277.3 ind. dm^{-3} and varied from 1.6 ind. dm^{-3}

Table 3

Average (x), scope of value (s) and standard deviation (SD) of abundance (ind. dm^{-3}) and biomass (mg. dm^{-3}) of zooplankton in examined sites

			Site 1			Site 2		
			2002	2003	2002–2003	2002	2003	2002–2003
ind. dm^3	Copepoda	x	75.8	159.8	119.6	43.6	31.5	37.3
		s	2.7–228.0	22.2–532.8	–	3.0–176.1	0.9–107.1	–
		SD	60.8	146.4	119.3	52.3	31.1	42.0
	Cladocera	x	66.8	73.9	70.5	8.6	6.5	7.5
		s	0.9–590.0	2.4–298.0	–	0.9–33.75	0.3–47.4	–
		SD	174.3	93.8	135.0	9.5	13.2	11.4
	Rotifera	x	766.9	336.8	542.5	121.9	231.5	179.1
		s	32.1–1965.3	24.9–782.4	–	28.8–353.2	33.0–1150.1	–
		SD	733.2	217.5	562.4	100.9	376.0	280.1
	Total	x	**909.5**	**570.4**	**732.6**	**174.1**	**278.3**	**223.8**
		s	**81.9–2225.9**	**129.2–891.0**	**–**	**40.6–369.7**	**12.0–1201.2**	**–**
		SD	**812.2**	**267.4**	**604.0**	**121.6**	**385.1**	**288.5**
mg dm^{-3}	Copepoda	x	0.6846	1.1742	1.0125	0.3187	0.3475	0.3337
		s	0.0322–3.5712	0.0670–4.8766	–	0.0525–1.3228	0.0008–2.6253	–
		SD	1.1346	1.3238	1.2212	0.5043	0.7420	0.6254
	Cladocera	x	0.3803	1.6130	1.0154	0.2068	0.0952	0.1486
		s	0.0045–1.8035	0.0729–6.7919	–	0.0106–1.0094	0.0300–0.5256	–
		SD	0.5362	2.1332	1.6773	0.3405	0.1446	0.2577
	Rotifera	x	0.3301	0.2933	0.2978	0.1058	0.1863	0.1478
		s	0.0295–0.7793	0.0130–0.8708	–	0.0534–0.2324	0.0035–0.8523	–
		SD	0.2332	0.2735	0.2493	0.0627	0.2715	0.2009
	Total	x	**1.3950**	**3.0805**	**2.3256**	**0.6312**	**0.6290**	**0.6301**
		s	**0.2063–4.9288**	**0.9534–8.7856**	**–**	**0.1152–2.4129**	**0.0982–1.1913**	**–**
		SD	**1.4526**	**2.6690**	**2.2740**	**0.8173**	**0.8969**	**0.8401**

cont. table 3

			Site 3			Site 4		
			2002	2003	2002–2003	2002	2003	2002–2003
ind. dm^3	Copepoda	x	7.1	5.3	6.2	4.4	4.4	4.4
		s	1.95–22.8	1.2–18.9	–	1.0–195	0.3–16.8	–
		SD	6.0	5.2	5.5	6.0	5.2	5.5
	Cladocera	x	4.8	1.3	3.0	0.9	0.9	0.9
		s	0.6–14.7	0.3–3.0	–	0.2–4.0	0.2–4.2	–
		SD	4.3	1.1	3.5	1.2	1.2	1.2
	Rotifera	x	125.0	70.4	96.5	60.9	24.5	41.9
		s	5.4–558.0	4.9–432.9	–	1.1–193.5	3.0–82.2	–
		SD	162.8	117.6	140.5	72.0	23.1	54.4
	Total	x	**136.9**	**77.0**	**105.6**	**66.2**	**29.8**	**47.2**
		s	**21.1–575.7**	**6.6–442.8**	**–**	**1.65–198.4**	**3.3–92.6**	**–**
		SD	**160.7**	**118.9**	**140.5**	**72.9**	**26.8**	**55.9**
mg dm^{-3}	Copepoda	x	0.0123	0.0207	0.0165	0.0077	0.0120	0.0100
		s	0.0004–0.0386	0.0007–0.1226	–	0.0004–0.0295	0.0001–0.0359	–
		SD	0.0131	0.0350	0.0261	0.0109	0.0131	0.0120
	Cladocera	x	0.0470	0.0180	0.0319	0.0064	0.0307	0.0191
		s	0.0091–0.2454	0.0033–0.0373	–	0.0011–0.0216	0.0015–0.2091	–
		SD	0.0737	0.0106	0.0500	0.0077	0.0615	0.0455
	Rotifera	x	0.0741	0.0423	0.0575	0.0436	0.0242	0.0335
		s	0.0135–0.2588	0.0030–0.0760	–	0.0031–0.1869	0.0013–0.1018	–
		SD	0.0735	0.0451	0.0611	0.0562	0.0349	0.0463
	Total	x	**0.1334**	**0.0793**	**0.1052**	**0.0577**	**0.0846**	**0.0744**
		s	**0.0638–0.6896**	**0.0390–0.4348**	**–**		**0.0052–0.2150**	**0.0029–0.2591–**
		SD	**0.1014**	**0.0577**	**0.0843**	**0.0651**	**0.0760**	**0.0625**

in November 2002 in the sample collected at site 4 to 2225.9 ind. dm^{-3} in August 2002 in the sample collected at site 1, below the eutrophic Lake Płoń (Figure 2). The highest mean number of animals of 732.6 ind. dm^{-3} was found in the sample collected at site 1. In the samples collected at all sites a strong dominance of the representatives of Rotifera was established, up to 89% in the sample collected at site 4. The domination of the Rotifera representatives was a consequence of a rather high abundance of *Keratella cochelaris*, found in the greatest number of 1791.6 ind. dm^{-3} (i.e. 91% of total number) in the sample collected in August 2002 at site 1.

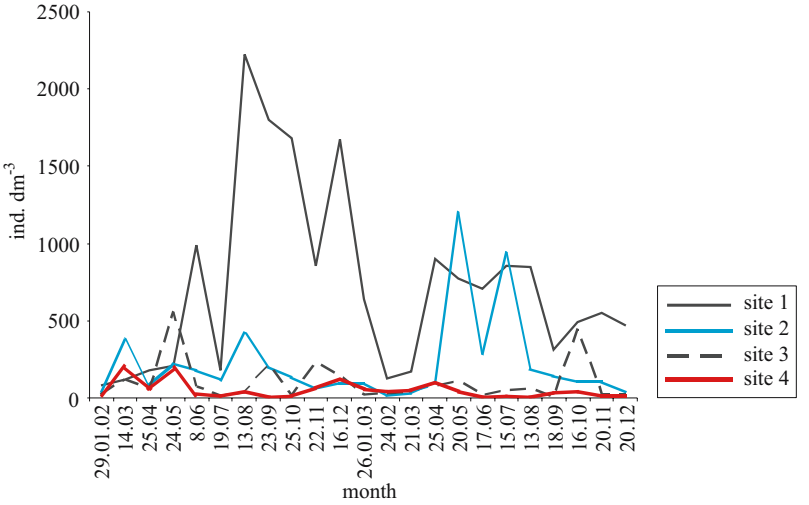

Fig. 2. Numbers (ind. dm^{-3}) of zooplankton in the river Płonia in 2002–2003

The highest abundance of the Crustacea was found at site 1, similarly as that of Rotifera. The mean value of Cladocera individuals at the site was 70.5 ind. dm^{-3}, while that of Copepoda was 119.6 ind. dm^{-3}. The maximum abundance of Cladocera was noted in Sept. 2002 (590 ind. dm^{-3}), with a profound dominance (93%) of *Chydorus sphaericus*. This species was abundantly represented only at site 1, at other sites no specific Cladocera species was particularly abundant. High abundance of Copepoda was a consequence of a large number of the larvae forms of Nauplii and Copepodites, making in the samples collected at each site over 60% of the density of Copepoda individuals. Low numbers of Copepoda were noted below the reolimnic reservoir (site 3) and the ones collected at site 4. With regard to the abundance of the zooplankton, statistically significant differences were found between the river water samples collected at different sites ($p < 0.05$).

The mean biomass of the zooplankton in the Płonia river was 0.7809 mg dm^{-3}, and varied from 0.0029 mg dm^{-3} at site 4 in Nov. 2002 to 8.7856 mg dm^{-3} at site 1 in Nov. 2003, below Lake Płoń (Figure 3). In the samples collected below the river outflows from the lakes and the reservoir the main contribution to the biomass came from Cladocera. It was particularly pronounced in the sample collected in Nov. 2003 at site 1, in which the contribution of *Bosmina coregoni* in the biomass of the total zooplankton was slightly over 77%. As regards the zooplankton biomass, the samples collected at different sites were found statistically significantly different ($p < 0.05$), except for those collected at sites 1 and 2. According to the agglomeration analysis the most similar in the zooplankton abundance and biomass were the samples collected at sites 3 and 4 (Figure 4 and Figure 5).

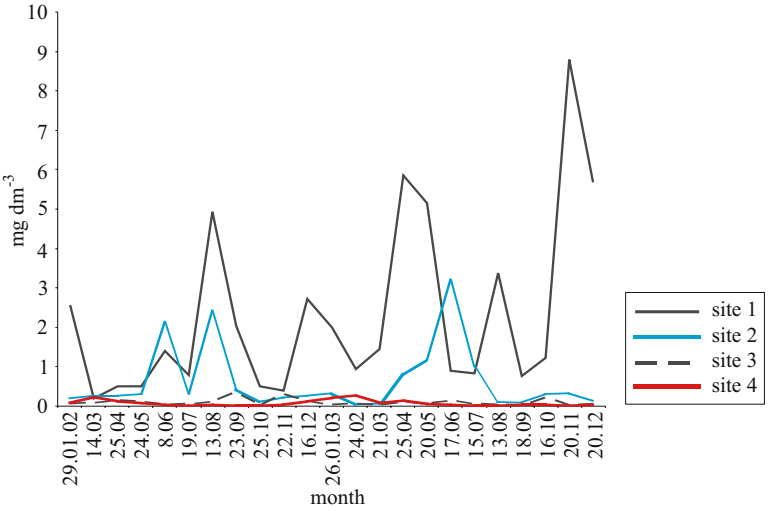

Fig. 3. Biomass (mg dm^{-3}) of zooplankton in the river Płonia in 2002–2003

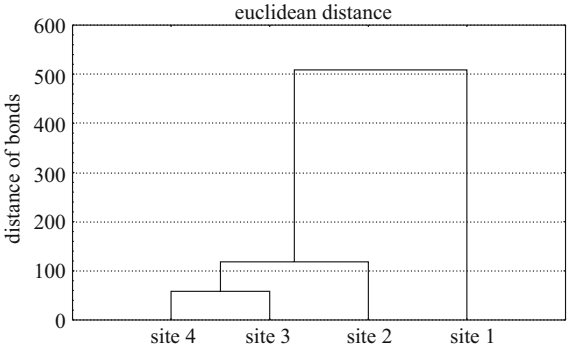

Fig. 4. Agglomerative analysis of zooplankton numbers in examined sites

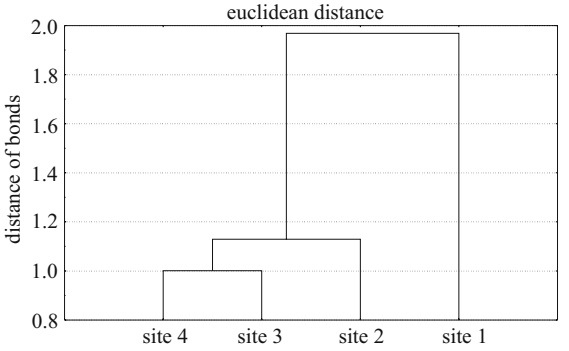

Fig. 5. Agglomerative analysis of zooplankton biomass in examined sites

Discussion

According to the number of taxa the dominant were Cladocera, while Rotifera dominated in numbers. Similarly, by SZLAUER (1977) reported 43 taxa, including 17 Cladocera, 10 Copepoda and 16 Rotifera in samples collected at the outflow of the river Płonia from Lake Płoń and Lake Żelewko. Similar results were reported by KARABIN and EJSMONT-KARABIN (1999), for the zooplankton from Lake Wigry. They found a relatively poor in species representation of the Rotifera, and rich of Crustacea. The species composition of communities collected at particular sites correlated to the trophic status of the reservoirs from which the zooplankton was carried out. In the studies of the zooplankton in the water of different trophic status, SZLAUER (1977), KARABIN (1985), GÓRNIAK, CHOCIAN (1999), EJSMONT-KARABIN1, KUCZYŃSKA-KIPPEN (2001), BOWSZYS (2004), CZERNIAWSKI (2004), reported finding species typical of a given trophic status. The zooplankton carried out of Lake Miedwie was characterised by a relatively high number of taxa and relatively small number, which can indicate a low but increasing eutrophication.

Below the river outflow from the reolimnic reservoir (i.e. site 3), according to the number of taxa represented the dominant were Cladocera, while according to the number of representatives the dominant were Rotifera. A considerable area of this reservoir is overgrown with both submerged and emerging macrophytes, creating conditions for habitation and reproduction of zooplankton species, particularly those typical of littoral, like *Alona* sp., *Bosmina* sp., *Chydorus* sp., *Brachionus* sp., and *Notholca* sp.. Few years ago domestic sewage was dumped directly into this reservoir (oral comm.), which has significantly increased the level of nutrients and zooplankton. CZERNIAWSKI (2004) reported the presence of the Cladocera species found in the Płonia river in the water carried out from the Drawa river floodplains. The river

floodplains and similar reolimnic reservoirs in their course are characterised by considerable coverage of their bottom with vegetation. According to many authors, the zones of reservoirs densely grown with macrophytes create favourable conditions for habitation and reproduction of zooplankton (KOWALCZYK 1998, KUCZYŃSKA-KIPPEN, NAGENGAST 2003, CZERNIAWSKI, PIASECKI 2004). The samples collected at site 4 were characterised by the lowest number of taxa and the lowest number of individuals. This site is not localised in the vicinity of any reservoir. This site the number of taxa representing Rotifera was higher than that representing Cladocera, which may be related to the food selection of the fish between site 3 and site 4. In the samples collected at site 4, the mean number of taxa, the mean abundance and biomass of all systematic groups analysed were lower than those characterising the samples from site 3. The differences in the zooplankton abundance between these two sites reached 406.5 ind. dm^{-3}. According to SZLAUER (1977), EJSMONT-KARABIN, WĘGLEŃSKA (1996), CZERNIAWSKI (2004), the abundance of the zooplankton carried out from a reservoir decreases significantly with the distance from this reservoir. A similar correlation was reported by ROMANOWICZ (1998) who studied the zooplankton of the rivers in the area if the Drawieński National Park, at the sites much distant from the river outflows. CHIA et al. (1984), EJSMONT-KARABIN, WĘGLEŃSKA (1996) reported that the zone near the river outflow is the region in which the abundance of the littoral zooplankton strongly decreased. The same situation was observed in this study.

Other reasons for the differences in the composition of the large size zooplankton between the sites could be the tendency to hide among the vegetation to avoid the feeding fry (SZLAUER 1977, JEPPESEN et al. 1997, LAURIDSEN et al. 1998, CZERNIAWSKI 2004) or the phenomenon of reotaxy (SZLAUER 1965, KAŹMIERCZUK, SZLAUER 1984). The above hypotheses seem to be confirmed by the abundance and taxonomic composition of the zooplankton in the samples collected at all the sites studied. The most abundantly represented were the Rotifera, while large size species occurred in small amounts.

The distance from the river outflow was also found to affect the taxonomic composition of the zooplankton, which was evidenced by the lowest taxonomic similarity index determined for comparison of the samples collected at site 1 and the most distant and different from it site 4.

It is expected that the density and taxonomic composition of the zooplankton is also related to the seasonal changes in the community in the reservoirs through which the river flows. This opinion is shared by KAŹMIERCZUK, SZLAUER (1984). Moreover, the composition, numbers and biomass of the zooplankton carried out by the river are also determined by the vertical distribution of the animals in the reservoirs. It is known that during the day zooplankton enters into deeper layers of the reservoirs, which has been

confirmed among others by the study of (SZLAUER 1960, 1977, PATALAS 1963). The samples studied were collected during the day, so taking into regard the fact that the river outflow collects mainly the surface layer of the water from the reservoir it passes through, (SZLAUER 1977), the amount of the zooplankton found in the samples was smaller than it would be if the samples were collected at night.

Translated by MARIA SPYCHALSKA

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STRUCTURE OF A POPULATION OF *TROLLIUS EUROPAEUS* L. AT A LOCALITY NEAR BARCZEWO IN THE OLSZTYN LAKELAND

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K e y w o r d s: *Trollius europaeus*, Olsztyn Lakeland, population structure, ecological concentration, specimen architecture.

A b s t r a c t

A population of *Trollius europaeus* whose locality was found near the town of Barczewo (NE Poland) was analyzed in the study. This species is a very rare component of flora in the Olsztyn Lakeland. The aim of the population survey conducted in 2005 and 2007 was to determine the abundance and density of the tested population, the type of its spatial structure, as well as selected characteristics of specimen architecture, such as the height and width of clumps of the European globeflower, and the number of vegetative and fertile shoots. The survey was carried out in three areas differing in terms of habitat conditions. In order to determine the spatial pattern of the population, the index of concentration was calculated as the variance-to-mean ratio. The following statistical measures were determined: arithmetic mean, standard deviation and mode.

The mean ecological concentration was 4.76. The highest number of specimens per m^2 was recorded in an open meadow, and the lowest – at the edge of a forest. The analysis of the spatial structure of the population showed that all three research areas were characterized by a uniform distribution of specimens, with a tendency towards a clustered distribution.

Differences in clump size between particular research areas were slight. The height of flowering plants ranged from 22 to 60 cm. The average number of vegetative shoots per clump was 21.34, while the number of fertile shoots varied from 0 to 15. Vegetative reproduction was predominant, as confirmed by the number of vegetative rosettes per fertile shoot in a clump. The most flowering specimens were recorded near a ditch, under high humidity conditions, whereas the fewest – in an open meadow.

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STRUKTURA POPULACJI *TROLLIUS EUROPAEUS* L. NA STANOWISKU W OKOLICY BARCZEWA NA POJEZIERZU OLSZTYŃSKIM

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S ł o w a k l u c z o w e: *Trollius europaeus*, Pojezierze Olsztyńskie, struktura populacji, zagęszczenie ekologiczne, architektura osobnika.

A b s t r a k t

Przedmiotem badań była populacja *Trollius europaeus* występująca na stanowisku w okolicy Barczewa. Gatunek ten jest bardzo rzadkim elementem flory Pojezierza Olsztyńskiego. Badania populacyjne przeprowadzono w roku 2005 i 2007. Zakres pracy obejmował określenie liczebności i zagęszczenia ekologicznego populacji, typu struktury przestrzennej oraz analizę wybranych cech architektury osobnika – wysokość i szerokość kęp pełnika europejskiego, a także liczbę pędów wegetatywnych i generatywnych. Badania przeprowadzono na trzech powierzchniach różniących się warunkami siedliskowymi. W celu określenia typu struktury przestrzennej populacji wyznaczono wskaźnik skupiskowości jako stosunek wariancji do średniej. W opracowaniu statystycznym uwzględniono średnią arytmetyczną, odchylenie standardowe oraz średnią modalną.

Średnie zagęszczenie ekologiczne wynosiło 4,76. Największą liczbę osobników na powierzchni 1 m^2 stwierdzono na otwartej łące, a najmniejszą na powierzchni pod lasem. Analiza struktury przestrzennej populacji wykazała, że wszystkie trzy powierzchnie badawcze cechują się równomiernym rozkładem osobników z tendencją do rozkładu skupiskowego.

Wielkość kęp na poszczególnych powierzchniach była zróżnicowana w niewielkim stopniu. Wysokość roślin kwitnących wahała się od 22 do 60 cm. Średnia liczba pędów wegetatywnych w kępie wynosiła 21–34, a liczba pędów generatywnych – 0–15. Stwierdzono, że dominował wegetatywny typ reprodukcji, o czym świadczyła liczba rozet wegetatywnych przypadająca na jeden pęd generatywny w obrębie kępy. Najwięcej osobników kwitnących odnotowano na powierzchni przy rowie, cechującej się największą wilgotnością, natomiast najmniej – na otwartej łące.

Introduction

The European globeflower (*Trollius europaeus)* grows wild across Europe, but in the south it can be found only in the mountains. It occurs also in the region of Caucasus and Arctic America (*Słownik botaniczny*. 2003). This species is widely distributed in Poland, both in the lowlands and highlands, where it occurs as an alpine subspecies, usually with greenish flowers (RUTKOWSKI 2005). Localities of the European globeflower are scattered across Poland. This species is very rare in some regions, reaching the greatest abundance in the south-eastern part of the country (*Atlas rozmieszczenia...* 1997).

The European globeflower prefers moist, eutrophic soils, such as peat (lowland moors), muck, steppe black, ground-gley and brown soils, whose

reaction is alkaline through neutral to slightly acid (HEGI 1975). It can be found at places protected against overdrying, but avoids too wet and frequently flooded sites. In the European Lowland this species typically occupies dump, fertile peaty meadows (often referred to as "globeflower meadows"), forest margins, peatlands, mid-forest clearings, well-lit shrub communities, head-stream habitats fed by seepage or small springs. Although in the Polish Lowland the largest populations of globeflowers have been reported from headwater areas, in the Białowieża Forest they are components of the herbaceous layer in oak woods. In the mountains globeflowers can be found in the alpine belt, where they grow in wet grasslands and on slopes (*Atlas rozmieszczenia...* 1997).

This species is characteristic of humid meadows classified as *Polygono bistortae-Trollietum europaei* (Hundt 1964) Bal.-Tul. 1981, belonging to the order *Molinietalia* (ĆWIKLIŃSKI, JASNOWSKI 1997, MATUSZKIEWICZ 2001). It is also encountered at the edge of thermophilous forests and scrubs of the order *Quercetalia pubescentis*. The European globeflower is also a component of the following plant communities: *Angelico-Cirsietum oleracei* R.Tx. 1937 em. Oberd. 1967 (*Cirsio-Polygonetum bistortae* R.Tx. 1951) (MATUSZKIEWICZ 2001), *Filipendulo-Geranietum palustris* Koch 1926 (NOWIŃSKI 1967) and *Juncetum subnodulosi* Koch 1926 (BACIECZKO 1996).

Under natural conditions this species usually forms numerous clusters covering large areas. Its populations consist of several to a few hundred specimens. The plant is protected and was recognized as a retreating species within the area of Wielkopolska, Western Pomerania and Kujawy. Investigations into the population structure of *Trollius europaeus* have been conducted to date only in the Wielkopolska-Kujawy Plain and in the Krajenska Upland (Wysoczyzna Krajeńska) in the Pomeranian Lakeland. The localities of this species were characterized in view of population density, spatial distribution of clumps, number of flowering shoots and seed mass (ANTKOWIAK 1999), as well as the structure and morphological variation of flowers (ANTKOWIAK, MACIEJEWSKA 1999). The inter-population variation of vegetative organs was also analyzed (ANTKOWIAK 2002).

In the Olsztyn Lakeland the European globeflower is very rare. This is a boreal species of European-Asian origin, which appeared in East Pomerania in the Tertiary period, together with a group of other boreal taxa (POLAKOWSKI 1963). Currently there are only two natural localities of this species in the Olsztyn Lakeland (Forest Division Wipsowo). The first of them is situated in the commune of Barczewo (Forest Administration Region Maruny). The population of *Trollius europaeus* at this locality is very abundant, in June 2007 it was composed of 648 clumps. The second locality is situated in the Forest Administration Region Cisy, in the ecological area "Klasztorne Łąki". The local

population consists of 32 clumps of *Trollius europaeus*. According to Professor Korniak (personal information), another locality of the European globeflower, most probably of anthropogenic origin, was found in 2004 in the village of Ruś located 10 km from Olsztyn. However, there are no detailed data on this locality and its existence has never been officially confirmed; it might have been destroyed by the owner of the local grassland.

Studies conducted in the past at the localities of the European globeflower in the Olsztyn Lakeland were fragmentary and focused primarily on estimating the population size at the locality near the village of Maruny (KLAROWSKI 1983). A more extensive survey was conducted in 2005 at a locality nearby the village of Maruny, commune of Barczewo, NE Poland (SZCZESNA 2006). The present paper completes earlier studies conducted on this locality in 2005. It was aimed at determining the abundance and density of the tested population, the type of its spatial structure, as well as selected characteristics of specimen architecture, such as the height and width of clumps of the European globeflower, and the number of vegetative and fertile shoots.

Characteristics of the study area

The research station is located in the commune of Barczewo (Forest Division Wipsowo, Forest Administration Region Maruny) – Figure 1. It has been registered as a nature monument, no. 527 (DĄBROWSKI et al. 1999). According to the physico-geographical regionalization of Poland (KONDRACKI 1998), the research area is situated in the mesoregion of Olsztyn Lakeland, western part of the macroregion of the Masurian Lakeland, province of East-Baltic Lowland, subprovince of Baltic Coastland. From the geobotanical perspective (MATUSZKIEWICZ J.M. 2001), the research area is located within the Northern Masurian-Belarussian Division, in the Masurian Region, West Masurian Subregion.

The analyzed locality of *Trollius europaeus* is situated in a mid-forest semi-natural meadow, covering an area of 2.25 ha, composed of the communities *Cirsio-Polygonetum bistortae* and *Polygono bistortae-Trollietum europaei*. The meadow developed spontaneously and for many years has been used extensively, which means that it has been mown sporadically and no mineral fertilizers have been applied. The meadow is located at a certain distance (approx. 3 km) from the village of Maruny and the access to this area is constrained due to steep slopes, which considerably limits human penetration and impact on the vegetation cover. In the west the meadow is surrounded by erosional hilly country, with mixed dry-ground forests composed of spruces, pines, birches, lindens, maples, hornbeams, ashes, trembling poplars and oaks. In the east

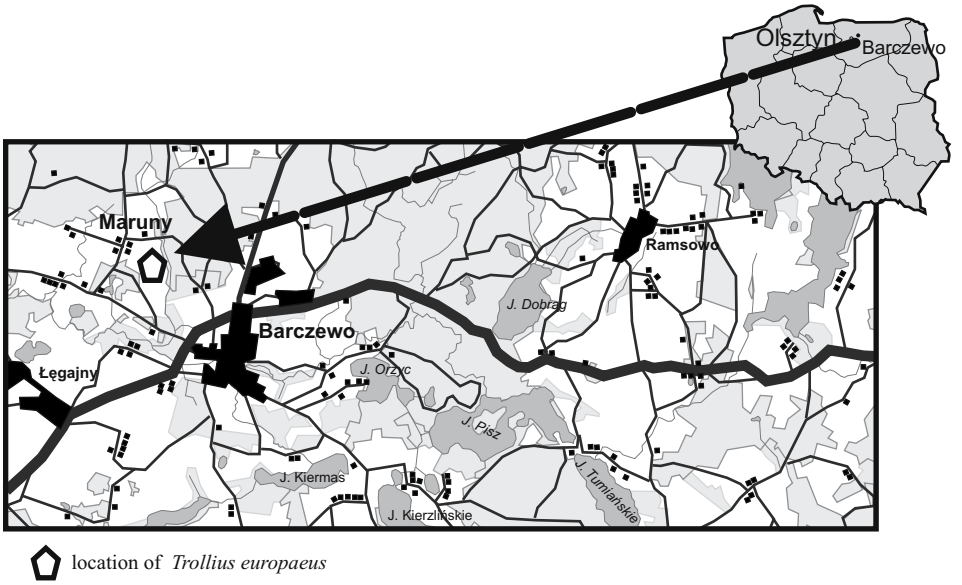

Fig. 1. Location of the study area

the country is undulating, with slightly podzolized soils. The slopes are overgrown with a deformed forest dominated by spruces, oaks and birches, with lindens and larches as admixture species. A nameless stream, approximately 10 km in length, cuts through the meadow and flows into the Pisa Warmińska River 1 km west of Barczewo (KLAROWSKI 1983).

Material and Methods

An important question in population surveys is the definition of the term "specimen". From the genetic-evolutionary perspective, an organism (genet) is an individual that descends from a zygote (HARPER 1977). According to this approach, a genet may be a seedling, but also a monocormonal (one-shoot), polycormonal (multi-shoot) and clonal individual. From the ecological perspective, a specimen is a separate organism, morphologically and physiologically integrated, consisting of a single rooted shoot or many shoots. Giving the status of a single specimen to clusters of shoots (clumps) is justified, since such an individual occupies a certain place in space, enters into various relations and interactions with other individuals of the same or other species, and produces generative and vegetative propagules, thus participating in reproduc-

tion. It follows that in this way single shoots as well as their clusters and clumps affect the structure and dynamics of a population (FALIŃSKA 1996).

Under natural conditions *Trollius europaeus* forms clumps. Their formation starts with the appearance of the first fertile shoot. Vegetative lateral shoots develop at the base of the fertile shoot, from perennating buds formed in the fall of the season preceding blooming. Further pre-generative tillering at the base of the shoot leads to the formation of clumps (TARANT 1997).

For the purposes of the present work it was assumed that a specimen is a clump composed of numerous vegetative and sterile shoots, morphologically distinct.

Field investigations were carried out in May and June of 2005 and in 2007, at the stage of full blooming within three research areas differing in terms of light and humidity conditions (Figure 2). Research area 1 (2 m x 5 m) was situated along a drainage ditch, research area 2 (4 m x 5 m) was located in the central part of the meadow, and research area 3 (2 m x 6 m) was located at the edge of a forest, under trees. The areas were divided into 1 m x 1 m squares using stakes. The number of specimens and their distribution were determined within each square. The density and spatial structure of population were estimated. Ecological concentration was determined as the number of clumps on the area of 1 m^2. In order to determine the spatial pattern of the population, the index of concentration was calculated as the variance-to-mean ratio (VMR), using the following formulas:

$$\text{variance} \frac{\Sigma\,(x)^2 - \frac{(\Sigma\,x)^2}{N}}{N-1}$$

$$\text{mean}\ \bar{x} = \frac{\Sigma\,(x)}{N}$$

where:
x – the number of individuals
N – the number of samples

According to Svedberg (KERSHAW 1978), VMR higher than 1 denotes a clustered distribution, while VMP lower than 1 denotes a uniform distribution.

A specimen architecture was also investigated. The analysis of the structure of *Trollius europaeus* clumps included the determination of the number of vegetative and fertile shoots, as well as the height and width of clumps, accurate to 0.5 cm. The measurements wee performed on 200 clumps growing

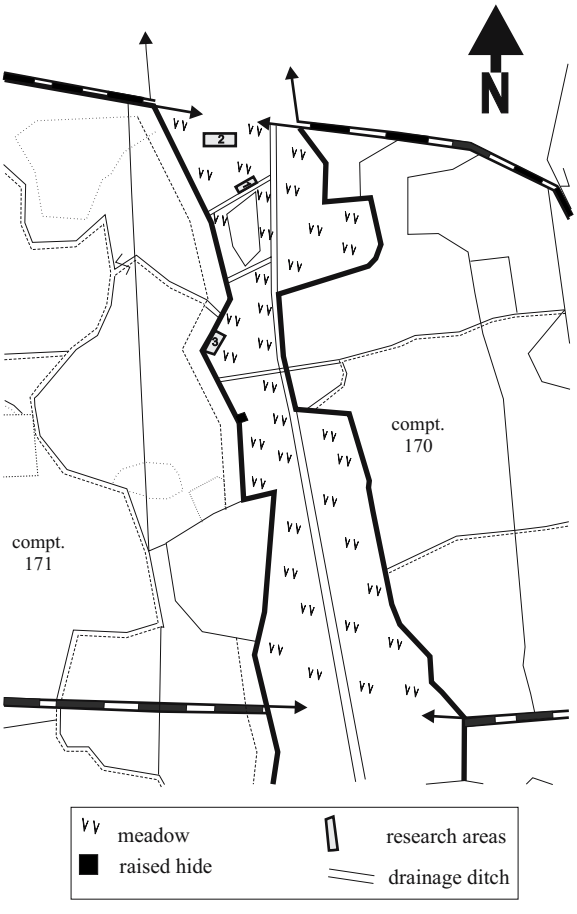

Fig. 2. Location of research areas at the locality of *Trollius europaeus* near Barczewo in the Olsztyn Lakeland

within the above research areas. The following statistical measures were determined: the minimal value, the maximal value, arithmetic mean, standard deviation and mode.

Results

Characteristics of the spatial structure of a population of *Trollius europaeus*

The estimation of the size of a population is a key methodological requirement in plant demography. Sometimes it is difficult to precisely determine the actual number of individuals forming a given population. Even when it is possible to single out all specimens and to set the range limits of a population, it is still necessary to estimate the number of plants per area unit, since plant density provides a basis for evaluating the properties of a population (FALIŃSKA 2002).

Ecological concentration was determined in distinct phytocenoses within plant communities, at selected sites within the area occupied by the tested population. 200 specimens were recorded over an area of 42 m^2. The mean ecological concentration was 4.76. Particular research areas differed slightly with regard to this parameter (Table 1). The highest number of specimens per m^2 was encountered in research area 2 (open meadow), while the lowest in research area 3 (edge of the forest).

Table 1

Spatial structure of the tested population of *Trollius europaeus*

Number of research area	Total number of specimens in the study area	Number of 1 m^2 x 1 m^2 squares	Ecological concentration	Index of concentration
1	51	10	5.10	0.93
2	105	20	5.25	0.96
3	44	12	3.67	0.31

The analysis of the spatial structure of the population, with the use of the index of concentration, showed that all three research areas were characterized by a uniform distribution of specimens. However, in research areas 1 and 2 the index of concentration was close to 1, which indicates a tendency towards a clustered distribution (Table 1). A detailed distribution pattern of *Trollius europaeus* clumps in particular research areas is presented in Figure 3. The number of specimens per m^2 ranged from 2 to 11 on research area 1, from 3 to 11 in research area 2, and from 2 to 5 in research area 3.

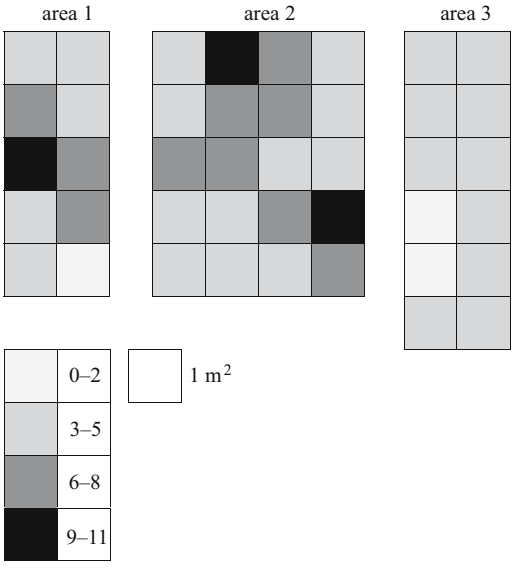

Fig. 3. Distribution of *Trollius europaeus* specimens in research areas

Characteristics of the architecture of *Trollius europaeus* specimens

Differences in clump size between particular research areas were slight. The height of flowering plants ranged from 22 to 60 cm, usually reaching 34 cm in research areas 1 and 3 and 40 cm in research area 2 (modal value), which gave an average of 38.18 cm (Table 2). The greatest differences in width of clump was noted in area 2, where it varied from 5 to 50 cm. In most cases clump diameter reached 30 cm in research areas 1 and 2, and 40 cm in area 3 (Table 2).

Table 2

Features of the architecture of specimens of *Trollius europaeus* in research areas

Number of research area	1					2					3				
Characteristics	min	max	$\bar{x}$	Mo	s	min	max	$\bar{x}$	Mo	s	min	max	$\bar{x}$	Mo	s
Clump height (cm)	24	60	38.39	34	7.55	22	50	37.44	40	5.80	28	52	38.70	34	6.27
Clump width (cm)	15	45	27.63	30	6.09	5	50	29.73	30	8.73	17	45	30.39	40	8.33
Number of vegetative shoots	7	50	20.59	15	9.90	1	66	20.85	15	13.09	11	42	22.57	23	7.67
Number of fertile shoots	0	15	2.24	0	3.37	0	14	2.37	0	2.94	0	7	1.80	0	2.05

$\bar{x}$ – arithmetic mean, Mo – mode, s – standard deviation

The average number of vegetative shoots per clump was 21.34, ranging from a minimum of 1 to a maximum of 66. The modal value (denoting the most common number of vegetative shoot) was 15 in research areas 1 and 2, and 23 in area 3 (Table 2).

Following the assumption that the number of fertile shoots is a relative indicator of biotic potential, the populations of *Trollius europaeus* with the highest number of fertile shoots may be expected to have the greatest biotic potential (ANTKOWIAK 1999). The number of fertile shoots varied from 0 to 15 in particular research areas. The most flowering specimens were recorded in research areas 1 and 2, whereas the lowest number of fertile shoots was observed in area 3 (Table 2).

Discussion

Few studies have examined the structure of *Trollius europaeus* populations in Poland. The locality in the commune of Barczewo, in the vicinity of the village of Maruny in the Olsztyn Lakeland, has been observed since 1979 (KLAROWSKI 1983). However, the data on the population size published to date were approximate only, and varied within a wide range of a few hundred to a thousand individuals (KLAROWSKI 1983). Current observations performed by the authors in 2007 confirmed the presence of 648 clumps of *Trollius europaeus* at this locality. The rare occurrence of this species in the Olsztyn Lakeland as well as its high abundance at the examined locality were important reasons for launching the present population survey.

The development of a certain spatial structure of population is dependent, to a great extent, on the biological properties of species and on the diversity of environmental conditions (FALIŃSKA 2002). Many authors, e.g. KWIATKOWSKA (1972), WILKOŃ-MICHALSKA (1976) and SYMONIDES (1979), share the opinion that a clustered distribution pattern is typical of the majority of plant species. A clustered distribution means that individuals occur in groups, so such a spatial pattern is characterized by a high number of empty squares, i.e. samples containing no specimens at all, as well as by a high number of squares within which plant density is considerable. A uniform distribution means that individuals are evenly scattered across the entire area (KERSHAW 1978). Under natural conditions, *Trollius europaeus* forms clumps that can be treated as elementary clusters of plants in a population (ANTKOWIAK 1999), which supports the hypothesis of hierarchical structure of clusters (KERSHAW 1978). Such a phenomenon is particularly noticeable in populations composed of a high number of plants, as observed by ANTKOWIAK (1999). Its occurrence was also confirmed by the results obtained in the Olsztyn Lakeland, where a strong tendency towards clustering was noted.

In the analysis of the group characteristics of the populations of *Trollius europaeus* found in the Wielkopolska-Kujawy Plain (ANTKOWIAK 1999) it was assumed, following RABOTNOV (1964), that a specimen is a shoot and not a clump. According to the definition adopted in the present study, a specimen is a morphologically distinct clump. Giving the status of a specimen to single shoots or clusters of shoots is justified (FALIŃSKA 1996), but the results concerning the ecological concentration of plants obtained in the Wielkopolska-Kujawy Plain and in the Olsztyn Lakeland are incomparable.

The European globeflower belongs to species that reproduce both generatively and vegetatively. The biotic potential of the European globeflower may be expressed as the number of flowering shoots in a sampling area (or in a clump) and as the number of vegetative lateral shoots that develop from perennating buds formed in the fall of the season preceding blooming (ANTKOWIAK 1999). According to FALIŃSKA (1977), the biotic potential of a population is the outcome of either genetic conditions or the limiting or stimulating impact of the natural environment. At the locality near Barczewo this potential was found to be different in particular research areas. Vegetative reproduction was predominant, as confirmed by the number of vegetative rosettes per fertile shoot in a clump. The most flowering specimens were recorded near a ditch, under high humidity conditions, whereas the fewest – in an open meadow. There was no correlation between the number of flowering shoots and clump size.

The size of specimens is of primary importance for the population because it may be a measure of living conditions (ANDRZEJEWSKI, FALIŃSKA 1986). FALIŃSKA (1990) demonstrated that adverse environmental conditions may result in poor development of specimens and in a uniform population size structure, while favorable environmental conditions may contribute to high variation in this parameter. The domination of medium-height plants in a population testifies to its stabilization. In the present study medium-sized specimens dominated in all research areas.

According to RABOTNOV (1950), habitat conditions can be considered favorable for population development if the growth rate of individuals is fast. The rate of development can be measured by the domination of specimens at the generative stage. This criterion of optimal development was not satisfied in the current study, since the numerical superiority of fertile shoots over vegetative ones was not observed in any of the research areas. The analyzed population shows features of a stable population.

Studies conducted in the Wielkopolska-Kujawy Plain revealed population variability related to habitat conditions (ANTKOWIAK 1999, 2002). The present observations were limited to one population, and testified to ecotype variation only, resulting from different light and humidity conditions at the examined locality.

Conclusion

1. The population of *Trollius europaeus* at the locality near Barczewo is a very rare and valuable component of flora in the Olsztyn Lakeland.

2. Observations concerning the population size, followed by an analysis of specimen architecture, shows that the tested population is stable.

3. Further population surveys, including an analysis of the chemical properties of soil, are necessary.

4. In order to preserve the analyzed locality of *Trollius europaeus,* it should be given the status of an ecological area and principles of sustainable land management should be established.

5. The meadow in which the locality of *Trollius europaeus* was found should be mowed periodically. Trees and shrubs should be removed, and the current water relations should be maintained.

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ASSEMBLAGES OF EPIGEIC *CARABIDAE* (*COL.*) IN A PEATBOG NATURE RESERVE SITUATED IN AN URBAN AREA

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K e y w o r d s: *Carabidae*, peatbog fauna, index species.

A b s t r a k t

This paper presents the species composition and number of epigeic carabid beetles (*Col.*, *Carabidae*) inhabiting Redykajny Peatbog Nature Reserve (UTM DE65). The reserve is one of Europe's largest forest complexes growing in town. Carabid beetles were captured in 2004–2005 at three sites in the nature reserve: on a margin of high bog, in a bog birchwood in the middle of Redykajny Reserve, and in a spruce wood, part of a humid mixed forest. Based on the quantity and quality composition of the populations of *Carbidae*, the authors made an attempt to evaluate the present state of the habitat. The results are at the same time the first inventory of epigeic *Carabidae* which occupy Redykajny Reserve.

ZGRUPOWANIA EPIGEICZNYCH *CARABIDAE* (*COL.*) REZERWATU TORFOWISKOWEGO ZLOKALIZOWANEGO W OBRĘBIE AGLOMERACJI MIEJSKIEJ

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Abstrakt

W przedstawionym opracowaniu autorzy prezentują skład gatunkowy i liczebność zgrupowań epigeicznych biegaczowatych (*Col., Carabidae*) rezerwatu torfowiskowego „Redykajny" (UTM DE65). Jest on częścią jednego z największych w Europie kompleksów leśnych zlokalizowanych w obrębie aglomeracji miejskiej. Odłowy *Carabidae* prowadzono w latach 2004–2005 w trzech stanowiskach rezerwatu: w strefie brzegowej torfowiska wysokiego, w brzezinie bagiennej występującej w środkowej części torfowiska oraz w borze świerkowym na siedlisku lasu mieszanego wilgotnego. W pracy przedstawiono próbę oceny kondycji badanego siedliska na podstawie analizy jakościowej i ilościowej zgrupowań *Carabidae*. Uzyskane wyniki są też pierwszą próbą inwentaryzacji epigeicznych *Carabidae* zasiedlających rezerwat „Redykajny".

Introduction

Urban areas are a permanent and important component of landscape. In the present-day spatial zoning, the main function of urban areas is to fulfill all needs of local residents as well as to provide good care of green areas. According to forecasts and current trends, the number of people living in towns and cities will continue to grow. Thus, the total area of urbanized land will also increase. This usually means inclusion of lands, often forested ones, which in proximity to urban areas. The current principles of spatial zoning for urban areas, which combine aspects of urbanization and ecology, presume that while such lands will be developed to provide man with housing, recreational and other facilities, great care will be taken to protect valuable plant assemblages (NIEMELA 1999). Implementation of such development plans will lead to the creation of small wooded areas, which will be like small oases and become a natural haven for many species, which will find suitable conditions for growth and expansion to nearby areas. Preservation of wooded areas in an urban spatial structure is often associated with an attempt to maintain the original character of a given area and to conserve valuable natural habitats.

Redykajny Peatbog Nature Reserve, which lies within the urban area of Olsztyn, is a part of the largest forest complexes in Europe located in town (DZIEDZIC 1998). Close vicinity of a town means that both plant and animal assemblages present in the reserve are under strong man-made pressure. The presence of transportation trails, forestry management, pollution of water and air – they all have an adverse effect on the peatbog habitat and can disturb its natural balance. Evaluation of the conditions and changes which occur in such natural areas can be based on the analysis of index species (RAINIO, NIEMELA 2003). Such species should reflect the intensity of abiotic and biotic factors which affect the habitat and should indicate the environmental changes which influence the habitats, the local species and the whole ecosystem (MCGEOCH 1998). Carabid beetles (*Col*., *Carabidae*) are often regarded as such index species (CZECHOWSKI 1981, SKŁODOWSKI 2001, SZYSZKO 2002). The objective

of the present study has been to assess the species composition and structure of land assemblages of carabid beetles dwelling in Redykajny Peatbog Nature Reserve. Another aim has been to assess the state of this assemblage of beetles. The results obtained during our tests will also be an initial attempt at creating an inventory of epigeic *Carabidae* which inhabit Redykajny Reserve.

Study Area and Methods

The carabid beetles were captured in the peatbog nature reserve located in the area of Olsztyn called Redykajny, which is situated in the south-western part of the Municipal Forest, on the northern outskirts of the town (UTM DE 65). The study covered three sites within the reserve:

Site I (S-I) – the marginal zone of high bog;

Site II (S-II) – a bog birchwood (assemblage of *Betula pubescens – Telypteris palustris*) growing in the middle of the peatbog. This wooded area is subboreal in character, which lies in north-eastern Poland;

Site III (S-III) – a spruce wood in a mixed humid forest.

Specimens of epigeic carabid beetles were captured in Redykajny Nature Reserve in the years 2004 (from 24th May to 24th October – 153 days) and 2005 (from 16th May to 25th October – 163 days). The beetles were caught into Barber traps. In each year, at three different sites in the reserve, 9 traps (3 traps at each site) were placed. Every trap, a plastic container measuring 90 mm in diameter and 1300 mm in height, was placed in ground so that the opening was at the level of undergrowth. The containers were half filled with 4% solution of formalin. The insects were removed from the traps every 10 days and preserved in 75% alcohol. The material was determined using species identification keys (HŮRKA 1996, PAWŁOWSKI 1974). The dominance structure was presented according to the model suggested by GÓRNY, GRÜM (1981), in which the following dominance classes were distinguished: superdominants (> 30%), eudominants (30–10%), dominants (10–5.1%), subdominants (5–2.1%), recedents (2–1.1%) and subrecedents (≥ 1%).

Results and Discussion

The peatog in Redykajny, an ecologically valuable habitat, has been given a complex description of its floral composition in the works of STEFFEN (1931), KOBENDZA (1949), POLAKOWSKI (1968), DZIEDZIC (1998). Studies on the fauna inhabiting this area were carried out in the 1950s and 1960s by WENGRIS (1962) and MIKOŁAJSKI (1962), and focused on the presence of ants and true

bugs (*Heteroptera*). Recognition of the species composition of other taxonomic groups of insects living on the bog at Redykajny will certainly be a valuable contribution to our knowledge on the presence of rare species, which will accord with the guidelines of the UE programme Nature 2000.

During our two-year-long observations, we captured 1905 specimens of *Carabidae*, which belonged to 49 species and 20 genera (Table 1). The number of the species caught was not very high as it represented about 20% of the species of this family which occur in north-eastern Poland (BURAKOWSKI et al. 1973, 1974, ALEKSANDROWICZ et al. 2003). This may have been due to two reasons: firstly, the insects were caught exclusively into ground traps and secondly, the sites were characterized by low natural diversity. The highest number of carabids (1223 specimens) were captured at a spruce forest. This site was adjacent to the high bog, yet it lay at a higher elevation and the undergrowth was less moist. This site was also the place were the biggest number of *Carabidae* species was captured (Table 1). A slightly smaller number of species was obtained from the marginal zone of high bog. However, the number of *Carabidae* specimens caught here was half the number of species obtained from a spruce wood in a mixed humid forest. The lowest species richness as well as the smallest number of individuals were determined at the area overgrown with a bog birchwood, situated in the centre of the nature reserve. This was most likely caused by the character of the site, where groundwater remained on a high level throughout the whole vegetative season.

Table 1

Species composition and number of specimens of *Carabidae* in Redykajny Nature Reserve in 2004–2005

	Species	2004			2005		
		S-I	S-II	S-III	S-I	S-II	S-III
1	2	3	4	5	6	7	8
1	*Amara aenea* (De Geer, 1774)						0.13
2	*Amara brunnea* (Gyllenhal, 1810)	0.25		5.78	0.47		0.26
3	*Amara communis* (Panzer, 1797)						0.13
4	*Amara plebeja* (Gyllenhal, 1810)		3.23				
5	*Amara similata* (Gyllenhal, 1810)	0.25					
6	*Bembidion lampros* (Herbst, 1784)						0.13
7	*Calathus fuscipes* (Goeze, 1777)				0.47		
8	*Calathus micropterus* (Duftschmid, 1812)	0.25		0.67			0.39
9	*Carabus arvensis* Herbst, 1784			1.56	0.47		0.39
10	*Carabus cancellatus* Illiger, 1798					2.33	
11	*Carabus convexus* Fabricius, 1775	0.50		2.22			0.65
12	*Carabus glabratus* Paykull, 1790	3.27	12.90	4.89	2.84	2.33	5.17
13	*Carabus granulatus* Linnaeus, 1758	16.62	3.23	9.11	16.59		1.29

cont. table 1

1	2	3	4	5	6	7	8
14	*Carabus hortensis* Linnaeus, 1758	17.88	3.23	26.89	35.55		63.78
15	*Carabus nemoralis* O.F. Müller, 1764			0.22	0.47		0.39
16	*Carabus violaceus* Linnaeus, 1758	1.26	3.23	1.56	1.42	6.98	1.16
17	*Cychrus caraboides* (Linnaeus, 1758)	1.51	3.23	1.56	1.90		0.78
18	*Dyschirius globosus* (Herbst, 1784)				0.95		
19	*Epaphius secalis* (Paykull, 1790)				0.47		
20	*Europhilus fuliginosus* (Panzer, 1809)	3.27		0.89	2.37		
21	*Harpalus griseus* (Panzer, 1797)	0.25					
22	*Harpalus latus* (Linnaeus, 1758)		3.23	0.22		2.33	
23	*Harpalus quadripunctatus* Dejean, 1829	0.25	3.23		0.47		0.26
24	*Harpalus rufipes* (De Geer, 1774)						0.91
25	*Leistus ferrugineus* (Linnaeus, 1758)			0.22			
26	*Leistus terminatus* (Hellwig, 1973)			0.22			
27	*Loricera pilicornis* (Fabricius, 1775)	0.25			0.47		0.13
28	*Microlestes maurus* (Sturm, 1827)						0.26
29	*Nebria brevicollis* (Fabricius, 1792)	0.25		1.11			1.16
30	*Notiophilus bigutatus(Fabricius, 1779)*			0.22			0.26
31	*Notiophilus palustris* (Duftschmid, 1812)			0.22			
32	*Oxypselaphus obscurus* (Herbst, 1784)	3.27			3.32		
33	*Patrobus atrorufus* (Stroen, 1768)	8.06		0.67	3.32		1.81
34	*Platynus assimilis* (Paykull, 1790)			0.00	0.47		
35	*Poecilus cupreus* (Linnaeus, 1758)		6.45	0.44			
36	*Poecilus versicolor (*Sturn, 1824)			0.22			0.13
37	*Pterostichus aethiops* (Panzer, 1797)			0.22	2.84	4.65	0.39
38	*Pterostichus anthracinus* (Illiger, 1798)	0.25			0.47		
39	*Pterostichus diligens* (Sturm, 1824)				0.47	2.33	0.13
40	*Pterostichus melanarius* (Illiger, 1798)	13.10		3.11	2.37		2.33
41	*Pterostichus minor* Gyllenhal, 1827	0.25			0.47		
42	*Pterostichus niger* (Schaller, 1783)	18.39	51.61	20.00	10.90	62.79	11.77
43	*Pterostichus nigrita* (Paykull, 1790)	0.50		0.22	0.47		0.39
44	*Pterostichus oblongopunctatus* (Fabricius, 1787)	6.55		12.89	4.27		4.14
45	*Pterostichus quadrifoveolatus* Letzner, 1852	1.76	3.23	2.89	0.95	4.65	1.16
46	*Pterostichus rhaeticus* Heer, 1838				0.47		
47	*Pterostichus strenuus* (Panzer, 1797)	1.01	3.23	1.56	4.27	11.63	0.13
48	*Pterostichus vernalis* (Panzer, 1796)	0.50					
49	*Stomis pumicatus* (Panzer, 1796)	0.25		0.22			
	Number of species	26	12	28	28	9	29
	Number of individuals	397	31	450	211	43	773
		878			1027		
	Shannon's coefficient J' (Pielou)	0.727	0.716	0.703	0.701	0.621	0.457
	Simpsons Diversity (D)	0.123	0.273	0.145	0.171	0.405	0.426
	Shannon Wiener's coefficient H'	2.368	1.779	2.342	2.336	1.363	1.538

The biggest divergence between the contribution of dominant species and the whole assemblage, as determined through an analysis of the dominance structure, was found at a bog birchwood (Table 1). The heavily moist surface of the highmoor bog was penetrated by 16 species of *Carabidae*, dominated, in both years of the study, by *Pterostichus niger*, which was classified as a superdominant species. In 2004, the second most numerous species in the birchwood was *Carabus glabratus* (12.9%), which was the only representative of the eudominant class. The group of dominants also consisted of a single species – *Poecilus cupreus*. The remaining 9 species belonged to subdominants. In the second year of the study, large disproportions were found between the dominance classes of *Carabidae*. Apart from *Pterostichus niger* mentioned above, *Pterostichus strenuus* (a eudominant) and *Carabus violaceus* (a dominant) occurred in large numbers. The class of dominants consisted of 6 species. In neither of the years was it possible to distinguish the class of recedents and subrecedents, which was most probably due to a small number of *Carabidae* specimens captured in this area. The edge of the highmoor bog was characterized by a uniform distribution of the dominance classes in both years, which is typical of properly developed ecosystems (TROJAN 1998). In 2004, the eudominants consisted of 4 species (*Pterostichus niger*, *Carabus hortensis*, *Carabus granulatus* and *Pterostichus melanarius*) and the dominants comprised 2 species (*Patrobus atrorufus* and *Pterostichus oblongopunctatus*). The remaining species caught in that year were classified as subdominants (3 species), recedents (4 species) and subrecedents (13 species). In the subsequent year, certain changes occurred in the pattern of dominance classes observed in the marginal zone of the bog. A superdominant species was found (*Carabus hortensis* – 35.55%). On the other hand, no species belonging to the dominant class were identified.

In 2004, the class of eudominants in the spruce wood consisted of three species: *Carabus hortensis* (26.89%), *Pterostichus niger* (20.00%) and *Pterostichus oblongopunctatus* (12.89%). The two former species were also classified as eudominants in the marginal zone of the high bog. The distribution of the other dominance classes of carabid beetles captured in the spruce wood was quite even – there were dominants (*Carabus granulatus* and *Amara brunnea*), subdominants (*Carabus glabratus*, *Pterostichus melanarius*, *Pterostichus quadrifoveolatus*, *Carabus convexus*), recedents (5 species) and subrecedents (15 species). The assemblages of carabids inhabiting this area in the following year were characterized by uneven distribution of dominance classes. This could have been caused by some forest care measures (tree felling), which were undertaken to protect the habitat from *Ips typographus*. This was the most likely reason why *Carabus hortensis* dominated (at 63.78% it became a superdominant species). The classes of eudominants and dominants were represen-

ted by single species: *Pterostichus niger* (11.77%) and *Carabus glabratus* (5.17%). Absence of *P. melanarius* on the high bog and its lower contribution, as a dominant species, at the other two sites may suggest that, unlike *P. niger*, this species prefers more abundant habitats (SKŁODOWSKI, POROWSKI 2000). At sites in the marginal zone of the high bog and in the spruce forest, situated higher than the bog, *Carabus hortensis* was a dominant species. This large zoophagous insect is one of the most frequently occurring species in European forests. In the marginal zone of the bog, other commonly captured carabids were *Carabus granulatus*, *Patrobus atrorufus* and *Pterostichus oblongopunctus*. Besides, in the spruce forest surrounding the bog, the dominant species also comprised *Carabus glabratus*, a mesophilous species, typical of various types of forests (ALEKSANDROWICZ 1991). ALESKANDROWICZ, KRZĘTOWSKI (2004), who studied assemblages of beetles on the banks of the Łyna River in close vicinity to Redykajny Nature Reserve, determined a similar composition of dominant carabid species.

Our analysis of the trophic structure and habitat penetration by carabids at the three sites suggests that the investigated habitats are in good condition. Zoophagous species dominated, both in terms of the quality (over 80%) and quantity (over 94%) – Table 2. Over 60% of the carabid species captured during the study were represented by the forms specific to forested and boggy areas, and the percentage of such species tended to be higher when the counts of specimens captured were analysed (Table 2). What may give rise to some concern is a rather high numerical proportion of (27.78%) of the species specific to open land determined at a spruce wood in a mixed humid forest, even though those species constituted barely 2.78% of the total count of the captured *Carabidae*. The occurrence of open land species at a spruce wood was most likely caused by the vicinity of felling areas in the forest, where those insects found favourable growth and development conditions. What is interesting in our study is the lack of tyrphobiontic species, typical of high moors (ALEKSANDROWICZ 2004). The hygrophilous species we found are actually tryphophilous, i.e. species specific for moist areas, which also occur in peat bogs. The three sites at Redykajny Reserve which we studied were different from one another in the soil moisture content. The moisture gradient ran from a bog birchwood, where the groundwater level was very high, through the marginal zone of the high bog, to the highest site – a spruce wood. As THIELE (1977) suggests, soil moisture content is one of the major abiotic factors that affect the species composition and structure of carabid assemblages. The area we analysed was dominated, both in terms of species composition and counts of beetles, by mesophilous species characterised by a wide range of tolerance to changeable moisture conditions (Table 2). A small percentage, both quantitatively and qualitatively, was made up of species associated with dry habitats.

Typical xerophilous species, such as *Amara aenea* and *Harpalus griseus*, occurred incidentally, most probably because of their ability to fly (ALEKSANDROWICZ 1991, HŮRKA 1996). Quite a numerous group of beetles in the whole assemblage of carabids comprised species which come in direct contact with water habitats (hygrophilous species) and species which prefer high moisture content habitats albeit not wet ones (mesohygrophilous species). In the high bog and the adjacent marginal zone, these species represented 37.14% and

Table 2
Ecological description of epigeic *Carabidae* captured in Redykajny Nature Reserve in 2004–2005

Ecological description	Qualitative aspect						Quantitative aspect					
	S-I		S-II		S-III		S-I		S-II		S-III	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Trophic structure												
Large zoophages	11	31.43	7	43.75	11	30.56	442	72.70	56	75.68	1012	82.75
Medium zoophages	13	37.14	4	25.00	12	33.33	103	16.94	7	9.46	151	12.35
Small zoophages	6	17.14	2	12.50	6	16.67	39	6.41	7	9.46	16	1.31
Hemizoophages	4	11.43	2	12.50	6	16.67	23	3.78	3	4.05	43	3.52
Phytophages	1	2.86	1	6.25	1	2.78	1	0.16	1	1.35	1	0.08
Total	**35**	**100**	**16**	**100**	**36**	**100**	**608**	**100**	**74**	**100**	**1223**	**100**
Habitat preferences												
Forest species	15	42.86	8	50.00	16	44.44	351	57.73	59	79.73	1059	86.59
Open area species	6	17.14	3	18.75	10	27.78	16	2.63	4	5.41	34	2.78
Peatbog species	8	22.86	3	18.75	6	16.67	161	26.48	8	10.81	79	6.46
Eurytopic species	6	17.14	2	12.50	4	11.11	80	13.16	3	4.05	51	4.17
Total	**35**	**100**	**16**	**100**	**36**	**100**	**608**	**100**	**74**	**100**	**1223**	**100**
Hygropreferences												
Xerophilic	1	2.86	0	0.00	1	2.78	1	0.16	0	0.00	1	0.08
Mesoxerophilic	4	11.43	1	6.25	4	11.11	13	2.14	1	1.35	49	4.01
Mesophilic	17	48.57	10	62.50	23	63.89	388	63.82	62	83.78	1086	88.80
Mesohygrophilic	3	8.57	3	18.75	2	5.56	116	19.08	8	10.81	59	4.82
Hygrophilic	10	28.57	2	12.50	6	16.67	90	14.80	3	4.05	28	2.29
Total	**35**	**100**	**16**	**100**	**36**	**100**	**608**	**100**	**74**	**100**	**1223**	**100**
Zoogeographical elements												
Holarctic	3	8.57	0	0.00	2	5.56	24	3.95	0	0.00	29	2.37
Palaearctic	19	54.29	9	56.25	20	55.56	104	17.11	20	27.03	183	14.96
Euroarctic	2	5.71	2	12.50	3	8.33	29	4.77	6	8.11	76	6.21
Euro-Siberian	6	17.14	3	18.75	6	16.67	296	48.68	45	60.81	298	24.37
Euro-Mediterranean	2	5.71	0	0.00	2	5.56	2	0.33	0	0.00	15	1.23
European Forest Province	3	8.57	2	12.50	3	8.33	153	25.16	3	4.05	622	50.86
Total	**35**	**100**	**16**	**100**	**36**	**100**	**608**	**100**	**74**	**100**	**1223**	**100**
Phenology												
Spring species	21	60	10	62.5	21	58.33	225	37.01	18	24.32	229	18.72
Autumn species	14	40	6	37.5	15	41.67	383	62.99	56	75.68	994	81.28
Total	**35**	**100**	**16**	**100**	**36**	**100**	**608**	**100**	**74**	**100**	**1223**	**100**

31.25%, respectively, of all the species captured. Hygrophilous species were most numerous (both the quality and quantity composition) in the marginal zone of the high bog. The species which inhabited this ecotone zone are able to migrate when the level of groundwater changes.

The fauna of *Carabidae* in Redykajny peatbog was represented by six zoogeographical elements (LEŚNIAK 1987). The highest numbers of carabids captured belonged to the Palaearctic and Euro-Siberian elements. They represented 55% and 18%, respectively, of all the species captured at the three sites (Table 2). Similar results were reported by KOSEWSKA (2004), who studied assemblages of epigeic *Carabidae* populating groups of trees on fields near Olsztyn. The results reported by BROWARSKI (2005), who described ground carabids beetles in Trofiaki Nature Reserve near Olsztyn, proved that such assemblages were mainly composed of species representing the Euro-Siberian and Holarctic types. However, the analysis of the quantity aspect shows that the assemblages of carabids in Redykajny Nature Reserve were dominated by individuals specific to the Euro-Siberian region and European Forest Province. The Euro-Siberian species, which are associated with the sphere of coniferous forests, occurred in Poland via migration from northern Siberia. A slightly different set of species was discovered at a bog birchwood, where the most numerous were the specimens which belonged to the Euro-Siberian (with *P. niger* being a dominant species) and Palaearctic elements. The spruce wood was most numerously inhabited by individuals which are specific to to the European Forest Province, with the superdominant species – *Carbus hortensis*.

Some information on the state of biocensosis can be derived from an analysis of the percentages of autumn and spring carabid beetles. GRÜM (1976) believes that autumn species are characterised by a higher contribution of assimilated biomass built into their biomass, as well as a lower rate of transmitting the biomass to higher trophic levels as compared to the species which belong to the spring development type. At the three sites in Redykajny Reserve, spring species were dominant (Table 2). However, the analysis of the numbers of carabid species captured implied that autumn species prevailed. In the marginal zone of the peatbog, autumn species represented 76% of *Carabidae* specimens; this proportion grew to 76% at the high bog and up to 82% in the spruce wood. The dominance of autumn species is characteristic of older woods and forests (FLIS, SKŁODOWSKI 1998). Spring species are more often encountered when beetles colonise new areas, including wooded lands, while autumn species are more common in thick woods. The high percentage of autumn species determined at the high bog, free from trees, resulted from the mass occurrence of *Pterostichus niger* and *Carabus glabratus*. These species are associated with wood habitats but can also be present in open areas adjacent to forests (LINDROTH 1985, SKŁODOWSKI 2002).

The biocenotic diversity of assemblages of beetles can be evaluated by using indices of the structure of assemblages (GÓRNY, GRÜM 1981). The Shannon--Wiener index of diversity (H') was the highest in both years for the carabids captured on the edge of the highmoor bog (S-I) and in the spruce wood. Compared to the data cited by other researchers, this index was rather high (CZECHOWSKI 1989, HURUK 1993). The species diversity determined for the bog birchwood was lower (1.779 in 2004 and 1.363 in 2005). The Shannon-Wiener index increases as the number of species in a given assemblage rises and the species occur in similar numbers of individuals. Simpson index (D), on the other hand, attaches less importance to rare species, infrequently found in a sample. The highest values of D index were reported for the carabid assemblages inhabiting the surface area of the birchwood (both years) and the spruce wood (2005) – Table 1. Pielou index of evenness (J') describes the ratio of actual species diversity of a given assemblage to the maximum attainable value. In 2004, all the examined assemblages of carabids were characterized by similar values of the evenness index. In the following year, the value of this index was similar to that obtained for the carabids inhabiting the marginal zone of the high bog and the bog birchwood, but it went down in the case of the spruce wood. This was most probably associated with the forest trimming treatments carried out in the spruce wood to protect the tree stand.

The similarity between the assemblages of *Carabidae* at the three sites was tested using Bray-Curtis formula, i.e. a group similarity dendogram (Figure 1). The dendogram presented in this paper shows two major clusters (30% similarity), which group the species living in the birchwood and on the edge of the high bog or in the spruce wood. The first cluster comprises the assemblages from the birchwood in both years of the research project, whereas

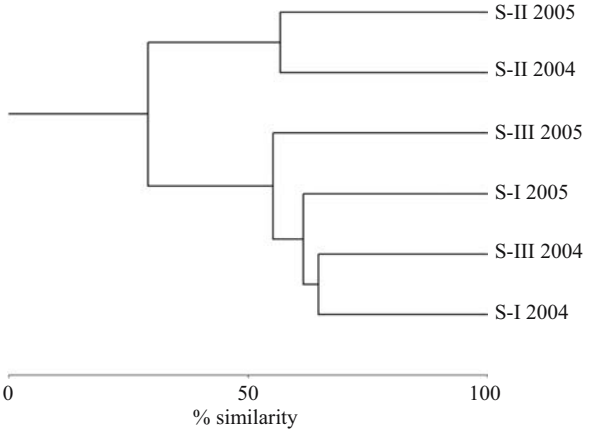

Fig. 1. Dendogram of similarity for the *Carabidae* assemblages at the three sites in Redykajny Nature Reserve analysed in 2004–2005

the other one encompasses the margin of the highmoor bog and the spruce wood. The first cluster reveals similarities between carabid assemblages depending on the year of the study.

Conclusions

1. During our two-year-long observations we captured 1 905 specimens of *Carabidae*, which were classified to 49 species. The highest number of carabid individuals and species was caught in the spruce wood at mixed humid forest.

2. The group of dominant species (*Carabus hortensis*, *Pterostichus niger*, *C. granulatus* and *P. oblongopunctatus*) made up 70% of all carabid species captured at the three sites, which may be indicative of certain disproportion between the most numerous species and the remaining carabid beetles in the assemblages. Assemblages of epigeic *Carabidae* inhabiting Redykajny Nature Reserve are characterised by a large proportion of wildly distributed species (Palaearctic and Euro-Siberian ones).

3. Forest and peatbog zoophagous beetles were dominant in the area of Redykajny Nature Reserve, and those are the species expected and preferred to occur in boggy areas. In addition, a high proportion of autumn species was discovered, which is characteristic of stable ecosystems.

4. Among the species of the family *Carabidae* discussed above, forest species (specific for mixed and riparian forests) including some tryphophilous species, characteristic for open wetlands, are dominant. Unfortunately, there are no stentobiotic peatbog species, which are valuable in natural environments.

5. The presence of a numerous group of mesophilous and mesoxerophilous species (*Harpalus rufipes*, *Harpalus griseus*, *Calathus fuscipes*, *Bembidion lamparos*, *Poecilus cupreus*, *Poecilus versicolor*, *Microlestes maurus*, *Amara aenea*, *Amara communis*, *Amara similata*) in the area under the investigation may suggest an early onset of anthropogenic succession, which causes degradation of the highmoor bog.

6. Several specimens of *Carabus convexus*, which in Poland is considered as a threatened species, were captured in Redykajny Nature Reserve.

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APPLICATION OF MITOCHONDRIAL DNA IN THE IDENTIFICATION OF DIVERSE CRAYFISH SPECIES

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K e y w o r d s: crayfish, indigenous and introduced species, RFLP marker, *cox1* gen.

A b s t r a c t

The populations of two indigenous Polish species, the noble crayfish (*Astacus astacus)* and the narrow-clawed crayfish (*Astacus leptodactylus*), were gradually reduced over the 20th century by extensive water pollution, the subsequent eutrophication over time of lakes and rivers, and the epizooty plague caused by the fungus *Aphanomyces astaci.* The crayfish populations were also adversely affected by the introduction of the American crayfish in 1890 (*Orconectes limosus*) and the signal crayfish (*Pacifastacus leniusculus*) in the late 1960s.

This work aims at finding molecular markers that would permit objective identification of crayfish species. This could facilitate monitoring the distribution and protection of crayfish species, both in Poland and throughout the world. To this end, the PCR-RFLP technique was used for the analysis of the mitochondrial *coxl* gene, using 5 restriction enzymes. The best molecular marker was the restriction enzyme *Alu*I, which proved efficient in differentiating all the studied crayfish. Five unique genotypes that occurred in a single species were observed, with most of them in the enzyme *Alu*I, which therefore may be regarded as a species-diagnostic marker.

WYKORZYSTANIE MITOCHONDRIALNEGO DNA W IDENTYFIKACJI RÓŻNYCH GATUNKÓW RAKÓW

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S ł o w a k l u c z o w e: raki, gatunki rodzime i introdukowane, marker RFLP, gen *cox1.*

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Abstract

Rak szlachetny (*Astacus astacus*) i rak błotny (*Astacus leptodactylus*) należą do rodzimych gatunków raków, których populacje pod względem ilości i liczebności ulegają systematycznemu zmniejszeniu od XIX w. Przyczyną tego jest postępująca eutrofizacja wód i ich zanieczyszczenie, powtarzające się epizoocje dżumy raczej oraz intensywne zasiedlanie stanowisk występowania raków przez dwa introdukowane z Ameryki Północnej gatunki, raka pręgowatego (*Pacifastacus leniusculus*) i sygnałowego (*Orconectes limosus*). Program ochrony i restytucji raków w Polsce wymaga opracowania skutecznych metod ich identyfikacji gatunkowej na różnych etapach rozwoju osobniczego. Przeprowadzone w pracy analizy PCR-RFLP dla genu *cox1* u czterech gatunków raków (*A. astacus*, *A. leptodactylus* oraz *P. leniusculus, O. limosus*) pozwoliły na opracowanie markerów RFLP do identyfikacji taksonomicznej. Najbardziej skuteczny okazał się enzym restrykcyjny *Alu*I, którego efekt trawienia jest diagnostyczny dla analizowanych gatunków. Z czterech badanych gatunków raków najłatwiejszy do identyfikacji okazał się *P. leniusculus* posiadający największą liczbę genotypów unikalnych.

Introduction

Base on body size one of the largest groups of invertebrates in Poland are the indigenous crayfish species, the noble crayfish, *Astacus astacus* (Linnaeus 1758), and the narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz 1823. They are very sensitive bioindicators of changes in the water environment due to their low tolerance to any alterations in environmental factors (GONDKO et al. 1992). Over the last two decades they have diminished in numbers in Polish lakes and rivers (STRUŻYŃSKI, ŚMIETANA 1999, MASTYŃSKI, ANDRZEJEWSKI 2001, ŚMIETANA 2001, ŚMIETANA et al. 2004, SCHULZ et al. 2006a). One of the reasons for the decrease in the crayfish populations is the contamination of waters with the pathogenous fungus *Aphanomyces astaci,* which causes crayfish plague (LEŃKOWA 1962, MASTYŃSKI, ANDRZEJEWSKI 2001). The situation is worsened by high pollution in waters and the subsequent increase in the eutrophication of lakes and rivers. Also, up until recently, water bodies in Poland were intensely stocked with large amounts of eel, a major predator of crayfish (LEŃKOWA 1962, MASTYŃSKI, ANDRZEJEWSKI 2001, SCHULZ et al. 2006a).

The gradual extinction of the crayfish was initially caused by the introduction of the non-indigenous spiny-cheek crayfish *Orconectes limosus* (Rafinesque 1817) in 1890, then the signal crayfish *Pacifastacus lenisculus* (Dana 1852) in the 1960s (KOSSAKOWSKI 1973, MASTYŃSKI, ANDRZEJEWSKI 2001). Both species, introduced from North America, are highly prolific, highly tolerant to environmental changes, and, most significantly, are carriers of the *A. astaci fungus* (PERSSON, SÖDERHÄLL 1983, PIEROŻYŃSKI 1951, STRUŻYŃSKI, ŚMIETANA 1999). Thus, both introduced species are very effective in the competitive exclusion of indigenous crayfish from their habitats. (STRUŻYŃSKI, ŚMIETANA 1999, MASTYŃSKI, ANDRZEJEWSKI 2001, ŚMIETANA 2001, MAIWALD et al. 2004, SCHULZ et al. 2006a).

Some scientists proposed that the protection of rare populations of the indigenous crayfish should be carried out by restocking the species and by inhibiting the spread of the epizooty crayfish plague, the latter by preventing the introduction of spiny-cheek crayfish and signal crayfish into new waters (LEŃKOWA 1962, STRUŻYŃSKI, ŚMIETANA 1999, ŚMIETANA 2001, SCHULZ et al. 2006a).

In order to monitor the distribution of species, it is necessary to be able to quickly identify them, both adults and especially young individuals, as their diagnostic characteristics are not fully developed yet. To that end, new molecular methods have been used for a few years now in invertebrate taxonomy (FOLMER et al. 1994, BALDWIN et al. 1996, FALNIOWSKI, WILKE 2001, SOROKA, GRYGIEŃCZO-RAŹNIEWSKA 2005).

The aim of this study is to determine the molecular markers for the objective identification of various species of crayfish. This, in turn, should facilitate their redistribution and protection, both in Poland and throughout the world.

Material and Methods

Material

The study involved four crayfish species – the noble crayfish (*Astacus astacus*), the signal crayfish (*Pacifastacus leniusculus*), the narrow-clawed crayfish (*Astacus leptodactylus*) and the spiny-cheek crayfish (*Orconectes limosus*) collected from Polish lakes, with the noble crayfish collected in Norway. The collection sites, dates, and the collecting scientists are provided in Table 1. A detailed description of the occurrence of the *A. astacus* in Graniczne Lake can be found in the study by SCHULZ et al. 2006a. Five specimens were considered for each species from Poland, apart from the narrow-clawed crayfish (2 specimens) and *A. astacus* from Norway (3 specimens).

Table 1

Characteristics of the collection sites

Species	Collection site	N	Collection date	Collecting scientist
Astacus astacus	Poland, Pomeranian voivodship, Graniczne lake	5	August 2000 and December 2001	P. Śmietana and A. Marczyński
	Nowary, Häresjön lake	3	August 2001	M. Świerczyński
Pacifastacus leniusculus	Poland, Pomeranian voivodship, Graniczne lake	5	December 2001	A. Marczyński
Astacus leptodactylus	Poland, Wielkopolskie voivodship, Gaj lake	2	March 2002	M. Świerczyński
Orconectes limosus	Poland, West Pomeranian voidvodship, Spore lake	5	September 2000	M. Soroka

DNA extraction

Genomic DNA was extracted from 3 mm × 3 mm muscle tissue fragments, which were removed with a scalpel and transferred onto microscopic slides; the tissue fragments were then placed in the homogenisation buffer solution STE 100 (0.1 M NaCl, 0.1 M EDTA, 0.05 M Tris-HCl, pH 8.0) and SDS (10% sodium dodecyl sulphate). The nucleic acid was purified by standard phenol/chloroform extraction. The DNA was precipitated in 96% ethanol; the precipitate was then washed with 70% ethanol, dried, and dissolved in a TE buffer (1mM EDTA, 0.01 M Tris-HCl, pH 8.0). The DNA extraction was carried out in sterile conditions.

Polymerase Chain Reaction (PCR)

DNA amplification was carried out with the primers LCO1490 and HCO2198, complementary to the fragment of the *cox1* gene (FOLMER et al. 1994). These primers produce a fragment of DNA approximately 730 bp long. The PCR reaction was carried out in a volume of 20 µl, consisting of: 0.8 to 1 µl of the isolated DNA as well as 0.5 units of *Taq* polymerase (Fermentas), 2 µl of 10 × buffer, 50 µM of nucleotide mixture, 5 pmol of each of the two primers, and 12.1 µl of sterilized distilled water. The final concentration of $MgCl_2$ in the PCR reaction was 3 mM. After initial denaturation (at 95°C for 90 secs), PCR was carried out for 5 cycles: denaturation at a temperature of 95°C (for 30 secs), annealing primers at 45°C (for 1 min) and extension at 72°C (for 90 secs). Subsequently, 27 cycles were run at the denaturation temperature of 95°C (for 30 secs), an annealing temperature of 55°C (for 45 secs), and an extension temperature of 72°C (for 1 min, but 7 min in the final cycle). PCR products were checked by electrophoresis in 2% agarose gel containing ethidium bromide and a TBE buffer (pH 8.0); the gels were visualized under UV.

Restriction endonuclease digestion

To perform the restriction analysis of the amplified *cox1* gene fragment, the following six restriction endonucleases were used: *Alu*I, *Bam*HI, *Bsi*ZI, *Csp*6I, *Eco*RI and ScrFI. The restriction enzyme digests consisted of 3 µl of the PCR product, 1 µl of 10 × buffer, 4 U of an enzyme, and 5.6 µl of sterilised distilled water. The reaction was run for 12 h at temperatures appropriate for each restriction enzyme. Subsequently, the whole reaction volume was loaded on 2% agarose gel with ethidium bromide. Electrophoresis was carried out for 2 h at

60 V in standard a TBE buffer. The gels were visualised under UV using BioCapt MW (Vilber Lourmat, France). The results were analysed with the computer programme Bio1D (Vilber Lourmat, France).

Results

The applied primers amplified fragments of the *cox1* gene with identical products, about 730 bp long, in all the analysed species. RFLP with different restriction enzymes applied to the previously amplified *cox1* gene fragments resulted in a similar total length of the restriction fragments, in terms of the number of base pairs, to those obtained as a result of amplification (about 730 bp). Certain discrepancies could have resulted from very small DNA fragments that were formed as a result of DNA digestion with restrictases and did not appear on the gel during visualization. When the endonuclease did not recognize any restriction site in the analysed fragment of the *cox1* gene, an identical genotype A with a length of 730 bp was observed (Table 2, Figure 1).

Table 2

Approximate sizes (bp) of mitochondrial *cox1* gene fragments generated by five restriction enzymes, and the obtained genotypes (letters) in four crayfish species

Species	*Alu*I	*Bam*HI	*Bsi*ZI	*Eco*RI	*Scr*FI	Multigenotype number
Astacus astacus	Genotype **K** 350 200 130	Genotype **A** 730	Genotype **A** 730	Genotype **A** 730	Genotype **B** 640 100	1
Pacifastacus leniusculus	Genotype **G** 450 190	Genotype **D** 650 50	Genotype **A** 730	Genotype **I** 410 300	Genotype **H** 420 150 100	2
Astacus leptodactylus	Genotype **C** 600 110	Genotype **A** 730	Genotype **D** 650 50	Genotype **C** 600 110	Genotype **B** 640 100	3
Orconectes limosus	Genotype **F** 500 430 130 110	Genotype **E** 500 200	Genotype **E** 500 200	Genotype **A** 730	Genotype **B** 640 100	4
The sum of genotypes	4	3	3	3	2	

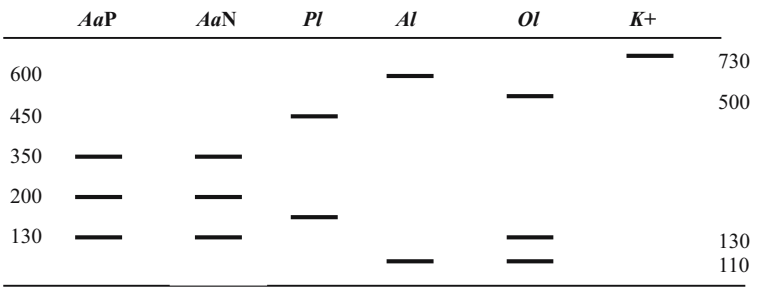

Fig. 1. Schematic diagram of fragment banding patterns generated by *Alu*I. Species abbreviations: *Aa*P – *Astacus astacus* from Poland, *Aa*N – *A. astacus* from Norway, *Pl* – *Pacifastacus leniusculus*, *Al* – *A. leptodactylus* and *Ol* – *Orconectes limosus*, *K*+ – undigested PCR product. Numbers represent base pair size. Note that the y-axis is nonlinear

RFLP involving five restriction enzymes did not reveal any variability within any of the four analysed crayfish species, with 2-5 individuals per species. Each species showed an enzyme-specific restriction pattern. The *A. astacus* specimens from Poland and Norway always showed identical endonuclease-specific restriction patterns.

Restriction analysis of the mitochondrial *cox1* gene with the enzyme *Alu*I identified 4 different genotypes (C, F, G and K) within the four examined crayfish species (Table 2, Figure 1). The endonuclease revealed from one in *A. leptodactylus* to three restriction sites in the remainder of the analysed crayfish species. The restriction enzyme *Bam*HI recognized one restriction site within the analysed fragment of the *cox1* gene only in *P. leniusculus* and *O. limosus,* but located in different parts, hence different genotypes were observed, respectively D and E (Table 2). Restrictase *Bsi*ZI digested the fragment of the *cox1* gene only in *A. leptodactylus* and *O. limosus* in one site which revealed genotypes D and E, respectively (Table 2). The analysed fragments of the *cox1* gene in two analysed crayfish species (*A. leptodactylus.* and *P. leniusculus*) each have one sequence recognizable by the restriction endonuclease *Eco*RI (G/AATT), but at different sites, and the obtained genotypes were respectively C and I. The restriction enzyme *Scr*FI revealed only two genotypes in the four analysed crayfish species. The genotype B (640 and 100 bp) was identical for all three species; *P. leniusculus* had a different genotype H (Table 2). The restriction endonuclease *Csp*6I did not result in characteristic patterns which may serve as molecular markers due to a large number of short DNA fragments.

Restriction analysis involving 5 restriction enzymes revealed 10 different genotypes (letter symbols) and certain genotypes reoccurred for more than one enzyme (Table 2). The most frequent was the genotype A (0.3), present three

times in *A. astacus* and once in each of the remaining species, however never for the enzymes *Alu*I and *Scr*FI. Genotype B (400 and 100 bp) reoccurred three times (0.15) and only for the enzyme *Scr*FI. Genotype C (600 and 100 bp) was found only for *A. leptodactylus* and genotype E (500 and 200 bp) for *O. limosus,* and both of them were observed twice in each of these species. Genotypes F, G, H, I and K occurred only once (0.05) and were most numerous along with the enzyme *Alu*I and for species *P. leniusculus*. The combined analysis of the four species, involving the 5 restriction enzymes, revealed 4 various multigenotypes (Table 2).

Discussion

Genetic research of various crayfish species, especially the rare and endangered ones, strikes immense interest among scientists. The aim of this research is to determine the level of variability within a population, as well as among different populations (BUSACK 1988, FEVOLDEN, HESSEN 1989, GRANDJEAN, SOUTY-GROSSET 1997, GRANDJEAN et al. 1997a, FETZNER, CRANDALL 1999, KRANE et al. 1999, SCHULZ 2000, SCHULZ et al. 2004, 2006b, ALARANTA et al. 2006). Many scientists argue it is absolutely necessary to identify the genetic structure of crayfish before any protection and restitution programme is started. Inadequate restitution or illegal introductions may irrevocably change the genetic pools of various species (FEVOLDEN et al. 1994, GRANDJEAN et al. 1997b).

Molecular research is also applied to the identification of origins of *A. astacus* and *Austropotamobius pallipes* in European countries. It is also used for the preparation of population markers for these species (LARGIADÈR et al. 2000, EDSMAN et al. 2002, ALARANTA et al. 2006).

In the process of protection and restitution of a species, it is very important to precisely identify the species at all stages of physical development. Molecular analyses are the perfect tool for such identification.

Initial genetic research of diverse crayfish species based on protein electrophoresis showed a low level of variability between European populations of freshwater crayfish (BUSACK 1988, FEVOLDEN, HESSEN 1989, AGERBERG 1990, FEVOLDEN et al. 1994). Studies on mitochondrial DNA carried out over the last decade have revealed a higher degree of variability and are applied successfully in population studies for several taxa of freshwater crayfish (CRANDALL et al. 1995, CRANDALL, FITZPATRIC 1996, GRANDJEAN, SOUTY-GROSSET 1997, SOUTY--GROSSET et al. 1997, FETZNER, CRANDALL, 1999, GRANDJEAN et al 2000).

Contemporary genetic studies on diverse species of crayfish involve analysis of protein electrophoresis, microsatellite loci, fragments of the ITS1 and

ITS2 regions and genes 18S and 28S rDNA (CRANDALL et al. 2000, GOUIN et al. 2000, HARRIS, CRANDALL 2000, LARGIADÈR et al. 2000, ALARANTA et al. 2006). The studies on mitochondrial DNA involve RFLP (Restriction Fragment Length Polymorphism) analysis of the 12S and 16S genes and their sequencing (LARGIADÈR et al. 2000, GRANDJEAN et al. 2000, 2002).

In 1994, Folmer et al. devised universal primers for the amplification of mitochondrial cytochrome c oxidase subunit I (gene *cox1*) in a polymerase chain reaction (PCR) for 11 classes of invertebrates, including Decapoda. Since then, the mitochondrial *cox1* gene has been widely applied in phylogenetic research and taxonomic identification of morphologically varied individuals at various stages of development. The PCR-RFLP method for the *cox1* gene may be efficiently used for both adult and young mussels of the species *Dreissena polymorpha* and *D. bugensis*, and their larvae forms (CLAXTON et al. 1997, CLAXTON, BOULDING 1998). This technique may be used in the systematic identification and monitoring of distributions of various animals. This method is easy to carry out in various laboratories and yields repeatable results, which is an additional advantage (SOROKA, GRYGIEŃCZO-RAŹNIEWSKA 2005).

PCR-RFLP analyses for the *cox1* gene in four crayfish species enabled the determination of molecular markers for their taxonomic identification. The most effective marker was the enzyme *Alu*I, which enabled identification of all four species. The signal crayfish was easy to identify, as it had the highest number of unique genotypes – three. Three enzymes (*Bam*HI, *Bsi*ZI and *Eco*RI) made it possible to identify two species: the signal crayfish (*P. leniusculus*) and the narrow-clawed crayfish (*P. leptodactylus*). The least effective enzyme was *Scr*FI, which differentiated only the signal crayfish from the rest. The greatest number of common genotypes (four) were in the noble and spiny-cheek crayfish.

The crayfish *A. astacus* from Poland and Norway had identical restriction patterns in all their analysed restriction enzymes. A similar lack of variability was observed between the mussels *Dreissena polymorpha* from Poland and Ukraine, as well as between *D. bugensis* var. shallow-water and var. profunda, and identical endonuclease-specific restriction patterns were always observed (BALDWIN et al. 1996, SOROKA, GRYGIEŃCZO-RAŹNIEWSKA 2005). The mitochondrial *cox1* gene examined with PCR-RFLP and 6 restriction enzymes in 9 species of freshwater bivalves showed an absence of individual variability within each species (SOROKA, GRYGIEŃCZO-RAŹNIEWSKA 2005).

The technique applied in this study did not detect any specimen variability within the species, which only proved the validity of the PCR-RFLP method for the *cox1* gene to differentiate between species, and to identify crayfish species both in Poland and throughout the world.

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Conclusions

One of the largest groups of invertebrates in Poland are the indigenous crayfish species, the noble crayfish, *Astacus astacus* (L.), and the narrow-clawed crayfish, *Pontastacus leptodactylus* (Esch.). In many cases they have diminished in numbers in Polish lakes and rivers over the last two decades. Initially, the gradual extinction of the crayfish was caused by the introduction of the non-indigenous spiny-cheek crayfish (*Orconectes limosus* Raf.) in 1890, and then the signal crayfish in the 1960s (*Pacifastacus lenisculus* Dana). Both species, introduced from North America, are highly prolific, highly tolerant to environmental changes, and, most significantly, are carriers of the *A. astaci* fungus that causes crayfish plague in our indigenous crayfish species. Both of these introduced species are very effective in the competitive exclusion of indigenous crayfish from their habitats. The aim this study was to determine molecular markers for the objective identification of four species of crayfish. PCR-RFLP analyses for the *cox1* gene revealed that the most effective marker was the enzyme *Alu*I, which enabled identification of all the studied species. The signal crayfish was easy to identify, as it had the highest number of unique genotypes – three.

The technique applied in this study did not detect any variability within the studied species, which suggest that the PCR-RFLP method for the *cox1* gene could be useful to differentiate between species and identify crayfish species both in Poland and worldwide.

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RESISTANCE OF SOIL FUNGI TO COPPER CONTAMINATION

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K e y w o r d s: soil fungi, copper, resistance.

A b s t r a c t

Copper(II) chloride dihydrate was to the soil (heavy loamy dusty sand) in two doses until the total copper content constituted weak (80 mg kg^{-1}) and strong (500 mg kg^{-1}) soil contamination. Throughout the experiment, a constant humidity of soil was maintained at the level of 50% of capillary water capacity. Morphologically differentiated fungal colonies, a few dozen from each type of soil, were isolated on day 1, 50, 100, 150, 250 and 350 after introducing copper chloride to the soil. These microorganisms were later transplanted on microbiological medium contaminated with copper and the same control medium. The growth of fungal colonies in both cultures was compared. Statistical analysis of the results revealed highly significant differences in copper resistance of fungi originating from soils of various degree of contamination with this metal. Generally, it can be assumed that the highest resistance to copper in microbiological medium was demonstrated by fungi isolated from the soil lightly contaminated with this metal and the lowest resistance by those that originated from heavily contaminated soil. However, it should be noted that on the last date of measurement, the origin of fungi (control soil or lightly contaminated soil) did not have any statistical effect on their resistance. Increased resistance of these fungal isolates that had a previous contact with copper in soil contaminated with a dose of 80 mg Cu^{2+} kg^{-1} was observed from day 100 to day 250 day of the experiment. Following this, fungal strains revealed a similar resistance, irrespective of their origin.

ODPORNOŚĆ GRZYBÓW GLEBOWYCH NA ZANIECZYSZCZENIE MIEDZIĄ

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S ł o w a k l u c z o w e: grzyby glebowe, miedź, odporność.

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Abstrakt

Do gleby (piasek gliniasty mocny pylasty) wprowadzono chlorek miedzi (dihydrat chlorku miedzi) w dwóch dawkach, tak by całkowita zawartość miedzi odpowiadała słabemu (80 mg Cu^{2+} kg^{-1}) i silnemu (500 mg Cu^{2+} kg^{-1}) zanieczyszczeniu gleby tym pierwiastkiem.

Przez cały okres trwania doświadczenia utrzymywano stałą wilgotność gleby na poziomie 50% kapilarnej pojemności wodnej. Zróżnicowane morfologicznie kolonie grzybowe, po kilkadziesiąt z każdej gleby, izolowano w terminach: 1, 50, 100, 150, 250, 350 doby od wprowadzenia chlorku miedzi do gleby. Mikroorganizmy te przeszczepiano następnie na podłoże mikrobiologiczne zanieczyszczone miedzią i to samo podłoże kontrolne. Porównywano wzrost kolonii grzybowych w obu hodowlach. Analiza statystyczna wyników wykazała wysoce istotne różnice między odpornością na miedź grzybów pochodzących z gleb o różnym stopniu skażenia tym metalem. Ogólnie można przyjąć, że największą odporność na miedź w podłożu mikrobiologicznym wykazywały grzyby izolowane z gleby słabo zanieczyszczonej tym metalem, a najmniejszą pochodzące z gleby silnie skażonej. Nie bez znaczenia jest jednak fakt, że w ostatnim terminie pomiaru pochodzenie grzybów (gleba kontrolna czy słabo zanieczyszczona) nie wpływało statystycznie istotnie na ich odporność. Podwyższona odporność izolatów grzybowych, mających wcześniej kontakt z miedzią w glebie zanieczyszczonej dawką 80 mg Cu^{2+} kg^{-1}, utrzymywała się od 100 do 250 doby doświadczenia, po tym okresie szczepy grzybowe wykazywały podobną odporność, niezależnie od pochodzenia.

Introduction

When factors harmful to soil environment reach a very high level, biological soil activity can be built only by those microorganisms that are resistant to xenobiotics. Tolerance and adaptation of microorganisms to heavy metals is a known phenomenon, particularly as regards certain strains of microorganisms (DÖNMEZ, AKSU 1999, GREEN, CLAUSEN 2003, HASTRUP et al. 2005, SPAIN 2003). However, the amount of existing data concerning the impact of heavy metals on soil microorganisms and referring to the whole population, to a given systematic group or the metabolic potential of soil, still appears to be unsatisfactory. Until now, it has not been definitively explained to what extent microorganisms can adapt to various types of contamination and whether certain systematic or physiological groups of microorganisms are capable of developing their adaptation abilities (GONZALEZ-CHAVEZ 2002, NIKLIŃSKA, CHMIEL 1997). Copper as a heavy metal has a destructive effect on microorganisms, including fungi, therefore this element was used as a component of the active substance of many fungicides, while on the other hand, copper is a microelement that is necessary for the development of all living organisms (BALAMURUGAN, SCHAFFNER 2006, GREEN, CLAUSEN 2003, GUILLEN, MACHUCA 2007, TOLER et al. 2005).

Fungal resistance to heavy metals results from various mechanisms, among which there are: the ability to passively extract metals from the cell, active transport of metal ions outside the cell, ability to mask metals by chelating them, enzymatic transformation of metal ions, creating vacuoles in which metal ions are gathered and immobilized in the form of polyphos-

phates, increased production of melanin and other pigments, ability to produce specific metal binding compounds inside the cell (BALAMURUGAN, SCHAFFNER 2006, GONZALEZ-CHAVEZ et al. 2002, OSTROWSKI, SKŁODOWSKA 1996). The ability to tolerate copper in the environment depends on many factors related to the characteristics of the strain itself or of its form, metal concentration, or pH of the environment (DÖNMEZ, AKSU 1999, GONZALEZ-CHAVEZ et al. 2002). Several researchers have confirmed the genetic basis of the phenomenon of developing resistance to heavy metals, when a gene providing resistance can be transferred in a way of conjugation or even transformation to susceptible strains (BIESZCZAD, SOBOTA 1999, CERVANTES, GUTIERREZ-CORONA 1994, SPAIN 2003).

Currently, the interest of researchers studying the impact of copper on soil fungi is related both to the positive and to the negative aspects of this activity. Many studies deal with the effect of copper ions on *Glomeromycota* (SILVA da et al. 2005, GONZALEZ-CHAVEZ et al. 2002, HOWE et al. 1999, TOLER et al. 2005). Due to the above mentioned ability of some copper resistant fungi to bioaccumulate this element (mainly in the cell wall but also inside the cell), ectomycorrhiza prevents this metal from penetrating into the plant tissues and as a result, to food. On the other hand, since *Glomerymycota* penetrate the root cells of the plant, they do not protect it against the accumulation, although this ability of maintaining elevated concentration of metal in the plant is used in phytoremediation (TOLER et al. 2005). A negative effect related to the toxic influence of copper to susceptible fungi is related to those species, the biomass of which is used for bioremediation of soil contaminated with petroleum originated compounds and in sewage treatment (DÖNMEZ, AKSU 1999, TOLER et al. 2005), and to soil fungi forming a part of the organic fraction of soil (KUCHARSKI et al. 2001, KUCHARSKI, WYSZKOWSKA 2004, WYSZKOWSKA, KUCHARSKI 2003). Numerous studies have been dedicated to the examination of fungi resistance to copper, in order to test the effectiveness of this metal, which as a component of fungicidal agents, is nowadays often replaced with more efficient ones. Wood decomposing fungi acquire the resistance to impregnate containing copper compounds. What is particularly widely described here is the ability of fungi (e.g. strains that are exceptionally resistant to copper: *Wolfiporia cocos, Laetiporus sulfureus*, *Aureobasidium pullulans, Rhizopus stolonifer*) to precipitate copper in the form of insoluble, biologically inactive copper oxalate (CERVANTES, GUTIERREZ-CORONA 1994, GUILLEN, MACHUCA 2007).

In the presented experiment, a simple microbiological parameter was developed and used – the so-called resistance index (IR), related both to individual fungal isolates and to the entire group of fungi (average IR). Based on the value of the resistance index (IR), the specimens under examination

were divided into four resistance groups (RG). Due to the complex system of soil microbiocenosis interrelations, it seems to be particularly important to acquire information on the effects of heavy metals, not on individual strains but on the entire population, or on given systematic or physiological group.

The aim of the experiment was to determine the resistance of soil fungi to copper contamination and to demonstrate whether fungi originating from polluted soil are more resistant to the addition of copper to the solid medium than fungi originating from uncontaminated soil.

Materials and Methods

Soil for analyses was collected from the humus horizon (0–10 cm). The soil was composed of dusty heavy loamy sand (58% sand fraction, 25% dust fraction and 17% fluming parts), and had a pH_{H2O} level of 6.5 with an organic substance content of 1.5%. Natural copper content in the soil was 6.1 mg kg^{-1}. The soil was dried and sieved through a 2 mm mesh sieve in order to remove skeletal parts and mechanical pollution. After determining the maximum water capacity, it was brought to 50% and such a condition was maintained for the whole experiment. Copper chloride was introduced into the soil in two doses, so that the total copper content corresponded to weak and strong (KABATA-PENDIAS, PENDIAS et al. 1993) contamination of the soil with this element. The terms of weak (80 mg Cu^{2+} kg^{-1}) and strong (500 mg Cu^{2+} kg^{-1}) copper contamination of soil were adopted after the Classification of Soils (according to IUNG) concerning copper content. Research material was incubated in PVC containers in one-kilo portions at 20°C, in three replications.

Using the method of surface inoculation of soil dilutions, 40 diversified fungal colonies (isolates) were isolated from soils (control, lightly and heavily contaminated) using selective medium of Martin (1950) with Rose Bengal and streptomycin. Afterwards, five-day old colonies were transplanted to the Martin medium with the addition of copper in a dose of 100 mg Cu^{2+} dm^{-3} and on the same substrate free of pollution (control), in four repetitions. After seven-day incubation at 25°C, "twin" fungal colonies (originating from one source or a "parent" colony) were obtained on two dishes.

HASTRUP et al. (2005) define the resistance to heavy metals as the ability of an organism to grow and function in its presence. The growth of twin colonies on the medium contaminated with copper and on the control medium was compared by measuring their diameters (BŁASZAK, NOWAK 2006). The resistance index (RI) was determined for each isolate as a relation of the diameter of the colony cultivated on the contaminated medium to the diameter of the colony incubated on control medium. An average resistance index was

related to all fungi isolated from the given soil. Four resistance groups (RG) were created based on diverse RI results:

– group I, RI = 0, completely susceptible microorganisms (no growth on the contaminated medium);

– group II, $0 < RI \leq 0.5$, very susceptible microorganisms (growth on the contaminated medium up to a half of the control colony size);

– group III, $0.5 < RI < 1$, microorganisms moderately susceptible (growth on the contaminated medium exceeds a half of the control colony size, but is not higher);

– group IV, $RI \geq 1$, resistant microorganisms (growth on the contaminated substrate is the same as on control medium or even higher).

Analysis dates (isolation and measurement) were set for day 1, 50, 100, 150, 250 and 350 of the experiment.

In order to determine statistically significant differences in the resistance of fungi originating from soils of a various degree of contamination and differences resulting form measurement days, a Duncan multiple comparisons test was applied at the significance level of 0.05 (STATSOFT, INC. 2007).

Results and Discussion

Statistical analysis revealed highly significant differences in copper resistance as regards fungi originating from soils of a various degree of contamination with this metal (Figure 1). The degree of fungi resistance (expressed as the average resistance index – RI and the percentage rate of fungi belonging to individual resistance groups – RG) isolated from the control soil was maintained at a relatively constant level during the experiment (Figure 1, Figure 2). No presence of fungi completely susceptible to copper (I group) was observed, apart from one date – day 150, when they constituted 2.5% of the whole isolated group. On the other hand, completely resistant fungi (group IV) constituted 15% on average, and this group was not subject to significant fluctuations as regards quantity (Figure 2). Moderately susceptible fungi – which did not reach the size of the control colonies, but were at least half of their size (III group) – prevailed in the total pool of the examined fungi of the control group (Figure 2).

Unlike fungi originating from the control soil, strains isolated from soils contaminated with Cu^{2+} revealed significant fluctuations as regards resistance to copper present in the microbiological medium (Figure 1). Light contamination of soil with copper immunized fungi for over half a year (up to day 250) – while in the same period completely and very susceptible to fungi (group I, II) – was the smallest part in the whole examined pool (Figure 3). The share

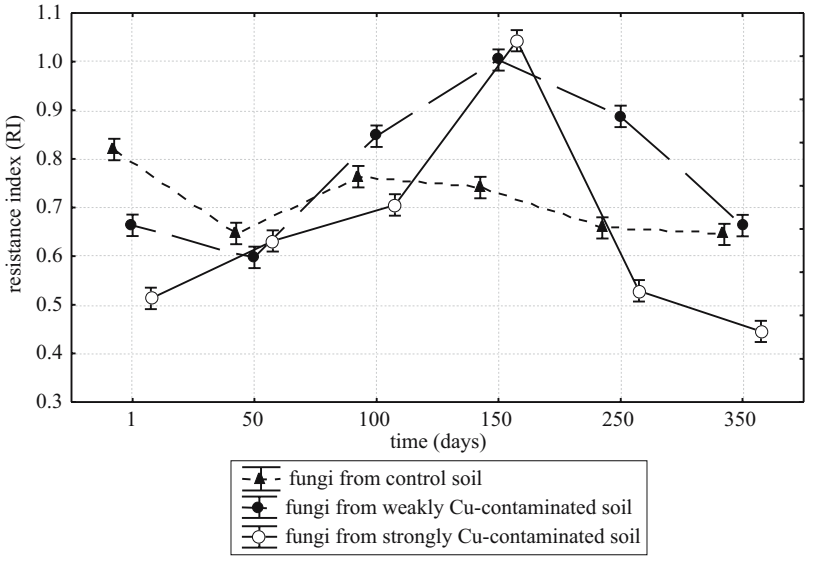

Fig. 1. Resistance index (RI) – the ratio of colony diameter of soil isolate cultured on copper--contaminated medium to colony diameter of the same isolate cultured on control medium. Fungi were isolated from control, weakly and strongly Cu-contaminated soil. There are averages of IR for all fungi which were isolated from each soil

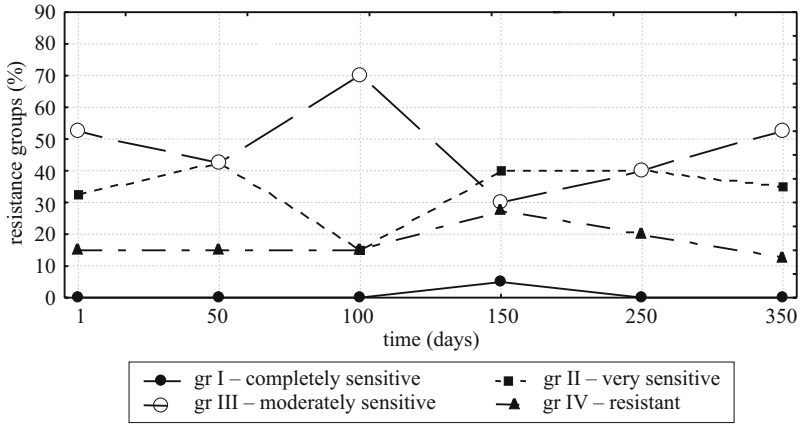

Fig. 2. Percentage participation of fungi from the control soil in particular resistance groups (RG). The value of resistance index (RI) decides upon the affinity of fungi to one of the four resistance groups (RG): I group, RI = 0 – completely sensitive; II group, $0 < RI \leq 0.5$ – very sensitive; III group, $0.5 < RI < 1$ – moderately sensitive; IV group, $RI \geq 1$ – resistant fungi

of susceptible fungi (group IV) gradually increased, and it was the greatest, at the level of 42% on day 150 of the experiment (Figure 3). RAJAPAKSHA et al. (2004) examined the effect of copper, among others, on the metabolic activity of fungi (directly, on breathing processes). Initially, this activity grew with the

increase in heavy metal concentration. After some time, the stimulating effect on fungal activity fell, brought about by adding metal, but the activity of this group of microorganisms was still higher in the contaminated soil than the control soil. BŁASZAK, NOWAK (2006) also observed better fungal growth on the medium contaminated with copper chloride (active substance of Miedzian 50 WP) in the case of those fungi which had previous contact with an increased dose of copper in the soil. The sizes of those fungal isolates were, on average, about 10% higher that those originating from soil with natural copper content.

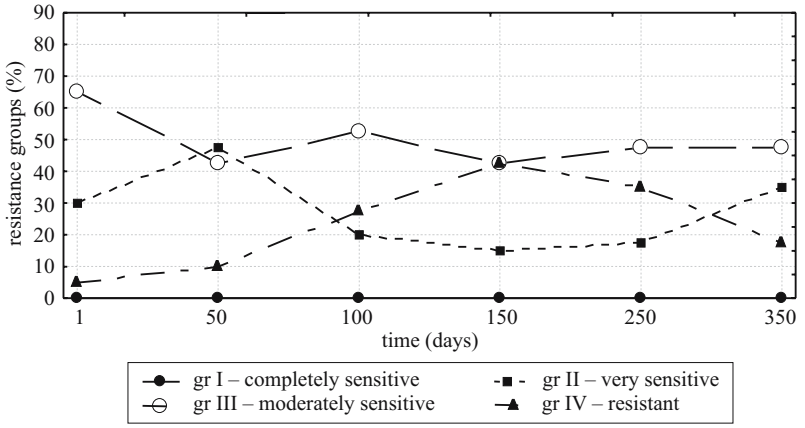

Fig. 3. Percentage participation of fungi from the weakly Cu-contaminated soil in particular resistance groups (RG). The value of resistance index (RI) decides upon the affinity of fungi to one of the four resistance groups (RG): I group, RI = 0 – completely sensitive; II group, 0 < RI ≤ 0.5 – very sensitive; III group, 0.5 < RI < 1 – moderately sensitive; IV group, RI ≥ 1 – resistant fungi

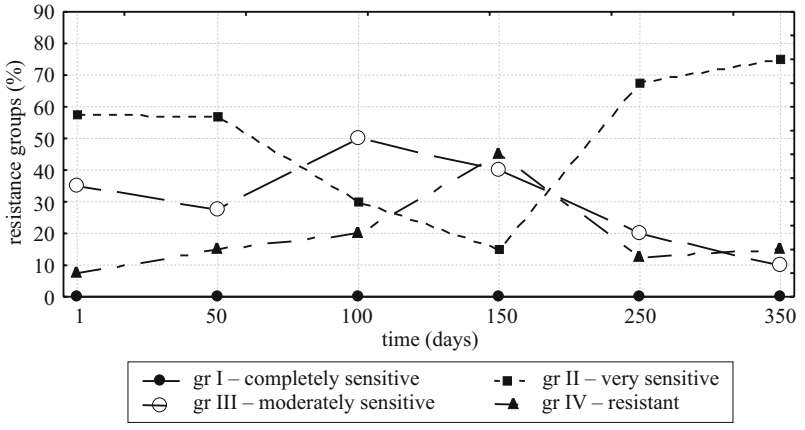

Fig. 4. Percentage participation of fungi from the strongly Cu-contaminated soil in particular resistance groups (RG). The value of resistance index (RI) decides upon the affinity of fungi to one of the four resistance groups (RG): I group, RI = 0 – completely sensitive; II group, 0 < RI ≤ 0.5 – very sensitive; III group, 0.5 < RI < 1 – moderately sensitive; IV group, RI ≥ 1 – resistant fungi

Fungi originating from the soil heavily contaminated with Cu^{2+} were represented mainly by very susceptible strains (group II). It was only on 150th day of the experiment that resistant strains – of group IV – dominated in isolated fungi (Figure 1, Figure 4), which resulted in a rapid growth of average RI (Figure 1). The above results demonstrate that heavy contamination of soil with copper did not bring about the effect of resistance to this metal under *in vitro* conditions. An experiment conducted by DÖNMEZ, AKSU (1999) examined the ability of fungal strains of *Ascomycetes* genus to grow on the nutrient medium containing Cu^{2+}. It was definitely established that in spite of a preselection as regards the resistance to copper, all four strains revealed a slow down or inhibition of growth in the presence of high copper content (300 mg Cu^{2+} dm^{-3}). Similarly, in another experiment (GUILLEN, MACHUCA 2007), the majority of the examined fungal strains did not grow in the presence of the increased concentration of copper in nutrient medium; only two out of ten: *Wolfiporia cocos* and *Laetiporus sulfureus* demonstrated resistance even to 10 mM of copper. Heavy metal tolerance is related very individually to a given microorganism strain, and the increase in the resistance of a certain group of fungi to copper could be accompanied with a gradual dying out of susceptible species DIAZ-RAVINA, BÅÅTH 1996). Likewise, NIKLIŃSKA, CHMIEL (1997), while comparing the rate of soil respiration (research concerned the release of CO_2 by soil microorganisms including fungi) from the area of the Copper Works in Głogów (copper content: 1120 mg kg^{-1}) and a control soil in the distance of about 30 km from this area (Cu content: 50 mg kg^{-1}) observed that the results for the control soil were by about 30% higher; however, after contaminating these soils with copper under laboratory conditions, a stronger reduction of breathing activity was obtained in the control soil. Still another experiment demonstrated that heavy pollution of the environment (bacteria and fungi were isolated from the area of coal mine Santa Catarina) did not always immunize microorganisms against heavy metals (CASTRO-SILVA i in. 2003).

Generally, it can be assumed that the highest resistance to copper in a microbiological medium was demonstrated by fungi isolated from soil that was weakly contaminated with copper and the lowest resistance was by those that originated from strongly contaminated soil. However; it should be noted that on the last measurement date, the origin of fungi (from the control or lightly contaminated soil) did not statistically influence their resistance (Figure 1). Increased resistance of fungi that had a previous contact with copper in the soil lightly contaminated lasted from day 100 to 250 of the experiment, and afterwards, fungal isolates revealed a similar resistance regardless of their origin (Figure 1). The decrease in the resistance to copper (after day 250) is difficult to explain, its direct cause was the reduction

of resistant fungi in the general pool of microorganisms (group IV), and the increase of the share of completely and very susceptible fungi (groups I, II). Not all of the examined fungi were able to immunize to copper, not all were capable of creating an appropriate resistance mechanism, since on each measurement date a group of microorganisms highly susceptible to the examined metal was observed.

Conclusions

1. Statistical analysis of results revealed highly significant differences in copper resistance as regards fungi originating from soils with various degrees of contamination with this metal.

2. The highest resistance to copper in the microbiological medium was demonstrated by fungi isolated from soil that was weakly contaminated with this metal (80 mg Cu^{2+} kg^{-1}) and the lowest by those that originated from strong contaminated soil (500 mg Cu^{2+} kg^{-1}). The increased resistance of fungal isolates that had a prior contact with copper in the soil weakly contaminated lasted from day 100 to 250 of the experiment, and after this period isolated fungi revealed a similar resistance, irrespective of their origin.

3. Strong contamination of soil with copper (500 mg Cu^{2+} kg^{-1}) did not cause the effect of increased resistance to this metal under *in vitro* conditions.

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PROBLEM OF FILAMENTOUS FOAMING OF ACTIVATED SLUDGE IN WASTEWATER TREATMENT PLANTS REMOVING BIOGENES IN THE WARMIA AND MAZURY PROVINCE, POLAND

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K e y w o r d s: activated sludge, foaming, filamentous microorganisms, *Microthrix parvicella.*

A b s t r a c t

Investigations were carried out in ten municipal wastewater treatment plants located in the Warmia and Mazury Province removing biogenes with the method of activated sludge. Extent and intensity of activated sludge foaming were determined and organisms accompanying that phenomenon were recognized. In 60% of the treatment plants in the autumn-winter season and in 90% of the treatment plants in the spring season stable brown foam was observed to cover surfaces of bioreactors. Microscopic picture of foam enabled detection of six types of filamentous bacteria and NALO actinomycetes (Nocardia amarae like organisms). In the entire experimental period, *Microthrix parvicella* was most often occurring in foam. The co-predominating microorganisms in two treatment plants, irrespective of the season, appeared to be Type 0092 and in one treatment plant the spring season predominating microorganisms were NALO. The high number of *Microthrix parvicella* in the activated sludge was not always linked with its foaming. The intensity of foaming, expressed by the Scum index, was statistically significantly negatively correlated with the concentration of ammonia nitrogen in the treated wastewaters.

PROBLEM NITKOWATEGO PIENIENIA OSADU CZYNNEGO W OCZYSZCZALNIACH ŚCIEKÓW USUWAJĄCYCH BIOGENY W WOJ. WARMIŃSKO-MAZURSKIM, POLSKA

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S ł o w a k l u c z o w e: osad czynny, pienienie, organizmy nitkowate, *Microthrix parvicella.*

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Abstrakt

Badania przeprowadzono w dziesięciu oczyszczalniach ścieków komunalnych w woj. warmińsko-mazurskim, usuwających biogeny metodą osadu czynnego. Oceniono rozmiar i intensywność pienienia się osadu czynnego oraz rozpoznano organizmy towarzyszące temu zjawisku. W 60% oczyszczalni w porze jesienno-zimowej i w 90% w porze wiosennej obserwowano trwałą brązową pianę pokrywającą powierzchnię bioreaktorów. W obrazie mikroskopowym piany stwierdzono sześć typów bakterii nitkowatych oraz promieniowce nokardiopodobne (NALO). Najczęściej występowała *Microthrix parvicella*. Współdominującymi mikroorganizmami okazały się w dwóch oczyszczalniach (niezależnie od pory roku) – Typ 0092 oraz w jednej oczyszczalni w porze wiosennej – NALO. Wysoka liczebność *Microthrix parvicella* w osadzie czynnym nie zawsze wiązała się z jego pienieniem. Intensywność pienienia, wyrażona indeksem piany, była istotnie statystycznie ujemnie związana ze stężeniem azotu amonowego w ściekach oczyszczonych.

Introduction

Foam of biological origin is a common phenomenon in most wastewater treatment plants operating with the method of activated sludge. In a microscopic picture, it is usually observed as bubbles of gas covered with flocks of activated sludge contained in the intersludge liquid. A predominating component of the flocks are filamentous organisms of bacteria. They are characterized by hydrophobicity of cell walls and/or production of surface-active compounds, owing to which they evoke adhesion of activated sludge to bubbles accompanying the wastewaters treatment process. This results in bulking and permanent foaming of the sludge. Not removed foam binds up to over 30% (WANNER 1994 cit. after HAO et al. 1988) of activated sludge contained in the system and becomes the site of proliferation for filamentous organisms colonizing it (FOOT et al. 1994). As a consequence, its thickness increases, which in turn impairs maintaining constant concentration of the sludge in bioreactors and in recirculate, decreases oxygen concentration in aeration chambers, poses safety problems in closed fermentation chambers (WESTLUND et al. 1998), increases health risks to treatment plant staff and causes a variety of other difficulties in everyday work of the treatment plant.

Recognition of factors stimulating excessive development of filamentous organisms predominating in foam is a prerequisite for elimination of causes of activated sludge foaming. Although the problem of biological foaming is known for several decades (BLACKALL 1991), it is still actual nowadays (WANNER et al. 1998, MADONI et al. 2000, KRHUTKOVÁ et al. 2002, KALISZ et al. 2005).

Therefore, the reported research was aimed at evaluating the extent and intensity of filamentous foaming of activated sludge used for wastewater treatment in then treatment plants removing biogenes as well as at recognizing organisms responsible for that phenomenon.

Materials and Methods

Experimental area

Investigations covered 10 treatment plants removing carbon, nitrogen and phosphorus, located in the Warmia and Mazury Province: Olsztynek, Łukta, Morąg, Kosyń, Biskupiec, Stawiguda, Ostróda, Lidzbark Warmiński, Pasym, and Jeziorany.

In two of them (Olsztynek and Pasym) wastewaters are treated biologically in sequencing batch reactors (SBR). In the others, integrated removal of carbon, nitrogen and phosphorus is carried out with the use of flow reactors.

Wastewater treatment plant throughput ranged from 300 to 7000 m^3 d^{-1}. In the case of five treatment plants only municipal wastewaters were discharged. The contribution of industrial sewage in wastewaters inflowing to the other treatment plants ranged from 16 to 60% of the total wastewater volume (Table 1).

Table 1

Characteristics of wastewater treatment plants

Treatment plant location	Treatment plant code	Treatment plant throughput		Reactor type	Contribution of industrial wastewaters (%)	Predominating industry
		designed (m^3 d^{-1})	real (m^3 d^{-1})			
Olsztynek	*A*	4200	2800	periodical	60	fruit processing
Łukta	*B*	1000	600	flow		
Morąg	*C*	4000	2330	flow	40	dairy
Kosyń	*D*	3300	1600	flow	16	
Biskupiec	*E*	5000	1700	flow	23	meat
Stawiguda	*F*	1200	450	flow		
Ostróda	*G*	12000	6855	flow	30	meat
Lidzbark Warm.	*H*	4000	3338	flow		
Pasym	*I*	600	320	periodical		
Jeziorany	*J*	1000	600	flow		

Material

One sample of activated sludge, foam (if occurred), influent and effluent wastewaters was collected for analyses from each treatment plant in the autumn–winter season (October– January 2004/2005) and in the spring season (May – June 2005).

In wastewater treatment plants in Olsztynek and Pasym, samples of foam for microscopic analyses were collected from the surface of sewage in a sequential biological reactor (SBR) during aeration phase, whereas in the other cases foam samples were collected from the surface of sewage in oxygen chambers. In addition, samples of activated sludge were collected from specified bioreactors.

Laboratory analysis

A microscopic analysis of activated sludge and foam samples was carried out in the material immediately after it reached the laboratory. Filamentous organisms were identified according to their morphology as well as the Gram and Neisser test following the method by JENKINS et al. 2004, EIKELBOOM (2000). The quantitative composition of the filamentous microorganisms in the samples of activated sludge was estimated using the widespread subjective scoring of filaments abundance proposed by JENKINS et al. (2004).

Intensity of the foaming problem was expressed by means of a Scum index (I_p) understood as a ratio of organic dry foam and activated sludge. Our previous observations demonstrate that $I_p > 1$ points to a serious foaming problem.

Determinations of the concentration of dry matter of activated sludge in chambers of biological treatment and those of contamination indicators in inflowing wastewaters were performed according to methodology of HERMANOWICZ (1999). Temperature of the inflowing wastewaters was measured at reactor's inlet.

Statistical analysis

In order to determine a dependency between the value of activated sludge loading with BOD_5, temperature of wastewaters inflowing to the reactor, concentration of ammonia nitrogen in treated wastewaters, and the Scum index, Pearson's correlation coefficients (r) were calculated. A relationship between concentration of ammonia nitrogen in treated wastewaters and the Scum index was additionally analyzed by means of a logistic regression model. The concentration of ammonia nitrogen in wastewater at treatment plant's outlet, expressed in mg dm^3, was adopted as an independent variable, whereas the value of the Scum index I_p, expressed in a binary system, as a dependent variable. If $I_p < 1$, then that value was recorded as 0, once $I_p > 1$ the value was recorded as 1.

Table 2

Physicochemical and technological parameters of the activated sludge process – autumn-winter season

Treatment plant code	COD mg O_2 dm^{-3}		BOD_5 mg O^2 dm^{-3}		P_{og} mg P dm^{-3}		N_{NH4} (mg N NH_4 dm^{-3})		Temperature °C	Sludge load (kg BOD/kg MLVSS)
	influent	effluent	influent	effluent	influent	effluent	influent	effluent	influent	
A	1485	59	475	13	16.0	2.4	12	0.0	18.1	0.146
B	984	42	653	16	23.6	11.1	49	0.1	13.2	0.056
C	2809	34	1863	4	27.4	0.6	78	1.8	14.3	0.086
D	604	54	473	7	14.9	2.0	36	1.4	11.0	0.043
E	598	72	400	10	16.7	6.8	50	2.5	11.0	0.024
F	710	41	524	6	20.5	9.4	53	0.0	8.2	0.081
G	888	97	507	24	26.8	1.8	65	0.7	13.4	0.144
H	848	41	558	6	20.2	1.2	36	2.8	11.6	0.142
I	385	56	197	7	13.2	0.6	32	3.8	6.6	0.004
J	1292	54	720	17	21.2	15.7	38	15.0	7.7	0.127

Table 3

Physicochemical and technological parameters of the activated sludge process – spring season

Treatment plant code	COD mg O_2 dm^{-3}		BOD_5 mg O^2 dm^{-3}		P_{og} mg P dm^{-3}		N_{NH4} (mg N NH_4 dm^{-3})		Temperature °C	Sludge load (kg BOD/kg MLVSS)
	influent	effluent	influent	effluent	influent	effluent	influent	effluent	influent	
A	1512	45	1200	10	17.2	2.9	27	2.4	13.3	0.111
B	961	41	766	13	29.0	2.5	84	2.8	11.2	0.072
C	920	51	676	12	23.0	1.4	51	2.6	14.1	0.026
D	417	77	231	16	7.8	2.0	32	3.7	10.9	0.040
E	2975	51	847	4	22.2	1.7	33	3.6	15.9	0.051
F	717	41	394	9	19.5	12.0	62	0.6	8.6	0.046
G	597	36	423	12	17.9	0.6	44	0.7	18.1	0.097
H	360	33	287	14	15.2	1.8	58	4.9	14.4	0.093
I	1229	92	1158	45	13.3	7.9	31	8.0	12.1	0.010
J	1014	48	494	5	25.4	11.2	39	2.8	12.7	0.112

Results

Physicochemical and technological parameters of the activated sludge process

Results of the physicochemical analysis of wastewaters and selected technological parameters were presented in Table 2 and Table 3. All treatment plants operated in a low range of activated sludge loading with a load inflowing wastewaters, i.e. from 0.004 to 0.146 kg BOD_5 kg^{-1} MLVSS d^{-1}. In the entire experimental period, in wastewaters inflowing to the treatment plants the value of COD ranged from 360 to 2975 mg O_2 dm^{-3}, and the value of BOD fluctuated from 197 to1863 mg O_2 dm^{-3}. Contents of total phosphorus ranged from 13.2-29.0. Ammonia nitrogen accounted for 12–84 NNH_4 dm^{-3}. Temperature of the inflowing wastewaters ranged from 6.6 to 18.1°C. Removal efficiency of COD, BOD_5, NNH_4, P_{og} was higher in the spring than in the autumn-winter season. The most significant differences were observed in the removal of nitrogen compounds, yet in the case of the other parameters decreased efficiency was noted as well.

In the activated sludge, the content of total suspended matter accounted for 3424–17136 mg smo dm^{-3}, and that of organic suspension for 2796–12688 mg smo dm^{-3}. In samples of foam contents of total suspension and organic suspension were as follows: from 1923 to 65886 mg smo dm^{-3}, and from 1679 to 53829 mg smo dm^{-3}, respectively (Table 4, Table 5).

Table 4
Concentration of activated sludge in biological treatment chambers and in foam – autumn-winter season

Treatment plant code	Sludge		Foam	
	total suspension	organic suspension	total suspension	organic suspension
A	7464	6184	–	–
B	6948	5058	–	–
C	6808	5216	2735	2411
D	8072	6008	1923	1679
E	8492	6148	39708	29968
F	6228	4567	–	–
G	3688	2792	–	–
H	3424	2796	10496	7376
I	6808	5028	52125	38969
J	6080	4612	38910	29450

Table 5
Concentration of activated sludge in biological treatment chambers and in foam – spring season

Treatment plant code	Sludge		Foam	
	total suspension	organic suspension	total suspension	organic suspension
A	7488	6204	11892	9900
B	6344	4584	46533	38411
C	8120	6516	65886	53829
D	4276	3184	38653	9093
E	8296	6272	15590	12160
F	8262	6377	5100	4216
G	4596	3484	–	–
H	5192	3852	20530	14850
I	17136	12688	49377	38274
J	5872	4272	43345	32697

Composition of filamentous organisms in the samples of activated sludge

In the entire experimental period, a total of 8 strains of filamentous microorganisms were identified in the samples of activated sludge: *Microthrix parvicella*, Type 0092, NALO (Nocardia amarae like organisms), Type 0041, Type 0675, *Nostocoida limicola* I, *Nostocoida limicola II*, *Thiothrix* sp. Irrespective of the experimental season, in all analyzed samples the predominating strain was *Microthrix parvicella* (count: 4–5 points in a 6-point scale by JENKINS et al). Co-predominating microorganisms in the activate sludge appeared to be: Type 0092 in the treatment plants in Morąg and Jeziorany both in the autumn-winter and spring seasons, and NALO in the treatment plant in Biskupiec in the spring season. The number of the accompanying strains ranged from 0 to 3 points (Table 6, Table 7).

Extent and intensity of activated sludge foaming with the share of filamentous microorganisms

The problem of sludge foaming was more wide-spread in the spring season. Brown foam covering surfaces of bioreactors was observed in that period in nine treatment plants, whereas in the autumn-winter season only in six treatment plants. Also the intensity of foaming was stronger in the spring season. In that period, the Scum index exceeding one, which indicates that the

Table 6

Composition of filamentous organisms – autumn-winter season

Treatment plant code	*Microthrix parvicella*		Typ 0092		NALO		Typ 0041		Typ 0675		*Nostocoida limicola I*		*Nostocoida limicola* II	
	AS	F	AS	F	AS	F	AS	F	AS	F	AS	F	AS	F
A	4		1				2							
B	4		2		1		3				3			
C	4	++	4	++			2	+					3	+
D	4	++	2	+								+		
E	4	++	2			+	3	+						
F	4		1				3		2					
G	4		1				2		1		3		1	
H	5	++	1			+	3	+	1		2	+	1	
I	5	++	3											
J	4	++	4	++	2	+								

AS – activated sludge; F – foam
(++) – dominant; (+) – accompanying
(1–5) – filament abundance

Table 7

Table 7. Composition of filamentous organisms — spring season

Treatment plant code	*Microthrix parvicella*		Typ 0092		NALO		Typ 0041		Typ 0675		*Nostocoida limicola I*		Thiothrix sp.	
	AS	F	AS	F	AS	F	AS	F	AS	F	AS	F	AS	F
A	4	++	3	+			2	+				+		
B	4	++	1			+	3	+			2	+		
C	5	++	5	++			3	+	1	+	1	+		
D	5	++	2	+			1							
E	4	++	1	+	4	++	2	+						
F	5	++					3	+						
G	4		2				1				3			
H	5	++	2	+			2	+	3	+	2	+		
I	5	++	3	+										
J	4	++	4	++		+			2					1

Explantations as in Table 6

concentration of dry organic matter in foam was higher than in the activated sludge, was reported in 89% of the samples examined, whereas in the autumn--winter season – in 67% of cases (Figure 1).

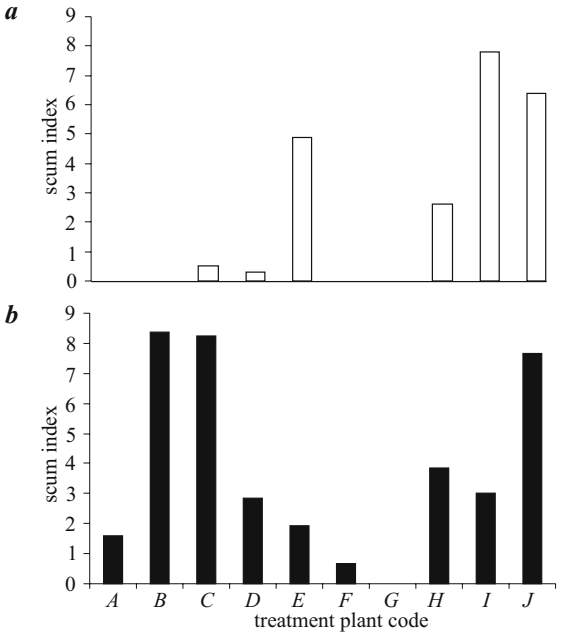

Fig. 1. Scum index: *a* – autumn-winter season; *b* – spring season

Foam components

As indicated by the microscopic picture, the foam consisted of bubbles of gas coated with hydrophobic particles of the sludge, composed mainly of filamentous microorganisms (Figure 2).

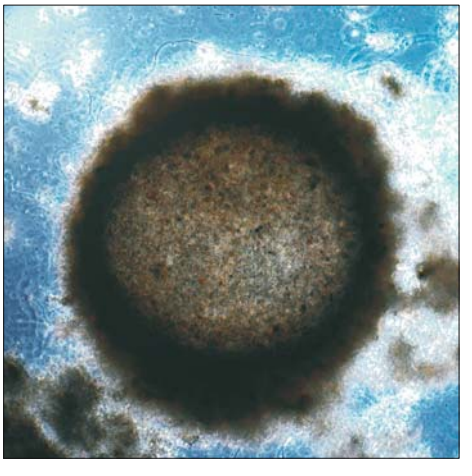

Fig. 2. Gas bubble coated with a suspension of activated sludge

The composition of filamentous organisms occurring in the foam, including their position in the community, was presented in Table 6, Table 7. Data obtained indicate that in the entire experimental period, the most frequently occurring and the most abundant in the foam were *Microthrix parvicella*. In two treatment plants, irrespective of the season, Type 0092 was found to predominate, whereas in one treatment plant in the autumn-winter season predominating organisms were NALO.

Summary and Discussion

The reported study carried out in the Warmia and Mazury Province demonstrated universality of the activated sludge foaming phenomenon linked with a strong growth of filamentous organisms. It confirms the significance of that problem in wastewater treatment plants removing biogenes with the method of activated sludge (KALISZ et al. 2005). The main biological components of the foam samples analyzed – type *Microthrix parvicella* – belongs to filamentous organisms commonly observed in foaming activated sludge (BLACKBEARD et al. 1986, SEVIOUR et al. 1990, PUJOL et al. 1991, WANNER et al. 1998, MADONI et al. 2000, KRHUTKOVÁ et al. 2002, KALISZ et al. 2005). It well develops at a low contamination load of activated sludge, high age of the sludge, low oxygen concentration and the presence of long-chain fatty acids in the inflowing wastewaters (JENKINS et al. 2004). An additional growth--promoting factor of those bacteria is temperature of wastewaters not exceeding 15°C (KNOOP, KUNST 1998).

Environmental conditions found in the treatment plants examined facilitated the proliferation of dominant *Microthrix parvicella*. All plants were treating municipal wastewaters containing products of fats and oils hydrolysis, at low values of activated sludge loading with pollutants, i.e. from 0.004 to 0.146 kg BOD_5 kg^{-1} MLVSS d^{-1}, and temperature of wastewaters usually not exceeding 15°C.

In the study, no simple correlation was observed between the values of load and Scum index (I_p) – the coefficient of correlation (r) was statistically insignificant ($r = -0.2077$; $n = 20$; $P > 0.05$). Both in the treatments plants operating at the loading < 0.01 and those with the loading > 0.1 kg BOD_5 kg^{-1} MLVSS d^{-1}, both intensive foam ($I_p > 1$) and its lack ($I_p = 0$) were observed. Activated sludge loading in the treatment plant in Stawiguda was lower than that kept in Jeziorany, however due to the season the intensity of foaming was remarkably higher in Jeziorany ($I_p = 6.3$; 7.6) than in Stawiguda ($I_p = 0$; 0.7). Although in the samples of activated sludge from the treatment plants compared, *Microthrix parvicella* was found to predominate (count: ≥ 4 points),

no foam was observed in Stawiguda in the spring-winter season. It is contradictory to findings of KNOOP and KUNST (1988), who claimed that abundant growth of that strain in the activated sludge was always linked with the generation of foam of biological origin on the surface of reactors of treatment plants operating with biological methods of nutrients removal.

In the experimental period, temperature of wastewaters inflowing to the reactors ranged from 6.6 to 18.1°C. No simple correlation was demonstrated between temperature of inflowing wastewaters and the value of the Scum index ($r = -0.3459$; $n = 20$; $p > 0.05$). Yet, always when the intensity of foaming was high ($I_p > 1$) the temperature of influent was not exceeding 16°C.

The concentration of ammonia nitrogen at the outlet of reactors of the treatment plants examined ranged from 0.0 to 15.0 mg dm^3. In the plants in which no foam was observed, the concentration of ammonia nitrogen in the treated wastewaters was ≤ 0.7 mg dm^{-3}. A statistically significant, simple, negative correlation was found between that parameter and Scum index ($r = -0.4661$; $n = 20$; $p < 0.05$; $y = 1.7463 + 0.244$ X). An analysis of the logistic regression model (Figure 3) demonstrated that the probability of intensive foam appearance ($I_p > 1$) exceeds 50% once ammonia nitrogen concentration in the treated wastewaters is > 2 mg dm^{-3}. Goodness of fit of that model accounts for $P = 0.0000$. It is common knowledge that ammonia nitrogen is an indispensable source of nitrogen for the growth of *Microthrix parvicella*. Its increased concentration at the outlets of the treatment plants examined might have occurred due to hypoxia of activated sludge contained in them.

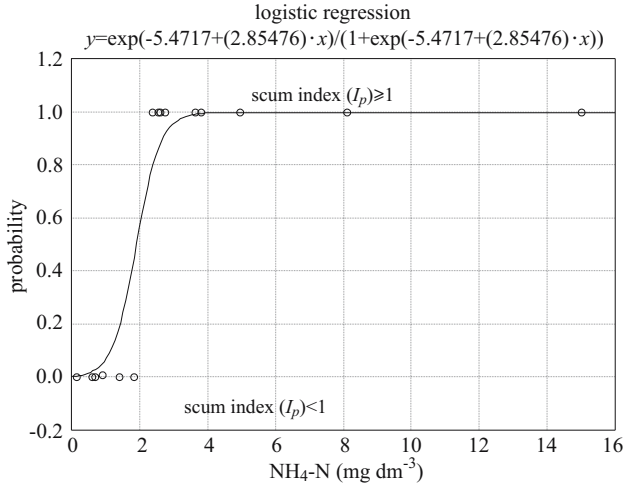

Fig. 3. Correlation between ammonia nitrogen concentration in treated wastewaters and scum index

Conclusions

1. In 60% of the treatment plants in the autumn-winter season and in 90% of the plants in the spring season foam of biological origin was observed.

2. In all foam samples analyzed the main biological component was *Microthrix parvicella.*

3. A higher number of *Microthrix parvicella* (count: 4 points) in the activated sludge in a load range of 0.004–0.146 kg BOD_5/kg MLVSS d was not always linked with the foaming of activated sludge.

4. Foaming intensity, expressed by the Scum index, was statistically significantly negatively correlated with the concentration of ammonia nitrogen in the treated wastewaters. A lack of foam was linked with $N\text{-}NH_4 \leq 0.7$ mg; whereas intensive foam ($I_p > 1$) with $N\text{-}NH_4$ concentration in the inflowing wastewaters > 2.0 mg dm^{-3}.

5. At intensive foaming, $I_p > 1$, temperature of wastewaters inflowing to the reactor did not exceed 16°C.

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THE REMOVAL OF C.I. REACTIVE BLACK 8 AND REACTIVE ORANGE 16 BY OZONATION

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K e y w o r d s: ozonation, reactive Black 8, reactive Orange 3R, kinetic constant.

A b s t r a c t

The study was undertaken to investigate the removal of reactive dyes: Orange 16 and Black 8, from aqueous solutions in the ozonation process. Experiments were carried under laboratory conditions at a concentration of both dyes reaching 100 mg dm^{-3} and at four doses of ozone – 16, 24, 32 and 40 g O_3 m^{-3}. Reaction rate constants were determined for each experimental series. The research demonstrated that both the dose of ozone supplied and chemical structure of dye affected the rate of the dye removal process. An increase in ozone dose evoked an increase in the reaction rate constant for both dyes examined. The reaction rate constants determined at a stable dose of ozone for Black 8 were ca. 2 times higher than the constants calculated for Orange 16.

USUWANIE C.I. REACTIVE BLACK 8 I REACTIVE ORANGE 16 W PROCESIE OZONOWANIA

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S ł o w a k l u c z o w e: ozonowanie, Black 8, Orange 16, stałe kinetyczne.

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Abstrakt

W pracy badano usuwanie barwników reaktywnych Orange 16 oraz Black 8 z roztworów wodnych w procesie ozonowania. Badania prowadzono w warunkach laboratoryjnych dla stężenia obu barwników 100 mg dm^{-3} oraz dla czetrech dawek ozonu – 16, 24, 32 i 40 g O_3 m^{-3}. Dla każdej przeprowadzonej serii badawczej wyznaczono stałe szybkości reakcji. Wykazano, że zarówno dawka doprowadzonego ozonu, jak i budowa chemiczna barwnika wpływały na szybkość procesu usuwania barwy. Wzrost dawki ozonu powodował wzrost stałej szybkości reakcji dla obu testowanych barwników. Stałe szybkości reakcji wyznaczone dla stałej dawki ozonu dla Black 8 były ok. 2-krotnie wyższe od wyznaczonych dla Orange 16.

Introduction

On the world-wide scale, the textile industry utilizes ca. 540.000 tones of dyes (SOLECKA, LEDAKOWICZ 2005). Dyes are enumerated amongst compounds resistant to biochemical decomposition, inducing skin irritations, allergies, often toxic, carcinogenic and mutagenic. They are applied mainly in the textile industry, for the production of paints and varnishes, in the leather, papermaking, cosmetic, food, and galvanizing industries, in tanneries as well as in biology, medicine, in the production of pharmaceutics, and many others (CZAJKOWSKI 2005, ZIMNICKI 2000).

In respect of chemical structure, dyes are usually aromatic derivatives of benzene, naphtalene, anthracene and heterocyclic compounds with active electrons loosely bound with a molecule. Reactive dyes are very good soluble in water, thus their removal from wastewaters is difficult with conventional physicochemical and biological methods (AKKAYA et al. 2007, BEYDILLI, PAVLOSTATHIS 2005). In the process of colouration and painting they bind with cellulose, thus forming covalent bonds. Such a dye-cellulose complex is very stable, due to which dyeing and printed cloth are characterized by a very good resistance to washing agents.

Treatment methods of wastewaters containing increased concentrations of dyes that would be characterized by a high effectiveness and economy price have been searched for a number of years. One of them consists in the application of ozone in order to oxidize color compounds occurring in wastewaters. Ozone is both a very strong oxidant and a disinfectant. Those characteristics enable its application in water treatment as a disinfecting agent or an oxidant (KOWAL, ŚWIDERSKA-BRÓŻ 2003). In literature, the ozonation process has been postulated as a potential, alternative method for decolourisation and improvement of biological treatment of sewage (SOARES et al. 2006). Apart from decolourisation, it is also used for the removal of bacteria and viruses, oxidation of dissolved iron and manganese, removal of flavor and odor, oxidation of organic and inorganic substances as well as for the removal of turbidity.

Theoretically, ozone is capable of oxidizing organic and inorganic matter to its highest oxidation degree, depending on selectivity of individual molecules and rate of their decomposition (CHU, MA 2000). Ozonation is an effective way of degradation for a wide variety of dyes in aqueous solutions because it destroys conjugated double bonds often associated with colour. Ozone leads to degradation of dyes and detergents, evokes destruction of aromatic systems and chromophore groups.

The paper reports on investigations into the removal of reactive dyes: Orange 16 and Black 8 differing in chemical structure, from aqueous solutions with the use of ozone. The research included the determination of the effect of dye structure and ozone dose on the effectiveness of the ozonation process. In addition, reaction rate constants were determined for the two dyes examined at various doses of ozone.

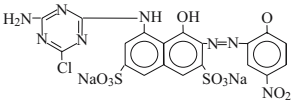
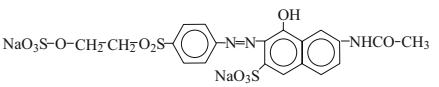
Materials and Methods

Characteristics and preparation procedure of the dyes examined

Dyes used in the study originate from the class of reactive dyes. Due to their chemical structures, they belong to chlorotriazine and vinyl groups. Analyses were carried out for the following dyes: Reactive Black 8 originating from the group of triazine dyes and Reactive Orange 16 from the group of vinylsulfone dyes. Molecular formulas of the dyes were presented in Table 1.

Table 1

Types of the dyes examined

Commercial name	CI	Reactive group	λ	Structural formula
Black DN	Reactive Black 8	chlorotriazine	580	
Brilliant Orange 3R	Reactive Orange16	vinylsulfone	490	

Solutions of the reactive dyes were prepared by weighing 1 g of the dye powder. Next the dye was quantitatively transferred into a measuring flask (with a capacity of 1 l) which was then filled up with distilled water at pH 7.0. The stock solution was then used to prepare working solutions. Dye's concentration in the working solutions was 50, 100 and 200 mg dm^{-3}.

Experimental station

Experiments were carried out in a reactor with a volume of 1 dm^3, containing a dye solution in an appropriate concentration (50, 100 or 200 mg dm^{-3}). Ozone was supplied to the reactor at five doses, i.e. 16, 24, 32 and 40 g O^3 m^{-3}. Several samples were taken at regular time intervals (5 min). In each of the experimental series, analyses were conducted until dye concentration in the reactor stabilized.

The efficiency of the process was calculated by monitoring the decolourisation. Dye concentration was measured spectrophotometrically using a UV-VIS Spectrophotometer SP 3000 apparatus.

Dyes Reactive Black 8 and Reactive Orange 16 were assigned a visual wavelength λ (Table 1) at which absorbance was measured in order to plot a standardization curve and to calculate conversion coefficient.

Ozone was produced by means of an OZOMATIC LAB 802 generator (WADECO, Poznań, Poland), using pure oxygen. At an overpressure of 0.5 bar, gas flow rate of 100 g Nm^{-3}, and temperature of 20°C, the production of ozone reaches 4 g h^{-1}. The maximum potential ozone concentration accounts for 250 g N m^{-3}.

Results and discussion

Time curves obtained in the 8 experimental series and depicting changes in the concentration of Reactive Black 8 and Reactive Orange 16 in the ozonation process in time were presented in Figure 1 and Figure 2. The obtained experimental results enabled determining reaction rate constants k.

In the description of experimental data obtained for changes of dye concentration C in time t, a good fit was demonstrated by the first-order kinetic model described by the following equation:

$$r = \frac{dC}{dt} = -k \cdot C \tag{1}$$

where k (min^{-1}) represents the first order rate constant and C (mg dm^{-3}) is dye concentration in each case. Integration of eq. (1) , considering $C = C0$ when $t = 0$, leads to:

$$\ln \frac{C}{C_0} = -k \cdot t \tag{2}$$

or

$$C = C_0 \cdot e^{-kt} \tag{3}$$

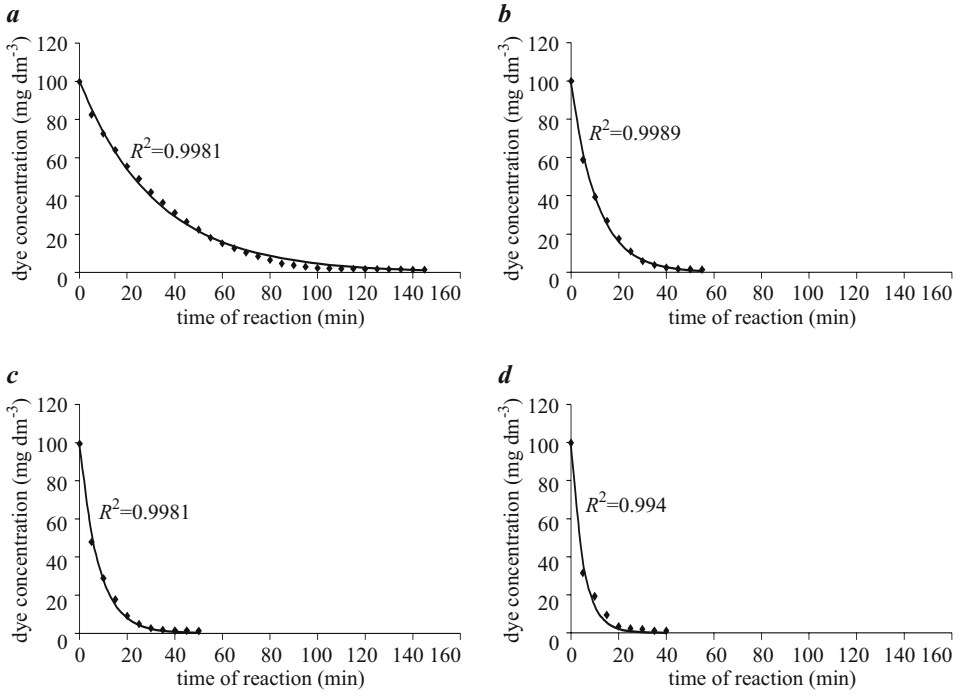


Fig. 1. Reactive Black 8 removal during ozonation versus ozon concentration and time of reaction: *a* – 16 g O_3 m^{-3}, *b* – 24 g O_3 m^{-3}, *c* – 32 g O_3 m^{-3}, *d* – 40 g O_3 m^{-3}

Table 2, Table 3 presents reaction rate constants determined with the method of non-linear regression for the two dyes analyzed. The study demonstrated that at a stable dye concentration depended on ozone dose applied and ranged from 0.01571 to 0.10551 min^{-1} for Reactive Orange 16 and from 0.03068 to 0.19242 min^{-1} for Reactive Black 8. The effect of an increasing dose of ozone from 16 to 40 g O_3 m^{-3} was comparable for both dyes and evoked an over 6-fold increase in the reaction rate constant. However, a strong effect of the chemical structure of dye on the rate of its removal was observed in the study. The dye with the chlorotriazine active group – Reactive Black 8 – was removed by ca. 2 times faster as compared to the dye containing the vinylsulfone active group.

The effect of the type of dye and its concentration in the solution on the effectiveness of dye removal has been earlier described by FARIA et al. (2005). They examined the kinetics of the ozonation process for three dyes belonging to various groups – acid (C.I. Acid Blue 113), reactive (C.I. Reactive Red 241) and basic (C.I. Basic Red 14), and demonstrated the impact of the type of dye on the kinetics of that process. Yet, the lowest reaction rate was obtained for the reactive dye, i.e. Reactive Red 241. It possessed a vinylsulfone group,

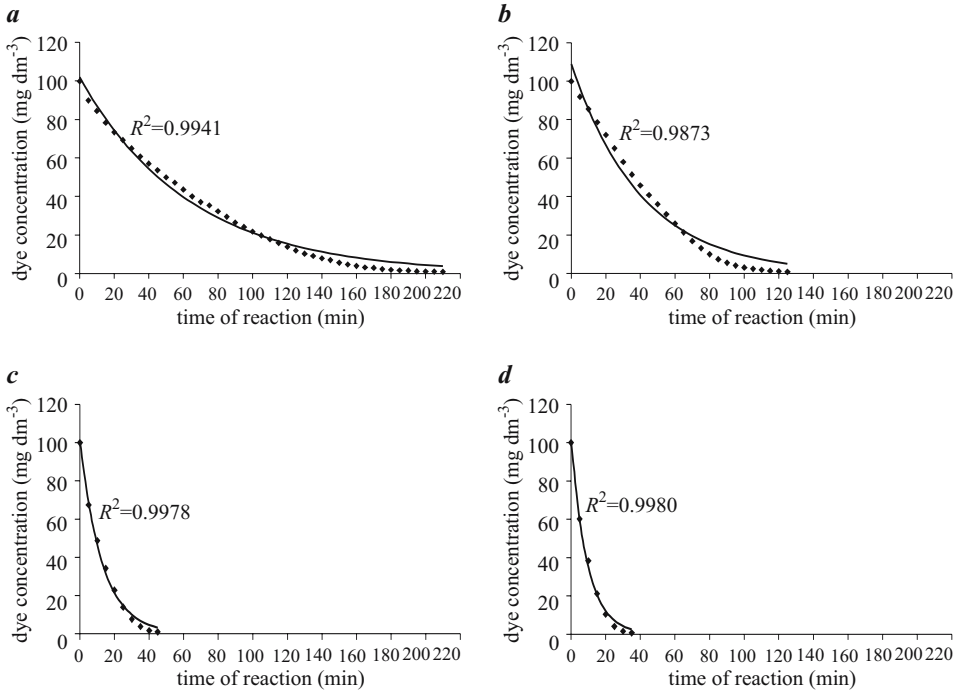


Fig. 2. Reactive Orange 16 removal during ozonation versus ozon concentration and time of reaction: *a* – 16 g O_3 m^{-3}, *b* – 24 g O_3 m^{-3}, *c* – 32 g O_3 m^{-3}, *d* – 40 g O_3 m^{-3}

Table 2

First order rate constants of decolourisation by ozonation at different concentrations of ozone

Dye type	Kinetics constants k (min^{-1})			
	16 g O_3 m^{-3}	24 g O_3 m^{-3}	32 g O_3 m^{-3}	40 g O_3 m^{-3}
Reactive Orange 16	0.01571	0.02457	0.0773	0.10551
Black 8	0.03068	0.09120	0.1249	0.19242

Table 3

Rate of decolourisation by ozonation at different concentrations of ozone

Dye type	Kinetics constants k (min^{-1})			
	16 g O_3 m^{-3}	24 g O_3 m^{-3}	32 g O_3 m^{-3}	40 g O_3 m^{-3}
Reactive Orange 16	1.57	2.46	7.73	10.55
Black 8	3.07	9.12	12.49	19.24

analogously to Reactive Orange 16 dye analyzed in our study. The reported research showed that within one group of reactive dyes the reaction rate constants are different and depend on the type of reactive group.

This has been confirmed by results described in a paper by DONG et. al. (2007). Those authors presented a mechanism and kinetic model of degradation of three synthetic dyes – Reactive Brilliant Blue KN-R, Reactive Brilliant Red X-3B and Acid Scarlet G. Reaction rate constants determined for those dyes accounted for: 0.0622 (Reactive Blue KN-R), 0.1454 (Reactive Red X-3B) and 0.2045 min^{-1} (Acid Scarlet GR). Out of the dyes tested, two belonged to a group of reactive dyes, yet Reactive Brilliant Blue KN-R contained a vinylsulfone active group – similarly to Reactive Orange 16 analyzed in our experiment, whereas Reactive Brilliant Red X-3B possessed a chlorotriazine active groups – analogously to Reactive Black 8 from our study. Likewise in the reported research, the reaction rate constant determined for the dye with the vinylsulfone active group was ca. twice as low as that calculated for the dye with the chlorotriazine group. The results obtained are likely to affect designing systems to be used in the dye removal process. The quantity of ozone assumed in the technology including ozonation should be adjusted to the type of dye and, in particular, to its chemical structure and reactive group.

Conclusions

1. Experiments demonstrated that the rate of dye removal depended on the type of dye. Reactive Orange 16 – a dye with a vinylsulfone active group was removed ca. 2 times slower than Black DN possessing a chlorotriazine group.

2. The effect of an increased dose of ozone on the increase in the rate of dye removal was comparable for both dyes examined. Increasing ozone dose from 16 to 40 g O_3 m^{-3} resulted in a 6.72-fold and 6.27-fold increase in the reaction rate for Reactive Orange 16 and Reactive Black 8, respectively.

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ANTHROPOGENIC IMPACT ON QUANTITATIVE DIFFERENTIATION OF NITROGEN CYCLING BACTERIA IN WATERS OF THE DRWĘCA RIVER

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K e y w o r d s: water, proteolytic bacteria, ammonifying bacteria, nitrifying bacteria, N-NO_3 to N-NO_2 reducing bacteria, denitrifying bacteria, *Clostridium pasteurianum*, *Azotobacter* sp.

A b s t r a c t

Water in the Drwęca River has been monitored in terms of differences in counts of bacteria which are active in transformations of nitrogen compounds depend on various man-made activity. In 2000–2001 samples of waters were assayed for counts of proteolytic, ammonifying, AOB, NOB, N-NO_3 to N-NO_2 reducing bacteria, denitrifying bacteria as well as anaerobic (*Clostridium pasteurianum*) and aerobic (*Azotobacter* sp.) atmospheric nitrogen binding bacteria, which differed within several orders (0–10^5 cfu 1 cm^{-3} or MPN 100 cm^{-3}) depending on the analysed physiological group of bacteria, sampling site or date. Statistically significant differences in counts of particular groups of microorganisms in the analysed water samples collected from the Drwęca River proved between the sampling dates only confirm that the river may be seasonally polluted by domestic sewage from towns and villages located in the river catchment or be associated with the cyclic nature of fisheries and/or agricultural production.

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ANTROPOGENICZNE ODDZIAŁYWANIE NA ILOŚCIOWE ZRÓŻNICOWANIE BAKTERII BIORĄCYCH CZYNNY UDZIAŁ W PRZEMIANACH ZWIĄZKÓW AZOTU W WODACH RZEKI DRWĘCY

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Słowa kluczowe: woda, bakterie proteolityczne, bakterie amonifikacyjne, bakterie nitryfikacyjne, bakterie redukujące N-NO_3 do N-NO_2, bakterie denitryfikacyjne, *Clostridium pasteurianum*, *Azotobacter* sp.

Abstrakt

Badano wody rzeki Drwęcy pod kątem zróżnicowania ilościowego bakterii biorących czynny udział w przemianach związków azotu, uwzględniając rodzaj działalności antropogenicznej. W latach 2000–2001 w próbach wód liczebność badanych bakterii proteolitycznych, amonifikacyjnych, nitryfikacyjnych I i II fazy, redukujących NO_3-N do NO_2-N, denitryfikacyjnych oraz wiążących azot atmosferyczny w warunkach beztlenowych (*Clostridium pasteurianum*) i tlenowych (*Azotobacter* sp.) różniła się o kilka rzędów wielkości (0–10^5 jtk 1 cm^{-3} lub NPL 100 cm^{-3}) w zależności od oznaczanej grupy fizjologicznej, stanowiska i okresu badawczego. Statystycznie istotne różnice w ilościowym występowaniu poszczególnych grup drobnoustrojów w badanych próbach wód, stwierdzono tylko między okresami badawczymi. Potwierdzają one możliwość sezonowego dopływu zanieczyszczeń pochodzenia allochtonicznego ze ścieków bytowo-gospodarczych z miejscowości położnych na terenie zlewni rzeki oraz związanych z cyklicznym charakterem gospodarki rybackiej i rolnej.

Introduction

Microbiological transformations of organic and mineral nitrogen compounds play a special role in aqueous ecosystems. The course and rate of such conversions depend primarily on the biochemical activity of dominant physiological groups of microorganisms (MUDRYK, DONDERSKI 1997). Particular assemblages of bacteria which participate in the nitrogen cycle differ in their physiology but together they constitute natural microflora of surface waters (LEWANDOWSKA et al. 2003, WIŚNIEWSKA et al. 2006). Composition of microbial flora, both qualitative and quantitative, can be affected by various environmental and antrophogenic factors (like: nature and properties of a water body and its catchment, atmospheric precipitations, seasons of the year, effects caused by man influx of domestic, intensive farming practice, increasing urbanisation) (KARPIŃSKI 1995, BOAVENTURA et al. 1997, LEWANDOWSKA et al. 2003, GOTKOWSKA-PŁACHTA et al. 2005, NIEWOLAK et al. 2005, NIEWOLAK

2006). Such fluctuations in assemblages of microorganisms are particularly unwanted in water bodies which are legally protected, and the upper course of the Drwęca River has been a water and ichthyological reserve since 1961.

Material and Methods

Research object

The Drwęca is a typical lakeland river. A right tributary of the Vistula River, the Drwęca is 207.2 km long and drains water from an area of 5343.5 km^2. The section of the Drwęca which flows through the area of the Warmia and Mazury Province is about 95 km long (*Raport o stanie środowiska* ... 2003). In its upper course, the river flows through a small lake called Ostrowin and a typical beeded lake known as Drwęckie (*Raport o stanie środowiska* .... 2000).

In 1961 the whole length of the Drwęca River was turned into a nature reserve. The water nature reserve covers 1888.4 ha from the river sources to its estuary into the Vistula River. This area of legally protected, called the 'Drwęca River Nature Reserve', was set up to protect the river's aqueous environment and its fish, in particular trout, salmon, lake trout and vimba. This is the longest ichthyological nature reserve in Poland and the total area covered by legal protection is 444.38 ha. Owing to large differences in elevation between the Drwęca and its tributaries, many of the river's sections are submontate in character. This favours the occurrence of rare species of fish and lampreys, which prefer highly oxygenated water (PETER 1997, 1999, 2003).

The river valley in the narrowest section of the upper course makes a gorge, which is 20–30 m deep and 8 km long. It is known as Czarci Jar (Devil's Gorge) and the Polish Angling Association has a fish hatchery there.

The major sources of point pollution for the Drwęca River are: household sewage and wastewater, industrial wastewater and post-industrial waters derived from the fish farms in Czarci Jar and Rychnowska Wola. In addition, polluted water is brought to the Drwęca by its tributary rivers such as the Gizela, the Iławka, the Sandela and the Wel, which receive wastewater from Bałcyny, Iława, Lubawa and Lidzbark Welski (*Raport o stanie środowiska*... 2003).

Sampling sites

The microbiological assays involved a 15-km long section of the upper Drwęca. Water samples were collected at 10 research sites set up in some characteristic parts of the river, from its sources up to Ostrowin Lake (Figure 1).

Fig. 1. Location sketch of sampling sites (I, II.X) in Drwęca River

– site I – 2 km away from the river sources;
– site II – before Fish Farm no. 1 (stocked with 20 tons of fish fry) in the village of Czarci Jar;
– site III – where the river flows from :the trout part; of Fish Farm no. 1 in the village of Czarci Jar;
– site IV – where the river flows from earth fish ponds of Fish Farm no.1 in the village of Czarci Jar;
– site V – 2 km away from Fish Farm no.1, in the village Gibała;
– site VI – in Rychnowska Wola, before Fish Farms no. 2 and 3;
– site VII – where the river flows from Fish Farm no. 2 (stocked with 54 tons of commercial trout) in Rychnowska Wola;
– site VIII – where the river flows from Fish Farm no. 3 (stocked with 58 tons of commercial trout) in Rychnowska Wola;
– site IX – 2.5 km away from Fish Farms no. 2 and 3 in Rychnowska Wola;
– site X – a bridge on the Drwęca before the river flows into Ostrowin Lake.

Sampling

Water samples from the Drwęca River were taken at 0.3–0.5 m depth in two-month intervals from January 2000 to December 2001. Water samples were collected directly to sterile bottles, according to the guidelines of the *Polish Norm (Woda i ścieki... PN-74/C-0462002)* and the *American Public Health Association* (1992). The samples were transported to a laboratory in an ice box at 4°C. The time which elapsed from the sampling to analyses did not exceed 6 hours.

In 2000–2001, 120 water samples from the River Drwęca underwent microbiological assays.

Microbiological assays

The microbiological assays involved the following determinations:

– counts of proteolytic bacteria (cfu 1 cm^{-3}) by Frazier's method (Rodina 1968) after 6-day incubation at 20°C (*Woda i ścieki... PN-74/C-04615, arkusz 17*);

– counts of ammonifying bacteria (MPN 100 cm^{-3}) on a broth medium supplemented with 3% peptone (pH 7.2) after 7-day incubation at 26°C (*arkusz 18*);

– counts of phase I nitrifying bacteria as AOB (MPN 100 cm^{-3}) on Meiklejohn's medium supplemented with $(NH_4)_2SO_4$ after 28-day incubation at 25°C, and counts of phase II nitrifying bacteria as NOB (MPN 100 cm^{-3}) on the same medium but supplemented with $NaNO_2$ after 28-day incubation at 25°C (SKERMAN 1967);

– counts of bacteria reducing NO_3-N to NO_2-N (MPN 100 cm^{-3}) on Giltay's medium in Durham's test tubes (RODINA 1968) after 7-day incubation at 25°C as well as counts of denitrifying bacteria reducing NO_3-N to N_2-N (MPN 100 cm^{-3}) on the same medium and with Durham's test tubes after 14-day incubation at 25°C;

– counts (MPN 100 cm^{-3}) of bacteria binding atmospheric nitrogen under anaerobic conditions (*Clostridium pasteurianum*) on Winogradsky's medium after 7-day incubation at 25°C (RODINA 1968);

– counts (cfu 1 cm^{-3}) of bacteria binding atmospheric nitrogen under aerobic conditions (*Azotobacter* sp.) on Fiodorow's medium supplemented with 2% mannitol after 7-day incubation at 25°C (RODINA 1968).

The determinations concerning numbers of proteolytic and aerobic atmospheric nitrogen fixing bacteria (*Azotobacter* sp.) were performed using the pour-plate method and the results were converted into cfu per 1 cm^3 of water.

In order to determine proteolytic bacteria, after 6-day incubation the plates were flooded with Frazier's reagent (RODINA 1968) and after 15 to 20 minutes, in order to count, all colonies with transparent zones (*Woda i ścieki... PN-75/C-04615, arkusz 17*). Characteristic large, cream white colonies were counted as *Azotobacter* sp. bacteria binding atmospheric nitrogen under aerobic conditions (RODINA 1968).

The MPN of ammonifying, AOB, NOB, bacteria reducing NO_3-N to NO_2-N, denitrifying bacteria and anaerobic atmospheric nitrogen binding (*Clostridium pasteurianum*) bacteria were determined by performing inoculations from 10-fold dilutions of a given water sample in 3 parallel replications. The results (MPN) were read from McCrady's tables (MEYNELL, MEYNELL 1970) and expressed per 100 cm^3 of water.

In order to determine MPN 100 cm^{-3} of ammonifying bacteria, changes such as cloudiness, coating or sediment at the bottom of a test tube with the medium were observed. In addition, the medium's reaction (pH) and presence of ammonia, made evident with an aid of Nessler's reagent, were tested. The test tubes with a visible growth of bacteria, presence of ammonia and the medium's alkaline reaction to about 8-9, which all proved that ammonification had taken place, were considered to be positive (*Woda i ścieki... PN-75/C-04615, arkusz 18*).

As regards nitrifying bacteria, after the incubation period, cultures were tested for the presence of NO_2-N (AOB) or NO_3-N (NOB) using Griess's dry reagent (RODINA 1968). NO_3-N was determined using a solution of diphenylamine in concentrated H2SO4, having first decomposed in the medium all the remaining, non-oxidised NO_2-N, using for this purpose ammonia and sulphuric acid. In addition, the samples in which the presence of NO_2-N or NO_3-N had been determined were examined under a microscope to find out if the proper nitrifying bacteria were present too.

In the case of bacteria reducing NO_3-N to NO_2-N and denitrifying bacteria, in samples where the medium had changed from green and blue to green and yellow, blue or yellow the following were tested: presence of nitrites using Griess's dry reagent (RODINA 1968), absence of ammonia using Nessler's reagent and absence of nitrates using diphenylamine in concentrated sulphuric acid (SKERMAN 1967), presence or absence of gas in Durham's test tubes.

The presence of bacteria binding atmospheric nitrogen under anaerobic conditions (*Clostridium pasteurianum*) was confirmed when the characteristic smell of butyric acid was perceptible and Durham's tubes contained gas. In addition, the dilutions which generated positive results were used to make microscopic preparations in order to confirm the occurrence of those bacteria (RODINA 1968).

Statistical analysis

In order to perceive relationships between counts of the assayed groups of bacteria: ammonifying, proteolytic, AOB, NOB, bacteria reducing NO_3-N to NO_2-N, denitrifying bacteria, binding atmospheric nitrogen under anaerobic (*Clostridium pasteurianum*) and aerobic (*Azotobacter* sp.) conditions, and the sampling sites as well as times of the year, a one-factor analysis of variance (ANOVA) was applied, which presumed that the variances in different groups are uniform (the same). The most powerful test to verify this hypothese was Leven's test. When Leven's test was significant (where $p < 0.05$), the hypothesis on uniform variance was discarded. Next Kruskal-Wallis test, a non-parametric equivalent to one-factor analysis of variance, was performed to verify the hypothesis that the samples compared had been obtained from a population characterised by the same distribution or distributions of the same median (STANISZ 2006).

Results

The results of determinations of counts of the following bacteria: proteolytic, ammonifying, AOB, NOB, bacteria reducing NO_3-N to NO_2-N, denitrifying bacteria and binding atmospheric nitrogen under anaerobic (*Clostridium pasteurianum*) and aerobic (*Azotobacter* sp.) conditions, in samples of water collected from the Drwęca River are presented in Table 1. Means and ranges of their counts differed within a few orders of magnitude depending on: the physiological groups of bacteria the sampling sites and sampling dates.

The mean counts of proteolytic bacteria varied from 359 cfu 1 cm^{-3} at site I to 22 902 cfu 1 cm^{-3} at site VIII. During the whole period covered by the research project, the smallest differences in quantities of those bacteria occurred at sites I and X (35–1000 cfu 1 cm^{-3} and 26–1000 cfu 1 cm^{-3}, respectively), whereas the largest differences were found at site VIII (160 to 100 000 cfu 1 cm^{-3}).

Ammonifying bacteria revealed similar trends in their lowest and highest counts (means and ranges) at the respective sampling sites. The mean numbers of these bacteria were up to 10-fold smaller than the counts of proteolytic bacteria and varied from 930 MPN 100 cm^{-3} (site I) to 14 000 MPN 100 cm^{-3} (site VIII). The smallest differences in amounts of ammonifying bacteria were observed at site I whereas the biggest differences occurred at site VIII. These differences were: 110–3000 and 200–70 000 MPN 100 cm^{-3}, respectively.

At most of the sampling sites, the mean numbers AOB and NOB were found to be similar. The mean of the quantitative occurrence of AOB varied

Table 1
The numbers (means and ranges) of the physiological groups of bacteria active in nitrogen cycling assayed in samples of water of the Drwęca River in 2000–2001

Sampling sites	Groups of microorganisms							
	proteolytic bacteria (cfu 1 cm^{-3})	ammonifying bacteria	AOB	NOB	bacteria reducing NO_3-N to NO_2-N	denitrifying bacteria	Bacteria fixing atmospheric nitrogen	
							Clostridium pasteurianum	*Azotobacter* sp.
		(MPN 100 cm^{-3})						(cfu 1 cm^{-3})
I	359 35–1000	930 110–3000	10 0–40	38 0–140	382 0–1100	366 70–700	6 0–30	23 0–75
II	709 27–2000	1215 300–3500	32 0–150	42 0–160	732 0–2500	490 0–2000	3 0–7	71 0–250
III	1418 20–5000	6314 2000–16 000	280 0–1400	127 0–140	6110 0–30 000	710 0–3 000	8 3–20	89 0–364
IV	2428 38–5300	7812 3500–20 000	224 0–1100	282 0–1400	7100 0–35 000	586 30–2000	11 4–35	60 0–250
V	1200 36–4589	2118 600–4000	125 0–650	143 0–1400	1050 40–4500	104 0–3500	9 0–40	50 2–98
VI	420 59–1030	3450 160–16 000	168 0–400	356 0–1400	490 0–1500	496 60–1100	15 2–45	28 0–115
VII	3798 20–15 000	6368 400–11 500	283 0–1400	284 0–1400	756 0–30 000	626 70–3500	5 0–16	86 4–320
VIII	22 902 160–100 000	14 000 200–70 000	320 0–1400	300 0–1100	2392 0–11 000	3202 400–14 000	3 0–7	98 10–340
IX	800 200–1600	3862 600–7000	253 0–1100	558 0–1100	506 0–1600	1252 60–4500	13 3–30	156 45–550
X	714 26–1 000	3581 9–11 000	305 0–1400	536 0–1400	216 0–750	410 0–1500	19 4–45	250 80–630

from 10 MPN 100 cm^{-3} at site I to 320 MPN 100 cm^{-3} at site VIII. In comparison, NOB were determined to vary from 38 MPN 100 cm^{-3} at site I to 558 MPN 100 cm^{-3} at site IX. During the whole duration of the experiment, the smallest differences in counts of AOB appeared at site I (0–40 MPN 100 cm^{-3}) and the biggest ones – at sites III, VII, VIII and X (0–1400 MPN 100 cm^{-3}). On the other hand, the smallest differences in counts of NOB were determined in water samples collected at sites I and III (0–140 MPN 100 cm^{-3}) and the biggest ones – at sites IV, V, VI, VII and X (0–1 400 MPN 100 cm^{-3}).

In waters of the Drwęca River, the mean quantities of bacteria which reduce NO_3-N to NO_2-N varied from 216 MPN 100 cm^{-3} at site X to 7100 MPN 100 cm^{-3} at site IV. Likewise, the smallest differences in counts of these bacteria were found at site X (0–750 MPN 100 cm^{-3}) and the biggest ones – at site IV (0–35 000 MPN 100 cm^{-3}).

In samples of water collected from the Drwęca, denitrifying bacteria (which reduce NO_3-N to N_2-N) occurred in numbers several-fold lower or similar to quantities of bacteria reducing NO_3-N to NO_2-N, depending on a sampling site. The mean, their lowest counts (104 MPN 100 cm^{-3}) were detected at site V and the largest ones (3202 MPN 100 cm^{-3}) – at site VIII. The minimum differences in ranges of their quantitative occurrence (70–7000 MPN 100 cm^{-3}) were determined at site I and the maximum ones (400–14 000 MPN 100 cm^{-3}) – at site VIII.

During the whole time period covered by the study, *Clostridium pasteurianum* occurred in the smallest counts (both in terms of means and ranges) among all atmospheric binding bacteria. The mean counts of *C. pasteurianum* varied from 3 MPN 100 cm^{-3} at sites II and VIII to19 MPN 100 cm^{-3} at site X. The smallest differences in counts of these bacteria were noted at sites II and VIII (0–7 MPN 100 cm^{-3}) and the biggest ones – at site X (4–45 MPN 100 cm^{-3}).

In turn, the mean numbers of *Azotobacter* sp., which bind atmospheric nitrogen under aerobic conditions, ranged from 23 cfu 1 cm^{-3} at site I to 250 cfu 1 cm^{-3} at site X. During the whole research period, the minimum and maximum differences in the quantities of these bacteria were also observed at the analogous sampling sites. They were 0–75 cfu 1 cm^{-3} (site I) and 80–630 cfu 1 cm^{-3} (site X), respectively.

The values of the coefficient of significance of differences (p) and statistical relationships between quantitative occurrence of the analysed bacteria, i.e. proteolytic, ammonifying, AOB, NOB, bacteria reducing NO_3-N to NO_2-N, denitrifying bacteria, binding atmospheric nitrogen under anaerobic (*Clostridium pasteurianum*) and aerobic (*Azotobacter* sp.) conditions, in waters of the Drwęca River between the sampling sites and dates are shown in Table 2. Kruskal-Wallis test revealed that statistically significant differences ($p < 0.05$) between counts of all the assayed groups of bacteria occurred only

between sampling dates. No such correlation was found between the sampling sites for any of the above groups of bacteria.

Table 2
Relationship between of the numbers of bacteria participating in the mineralization processes of nitrogen compounds and sampling sites and periods of study in River Drwęca based on test of the Kruskal-Wallis

Group of microorganisms	Coefficient correlation (p) between numbers of studied groups of microorganisms	
	at sampling sites	in periods of study
Proteolytic bacteria	0.0642	0.0352*
Ammonifying bacteria	0.1014	0.0127*
AOB	0.7969	0.0000*
NOB	0.1989	0.0250*
Bacteria reducing NO_3-N to NO_2-N	0.9972	0.0000*
Denitrifying bacteria	0.2175	0.0040*
Bacteria *Azotobacter* sp. fixing atmospheric nitrogen under aerobic conditions	0.3076	0.0136*
Bacteria *Clostridium pasteurianum* fixing atmospheric nitrogen under anaerobic conditions	0.2820	0.0098*

* – satistically important differences ($p < 0.05$)

Discussion

Processes of mineralization of organic matter, which is produced in water bodies or which reaches surface waters from their basins, are largely shaped by the presence and activity of various physiological groups of microorganisms (MUDRYK, DONDENRSKI 1997, WIŚNIEWSKA et al. 2006). This specially concerns bacteria which play an active role in transformations of organic and inorganic nitrogen compounds (BOTHE et al. 2000, BOLLMANN, LAANBROEK 2001, YE, THOMAS 2001). In waters of the section of the Drwęca covered by the present study, counts of the analysed bacteria, i.e. proteolytic, ammonifying, AOB, NOB, bacteria reducing NO_3-N to NO_2-N, denitrifying bacteria, binding atmospheric nitrogen under anaerobic (*Clostridium pasteurianum*) and aerobic (*Azotobacter* sp.) conditions, differed within the range of a few orders of magnitude, depending on: a physiological group of bacteria, sampling site and date. Counts of proteolytic, ammonifying and AOB reached the lowest values at site I and the highest ones – at site VIII. Such tendencies in quantitative occurrence of the above bacteria may have been caused by a low content of organic matter in water near the river's sources. In contrast, higher

counts of these bacteria determined at sites III and IV (behind the Fish Farm in Czarci Jar) as well as their highest amounts determined at sites VII and VIII (behind the Fish Farms in Rychnowska Wola) suggested there was some influx of organic matter associated with intensive pisciculture (BOAVENTURA et al. 1997). Counts of proteolytic and ammonifying bacteria are closely connected with amounts of organic nitrogen whereas development of nitrifying bacteria is conditioned by bioavailability of ammonia (BOTHE et al. 2000, BOLLMANN, LAANBROEK 2001). Thus, in samples of water collected from the Drwęca at sites located behind fish farms, these bacteria could advance better owing to organic matter supplied from the fish farms in the form of unused fish fodder and fish excrement (ENNEL 1995, KARPIŃSKI 1995, BOAVENTURA et al. 1997). An additional factor which could have modified quantitative composition of the above bacteria was the water from atmospheric precipitations which leached organic compounds and microorganisms from the soil into the river (NIEWOLAK et al. 2005).

In most of the samples from the Drwęca, quantitative presence of NOB was similar to that of AOB, which could be attributed to the growth of these bacteria stimulated by the occurrence of nitrate ions produced during the first phase of nitrification. This process means some preservation of the continuity in transformations of nitrogen compounds taking place in due time periods (GOTKOWSKA-PŁACHTA et al. 2005). This fact is further confirmed by the observed gradual and continuous increase in counts of NOB at subsequent sampling sites. The highest numbers (1100 to 1400 MPN 100 cm^{-3}) of both groups of those chemoautotrophic nitrifying bacteria, determined at most of the research sites on the Drwęca, suggest that new cells of nitrifying bacteria adsorbed on mineral and organic particles in the river;s catchment may have been leached by rainwater (AAKRA et al. 2000).

The amounts of microorganisms reducing NO_3-N to NO_2-N and denitrifying bacteria in waters of the Drwęca River, which ranged between 0 to 104 MPN 100 cm^{-3}, were similar to those detected in other water bodies (LEWANDOWSKA et al. 2003, GOTKOWSKA-PŁACHTA et al. 2005, NIEWOLAK 2006). Differences in the counts of these bacteria found at particular sampling sites could have been due to different oxygen content in water, temperature (time of the year), possible influx of allochtonous substance as well as natural interaction between oxygenating and nitrogen reducing bacteria (KOTLAR et al. 1996, BOTHE et al. 2000, LEWANDOWSKA et al. 2003).

The quantitative occurrence of bacteria binding atmospheric nitrogen under aerobic or anaerobic conditions in waters of the Drwęca River was congruent with the relevant data cited by references (NIEWOLAK et al. 2005, WIŚNIEWSKA et al. 2006). Counts of bacteria belonging to *Azotobacter* sp. Were lower by 2 to 3 orders of magnitude than numbers of *Clostridium*

pasteurianum, which may have been an effect of different water soluble oxygen concentrations (GOTKOWSKA-PŁACHTA et al. 2005, NIEWOLAK et al. 2005).

Differences in the counts of particular groups of microorganisms which take part in nitrogen cycling found in the analysed samples of water collected from the Drwęca were proved to be statistically significant only between the time periods studied. This suggests that there could have been some seasonal influx of allochtonous pollutants (BOAVENTURA et al. 1997). Increased concentrations of such organic and mineral substances may be associated with the cyclic nature of intensive fish farming carried out in the area covered by the research. Besides, another factor which could have modified bacterial assemblages in waters of the Drwęca was the pollution caused by intensive agricultural production. Such pollutants reach the river from farms and villages in its basin together with atmospheric precipitation. Both surface flows and water percolating through soil result in quantitative and qualitative modifications of bacterial assemblages which participate in the cycling of nitrogen in nature and make up natural microflora of surface waters (LEWANDOWSKA et al. 2003, WIŚNIEWSKA et al. 2006). Under the influence of long-term pollution originating from local sources, the general and microbial quality of waters of the Drwęca River, a nature reserve, can change.

Conclusions

1. The quantitative occurrence of proteolytic, ammonifying, AOB, NOB, bacteria reducing NO_3-N to NO_2-N, denitrifying bacteria as well as bacteria binding nitrogen under anaerobic (*Clostridium pasteurianum*) and aerobic (*Azotobacter* sp.) conditions, determined in waters of the Drwęca River, varied within a few orders of magnitude depending on: the physiological group of studied microorganisms sampling site and date.

2. Increased counts of most of the analysed groups of microorganisms found at sites III and IV (behind the Fish Farm in Czarci Jar) and at sites VII and VIII (behind the Fish Farms in Rychnowska Wola) suggest that there is some seasonal and/or local influx of organic matter associated with intensive aquaculture.

3. Statistically significant differences in counts of particular groups of bacteria in the analysed samples of water collected from the Drwęca River, which were detected only between the sampling dates, confirm seasonal influx of allochtonous pollutants from domestics sewage from towns and villages located in the river;s catchment as well as those associated with the cyclic nature of fish farming and/or agricultural production.

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INTERACTIONS OF ALUMINUM AND IRON(III) SALTS WITH HUMIC ACIDS IN A MODEL ALKALINE SOLUTION

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K e y w o r d s: humic acids, aluminum salts, iron(III) salts, coagulation.

A b s t r a c t

Both ferric and aluminum salts show good coagulation and complex-forming ability in relation to water-dissolved organic matter. The aim of this study was to characterize the reactions of aluminum salts ($AlCl_3$) and iron(III) salts ($FeCl_3$) with humic acids (HA) in a model alkaline solution as dependent on the type and concentration of coagulant. Conductometric and pH-metric titrations and coagulation jar tests were performed. Changes in COD, color and streaming potential were measured in coagulated samples.

It was found that the characteristic changes in pH and electrolytic conductivity of solutions during titration with Al and Fe(III) salts are indicative of a gradual course of humic acids coagulation process. Depending on the salt used (aluminum or iron(III)), humic acids removal is a result of coagulation proceeding via various mechanisms. Precipitation was observed at a dose of 3.2 mmol dm^{-3} and $pH < 6.4$ in the case of $AlCl_3$, and at a dose of 3.0 mmol dm^{-3} and $pH < 4.8$ in that of $FeCl_3$. COD and color removal efficiency was equal to approximately 97% and 99% after coagulation with both $AlCl_3$ and $FeCl_3$. The increase in streaming potential at a salt dose of 2.4–3.6 mmol dm^{-3} was probably related to the binding of positively charged products of salt hydrolysis by the functional groups of humic acids molecules, followed by the neutralization of their charge.

ODDZIAŁYWANIE SOLI GLINU I ŻELAZA(III) Z KWASAMI HUMINOWYMI W ALKALICZNYM ROZTWORZE MODELOWYM

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S ł o w a k l u c z o w e: kwasy huminowe, sole glinu, sole żelaza(III), koagulacja.

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Abstrakt

Sole żelaza i glinu wykazują wysoką zdolność koagulacyjną oraz kompleksotwórczą w stosunku do rozpuszczonej w wodzie materii organicznej. Celem badań było scharakteryzowanie oddziaływania soli glinu ($AlCl_3$) oraz żelaza(III) ($FeCl_3$) z kwasami huminowymi (KH) w alkalicznym roztworze modelowym w zależności od rodzaju i stężenia użytego koagulantu. Przeprowadzono miareczkowania konduktometryczne i pH-metryczne oraz testy koagulacyjne – „jar testy". W próbach poddanych koagulacji mierzono zmiany: ChZT, barwy i potencjału przepływu.

Wykazano, że charakterystyczne zmiany pH oraz przewodności elektrolitycznej roztworów podczas miareczkowania za pomocą soli Al i Fe(III) świadczą o stopniowym przebiegu procesu koagulacji kwasów huminowych. W zależności od rodzaju użytej soli – glinu lub żelaza(III) usuwanie kwasów huminowych jest wynikiem koagulacji zachodzącej wg różnych mechanizmów. Wytrącanie się osadu stwierdzono po dawce 3.2 mmol dm^{-3} przy pH < 6.4 w przypadku $AlCl_3$ oraz po dawce 3.0 mmol dm^{-3} i pH < 4.8 w przypadku $FeCl_3$. Po koagulacji za pomocą zarówno $AlCl_3$, jak i $FeCl_3$ stwierdzono ok. 97% efektywność usuwania ChZT oraz ok. 99% efektywność usuwania barwy. Zwiększenie wartości potencjału przepływu w zakresie dawek soli 2.4–3.6 mmol dm^{-3} jest prawdopodobnie wynikiem wiązania dodatnio naładowanych produktów hydrolizy soli przez grupy funkcyjne cząsteczek KH i neutralizacji ich ładunku.

Introduction

The main component of water-dissolved organic matter are humic substances, which account for 50% to 70% of its total content (GÓRNIAK 1996). Studies on the chemical structure of humic substances showed that they are chemically non-homogenous, macromolecular organic compounds with a complex skeleton composed primarily of aromatic rings bound to organic functional groups (SHULTEN 2001).

From the perspective of economic purposes of water use for, the presence of these substances is undesirable. They have an adverse impact on the physicochemical properties of water, including color and COD, and may increase the risk of formation of disinfection by-products (ZHANG, MINEAR 2002). Due to their chemical structure, humic substances do not undergo fast microbial decomposition, and their elimination requires the use of physicochemical methods. They can be effectively removed by coagulation (O'MELIA et al. 1999). This process is based on interactions between humic acid molecules and molecules of hydrolyzing salts. Organic colloid coagulation may be induced using chemical reagents characterized by strong destabilization ability. Metal ions found in surface waters are capable of binding organic ligands ($Ca^{2+} < Al^{3+} < Fe^{3+}$), and their concentration affects the organic matter content of surface waters (KAISER 1998). The stability of colloidal particles may decrease as a result of their reactions with molecules carrying opposite charges. According to Model VI (TIPPING et al. 2002), humic acids are able to bind both metal ions and their first hydrolysis products – $MeOH^{2+}$, whose concentration in the solution depends on pH. A better understanding of interactions between humic acids and aluminum salts and iron(III) salts during coagulation may contribute to the optimization of this process.

The aim of this study was to characterize the interactions of aluminum salt ($AlCl_3$) and iron(III) salt ($FeCl_3$) with humic acids (HA) in a model alkaline solution as dependent on the type and concentration of coagulant.

Materials and Methods

A model solution of humic acids was used (Table 1). 1 dm^3 of the solution was prepared using 500 mg of humic acids (Fluka No. 53680). The analytical sample KH dried to constant mass at 50°C was dissolved in 100 cm^3 of 0.1 mol dm^{-3} NaOH solution and then was diluted to 1 dm^3 with redistilled water. The solution for experiments was 500 mg KH in 0.01 mol dm^{-3} NaOH solution.

Table 1

Characteristics of a model solution of humic acids

pH	11.96 ± 0.02
Electrolytic conductivity (μS cm^{-1})	1325 ± 5
Color – PtCo (mg dm^{-3})	795 ± 12 (dilution 1:25)
Turbidity – FTU (mg dm^{-3})	91 ± 5 (dilution 1:25)
Suspended solids (mg dm^{-3})	289 ± 24
COD (mg O_2 dm^{-3})	795 ± 10
C org. (mg C dm^{-3})	298 ± 4

Total functional group (TA – total acidity) content and carboxylic group (COOH) content was determined with $BaCl_2$ (Kononowa 1968) and $Ca(CH_3COO)_2$ (KONONOWA 1968, SCHNITZHER, KHAN 1972), respectively (Table 2). The content of phenolic groups (OH) was calculated as the difference: *TA* – COOH = OH-phenolic (SCHNITZER, KHAN 1972, SOUZA SIERRA DE et al. 2001).

Table 2

Functional group content of humic acids

Total functional group (TA)	6.18 ± 0.23 meq g^{-1} ashless dry matter of HA
Carboxylic group (COOH)	3.60 ± 0.07 meq g^{-1} ashless dry matter of HA
Phenolic group (OH)	2.58 ± 0.21 meq g^{-1} ashless dry matter of HA

The humic acids solution was coagulated with solutions of aluminum chloride and iron(III) chloride at a concentration of 0.2 mol dm^{-3}. Coagulant solutions were prepared from analytical samples of solid salts, $AlCl_3 \cdot 6H_2O$ p.a. and $FeCl_3 \cdot 7H_2O$ p.a., respectively.

Conductometric and pH-metric titration of 100 cm^3 of humic acids solution and reference solution (NaOH 0.01 mol dm^{-3}) was performed. The coagulant solution was applied at doses of 0.05 cm^3. 1 cm^3 of coagulant solution corresponded to 2 mmol of salt per 1 dm^3 HA solution.

Coagulation efficiency was determined by a jar test procedure. In beakers containing 200 cm^3 of humic acids solution, used increasing coagulant doses. 1 min. of rapid mixing (300 rpm) was followed by 10 min. of slow mixing (30 rpm) and 1 h of sedimentation. COD – Cr (mg O_2 dm^{-3}), color – PtCo (mg dm^{-3}) and pH values (APHA, AWWA and WEF 1995, HERMANOWICZ et al. 1999) were determined in the coagulated solution. Streaming potential (mV) was measured in samples of the humic acids solution during coagulation tests. A PCD 03 detector (Mutek Analytic Gmbh) was used.

Results and Discussion

The curves in Figure 1b and Figure 2b show the results of pH-metric and conductometric titration of a model humic acids solution with 0.2 mol dm^{-3} solutions of aluminum chloride and iron(III) chloride. For the purpose of comparison, pH and electrolytic conductivity were measured during titration of a 0.01 mol dm^{-3} NaOH solution containing no HA (Figure 1a and Figure 2a). There were considerable differences in the course of titration curves obtained for a NaOH solution and an alkaline HA solution. Differences stemming from the type of coagulant were also observed. The reactions taking place in titrated samples and in samples used for jar tests are affected by hydrolysis, formation of complex compounds, adsorption, electrokinetic phenomena, etc. (DUAN, GREGORY 2003). At assumption of simplified scheme of reactions of salt hydrolysis, the obtained products may be $Al(OH)_3$ as well as Al^{3+}, $Al(OH)^{2+}$, $Al(OH)^{2+}$ and $Al(OH)^{4-}$ ions. The equilibrium state between the concentrations of the above ions depends on the pH of the solution (BOTTERO, BERSILLON 1989). At pH > 9, in the state of equilibrium with amorphous $Al(OH)_3$, $Al(OH)^{4-}$ ions dominate in the solution, while the pH range of 5–8 promotes $Al(OH)_3$ precipitation. At pH < 7 the concentrations of cationic hydrolysis products increase significantly. The hydrolysis of iron(III) salts produces ions with a similar structure, but their dependence on the pH of the solution is different, as compared to aluminum salts (MARTIN 1991).

The curves in Figure 1a illustrate three ranges of changes in pH and electrolytic conductivity, corresponding to successive stages of reaction with the coagulant, i.e. pH 11.9–9.8, pH 9.8–5.4 and pH < 5 (towards the end of titration). At first sodium hydroxide reacted with acid formed by hydrolysis of $AlCl_3$, which caused a decrease in pH accompanied by a gradual increase

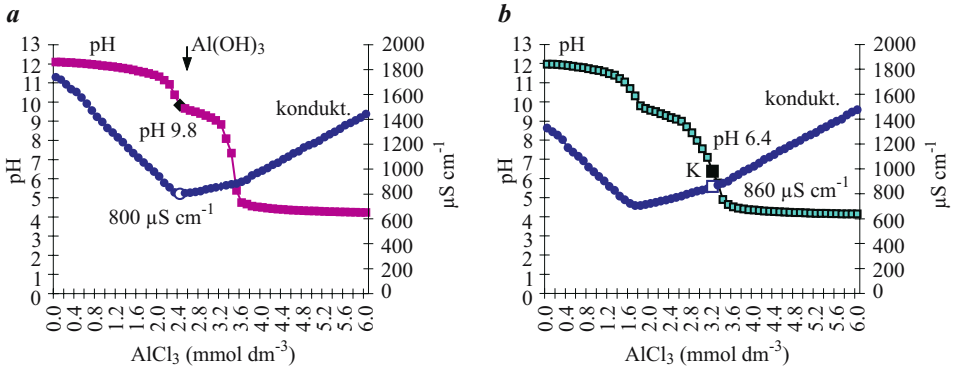

Fig. 1. Titration of 0.01 mol dm^{-3} NaOH (a) and a model HA solution (b) with $AlCl_3$

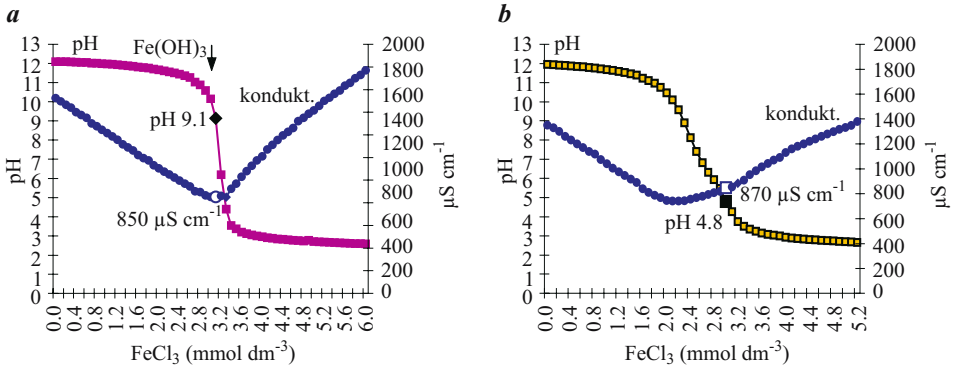

Fig. 2. Titration of 0.01 mol dm^{-3} NaOH (a) and a model HA solution (b) with $FeCl_3$

in the concentration of $Al(OH)^{4-}$. Precipitation was initiated when a molar ratio of hydroxide to aluminum salts (OH/Al) reached approximately 2.8–2.9 at pH = 9.8. Further changes in pH, within the second range, resulted from precipitation of amorphous aluminum hydroxide at pH < 7, followed by its dissolution and formation of aluminum hydroxy cations at pH < 6.0. At pH < 5.0 (third range) Al^{3+} ions could be already observed in the solution.

Similarly as in the case of curves in Figure 1a, the curve representing titration of a HA solution (Figure 1b) may be divided into three parts, corresponding to three pH ranges. The first pH range corresponded to a gradual reaction of neutralization of sodium hydroxide, from pH 12 to 9.8 at a dose of 1.8 mmol $AlCl_3$ dm^{-3}, and further neutralization of OH^- ions formed by hydrolysis of sodium humate. No precipitation was observed at pH 9.8. These

reactions were accompanied by an increase in the concentration of $Al(OH)^{4-}$ ions. Precipitation was initiated at pH 6.38 and a dose of 3.2 mmol $AlCl_3$ dm^{-3}. The value of the isoelectric point of amorphous $Al(OH)_3$ corresponds to pH ~ 8.3 (BOTTERO, BERSILLON 1989). Aluminum hydroxide shows the lowest solubility, about 10^{-6} mol dm^{-3}, at pH ~ 6 (WESOŁOWSKI, PALMER 1994). The experimentally determined dose of $AlCl_3$ initiated coagulation and induced precipitation of $Al(OH)_3$ whose colloidal molecules carry a positive electrical surface charge in a slightly acid environment, which is conducive to the adsorption of negatively charged organic colloids. The mechanism of the so called sweep flocculation may be assumed in this case, according to which an organic colloid binds to the surface of the newly-formed hydroxide during rapid and efficient flocculation (DUAN, GREGORY 2003).

A further increase in the dose, above 3.2 mmol $AlCl_3$ dm^{-3}, was followed by an increase in the acidity of the solution (pH < 6.4) and probably contributed to the precipitation of metal-humic complexes in consequence of reactions of aluminum hydroxo cations and Al^{3+} ions with negatively charged HA molecules (LU et al. 1999). At the final stage of titration (pH < 5) the precipitate was in equilibrium with Al^{3+} ions coming from successive coagulant doses. The characteristic points of inflection and precipitation (point K) on curves representing pH-metric titration are close to those on curves representing conductometric titration.

Figure 2 present curves of titration of the experimental solutions with $FeCl_3$ 0.2 mol dm^{-3}. The courses of these curves are noticeably different. In view of data shown in Figure 2a (NaOH solution), precipitation began at pH ~ 9.1 at a dose of 3.1 mmol $FeCl_3$ dm^{-3}. Next coagulant doses caused a rapid change in pH, which decreased to 4.4. In the case of a HA solution (Figure 2b) precipitation began in an acid environment at pH 4.8 and a comparable (3.0 mmol) coagulant dose. Successive doses of $FeCl_3$ solution intensified precipitation at pH < 4. The changes observed in pH and electrolytic conductivity resulted from reactions of the products of $FeCl_3$ hydrolysis and solution ingredients. Hydrolysis of $FeCl_3$ leads to the formation of $Fe(OH)_3$ and various forms of Fe(III) ions. Cationic products of hydrolysis dominate at pH < 6. The $Fe(OH)_3$ precipitate shows the lowest solubility, about 8^{-10} mol dm^{-9}, at a wide pH range of 6–10 (FLYNN 1984). Iron(III) hydroxide precipitated from $FeCl_3$ and hydroxide precipitated from $Fe_2(SO_4)_3$ have their isoelectric points at pH < 8 and pH ~ 6 (JIANG, GRAHAM 1998). This suggests that at a coagulant dose (determined experimentally) required to initiate precipitation, iron(III) hydroxy cations probably reacted with negatively charged molecules of organic colloids, still present at pH 4.5.

Precipitation in titrated solutions is an important observation in view of the stability of colloidal solutions. It was demonstrated that in a HA

solution, compared with a NaOH solution containing no humic acids, precipitation takes place at lower pH values, following the addition of 3–3.2 mmol Me^{3+} dm^{-3}.

Value of pH at point K are also dependent on the type of salt – aluminum or iron(III), added to the solution. According to BENEGAS et al. (2003), the reason for humic acids precipitation is metal ion binding by functional groups capable of ionization.

The ratio between the functional group content of humic acids in 1 dm^3 of a model solution (2.87 meq), calculated based on their total content (Table 2), and the amount of metal ions (3.2 mmol Al^{3+}) bound at point K is 0.9. In the case of carboxylic groups (1.67 meq) this ratio is as low as 0.52. KINNIBURGH et al. (1999) reported that both carboxylic groups -COOH and phenolic groups -OH may serve as binding sites for metal ions in HA molecules. The ratio between the total content of functional groups in HA to the amount (mmol) of Me^{3+} ions bound at point K, determined in this study, is much lower than 3:1. In the case of fulvic acids, BENSCHOTEN, EDZWALD (1990) proposed a stoichiometric formula of a complex compound formed in a solution at pH 5–7, $AlOH_{2,7}FA_{0,64}$.

The curves in Figures 3 and 4 present changes in the efficiency of humic acids removal, reflected in a percentage decrease in color and COD at a given coagulant dose, and changes in streaming potential (mV) in coagulated samples. It was found that the optimum dose of aluminum chloride and iron(III) chloride was 3.2–3.6 mmol dm^{-3}. The application of aluminum salts resulted in a maximum removal efficiency of about 99.5% for color and about 97% for COD. In the case of ferric coagulant the respective maximal values were 99.8% and 97.3%.

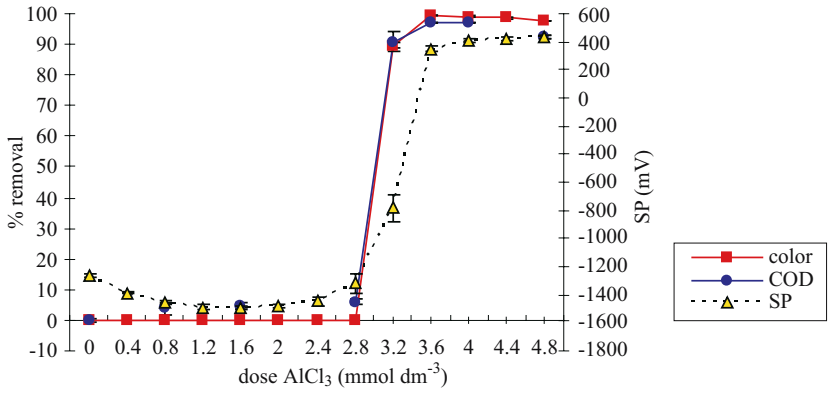

Fig. 3. Efficiency of color and COD removal and changes in streaming potential as dependent on $AlCl_3$ dose

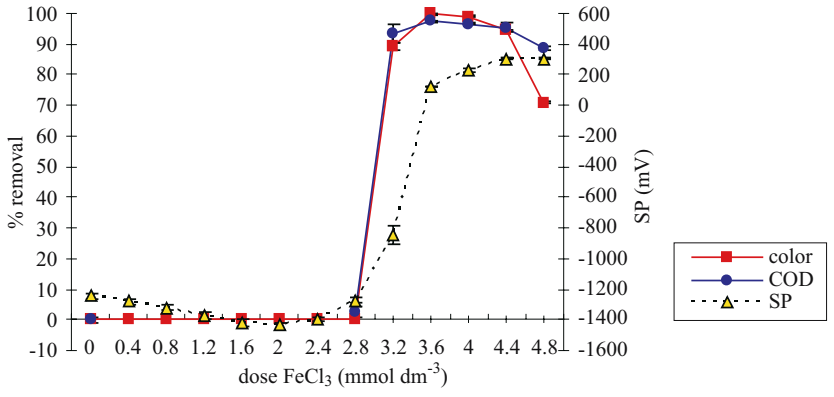

Fig. 4. Efficiency of color and COD removal and changes in streaming potential as dependent on $FeCl_3$ dose

Other authors also confirmed the high (90–95%) efficiency of humic substances coagulation with aluminum salts and iron(III) salts (LU et al. 1999, CHENG 2002). Figure 3 and Figure 4 present also changes in streaming potential (SP). The greatest changes in SP levels were recorded within a dose range of 2.8–3.6 mmol dm^{-3}, and the zero value of SP was observed for 3.2–3.6 mmol dm^{-3}.

Among the physicochemical phenomena associated with coagulation there are electrokinetic phenomena, such as zeta potential, streaming potential or electrophoretic mobility of particles. Reliable measures of changes in the charge of colloidal particles in a solution are changes in the values of streaming potential. In our previous studies on coagulation of pulp and paper mill effluents we observed the most significant changes in SP at the optimum doses of $FeCl_3$ and $AlCl_3$ (DZIEJOWSKI et al. 2005). BACHE et al. (1999) applied streaming potential measurements and a jar test procedure in studies on coagulation of a HA in a 0.01 mol dm^{-3} NaOH solution with $Al_2(SO_4)_3$, and recorded SP ~ 0 at the optimum dose. The value of electrokinetic potential in a HA solution depends on ionic strength, pH and the type of humic acids (AVENA et al. 1999). According to BACHE et al. (1999), the ability of functional groups to dissociate at a given pH of the solution decides about the charge of humic acid particles. A decrease in the pH of a solution is accompanied by reduced dissociation of functional groups in molecules, which affects the value of surface charge and changes in SP. Therefore, interactions between HA molecules with positively charged products of hydrolysis of aluminum salts and iron(III) salts are of electrostatic character, and a decrease in the negative charge of organic colloid particles may lead to the disappearance of intermolecular repulsion forces, followed by aggregation (EDWARDS, AMIRTHARAJAH 1985).

DUAN et al. (2002), BENSCHOTEN, EDZWALD (1990) observed the highest efficiency of coagulation of humic acids with aluminum salts at pH ~ 6.0, conducive to minimal solubility of aluminum hydroxide. This suggests that organic substances removal from water by coagulation is related not only to colloidal charge neutralization, but adsorption of these substances on flocs of the aluminum hydroxide precipitate is also possible (LU et al. 1999).

A greater ability of iron(III) salts, compared with aluminum salts, to form complexes with water-dissolved organic matter was demonstrated by JANSEN et al. (2002). According to LEFEBVRE, LEGUBE (1993), the possible mechanisms of coagulation with iron(III) salts are as follows:

1) ligand exchange on the surface of amorphous iron(III) hydroxide,

2) complex formation or ligand exchange between dissolved iron(III) hydroxo cations and organic matter molecules.

Further research is needed to explain humic acids coagulation with aluminum salts and iron(III) salts. The analysis should focus, among others, on the structure of intermediate and final products as well as on electrokinetic phenomena.

Conclusions

1. Salt doses that initiate coagulation were determined by a titration method. They appeared comparable to the optimal doses determined by a jar test procedure.

2. The optimal doses of aluminum and ferric coagulants enabled almost total removal of COD and color from a given solution.

3. The coagulation process of humic acids runs at a pH level below 4.8 in case of iron(III) salt and at a pH below 6.4 in case of aluminum salt.

4. The streaming potential (SP) for investigated systems was close to zero after introducing of optimum coagulant doses.

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CELLULOLYTIC, LECITHIN-MINERALIZING, TRIBASIC CALCIUM PHOSPHATE-SOLUBILIZING AND SULFATE-REDUCING BACTERIA IN MEADOW SOILS IRRIGATED WITH BIOLOGICALLY TREATED SEWAGE*

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K e y w o r d s: soil, sewage, irrigation, mineral fertilization, cellulolytic bacteria, lecithin-mineralizing bacteria, tribasic calcium phosphate-solubilizing bacteria, sulfate-reducing bacteria.

A b s t r a c t

The effect of irrigation (fresh water, treated sewage, treated sewage stored in a biological pond) and mineral fertilization (NPK) of meadow soils on the counts of aerobic and anaerobic cellulolytic bacteria, lecithin-mineralizing bacteria, tribasic calcium phosphate-solubilizing bacteria, anaerobic sulfate-reducing bacteria was examined. The studies were performed in 1996 and 1997 in 8 different variants of irrigation and fertilization on 32 plots in the vicinity of the treatment plant in Olsztynek. Aerobic and anaerobic cellulose-degrading bacteria generally occurred in greater numbers in the soil from the plots irrigated with fresh water and/or with treated sewage stored in biological pond (particularly for the maximum dose). They were sporadically recorded in NPK fertilized soil. Lecithin-mineralizing bacteria occurred in great numbers both in non-irrigated, non-fertilized soil and in soil irrigated with fresh water and/or treated sewage. Tribasic calcium phosphate-solubilizing bacteria were sometimes recorded in greater numbers in NPK fertilized soil. Sulfate-reducing bacteria usually occurred in small numbers in the soils irrespectively of the irrigation or fertilization variant. The bacteria groups under study were reported in greater numbers in the 0-10 cm soil layer with the exception of anaerobic cellulose-degrading bacteria which were more numerous in the 15–25 cm and the 30–50 cm soil layers.

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BAKTERIE CELULOLITYCZNE MINERALIZUJĄCE LECYTYNĘ, ROZPUSZCZAJĄCE FOSFORAN TRÓJWAPNIOWY I REDUKUJĄCE SIARCZANY W GLEBACH ŁĄKOWYCH NAWADNIANYCH BIOLOGICZNIE OCZYSZCZONYMI ŚCIEKAMI

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S ł o w a k l u c z o w e: gleba, ścieki, nawodnienia, nawożenie mineralne, bakterie celulolityczne, bakterie mineralizujące lecytynę, bakterie rozpuszczające fosforan trójwapniowy, bakterie redukujące siarczany.

A b s t r a k t

Zbadano wpływ nawadniania (wodą, oczyszczonymi ściekami, oczyszczonymi ściekami retencjonowanymi w stawie biologicznym) i nawożenia mineralnego NPK gleb łąkowych na liczebność bakterii rozkładających błonnik w warunkach tlenowych i beztlenowych, bakterii mineralizujących lecytynę i rozpuszczających fosforan trójwapniowy oraz beztlenowych bakterii redukujących siarczany. Badania przeprowadzono w latach 1996–1997 w 8 wariantach nawodnieniowo-nawożeniowych na 32 poletkach przy oczyszczalni ścieków w Olsztynku. Bakterie rozkładające błonnik w warunkach tlenowych i beztlenowych występowały z reguły liczniej w glebach nawadnianych wodą i/lub ściekami oczyszczonymi i następnie retencjonowanymi w stawie biologicznym, a niekiedy również w glebach nawożonych mineralnie NPK. Bakterie mineralizujące lecytynę występowały równie licznie zarówno w glebach nienawadnianych i nienawożonych, jak i w nawadnianych wodą i/lub oczyszczonymi ściekami. Liczba bakterii redukujących siarczany była natomiast znacznie niższa w glebach wszystkich wariantów nawodnieniowo-nawożeniowych. Badane grupy bakterii występowały na ogół liczniej w warstwie gleby 0–10 cm. Wyjątkiem były bakterie rozkładające błonnik w warunkach beztlenowych, które dominowały w głębszych warstwach (15–20 cm i 30–50 cm).

Introduction

Cellulose is the most common polymer of a cellular wall of a plant. Together with chemicelluloses it is the main biodegradable component in natural environments. Utilization of solid wastes containing cellulose and plant remnants in soil is closely connected with a biochemical activity of cellulolytic microorganisms (ACEA, CARBALLAS, 1988, 1996, BARLAZ et al. 1992, QIAN, BARLAZ 1996, RAI, SRIVASTAVA 1983, SCHRÖDER, Urban 1985, SINSABAUGH, LINKINS 1988, TATENO 1988). Active microorganisms in those processes belong to different physiological and systematic groups of the order *Actinomycetales* and fungi (ERICKSSON et al. 1992, GOTTSCHALK et al. 1981, JOLIFF et al. 1989, JOHANSEN, BINNERUP 2002, SCHWARTZ 2004) with differentiated biochemical abilities (LI 1997, ULRICH, WIRTH 1999, WIRTH, ULRICH 2002). They are common in soil sewages, bottom sediments of tanks, manure dung, compost,

wood fermenter, pig intestine, paper mill, hot springs (CHEN, WEIMER 2001, MORVAN et al. 1996, POUCHER et al. 2001, QIAN, BARLAZ 1996, SCHWARTZ 2004, WEIMER et al. 1997). In soil cellulose degradation processes run slowly and depend on concentration, localization and mobility of microorganisms and their cellulases (HAYANO 1986), kind of substrate (straw, paper, wood), pH, temperature, water content (KASHATTRIYA et al. 1992, SINSABAUGH, LINKINS 1988). Intensity of the processes increases at pH 5–6 and at 30–50°C (HOPE, BURNS 1987). Agrotechnical procedures, fertilization, season of the year, kind of cultivation are important as well (KISS et al. 1978). Released in the process of cellulose degradations to monosaccharides and their further digestion to organic acids may stimulate the development and activity of other microorganisms being important from the point of view of soil productivity. Bacteria mineralizing phosphorus compounds and dissolving its mineral compounds are significant (BOINOVA et al. 1997, DOMEY 1992, FÖRSTER, FREIER 1988, FREIER 1987, ILLMER et al. 1995, ILLMER, SCHINNER 1995, JONES et al. 1991, MIKANOVA, NOVÁKOVÁ 2002, RODRIGUEZ, FRAGA 1999). Most phosphorus found in soils are non- assimilate by plants. In case of fertilizing soil superphosphate 70–90% of phosphorus is fixed with oxides and hydroxides of Al and Fe (in acid soils) or Ca (in alkalic soils). In all these cases effectiveness of phosphorus fertilization is low (IGUAL et al. 2001). Ability of dissolving such difficult decomposable mineral compounds of phosphorus by microorganisms is attributed to organic acids secretion (lactic, isovaleric, isobutyric, glycolic, oxalic, malonic, succinic). Such microorganisms are particularly active in the upper layer of soil, in plant root system (CHUNG et al. 2005). They inhabit highly competitive niches on the border of roots- soils sphere (rhizosphere) where they are supplied with nutriens and ecological space (PARRET et al. 2003). Cellulose degradation by cellulolytic microorganisms supplies the sources of carbon and energy for many other microorganisms, for example, bacteria reducing sulphates. Those bacteria regulate different processes especially in water-logged soil, including organic matter turnover, biodegradation of chlorinated organic compounds, mercury methylation in anaerobic soils and in bottom sediments of reservoirs (CASTRO et al. 2001). There are also known phenomena of coexistence of bacteria reducing sulphates with bacteria decomposing cellulose in a masseter of ruminants (MORVAN et al. 1996). The ability of occupancy of different anaerobic environments causes that these bacteria have become of great interest for past ten years. The number of bacteria decomposing cellulose, liberating phosphorus from mineral compounds are reducing sulfates may range within few orders of magnitude per 1 g of soil dry mass depending on local conditions. Biological development and activity of these microorganisms in soil is strictly connected with water content. Therefore it is advisable to irrigate sand soils during drought. Utilization

of sewage effluents from a treatment plant is used for the reduction of the amount of sewage discharged into surface waters and for the soil enrichment in biogenic compounds assimilated by plants and microorganisms (NIEWOLAK, TUCHOLSKI 2001, NIEWOLAK et al. 2001). This paper presents the results of research on the number of bacteria decomposing cellulose in aerobic and anaerobic conditions, mineralizing lecithin, dissolving calcium phosphate and reducing sulfates in meadow waters irrigated and/ or fertilized (NPK). Processes of mineralization of organic substances in soil, liberation of nutrients for plants (P) and degradation of harmful substances of sewages are connected with biological activity of these bacteria.

Materials and Methods

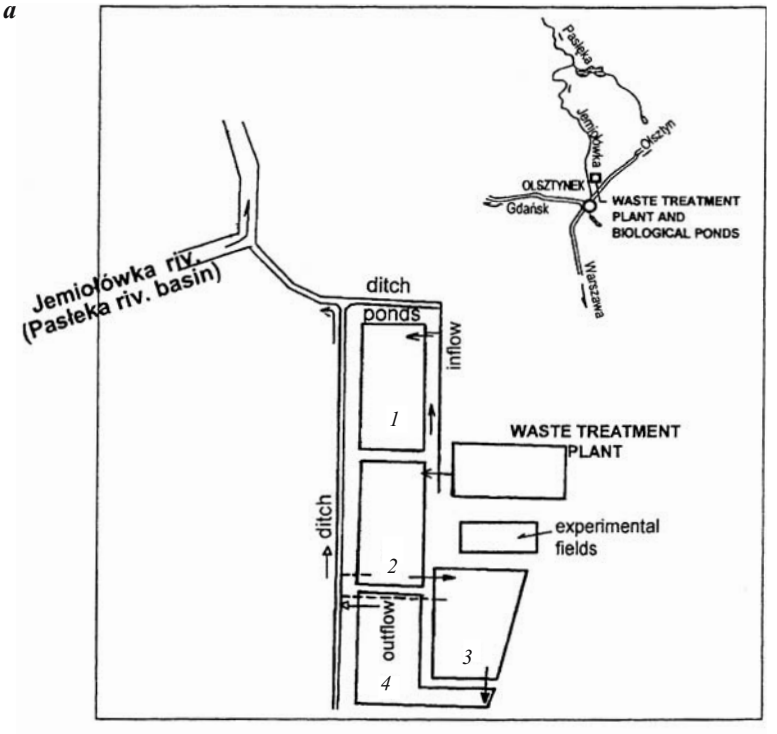
The area of research

Studies were carried out during the vegetation periods in 1996 and 1997 in the area belonging to the household and fruit and vegetable processing plant in Olsztynek. The meadow under study was characterized by light soil composed of light clay sand with some clay layers. Soil quality corresponded to IV b class (good rye complex). The distribution of plant species was uniform *Dactylis*, *Poa, Agropyrum* and *Taraxacum* dominated (TUCHOLSKI et al. 1998). The meadow was divided into 32 plots of 15.75 m^2 (Figure 1). The experiment was carried out in 8 variants described in table 1, each in 4 replications.

Table 1.
Experimental variants of irrigation and fertilization of meadow soils near a treatment plant in Olsztynek

Experimentall variants	Irrigation and fertilization variants
A	control, without irrigation and fertilization
B	irrigation with clean water (basic dose)
C	irrigation with biologically treated sewage (discharged directly by the treatment plant), basic dose (243.7 mm in 1996 , 258.4 mm in 1997)
D	irrigation with biologically treated sewage after retention in a biological pond, basic dose
E	irrigation with biologically treated sewage after retention in a biological pond, 150% of the basic dose
F	irrigation with biologically treated sewage after retention in a biological pond, 200% of the basic dose
G	mineral fertilization, dose in 1996: N – 90 kg ha^{-1}; P – 43.6 kg ha^{-1}; K – 112.1 kg ha^{-1}. Dose in 1997: N – 120 kg ha^{-1}; P – 43.6 kg ha^{-1}; K – 149.4 kg ha^{-1}
H	mineral fertilization as in variant *G* and irrigation as in variant *B*

a



b

C 25	*B* 26	*G* 27	*F* 28	*D* 29	*A* 30	*H* 31	*E* 32
A 17	*D* 18	*E* 19	*B* 20	*G* 21	*H* 22	*F* 23	*C* 24

H 9	*G* 10	*C* 11	*D* 12	*E* 13	*F* 14	*A* 15	*B* 16
B 1	*E* 2	*F* 3	*H* 4	*A* 5	*C* 6	*D* 7	*G* 8

For explanation see Table 1

Fig. 1. Scheme of ponds (*a*) and experimental fields (*b*) in Waste Treatment Plant in Olsztynek

Sampling procedure

Samples of soils for microbiological measurements were collected after cropping the grass in sunny weather on 11 June, 31 July and 15 August 1996 and on 14 March, 2 June, 30 July and 7 October 1997. In non-irrigated and non-fertilized (*A*) and irrigated with treated sewage stored in a biological pond (*D*) soil samples were collected from 0–10 cm, 15–25 cm and 30–50 cm layers. In the plots of the remaining experimental variants (*B, C, E, F, G, H*) the soil samples were collected from 0–10 cm (Figure 2). 12 soil samples were collected from each plot (in total 48 samples in a given variant, of approx. 1 kg) to a sterile cuvette made of enamel metal, thoroughly mixed in situ and in sterile glass dishes were carried to a laboratory. Before microbiological analysis, the soil was mixed again in the same cuvettes, 10 g samples were weighed and put into Erlenmeyer's flasks with 90 cm^3 sterile physiological solution of NaCI (0.85%). They were homogenized for 15 min on a magnetic stirrer and diluted to the following degree: 1:10, 1:100, 1:1000, 1:10 000 in the same physiological salt NaCl. 1 cm^3 of each dilution was transferred onto sterile Petri dishes and test tubes with a selective medium. At the same time, additional 10 g soil portions (in 2 replications) were dried at 105°C for 24 hours to determine dry mass.

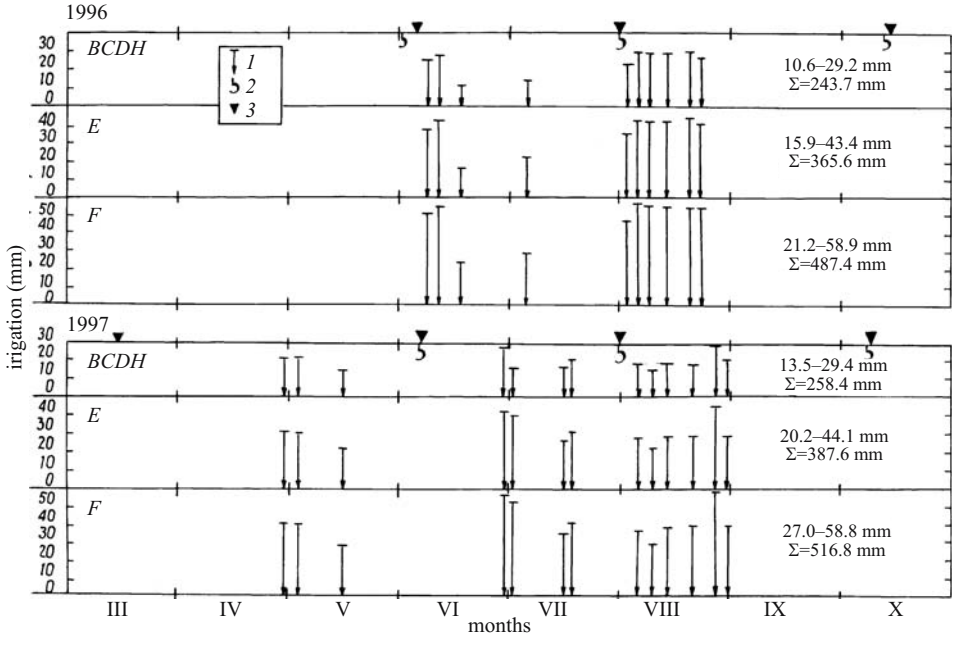


Fig. 2. Timetable of meadow fields irrigation and used dose of water/biological treated sewage: 1 – dose of water/biological sewage; 2 – hay-making; 3 – soil sampling

Microbiological determinations

The following determinations were performed in the collected soil samples: 1) number (MPN/g d. m.) of aerobic cellulose-degrading bacteria on Hutchinson liquid medium (RODINA 1968) after 21 days of incubation at 25°C; 2) number (MPN/g d. m.) of anaerobic cellulose degrading bacteria on Omelianski liquid medium (RODINA 1968) after 28 days of incubation at 25°C; 3) number (cfu/g d.m.) of lecithin-mineralizing bacteria on a Mienkina (Difco) agar thickened medium after 14 days of incubation at 25°C; 4) number (cfu/g d.m.) of tribasic calcium phosphate-solubilizing bacteria $Ca_3(P0_4)_2$ on Bunt and Rovira (Difco) agar thickened medium after 14 and 21 days of incubation at 25°C; and 5) number (cfu/g d. m.) of anaerobic sulfate-reducing bacteria on Tauson (Difco) agar thickened medium (modified by Szturm) after 7 days of incubation at 25°C. The methodology applied in this study complied with the recommendations given in the manual by Rodina (RODINA 1968).

All microbiological determinations were carried out in 3 parallel replications of the same soil sample. Number (cfu/g d. m. of soil) of lecithin--mineralizing bacteria, tribasic calcium phosphate-solubilizing bacteria and sulfate-reducing bacteria were given in colony forming units. The most probable numbers of aerobic and anaerobic cellulose-degrading bacteria (MPN/g d.m. of soil) were read out from the MacCrady chart. The presence of aerobic cellulose-degrading bacteria was detected basing on decomposition of filter paper strips (10 x 1 cm) at the water-air phase, while the presence of anaerobic cellulose-degrading bacteria was detected on base of decomposition of filter paper strips (5 x 1 cm) immersed in a medium poured into test tubes and filled up to 2/3 of the height. For lecithin-mineralizing and tribasic calcium phosphate-solubilizing bacteria, colonies circled with transparent zones of broken down substrate on selective medium in Petri dishes were counted. For sulfate-reducing bacteria, black colonies in an agar medium were poured into test tubes up to 2/3 of the height.

Results

Aerobic cellulose-degrading bacteria were usually more numerous in the soil irrigated with effluents from mechanical-biological waste treatment plant (Table 2). Exceptionally great numbers of these bacteria were recorded in the soil from non-irrigated and non-fertilized plots (control) in July 1996, and June and July 1997 and from NPK fertilized and fresh water irrigated plots in March 1997. Greater numbers of these bacteria in the soil from non-irrigated and non-fertilized (control) plots and in the plots irrigated with the basic dose

Table 2

Number of aerobic and anaerobic cellulolytic bacteria in soil irrigated with sewage from a mechanical-biological treatment plant in Olsztynek, 1996 and 1997

Variant	Depth (cm)	Aerobic cellulolytic bacteria							Anaerobic cellulolytic bacteria						
		1996			1997				1996			1997			
		10.06	31.07	15.10	14.03	02.06	30.07	07.10	11.06	31.07	15.10	14.03	02.06	30.07	07.10
		MPN 10^3 kg^{-1}*													
A	0–10	492	127400	1343	1127	62	846	125	192	683	81	69	12460	846	374
	15–25	–	2805	4050	146	1870	25	81	–	103	405	195	1870	9955	407
	30–50	–	4230	898	198	80	10570	27	–	1410	66	150	1335	113	860
B	0–10	893	38070	42300	297	9185	1273	56	28	803	28	150	11690	1275	72
C	0–10	30	114800	12460	100	9295	765	125	143	615	2225	100	1690	765	790
D	0–10	724	2088	1215	140	11340	187	54	30	33	162	68	2430	888	154
	15–25	2037	104	1388	197	140	320	2775	194	1087	185	936	13020	4095	415
	30–50	30	140	6113	90	140	137	26	860	233	130	1980	698	4095	80
E	0–10	27	1840	10178	1455	11004	162	2088	405	184	310	1455	8645	770	793
F	0–10	694	205	4253	1485	1838	285	1988	1388	950	4253	10890	10290	2445	755
G	0–10	130	40	9315	62	2745	117	2163	700	194	243	9735	914	156	390
H	0–10	138	4163	375	1455	10220	170	2038	368	185	793	43850	292	1275	122

* most probable number

of effluents stored in a biological pond were usually recorded in the upper layers of 0–10 cm and/or 15–25 cm. Anaerobic cellulose-degrading bacteria (*Clostridium* sp.) usually occurred in greater numbers in those soils irrigated with the maximum dose of effluent stored in a biological pond. Exceptionally great numbers of these bacteria were reported in March 1997 and also in the NPK fertilized and fresh water irrigated plots in June and July 1997 in both non-irrigated and non-fertilized (control) soil and irrigated with fresh water and the basic dose of treated waste effluents (Table 2). Greater numbers of these bacteria were also reported in July 1997 in non-irrigated and non--fertilized soil (control) and in the soil irrigated with the basic dose of effluents stored in a biological pond. In principal anaerobic cellulose-degrading bacteria were found in greater numbers in the soil sampled from deeper layers of the control plots and the plots irrigated with the basic dose of effluents stored in a biological pond.

The number of lecithin-mineralizing bacteria, occurred in soil samples collected from plots representing all irrigation and fertilization variants was usually 10-100-fold greater than the number of tribasic calcium phosphate--solubilizing bacteria. There were no greater differences in the number of these bacteria recorded in the non-irrigated and non-fertilized (control) plots and the plots irrigated with fresh water, treated waste or effluents stored in a biological pond and minerally fertilized (NPK).Varied numbers of these bacteria were reported at different depths of the soil profile in different months (Table 3). Larger populations of tribasic calcium phosphate-solubilizing bacteria occurred usually in the NPK fertilized soil or additionally irrigated with fresh water, smaller populations of these bacteria were found in the soil from non-irrigated and non-fertilized (control) plots and the plots irrigated exclusively with fresh water, treated sewage or effluents stored in a biological pond. The maximum numbers of these bacteria in the soil from non-irrigated and non-fertilized (control) plots and from the plots irrigated with the basic dose of effluents stored in a biological pond were usually recorded at the same depths as the lecithin-mineralizing bacteria (Table 3).

Sulfate-reducing bacteria were found in the majority of the soil samples. The differences in their numbers between the non-irrigated and non fertilized (control) plots and these irrigated with fresh water, treated sewage, effluents stored in a biological pond or NPK fertilized were ambiguous (Table 4). On the control plots and the plots irrigated with the basic dose of effluents stored in a biological pond, these bacteria were usually more numerous at the depth of 0–10 cm.

Table 3

Number of lecithin-mineralizing and tribasic calcium phosphate-solubilizing bacteria in soil irrigated with sewage from a mechanical-biological treatment plant in Olsztynek, 1996 and 1997

Variant	Depth (cm)	Lecithin-mineralizing bacteria						Tribasic calcium pfosphate-solubilizing bacteria					
		1996		1997				1996		1997			
		31.07	15.10	14.03	02.06	30.07	07.10	31.07	15.10	14.03	02.06	30.07	07.10
		cfu 10^6 kg^{-1}*											
A	0–10	–	18	5880	–	9345	4980	0	0.9	0	1.8	31.2	37.4
	15–25	–	54	5020	–	9050	5520	0	0	9.8	4.2	22.6	31.7
	30–50	–	9	10890	–	680	7240	0	0.9	19.8	2.7	0	18.1
B	0–10	68	0	1060	–	17000	8855	84.5	1.4	0	2.5	17	16.1
C	0–10	0	0	39267	–	1208	10126	8.9	0	18.2	1.7	0	29.1
D	0–10	3590	0	4594	–	842	6545	8.3	4.1	0	0.8	37.4	38.5
	15–25	0	19	10835	–	364	7724	18.9	0	0	3.3	22.8	32.4
	30–50	626	24	4604	–	410	8269	9.4	0	19.8	2.3	9.1	61.3
E	0–10	1800	0	2086	–	5954	6930	0	0	0	2	2.8	25.1
F	0–10	5530	0	11880	–	8150	7235	0	0	44.6	4.4	0	15.9
G	0–10	2320	0	1770	–	2730	7785	1008.8	243	0	3.2	35.1	51.9
H	0–10	0	0	4850	–	425	7417	50.9	125.3	0	2.5	51	57.1

* colony forming units

Table 4
Number of sulfate-reducing bacteria in soil irrigated with sewage from a mechanical-biological treatment plant in Olsztynek, 1996 and 1997

Variant	Depth (cm)	1996			1997			
		11.06	31.07	15.10	14.03	02.06	30.07	07.10
		cfu 10^3 kg^{-1}*						
A	0–10	–	410	295	440	27	36	25
	15–25	–	37	297	145	37	27	27
	30–50	–	38	312	0	36	23	27
B	0–10	–	210	310	40	210	26	24
C	0–10	–	245	98	36	76	24	25
D	0–10	30	33	40	0	203	84	23
	15–25	58	66	65	443	37	64	28
	30–50	29	280	33	10	37	82	26
E	0–10	80	230	53	243	70	90	33
F	0–10	28	74	40	150	184	90	72
G	0–10	65	243	73	0	200	30	26
H	0–10	28	37	58	145	548	77	24

* colony forming units

Statistical evaluation of the results. Parametric test ANOVA Kruskal--Wallis', which corresponds to one- factor analysis of variance, was used to obtain information if the number of the examined groups of microorganisms differs between each other for particular variants of experiments and for particular research period (months/years) because there is a lack of normality in the distribution of variables. Hypothesis of means equality (H: $x_1 = x_2 ... = x_5$) on the level of significance $\alpha = 0{,}05$ was verified. In case when the test was significant, verified hypothesis was rejected (STANISZ 1998). Significant differences in the number of isolated bacteria from waters time and place independent were not found.

Discussion

The number of bacteria decomposing cellulose in aerobic condition found in meadow soil samples being not irrigated and not fertilized (control) was generally lower than being irrigated and fertilized (KUCHARSKI et al. 2001) for brown soil (leached) made of clay light dusty sand being fertilized with mineral salts at constant moisture (60%). Similar number of these bacteria was found by ACEA and CARBALLAS (1996) for forest soil with pine (*Pinus pilaster* Sol.) in the North-West Spain. Generally higher amounts of these organisms were

found in the samples of meadow soil irrigated with effluents from a mechanical- biological waste treatment plant in Olsztynek, rather than with effluents additionally stored in a biological pond because they may stay in a compound due to moisture conditions and the presence of small amounts of bygones (N,P) in sewages. The presence of moisture in soil is one of the main factors (including cellulose as substratum) controlling the development and biological activity of bacteria decomposing cellulose. Environmental conditions (soil, pH, temperature, kind of crops) play a significant role; the amount of cellulose getting to soil depends on the season of year (HOPE BURNS 1987, KISS et al. 1978). Larger amounts of bacteria decomposing cellulose in aerobic conditions are generally found in soil layers at 0–10 cm depth, sometimes at 15–25 cm and it may be attributed to advantageous aerobic conditions, greater availability of substratum (cellulose) and root system of grasses conditioning proper structure of soil. Oxygen from air is transported through a root system. Oxygen transported by plants to the root system creates oxygen microsites adhering to roots and root hairs in the reduced substrate. In the microsites ammonium ions could be oxidized by nitrifying bacteria into nitrates (NIEWOLAK et al. 2005). The latest ones as well as the most active organic fractions of roots secretions of plants (aminoacids, and other organic acids) could be a source for cellulolytic bacteria. Additional amounts of nitrogen necessary for these bacteria could have been carried out with the sewage effluents used for irrigation (NIEWOLAK, TUCHOLSKI 2001). The decrease of the number of bacteria decomposing cellulose in aerobic conditions in soil layers taken from 30–50 cm (variants *A* and *D*) can be attributed to disadvantageous aerobic conditions, smaller amount of substrate and biogenic chemical elements (N, P), for example, far from the roots, nitrates can be denitrified under anaerobic conditions and release gaseous nitrogen into the atmosphere (NIEWOLAK et al. 2005). Larger amounts of bacteria decomposing cellulose in aerobic conditions in July (variants *A,B,C,D*) and October 1996 (variants *B,C,E,F,G*), in June (variants *B,C,D,E,F,G,H*) and October 1997 (variants D,E,F,G,H) could have been attributed to thermal conditions (in summer) and availability of substrate (in autumn). In autumn larger amounts of cellulose from decayed plants and/or remnants of cut grasses get into the soil; in summer non- decomposed cellulose from autumn (due to low temperature in winter and early spring) can be available for cellulolytic bacteria when soil temperature reaches the values close to the optimal ones (HOPE, BURNS 1987). Larger amounts of these microorganisms found in October in 1996 and 1997 in meadow soil samples from different variants of irrigating-fertilizing experiments can be an effect of dying antagonistic organisms (protozoa, earthworms, and other) grazing on bacteria and fungi. Larger amounts of bacteria decomposing cellulose in anaerobic conditions (*Clostridium* sp.) in meadow soil samples

from control plot and meadow soils irrigated with biologically treated sewages stored in a biological pond (variant A and D) taken from the levels: 25–30 and/or 30–50 cm can be attributed to limited availability of oxygen. Probably in the layer of soil at 0–10 cm there are anaerobic niches where these bacteria may exist in larger amounts in all variants of irrigating-fertilizing experiments.

The number of bacteria mineralizing lecithin (organic phosphorus compound) in meadow soils samples of different variants of irrigating-fertilizing experiments (0–39.2 · 10^3 cfu/d.m. soil) did not differ from the previous one found in the soils of Kaszuby Lake District being fertilized with sewage from a swine farm earlier treated in a specially constructed pound (NIEWOLAK, KOC 1995). They were from few to several dozen per cent of a total number of heterotrophic bacteria capable of growth on artificial substrates and were comparable to the number of proteolytic and ammonifying bacteria (NIEWOLAK, TUCHOLSKI 2001, NIEWOLAK et al. 2001) found in the same research period. It is generally accepted (KRISTIANSEN 1982a,b) that soil irrigation causes the increase of the number of heterotrophic bacteria, in the case of bacteria mineralizing lecithin it was not so obvious. The number of bacteria mineralizing lecithin reached the values twice higher than in the samples of soils taken from 0–10 cm being non-irrigated and non-fertilized (variants *A* and *C*) only in samples of soils biologically irrigated with treated sewage taken in October 1997. In case of bacteria dissolving calcium phosphate mineral fertilization NPK without (variant *G*) or with additional irrigation with tap water (variant *H*) was of significance. Apart from the samples of soil fertilized NPK (variant *G*) in July 1996 they did not reach the values (DOMEY 1992) for a given type of soils in Germany.

Greater amount of such bacteria was found in a surface layer of soil (0–10 cm), except the samples of meadow soil taken in July 1996 and March 1997 irrigated with sewage effluents stored in a biological pond at a sewage treatment plant in Olsztynek (variant *D*) where bacteria reducing sulfates were numerous in a layer 30–50 cm and 15–25 cm. Although, anaerobic bacteria in the presence of oxygen cannot reduce sulfates (FAKUI, TAKII 1990), however, some of them can survive oxygenation (HARDY, HAMILTON 1985). It is thought that bacteria reducing sulfates can be physiologically active inside microniches not containing oxygen but on the other hand in aerobic environments (CYPIONKA et al. 1985). Some of them such as *Desulfovibrio desulfotomaculum, Desulfobulbus* sp. are found near oxic-anoxic interfaces or even in oxic layers belonging to incompletely oxidizing organic substrates to acetate. Only filamentous species of the genus *Desulfonema* oxidizing organic substrates completely are found to be abundant in the oxic zone of microbial mats (SASS et al. 2002, SASS, CYPIONKA 2002). The appearance of these microorgan-

isms in a soil layer from 0–10 cm can be attributed to a larger amount of organic substance (monosaccharides, organic acids) originated from cellulose decomposition, being a source of carbon and energy. Sulfates and soil pH (inactive alkalic) play an important role in the development of these bacteria. Apart from aerobic conditions and the content of organic substance these factors could control the number of bacteria reducing sulfates in the examined samples of soils. Comparing the number of bacteria reducing sulfates in particular months of the research period in soils samples being non-irrigated and non-fertilized to the soils samples of different irrigating-fertilizing combinations an univocal answer concerning the influence of these procedures on these microorganisms cannot be given. Similarly as in the case of cellulolytic bacteria, mineralizing bacteria of lecithin and dissolving calcium phosphates, the lack of significant differences among the number of bacteria reducing sulfates in non-irrigated and non-fertilized soils (control) and in soils with different irrigating-fertilizing combinations are explained by a constant tendency of a fast reversion of soil bacteria population to the proper values for a given type of soils. It can be a result of a quick consumption of one or many significant "nutrients", antibiosis phenomena, gathering of toxic substances in soil environment or digestion by protozoa and other which control the population of microorganisms in natural environments maintaining them on a constant low lever. Besides the fact that the samples for microbiological examination were taken just after hay harvesting and after a longer break in irrigation should be taken into consideration. It is known from literature that the largest growth of the number of bacteria in soils irrigated with sewages takes place after this procedure. Therefore there were no significant differences in the number of the examined groups of physiological bacteria in soil samples from different variants of irrigating-fertilizing experiments.

Conclusions

1. The effect of irrigation (fresh water, biologically treated sewage, biologically treated sewage stored in a biological pond) and/or minerally fertilized (NPK) on the count of cellulose-degrading (aerobic and anaerobic), lecithin--mineralizing, tribasic calcium phosphate-solubilizing or sulfate-reducing bacteria in meadow soils depended on the physiological group of microorganisms and a research period.

2. Irrigation had a greater effect on the number of aerobic and anaerobic cellulose-degrading bacteria than on the number of lecithin-mineralizing bacteria, tribasic calcium phosphate-solubilizing bacteria or sulfate-reducing. This effect on the three latter types of bacteria was small or non-existing.

In some periods, NPK mineral fertilization with or without fresh water irrigation had a more beneficial effect.

3. In principal greater numbers of aerobic cellulose-degrading, lecithin--mineralizing, tribasic calcium phosphate-solubilizing and sulfate-reducing bacteria in non-irrigated and non-fertilized soils (control) and in the soil irrigated with a basic dose of sewage effluents stored in a biological pond and sampled from the depth of 0–10 cm, their lower numbers in soil samples originating from the depths of 15–25 cm and 30–50 cm may be related with the accumulation of greater amounts of organic substance in the surface layers of the soil. In the case of sulfate-reducing bacteria, they may utilize sulfur compounds possibly present in the soil surface.

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ROLE OF ACTINOMYCES OF THE GENUS *STREPTOMYCES* IN ALLEVIATING THE EFFECTS OF SOIL CONTAMINATION WITH DIESEL OIL

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K e y w o r d s: diesel oil, enzymatic activity, microorganism counts, *Streptomyces*.

A b s t r a c t

A pot experiment was conducted to investigate the role of three species of actinomyces in alleviating the effects of soil (light loam) contamination with diesel oil. The variables in the experiment were: soil contamination with diesel oil in the amount of 0 or 10 cm^3 kg^{-1} and soil inoculation with spores of the following actinomyces: *Streptomyces longisporoflavus, Streptomyces odorifer* and *Streptomyces viridis*. Actinomyces activity was compared against the results reported for non-inoculated soil.

The experiment revealed that soil contaminated with diesel oil was biologically unbalanced due to an increase in the microorganism population, which stimulated the activity of dehydrogenases, urease and alkaline phosphatase. The applied inocula comprising spores of the following actinomyces: *Streptomyces longisporoflavus*, *Streptomyces viridis* and *Streptomyces odorifer* proved to be relatively ineffective in detoxifying soil contaminated with diesel oil. Further research into the use of actinomyces in the reclamation of soil contaminated with diesel oil is required to investigate the suitability of other actinomyces species as inocula, and to determine the quantity of by-products of oil biodegradation.

ZNACZENIE PROMIENIOWCÓW Z RODZAJU *STREPTOMYCES* W ŁAGODZENIU SKUTKÓW ZANIECZYSZCZENIA GLEBY OLEJEM NAPĘDOWYM

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S ł o w a k l u c z o w e: olej napędowy, aktywność enzymów, liczebność drobnoustrojów, *Streptomyces*.

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Abstrakt

W doświadczeniu wazonowym badano przydatność 3 gatunków promieniowców w łagodzeniu skutków zanieczyszczenia gleby (glina lekka) olejem napędowym. Czynnikami zmiennymi w badaniach były: zanieczyszczenie gleby olejem napędowym w ilości 0 lub 10 cm^3 kg^{-1} oraz szczepienie gleby zarodnikami promieniowców: *Streptomyces longisporoflavus, Streptomyces odorifer* lub *Streptomyces viridis*. Działanie promieniowców porównywano z glebą nieszczepioną.

Stwierdzono, że w glebie zanieczyszczonej olejem napędowym nastąpiło naruszenie równowagi biologicznej w wyniku zwiększenia liczebności drobnoustrojów, co w efekcie przyczyniło się do podwyższenia aktywności dehydrogenaz, ureazy i fosfatazy alkalicznej. Zastosowane szczepionki, składające się z zarodników promieniowców: *Streptomyces longisporoflavus*, *Streptomyces viridis* i *Streptomyces odorifer*, okazały się mało przydatne w detoksykacji zanieczyszczonej gleby. Badania nad wykorzystaniem promieniowców w przywracaniu sprawności glebom zanieczyszczonym olejem napędowym powinny być kontynuowane i rozszerzone o większą liczbę ich gatunków w szczepionkach oraz pogłębione o ilościowe oznaczanie powstających produktów pośrednich w trakcie biodegradacji oleju.

Introduction

Oil derivative products which penetrate into the natural environment not only contribute to soil degradation through deterioration of its air-water and chemical properties, but also have an adverse effect on soil-dwelling microorganisms and plants (BUDNY et al. 2002, DELILLE, PELLETIER 2002, WYSZKOWSKA, KUCHARSKI 2005). Nevertheless, their impact on soil microbes and enzymes has not been clearly demonstrated. Microorganisms and enzymes respond differently to contamination with petrol, compared to diesel oil (KAPLAN, KITTS 2004, WYSZKOWSKA, KUCHARSKI 2000, 2001, 2005, WYSZKOWSKA et al. 2006). Regardless of its type, petrol usually reduces bacterial counts (WYSZKOWSKA, KUCHARSKI 2001) and inhibits enzymatic activity (WYSZKOWSKA, KUCHARSKI 2000), while diesel oil may stimulate the proliferation of some microorganisms and the activity of such enzymes as dehydrogenases and urease (WYSZKOWSKA, KUCHARSKI 2005, WYSZKOWSKA et al. 2006). The varied effect of petrol and diesel oil is due to the differences in the chemical composition of those products, which in turn determines their physical and chemical properties (*Przetwory naftowe...* PN-C 96025:1999, *Przetwory naftowe...* PN-EN 590:1999).

Although it may stimulate the activity of some microorganisms, diesel oil always has an adverse effect on plant growth and development. The proliferation of selected microorganisms in soil contaminated with diesel oil could be used to accelerate the biodegradation of diesel oil that has spread to the soil in consequences of a failure (DELILLE, PELLETIER 2002, WYSZKOWSKA et al. 2002). Actinomyces actively break down even those organic substances which are difficult to degrade. For this reason, a study was conducted to investigate the effectiveness of certain actinomyces in the reclamation of soil contaminated with diesel oil.

Materials and Methods

The experiment was carried out in the greenhouse of the University of Warmia and Mazury in Olsztyn. Every plastic pot was filled with 3.1 kg of soil. Under natural conditions, this was leached brown soil developed from light loam with pH_{KCl} 6.6, hydrolytic acidity of 9.5 mmol(H^+) kg^{-1}, sum of exchangeable basic cations of 81.0 mmol(+) kg^{-1} and total organic carbon content of 7.5 g kg^{-1}. Prior to placement in the pots, the soil was fertilized with the following macro- and micronutrients, in mg kg^{-1} (in terms of pure nutrients): N – 200 [$CO(NH_2)_2$]; P – 70 [K_2HPO_4]; K – 100 [K_2HPO_4 + KCl]; Mg – 30 [$MgSO_4 \cdot 7H_2O$]; Zn – 4 [$ZnCl_2$]; Cu – 4 [$CuSO_4 \cdot 5H_2O$]; Mn – 4 [$MnCl_2 \cdot 4H_2O$]; Mo – 4 [$Na_2MoO_4 \cdot 2H_2O$]; B – 0.2 [H_3BO_3]. Mineral fertilizers were applied once, pre-sowing and were thoroughly mixed with the soil material to be placed in a single pot. At the same time, soil was contaminated with diesel oil and actinomyces spores were added. The experiment involved 4 replications with the following variables:

1. soil contamination with diesel oil in the amount of 0 or 10 cm^3 kg^{-1},

2. soil inoculation with the spores of the following actinomyces: *Streptomyces longisporoflavus, Streptomyces odorifer* or *Streptomyces viridis*. Actinomyces activity was compared against the results reported for non-inoculated soil.

Actinomyces, obtained from the collection of the Department of Microbiology, University of Warmia and Mazury in Olsztyn, were grown on slants at a temperature of 28°C for 7 days. Next the cultures were rinsed with a saline solution (3 cm^3 per slant). Eighty slant cultures were placed in a 1 dm^3 conical flask and mixed. A suspension sample of 5 cm^3 was taken per pot (3.1 kg of soil). The culture medium consisted of: soluble starch – 10.0 g, casein – 0.3 g, KNO_3 – 2.0 g, NaCl – 2.0 g, K_2HPO_4 – 2.0 g, $MgSO_4 \cdot 7H_2O$ – 0.05 g, $CaCO_3$ – 0.02 g, $FeSO_4$ – 0.01 g, agar – 20.0 g, distilled water – up to 1 dm^3, pH – 7.0.

Soil placed in pots was brought to a moisture content corresponding to 60% of capillary water capacity. This humidity level was maintained throughout the entire experiment (62 days). On day 12, soil samples were taken for microbiological, biochemical and physicochemical analyses, and spring barley cv. Start was sown (14 plants per pot). Barley was harvested at the flowering stage, i.e. on day 62. At that moment soil samples were taken again for the same analyses which were performed on day 12 of the experiment.

Microbiological analyses involved the determination of the following bacterial counts: oligotrophic bacteria, spore-forming oligotrophic bacteria, copiotrophic bacteria and spore-forming copiotrophic bacteria on the ONTA and HATTORI medium (1983), *Azotobacter* spp. by the method proposed by FENGLEROWA (1965), cellulolytic bacteria on a medium described by WYSZKOWSKA and KUCHARSKI (2005), actinomyces on the Kuster and William medium with

the addition of nystatin and actidione (PARKINSON et al. 1971), and fungi on the MARTIN medium (1950). Microorganisms were incubated on Petri dishes, at 28°C, from 2 (*Azotobacter*) to 21 (oligotrophic bacteria) days. Spore-forming oligotrophic and copiotrophic bacteria were determined in material pasteurized for 15 minutes at 85°C. The number of colony-forming units (cfu) was determined with the use of a bacterial colony counter.

Biochemical analyses were performed to determine the activity of: dehydrogenases – with TTC substrate (ÖHLINGER 1996), urease – as described by ALEF and NANNIPIERI (1998), acid phosphatase (Pac) and alkaline phosphatase (Pal) – by the method developed by ALEF et al. (1998). The activity of dehydrogenases was expressed in cm^3 H_2, required for TCC reduction to TFP, the activity of urease – in mg $N\text{-}NH^{4+}$ produced from hydrolyzed urea, and the activity of phosphatases – in mmol p-nitrophenol (PNP) produced from sodium 4-nitrophenylphosphate.

At each stage of the experiment soil samples were also analyzed to the determine the following: pH_{KCl}, organic carbon content, hydrolytic acidity, sum of exchangeable basic cations and soil saturation with basic cations. The above analyses were performed in line with the procedures described previously (WYSZKOWSKA, KUCHARSKI 2005). The results of physicochemical analyses are not presented in this paper because none of the tested actinomyces significantly modified the investigated parameters, and diesel oil contamination contributed only to an increase in the relative carbon content by 17%.

The obtained results were processed statistically with Duncan's multiple range test and a three-factorial analysis of variance (StatSoft, Inc....2005).

Results and Discussion

The data presented in Table 1 indicate that the bacterial count was determined by many variables applied in the experiment, including the date of the analysis. In soil samples analyzed on day 12 of the experiment, compared to day 62, the count of total oligotrophic bacteria, total copiotrophic bacteria and cellulolytic bacteria was 1.3-fold, 1.1-fold and 3.8-fold higher, respectively. A reverse trend was reported with regard to the remaining microbial groups. At the end of the experiment, compared to the initial stage of the study, the count of *Azotobacter,* fungi, actinomyces, spore-forming oligotrophic bacteria and of spore-forming copiotrophic bacteria was 27.7-fold, 15.4-fold, 5.5-fold, 5.1-fold and 1.6-fold higher, respectively.

The bacterial count was equally affected by soil contamination with diesel oil as by the date of analysis. It should be noted that except for spore-forming oligotrophic and copiotrophic bacteria, microorganisms showed a positive

Table 1

Effect of soil contamination with diesel oil on the counts of soil microorganisms (cfu kg^{-1} soil dry matter)

Dose ON (cm^3 kg^{-1} of soil)	Olig · 10^9		$Olig_p$ · 10^7		Cop · 10^9		Cop_p · 10^8		Cel · 10^7		Az · 10^3		Act · 10^9		Fun · 10^7	
	date of analysis															
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
without actinomyces																
0	4.5	40.6	8.5	32.5	20.1	38.2	3.2	4.4	6.4	1.7	0.7	0.7	2.1	24.0	3.7	14.4
10	152.9	90.8	6.4	43.4	144.7	123.2	4.1	3.4	14.7	2.1	3.0	62.0	10.5	38.7	4.1	131.4
with *Streptomyces longisporoflavus*																
0	4.9	25.4	11.6	50.4	21.8	52.0	2.5	3.1	15.0	1.9	0.4	0.8	3.5	44.3	5.7	16.8
10	240.9	135.2	10.1	40.4	213.9	108.3	2.7	5.3	16.4	2.6	1.9	73.1	21.4	75.1	6.5	213.6
with *Streptomyces odorifer*																
0	5.06	34.9	8.3	57.8	26.1	25.8	2.1	7.2	10.5	5.9	1.5	1.1	3.8	42.1	3.2	12.8
10	163.1	119.7	6.6	39.4	140.3	158.6	2.6	4.7	15.0	6.5	1.0	63.5	23.1	117.2	5.6	57.1
with *Streptomyces viridis*																
0	5.5	21.1	10.3	49.5	53.7	35.8	2.6	5.1	9.5	1.3	0.3	1.4	4.1	40.4	4.5	13.9
10	159.4	82.5	9.0	46.3	122.4	136.5	3.0	3.6	11.0	3.8	0.8	62.8	18.4	99.2	4.9	126.9
$LSD_{0.05}$	a – 10.2 b – 14.5 c – 10.2 $a \cdot b$ – 20.5 $a \cdot c$ – n.s. $b \cdot c$ – 20.5 $a \cdot b \cdot c$ – 28.9		a – 2.5 b – 3.6 c – 2.5 $a \cdot b$ – n.s. $a \cdot c$ – 3.6 $b \cdot c$ – 5.1 $a \cdot b \cdot c$ – 7.26		a – 12.2 b – 17.3 c – n.s. $a \cdot b$ – 24.5 $a \cdot c$ – 17.3 $b \cdot c$ – n.s. $a \cdot b \cdot c$ – 34.7		a – 0.6 b – 0.9 c – 0.6 $a \cdot b$ – 1.3 $a \cdot c$ – 0.9 $b \cdot c$ – n.s. $a \cdot b \cdot c$ – 1.8		a – 1.1 b – 1.6 c – 1.1 $a \cdot b$ – 2.2 $a \cdot c$ – 1.6 $b \cdot c$ – 2.2 $a \cdot b \cdot c$ – 3.1		a – 3.8 b – 5.3 c – 3.8 $a \cdot b$ – n.s. $a \cdot c$ – 5.3 $b \cdot c$ – 7.6 $a \cdot b \cdot c$ – 10.7		a – 4.9 b – 7.0 c – 4.9 $a \cdot b$ – 9.9 $a \cdot c$ – 7.0 $b \cdot c$ – 9.9 $a \cdot b \cdot c$ – 14.1		a – 3.6 b – 5.1 c – 3.6 $a \cdot b$ – 7.3 $a \cdot c$ – 5.1 $b \cdot c$ – 7.3 $a \cdot b \cdot c$ – 10.3	

ON – diesel oil, I – before sowing spring barley, II – after harvest spring barley, Olig – oligotrophic bacteria, Cop – copiotrophic bacteria, $Olig_p$ – spore-forming oligotrophic bacteria, Cop_p – spore-forming copiotrophic bacteria, Az – *Azotobacter* spp., Cel – cellulose-decomposing bacteria, Act – actinomyces, Fun – fungi

LDS for: a – diesel oil dose, b – actinomyces application, c – date of analysis

n.s. – non-significant

response to contamination, and their number increased many-fold in comparison with uncontaminated soil. The total count of oligotrophic bacteria increased more than 8-fold, total copiotrophic bacteria – 4-fold, cellulolytic bacteria – 1.4-fold, actinomyces – 2.5-fold, fungi – 7-fold, *Azotobacter* bacteria – 38-fold.

The applied actinomyces inoculum had a much weaker effect on soil microbes than diesel oil. All of the actinomyces species contributed only to an increase in the population size of spore-forming oligotrophic bacteria and in the total count of actinomyces. The growth of oligotrophic, copiotrophic, cellulolytic bacteria and fungi was also stimulated by *Streptomyces longisporoflavus,* while the remaining species exerted no significant influence on oligotrophic, copiotrophic, cellulolytic bacteria or fungi. None of the tested species affected the *Azotobacter* population.

Similarly to microorganism counts, also the activity of soil enzymes varied over time (Table 2). On day 12 of the experiment, the activity of dehydrogenases was 43% higher than on day 62, the activity of urease was 211% higher, the activity of alkaline phosphatase was 86% higher, while the activity

Table 2
Effect of soil contamination with diesel oil on the activity of soil enzymes in 1 kg soil dry matter

Dose ON (cm^3 kg^{-1} of soil)	Dehydrogenases (cm^3 H_2 d^{-1})		Urease (mg N-NH_4 h^{-1})		Phosphatase (mmol PNP h^{-1})			
					alkaline		acid	
	I	II	I	II	I	II	I	II
without actinomyces								
0	6.13	4.13	12.68	10.42	1.20	0.45	1.58	2.02
10	18.05	5.67	117.16	24.87	1.57	1.01	1.64	1.56
with *Streptomyces longisporoflavus*								
0	5.95	6.39	10.73	9.57	0.91	0.46	1.47	2.54
10	17.53	17.76	122.02	33.93	1.24	0.75	2.03	1.35
with *Streptomyces odorifer*								
0	5.94	5.25	14.57	7.79	1.04	0.47	1.47	2.00
10	19.43	10.25	123.77	37.13	1.55	0.90	1.53	1.47
with *Streptomyces viridis*								
0	8.88	4.14	34.64	7.52	0.88	0.41	1.56	1.97
10	16.68	15.12	93.33	38.56	1.50	0.86	2.14	1.23
$LSD_{0.05}$*	a – 0.11 b – 0.15 c – 0.11 $a \cdot b$ – 0.22 $a \cdot c$ – 0.15 $b \cdot c$ – 0.22 $a \cdot b \cdot c$ – 0.31		a – 0.31 b – 0.44 c – 0.31 $a \cdot b$ – 0.62 $a \cdot c$ – 0.44 $b \cdot c$ – 0.62 $a \cdot b \cdot c$ – 0.88		a – 0.02 b – 0.03 c – 0.02 $a \cdot b$ – 0.04 $a \cdot c$ – 0.03 $b \cdot c$ – 0.04 $a \cdot b \cdot c$ – 0.06		a – 0.02 b – 0.03 c – 0.02 $a \cdot b$ – 0.04 $a \cdot c$ – 0.03 $b \cdot c$ – 0.04 $a \cdot b \cdot c$ – 0.06	

* explanations as in Table 1.

of acid phosphatase was lower by more than 5%, but the resulting difference was statistically significant. Diesel oil significantly affected the activity of the investigated enzymes. It increased the activity of dehydrogenases by 157%, of urease – by 447% and of alkaline phosphatase – by 61%, and it decreased the activity of acid phosphatase by 11%. Enzymatic activity was also significantly affected by actinomyces applied in the study. On average, all actinomyces species stimulated the activity of dehydrogenases and urease, while inhibiting the activity of alkaline phosphatase. Two species, *Streptomyces longisporoflavus* and *Streptomyces viridis*, stimulated the activity of acid phosphatase, while *Streptomyces odorifer* significantly reduced its activity. The enzyme-stimulating effect of actinomyces was observed mostly in soil samples contaminated with diesel oil.

The positive effect of actinomyces on selected soil enzymes was not reported in respect of spring barley yield (Figure 1). In soil contaminated with diesel oil, the yield of spring barley decreased 2.7-fold, and the applied inoculum of actinomyces spores enhanced the adverse impact of diesel oil on the growth and development of barley plants. The intensified negative effect of diesel oil on spring barley grown in soil which was inoculated with actinomyces could point to faster oil biodegradation in those treatments, a fact which requires additional investigation through an analysis of diesel oil residues and by-products of its transformation. Those by-products probably further inhibited the growth and development of spring barley. Better results might have been obtained if actinomyces inocula combining several, and not a single, species were applied. The applied inocula had a varied effect on bacterial counts and the activity of soil enzymes, and a more cumulative effect could be produced if an inoculum composed of three species were used.

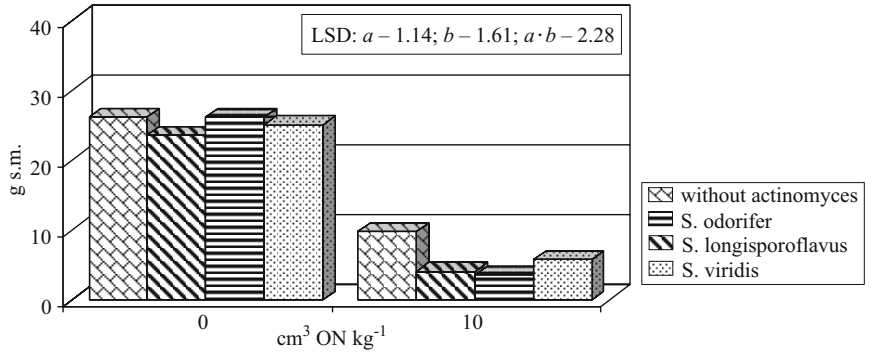

Fig. 1. Effect of diesel oil on the yield of spring barley dry matter (g pot^{-1}) LDS for: *a* – diesel oil dose, *b* – actinomyces application

According to numerous authors (LISTE, PRUTZ 2006, PICHTEL, LISKANEN 2001, WYSZKOWSKA, KUCHARSKI 2000, WYSZKOWSKA et al. 2002, 2006), oil derivative products have a clearly negative impact on plants, while – as presented in this study – their effect on bacterial counts and enzymatic activity varies, subject to the type of the contaminant (BUDNY et al. 2002, LISTE, PRUTZ 2006, PALMROTH et al. 2005, PICHTEL, LISKANEN 2001, WYSZKOWSKA, KUCHARSKI 2001, 2005). Diesel oil is a source of carbon for certain microorganisms. As a result, their populations in diesel oi-contaminated soil may grow, which in turn may affect enzymatic activity (BIELIŃSKA, TOMASZEWICZ 2006, WYSZKOWSKA et al. 2006). The above hypothesis can be supported by a 17% increase in the organic carbon content of soil contaminated with diesel oil, where spring barley was grown.

Conclusions

1. Soil contaminated with diesel oil was biologically unbalanced due to an increase in microbial counts and, consequently, higher activity of dehydrogenases, urease and alkaline phosphatase.

2. The applied inocula comprising spores of the following actinomyces: *Streptomyces longisporoflavus*, *Streptomyces viridis* and *Streptomyces odorifer* proved to be relatively ineffective in detoxifying soil contaminated with diesel oil.

3. Further research into the use of actinomyces in the reclamation of soils contaminated with diesel oil is required to investigate the suitability of other actinomyces species as inocula, and to determine the quantity of by-products of oil biodegradation.

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FACTORS THAT STIMULATE CONSUMER BEHAVIOR IN PURCHASING DAIRY PRODUCTS

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K e y w o r d s: dairy products, consumer behavior, questionnaire survey.

A b s t r a c t

The aim of this study was to establish the hierarchy of factors which influence consumer behavior in purchasing dairy products, with special emphasis on the information provided on the packaging. The survey involved a direct questionnaire which was distributed to 500 respondents. The results of the survey indicate that when purchasing dairy products, consumers are guided mainly by their own experience and the product's price. For 30% of respondents, labeling was an important source of information on the product and an equally important factor which stimulated the consumers' decision to buy dairy products.

CZYNNIKI KSZTAŁTUJĄCE ZACHOWANIA KONSUMENCKIE PRZY ZAKUPIE WYROBÓW MLECZARSKICH

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S ł o w a k l u c z o w e: wyroby mleczarskie, zachowania konsumenckie, badania ankietowe.

A b s t r a k t

Celem badania było określenie hierarchii czynników wpływających na zakup produktów mleczarskich, ze szczególnym uwzględnieniem informacji znajdującej się na ich opakowaniach. W badaniach przeprowadzonych na grupie 500 respondentów posłużono się kwestionariuszem ankiety bezpośredniej. Wykazano, że konsumenci, kupując wyroby mleczarskie, kierują się głównie własnymi doświadczeniami i ceną towaru. Informacja znajdująca się na opakowaniu stanowiła ważne źródło wiedzy o produkcie, a tym samym ważny czynnik wyborów konsumenckich dla grupy ok. 30% badanych.

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Introduction

Consumer preferences regarding food products are shaped by numerous factors which are related both to the product and the consumer making a purchasing decision. Dairy products are primary products which is why the majority of decisions made in the purchasing process are planned or impulse. When buying dairy products consumers are likely to follow a certain routine, where personal experiences and habits play an important role, while impulse buying is usually due to a sudden urge caused by emotions, hunger, special offers, eagerness to try out a new product, adherence to popular trends, etc. (GABRIELSEN 2001, GRUNERT et al. 2000).

The labeling of food products is a direct method of informing consumers about the products they are buying. Clear and comprehensive label information enables the consumers to make well-informed decisions on the purchased products and protects the consumers.

In view of the above, the aim of this study was to establish the hierarchy of factors and stimuli which influence the purchasing decisions made by consumers and the extent to which consumers rely on particular sources of information in their decisions to buy dairy products, with special emphasis on the information provided on the packaging.

Methods

The survey was carried out in the second half of 2005 and it involved a group of 500 Olsztyn residents who shop in hypermarkets, supermarkets and small local shops. The survey was conducted based on a direct questionnaire comprising two parts. In the first part, the respondents were asked to evaluate the degree to which the indicated social and economic factors affected their decisions to buy the preferred dairy products. The second part featured questions on the respondents' sex, age, occupation, place of residence and monthly income per person in the household.

Respondent characteristics

The characteristic features of the surveyed group are presented in Table 1. Despite the fact that women slightly outnumbered men in the respondent group, the age structure of the surveyed female and male population was similar. The majority of respondents were aged from 21 to 40. When the surveyed population was analyzed in view of the occupational criterion, the

researchers found that the vast majority of respondents were white collar workers who had a 44.4% share of the surveyed group. Students (22.6%) and blue collar workers (19%) were less well represented, while old age and disability pensioners and the unemployed (14%) had the smallest share of the respondent group.

Table 1

Respondent characteristics

Respondents		Population	(%)
Sex	female	277	55.4
	male	223	44.6
	total	**500**	**100.0**
Age in years	up to 20	38	7.6
	21–40	244	48.8
	41–60	186	37.2
	Above 60	32	6.4
	total	**500**	**100.0**
Occupation	pupil/student	113	22.6
	blue collar worker	95	19.0
	white collar worker	222	44.4
	old age/disability pensioner	58	11.6
	unemployed	12	2.4
	total	**500**	**100.0**
Place of permanent residence	rural area	74	14.8
	city < 50 000	38	7.6
	city 50 000–100 000	49	9.8
	city > 100 000	339	67.8
	total	**500**	**100.0**
Monthly income per person	under PLN 300	37	7.4
	PLN 301–900	122	24.4
	PLN 601–900	139	27.8
	PLN 901–1200	83	16.6
	above PLN 1200	119	23.8
	total	**500**	**100.0**

The vast majority of respondents resided in cities with a population higher than 100 000 (67.8%). A smaller percentage share was reported in respect of residents from smaller cities (17.4%), while only around 15% of the polled subjects resided in rural areas. This configuration of the respondent structure, subject to the place of permanent residence, was nearly identical for both sexes.

The largest group of respondents, accounting for nearly 60% of the entire surveyed population, comprised persons generating monthly incomes of up to PLN 900 per person in the household.

Results and Discussion

Survey results were used to establish the hierarchy of factors which stimulate purchases and the extent to which consumers rely on particular sources of information in their decisions to buy dairy products.

The researchers concluded that personal experience is the most important factor which influences the consumer's decision to buy a given dairy product (Figure 1). Previous experience was indicated by more than 80% respondents as an important and very important factor in the decision-making process. These results support the theory that the purchase of primary products is a planned and routine process. According to the Howard-Sheth model of consumer behavior which relies on previous purchasing experience, consumers need very limited information to make a purchase and in this case, the decision is made very quickly (SMYCZEK 2003a,b).

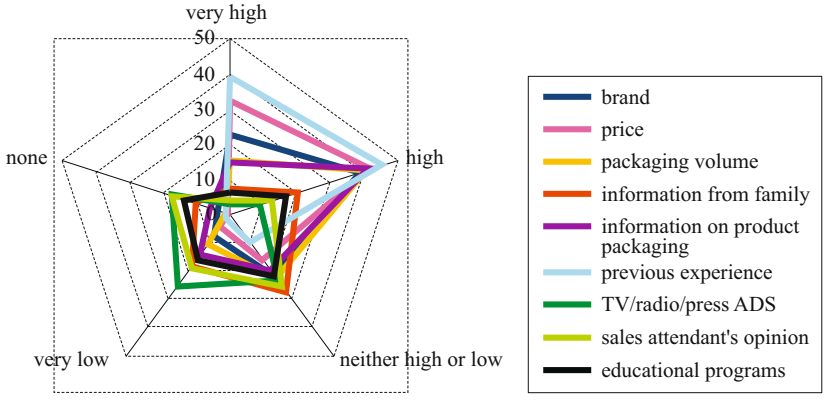

Fig. 1. Influence of social and economic factors on the consumers' decision to buy dairy products (% indications)

Survey results, verified with the use of the χ^2 test, showed a significant relationship between the influence of previous experience on the decision to buy and the respondents' age and place of residence (Table 2). Previous experience was the most important factor influencing the purchasing decision in the group of respondents aged up to 20 and respondents in the 21–40 age group. Experience was indicated as the most important factor by respondents aged over 60 and respondents in the 41–60 age group. Previous experience was most frequently indicated as the factor stimulating the decision to buy dairy products by respondents from cities with a population higher than 50 000. The significance of experience was a less decisive factor in the purchasing process

for respondents residing in rural areas. Only a small fraction of the entire surveyed group (5.8%) pointed to previous experience as a relatively unimportant and completely insignificant factor stimulating their decisions to buy dairy products (Figure 2a and Figure 2b).

Table 2
Significance of selected social and economic factors in the consumers' decisions to buy dairy products subject to: sex, age, place of permanent residence, occupation and monthly income per person (value of χ^2 test)

Specification	Sex	Age	Place of permanent residence	Occupation	Monthly income per person
Brand	2.74	43.05*	26.89	32.86	28.72
Price	2.62	54.85*	17.37	34.42	22.32
Packaging volume	5.89	25.54	39.58*	20.61	18.51
Information from family/friends	9.32	20.02	19.54	21.90	13.88
Information on product packaging	4.47	31.05*	16.80	21.66	25.66
Previous experience	2.75	32.45*	35.42*	18.90	15.87
TV/radio/press ads	2.50	36.84*	17.98	14.83	28.02
Sales attendant's opinion	5.82	32.42*	13.67	28.68	18.91
Educational programs	2.56	18.90	33.74*	45.23*	11.57

* – significant value at $\alpha = 0.01$

As an inseparable product feature, the price was the second most important factor after previous experience in the hierarchy of consumer decisions to buy dairy products. The obtained results, verified with the use of the χ^2 test, pointed to a significant dependency between the influence of the price on the decision to buy dairy products and the respondents' age. Price was a the most important factor (in comparison with other purchase stimulating factors) in the group of respondents older than 60 (Table 2, Figure 3a and Figure 3b). A similar view is postulated by KUŚMIERCZYK (2000) whose research indicates that in comparison with other age groups, elderly consumers are more likely to be guided by the price in their purchasing decisions and this dependency intensifies with age. The researchers also concluded that the price is a more important factor determining the purchase of dairy products for less educated respondents, blue collar workers, the unemployed and students. In a study to determine the significance of information on product packaging, OZIMEK (2002) classified price as the second most important factor in view of the significance of particular components of information provided on product packaging. It should be noted, however, that in most food products, including dairy products, price is not a permanent feature of product packaging.

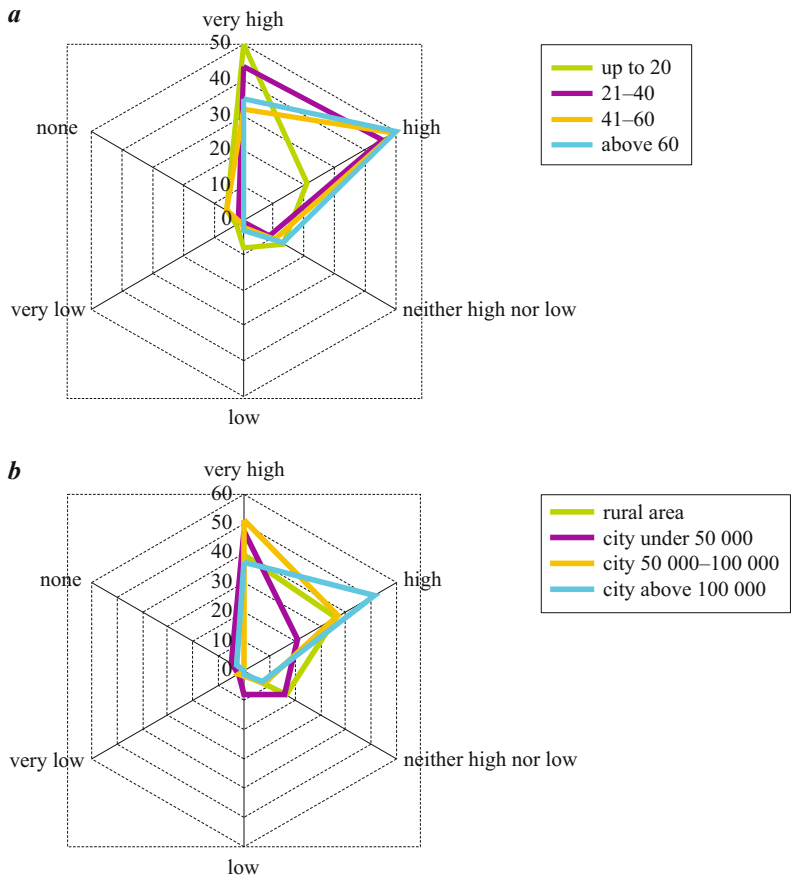

Fig. 2. Influence of previous experience on the consumers' decision to buy dairy products: *a* – subject to age, *b* – subject to place of residence (% of indications)

Brand is a guarantee of product quality for consumers, and the choice of a given brand is an expression of the consumers' trust vested in a given product and the brand proprietor. In a market with an enormous number of competing dairy producers, customers are increasingly likely to focus on products offered by a given company (GÓRSKA-WARSEWICZ 2006, JAKOWSKI 2002). A renowned product brand and the producer's trademark ranked third in the hierarchy of factors which affect the purchasing decisions of the surveyed group. Product brand was indicated as an important stimulating factor by more than 60% of respondents. The popularity of specific brands among consumers make the brand an inestimable and the most valuable intangible asset of its proprietor. According to the results of the survey, most consumers identified the brand as an important factor which affects their

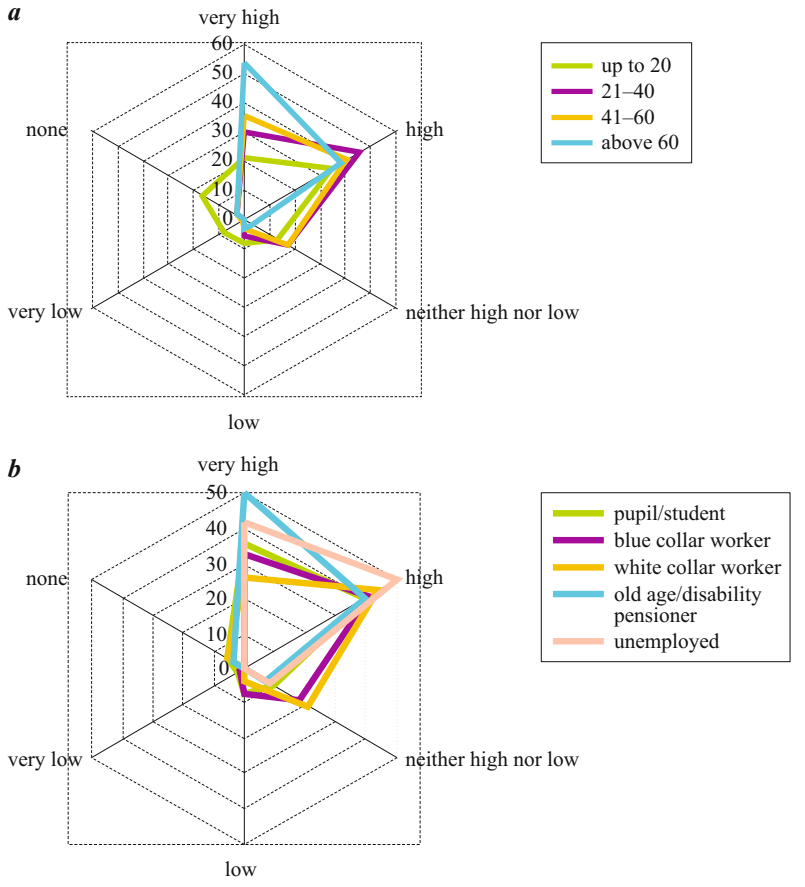

Fig. 3. Influence of price on the consumers' decision to buy dairy products: *a* – subject to age *b* – subject to occupation (% of indications)

decision to buy dairy products regardless of the respondents' sex, occupation, place of residence or income per person in the household. In view of the obtained data, verified with the use of the χ^2 test, the only statistically significant relationship was observed when the significance of product brand was analyzed in view of the respondents' age. The highest interest in the brand criterion was demonstrated by persons in the 41–60 age group, while the brand was perceived as the least influential factor determining the decision to buy dairy products among the respondents aged up to 20 (Figure 4).

A slightly different view on the brand's significance in the choice of dairy products was presented by KUŚMIERCZYK (2000) whose research, carried out at the Institute of Home Market and Consumption on a group of 700 urban households, indicated that dairy product brands do not have a significant

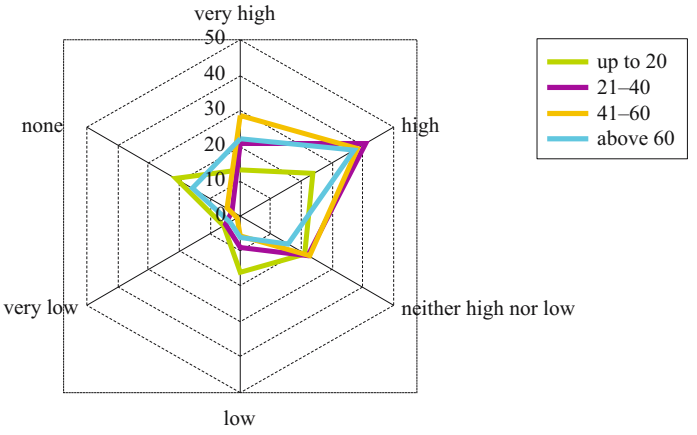

Fig. 4. Influence of brand on the consumers' decision to buy dairy products subject to age (% of indications)

impact on the consumers' purchasing decisions. Despite the above, a significant fraction of the surveyed respondents claimed to have preferred brands and were able to indicate brands which they purchased most often.

The study analyzing the influence of social and economic factors in shaping the preferences of dairy product consumers indicates that the volume of unitary packaging of dairy products and the information on product packaging are equally responsible for stimulating purchasing decisions. Positive responses (very significant and significant) regarding the impact of those factors on the decision to buy dairy products were given by around 30% of respondents on average. Around 20% of the surveyed population did not recognize the above factors as important purchase stimuli (Table 1).

The role of packaging volume in the process of purchasing dairy products proved to be particularly significant for respondents residing in cities with population of 50 000–100 000. Residents of large cities with population above 100 000 as well as residents of rural areas and smaller cities with population below 50 000 had similar opinions on the impact of packaging volume on dairy product purchases. Around 40% of respondents from the above groups claimed that packaging volume is an important factor which affects their choice of a given product (Figure 5).

Information plays a very important role on the contemporary market where consumers can choose from a wide variety of dairy products. Consumers have access to extensive information from various sources, including the information displayed on product packaging or labels (OZIMEK 2002).

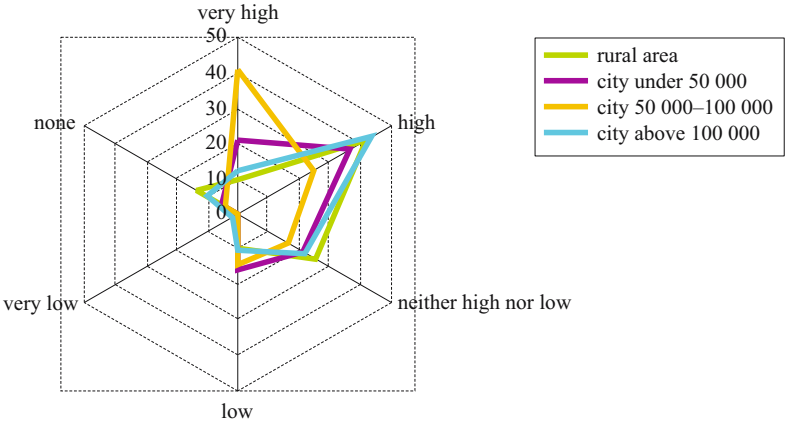

Fig. 5. Influence of packaging volume on the consumers' decision to buy dairy products subject to place of residence (% of indications)

According to the survey, the information displayed on product packaging was regarded as a very significant factor which influences purchasing decisions by only 14.6% of the respondents, while 41.2% surveyed persons were of the opinion that it is a significant factor. The results obtained in respect of the above, verified with the use of the χ^2 test, pointed to a significant relationship between the information on product packaging and the respondents' age. It was concluded that the significance of information displayed on dairy products grew with the respondents' age (Figure 6).

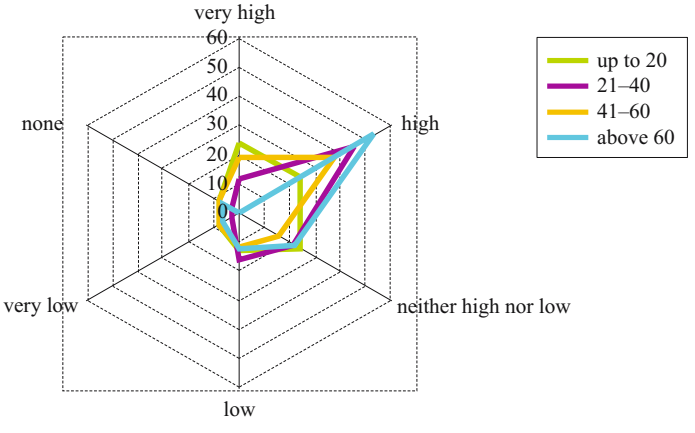

Fig. 6. Influence of packaging information on the consumers' decision to buy dairy products subject to age (% of indications)

Age was also an important factor determining the behavior of consumers whose purchases are influenced by advertising (TV/radio/press). Yet in this case, a reverse trend to that reported in respect of the role of product information was observed. A much higher number of respondents aged under 20 claimed that advertising had a significant and very significant impact on their decisions to buy dairy products (Figure 7).

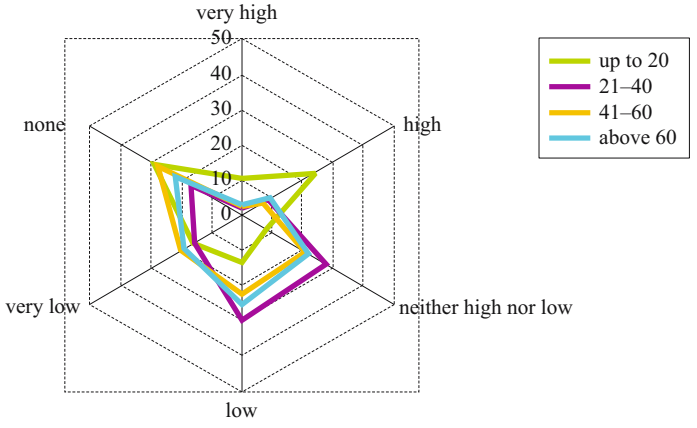

Fig. 7. Influence of TV/radio/press advertising on the consumers' decision to buy dairy products subject to age (% of indications)

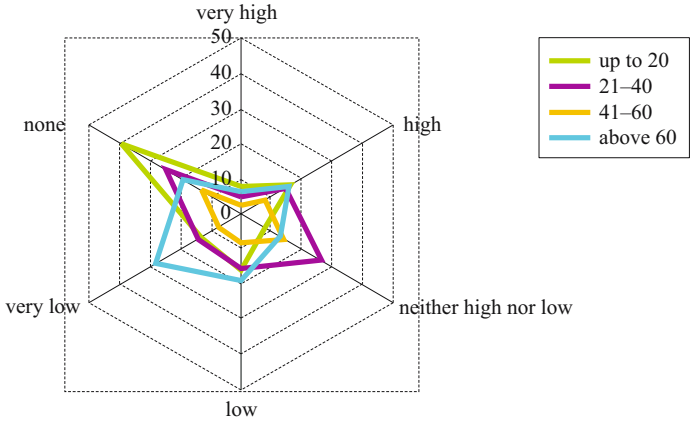

Fig. 8. Influence of sales attendant's opinion on the consumers' decision to buy dairy products subject to age (% of indications)

From among other factors shaping dairy product purchases, only around 13.8% respondents on average declared that information obtained from family members and friends played a significant or very significant role in their choice of product brand (Figure 1).

According to more than 40% of respondents aged up to 20, the sales attendant's opinion was of absolutely no relevance to their decision to buy a specific dairy product brand. More than 30% of respondents older than 60 claimed to take into account the sales attendant's suggestions regarding the choice of product (Figure 8).

Conclusions

The results of the survey lead to the following conclusions:

1. Consumers are most likely to rely on previous purchasing experience when choosing a dairy product.

2. The price of the product proved to be an important factor shaping the consumers' choice, especially among older respondents and respondents in the lower income group.

3. Brand was recognized as an important factor which stimulates purchasing decisions by 32.5% of the overall respondent population regardless of sex, occupation, place of residence or income.

4. For 30% of the surveyed respondents, the information on product packaging was an important source of product information and a significant factor which determined their choice of dairy products.

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