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**ASSESSMENT OF GRASSLAND HABITATS  
ON THE POPIELNO PENINSULA  
BY PHYTOINDICATION METHODS**

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**Key words:** phytoindication, indicator values, grassland.

**A b s t r a c t**

Grassland habitats located in a part of the Popielno Peninsula that is used for agricultural purposes (Popielno and Wierzba), under conditions of spatial isolation, were evaluated based on phytosociological relevés. A phytoindication method based on ecological indicator values, developed by ZARZYCKI et al. (2002), and simple statistical methods were employed in the study.

The analyzed grasslands are managed as hay meadows and pastures. Vegetation is dominated by sown grasses, primarily *Dactylis glomerata*, *Poa pratensis* and *Festuca rubra*. The local plant communities belong to the classes *Molinio-Arrhenatheretea* and *Phragmitetea*. A total of 200 species of vascular plants were identified in the research area, including 197 species described by ZARZYCKI et al. (2002) with the use of indicator values.

Grassland vegetation in Popielno and Wierzba prefers moderate light and temperature conditions. On a 5-point scale, the values of light and temperature reached 3.47 to 4.65 and 3.48 to 3.99, respectively. The soils in the area are fertile, nutrient-abundant (trophy of 3.32 to 4.20) and neutral (acidity of 3.56 to 4.79). Soil moisture content in the examined habitats ranged from 2.83 to 5.89 on a 6-point scale.

## FITOINDYKACYJNA OCENA SIEDLISK UŻYTKÓW ZIELONYCH PÓŁWYSPU POPIELNIAŃSKIEGO

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**Słowa kluczowe:** fitoindykacja, liczby wskaźnikowe, użytki zielone.

### Abstrakt

W oparciu o zestaw zdjęć fitosocjologicznych oceniono siedliska pod trwałymi użytkami zielonymi, zlokalizowanymi w warunkach izolacji przestrzennej na rolniczej części Półwyspu Popielniańskiego (Popielno i Wierzba). Wykorzystano metodę fitoindykacyjną liczb ekologicznych wg ZARZYCKIEGO i in. (2002) oraz proste metody statystyczne.

Roślinność użytków zielonych analizowanego obszaru ma charakter łąk i pastwisk zagospodarowanych, z dominacją traw pochodzących z siewu, głównie: *Dactylis glomerata*, *Poa pratensis*, *Festuca rubra*. Zbiorowiska kształtujące się tu zakwalifikowano do klas *Molinio-Arrhenetheretea* i *Phragmitetea*. Zidentyfikowano 200 gatunków roślin naczyniowych, 197 zostało opisanych przez ZARZYCKIEGO i in. (2002) liczbami wskaźnikowymi.

Roślinność użytków zielonych Popielna i Wierzby preferuje umiarkowane warunki świetlne i ciepłe. Wartości wskaźnika świetlnego wynosiły 3,47-4,65, a termicznego 3,48-3,99 (skala 5-stopniowa). Gleby badanego terenu określono jako zasobne (wskaźnik trofizmu – 3,32-4,20) i obojętne (wskaźnik kwasowości – 3,56-4,79). Badane siedliska reprezentują szerszy zakres wilgotności: wskaźniki wilgotności w 6-stopniowej skali osiągały wartości 2,83-5,89.

## Introduction

Phytoindication methods for the assessment of habitat conditions have been gaining increasing interest recently due to both their theoretical and practical aspects (WÓJCIK 1983, BOROWIEC 2003, ROO-ZIELIŃSKA 2004). The theoretical and methodological foundations of this kind of assessment were laid by ELLENBERG (1950, 1974), who presented the requirements of a wide variety of species with regard to certain ecological factors in the form of the so called indicator values for Central Europe. In Poland a list of the ecological indicator values for native and naturalized (fully domesticated) species was published by ZARZYCKI (ZARZYCKI 1984, ZARZYCKI et al. 2002). According to KOZŁOWSKA (1991), the use of a 10-point scale (ELLENBERG 1979) and a 5-point scale (ZARZYCKI 1984) provides comparable results.

The main advantage of phytoindication methods over other methods is that they are inexpensive and easy to apply (BOROWIEC 2003). Ecological indicator values have a wide range of applications. They can be easily compiled, grouped

and calculated. The habitats assessed by means of indicator values can be compared in terms of changes taking place in space and time. However, specialists have also pointed out some disadvantages of these methods (ZARZYCKI et al. 2002, BOROWIEC 2003). Professional literature on the subject, both international (e.g. LAWESSON, MARK 2000, ROY et al. 2000, CORNWELL, GRUBB 2003, SCHMIDTLEIN, EWALD 2003) and Polish (e.g. STUPNICKA-RODZYNKIEWICZ et al. 1986, BOROWIEC et al. 1989, HOŁDYŃSKI 1989, KAPELUSZNY, JĘDRUSZCZAK 1994, AFFEK-STARCZEWSKA, SKRZYZYŃSKA 2003, KAPELUSZNY, HALINIARZ 2003, ŁABZA et al. 2003, ROLA et al. 2003, TRĄBA et al. 2004), refers primarily to the works of Ellenberg. The present study is based on the indicator values developed by ZARZYCKI et al. (2002).

The objective of this study was to evaluate permanent grassland habitats located in an open part of the Popielno Peninsula that is used for agricultural purposes, under conditions of spatial isolation. A phytoindication method based on ecological indicator values, proposed by Zarzycki (ZARZYCKI et al. 2002), and simple statistical methods were employed. The area under analysis, although small, is ecologically interesting due to its diversified relief. It is mostly farmland used as pasture, surrounded by lakes, which provides habitat for conservation herds of Konik Polski and Polish Red cattle.

## Materials and Methods

The study was based on 194 phytosociological releves made on permanent grassland located in the northern part of the Popielno Peninsula. This peninsula is surrounded on three sides by lakes: Beldany, Mikołajskie, Śniardwy and Warnołty (Figure 1). According to physiographic criteria, it belongs to the mesoregion of Great Masurian Lakes and to the macroregion of Masurian Lakeland (KONDRACKI 2001). According to the geobotanical division of Poland (SZAFER 1977), this peninsula is located along the south-western boundary of the Northern Division, within the range of spruce (*Picea abies*) and beyond the range of beech (*Fagus sylvatica*). This is a transition zone influenced by the Atlantic and continental climate (WOŚ 1999), and the close vicinity of lakes contributes to its specific microclimate. The geomorphological features of this peninsula were formed mostly during the first phases of the Würm Glaciation. Surface topography includes morainal ridges and hills. The investigations were conducted on meadows and pastures belonging to the villages Popielno (eastern part on the side of Lake Śniardwy) and Wierzba (north-western part). In the south they border on extensive forest communities, and on the side of lakes they partly turn into rush communities. The area is dominated by gray-brown podsolic soils developed from boulder

clay, with a sand layer to a depth of 40-60 cm. Organic (peat and alluvial-muck) and deluvial soils can be found in morainal depressions. The analyzed grasslands are managed as hay meadows and pastures. Vegetation is dominated by sown grasses, primarily *Dactylis glomerata*, *Poa pratensis* and *Festuca rubra* (JUTRZENKA-TRZEBIATOWSKI, HOŁDYŃSKI 1996).

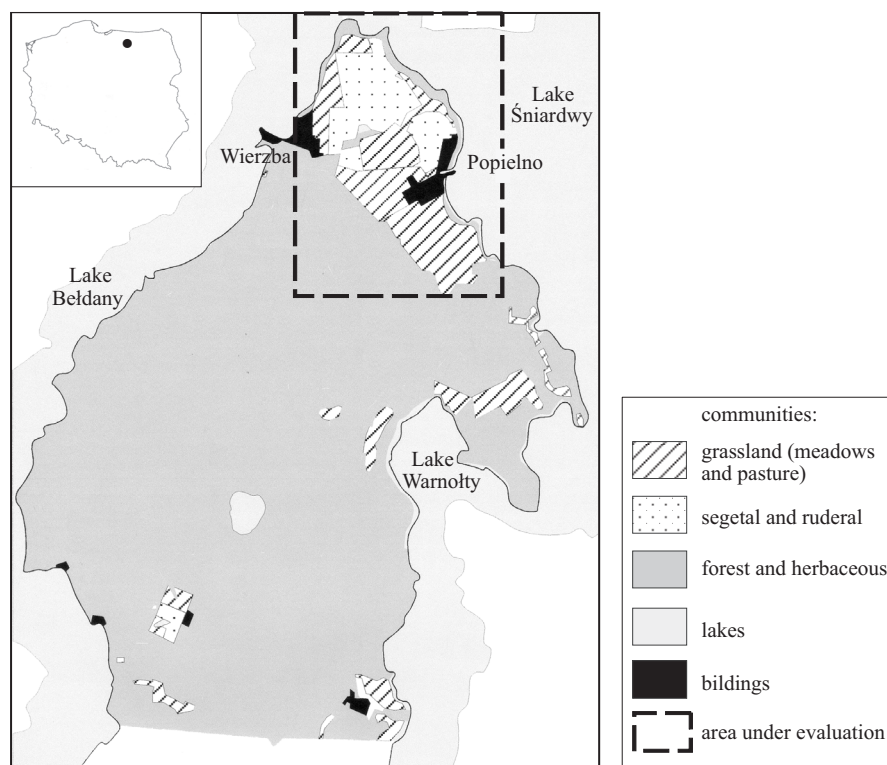


Fig. 1. Research area — the Popielno Peninsula; note grassland communities assessed by phytoindication methods

Phytosociological relevés were made by the Braun-Blanquet method, in the summer months (June, July, August). The area of a single relevé varied from 9 to 100 m<sup>2</sup>, depending on phytocenosis size. The obtained analytical materials provided a basis for calculating mean indicator values for climate (*L* – light, *T* – temperature, *K* – continentality) and edaphic conditions (*W* – soil moisture content, *Tr* – soil (water) trophic, *R* – soil acidity, *D* – soil granulometric composition, *H* – soil organic matter content). Ecological indicator values,



proposed by ZARZYCKI et al. (2002) for vascular plants in Poland, were used in the study. In most cases indicator values were estimated on a 5-point scale (except for *W* – a 6-point scale, *H* – a 3-point scale), with the intensity of a factor increasing from 1 to 5(6). Mean values of all indicators were calculated according to the formula:

$$X = \frac{\sum X_n \cdot i_n}{\sum i_n}$$

where:

*X* – mean value of a habitat indicator (*L*, *T*, *K*, *W*, *Tr*, *R*, *D*, *H*);

*X<sub>n</sub>* – indicator value for a given species;

*i<sub>n</sub>* – relative abundance of a given species according to the Braun-Blanquet scale (JANKOWSKI 1994).

For abundance + the value 0.1 was adopted in calculations (the middle of the cover range corresponding to the relative abundance determined by this rank). If the amplitude of the requirements of a species concerning a given factor was not defined by a single indicator value, the following principles were applied:

- if the requirements of a species were defined within an interval of two neighboring numbers (e.g. 1-2, 4-5) or in-between neighboring numbers (e.g. 1/2, 4/5), the average value of these two numbers was adopted as an indicator value;

- in the case of wider intervals (e.g. 2-5, (2)3-4) a given species was not treated as an indicator for a certain habitat factor and was excluded from further calculations.

The calculated mean indicator values for particular relevés were subjected to further generalization. Four times, independently, they were grouped according to the criteria given in Table 1. Some relevés were disregarded since they could not be classified in accordance with the adopted assumptions. It should be noted that communities of the class *Phragmitetea* were formed primarily on organic soils used for various purposes; they were determined in both villages.

Applying the established criteria, the following simple statistical measures were calculated for the entire research area and for particular groups: mean, coefficient of variation, median, mode and mode size. Histograms of abundance are presented as intervals whose limits were determined based on the 1-5 scale (1-6 for *W*, 1-3 for *H*), so as to round the values within an interval to the integer number being the middle of this interval; fractional numbers were rounded down. Within each criterion, means of groups were compared by the t-test for independent samples. Relationships between indicator values are presented as coefficients of simple correlation.

Table 1

Criteria for the division of the experimental material into groups

Criteria	Group	Number of releves in a group
Location	Popielno	178
	Wierzba	16
Soil type	Organic	80
	Mineral	114
Land use type	Pasture	129
	Meadow	51
Plant community (phytosociological class)	<i>Molinio-Arrhenetheretea</i> (Mol-Arr)	157
	<i>Phragmitetea</i> (Phragm)	37

## Results

Among a total of 200 species of vascular plants identified in the analyzed area, 197 were described by ZARZYCKI et al. (2002) using indicator values. Not all of these species were taken into account while evaluating a given habitat with regard to particular ecological factors. The species whose ecological amplitude of requirements concerning a specified factor was too wide to consider them indicator species were disregarded, as mentioned in the Materials and Methods section. Some species, e.g. members of the genus *Lemna*, were not described in terms of their soil texture (granulometric composition) requirements due to their ecological specificity. The total number of species excluded from the analysis for this reason was as follows: light conditions – 9 species, temperature conditions – 49 species, continentality – 2 species, soil moisture content – 6 species, trophy status – 5 species, soil acidity – 30 species, soil granulometric composition – 52 species, soil organic matter content – 6 species.

The indicator values for light ranged from 3.47 to 4.65, at variation as low as 3.6% (Table 2). 98% of all releves remained within the 3.5-4.5 interval (Figure 2). Taking into account the fact that the factor was evaluated on a 5-point scale, this is a relatively small range. The values of mean, median and mode were at a similar level, i.e. 4.07, 4.05 and 4.00, respectively, which resulted from the numerical superiority of species preferring moderate light conditions, such as *Dactylis glomerata*, *Festuca rubra*, *Lolium perenne*, *Phalaris arundinacea*, *Taraxacum officinale*, *Trifolium repens*, members of the genera *Alopecurus* and *Carex*. The data in Table 2 show that the mean indicator values for light in particular groups formed based on four independent criteria differed highly significantly. Higher indicator values were recorded in Popielno, on mineral soil, on pastures and in communities of the class *Molinio-Arrhenetheretea*.

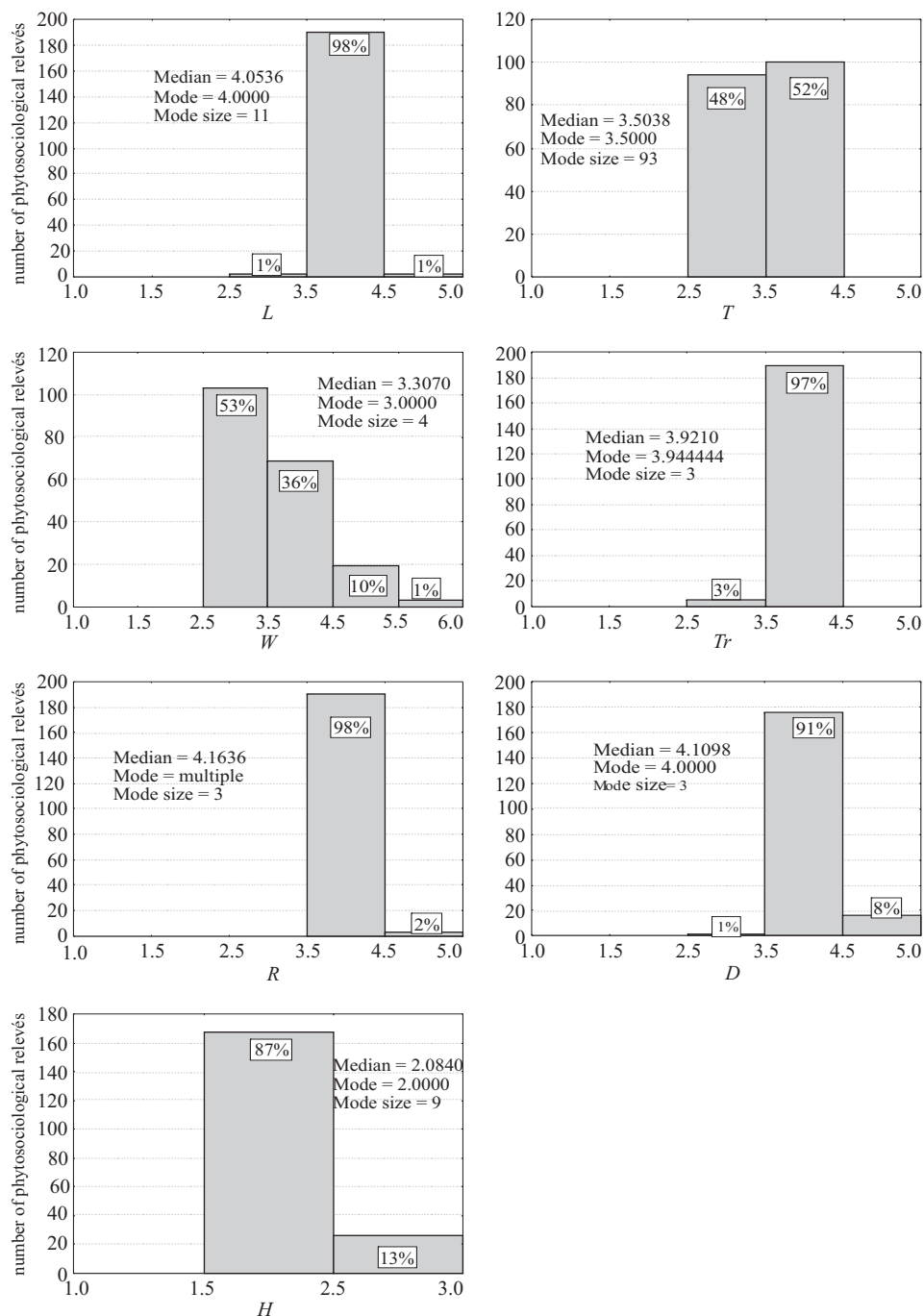


Fig. 2. Histograms of distribution of phytosociological relevés in intervals of ecological indicator values for climate and edaphic conditions, according to ZARZYCKI et al. (2002)

Table 2

Statistical characteristics for light value (*L*)

Specification	Mean	Range of variation	Coefficient of variation (%)
Entire research area	4.07	3.47-4.65	3.6
Location – Popielno	4.09 <sup>A</sup>	3.66-4.65	3.1
– Wierzba	3.87 <sup>B</sup>	3.47-4.15	5.6
Soil type – organic	4.02 <sup>A</sup>	3.47-4.35	4.2
– mineral	4.10 <sup>B</sup>	4.00-4.65	3.0
Land use type – pasture	4.09 <sup>A</sup>	3.70-4.57	2.8
– meadow	3.99 <sup>B</sup>	3.47-4.21	4.3
Plant community – Mol-Arr	4.09 <sup>A</sup>	3.65-4.65	3.2
– Phragm.	3.99 <sup>B</sup>	3.47-4.25	4.8

*AB, ab* — significance of differences between means: values within categories followed by the same letters do not differ significantly at  $p \leq 0.05$  (small letters) or  $p \leq 0.01$  (capital letters)

The indicator values for temperature remained within a very narrow range of 3.48 to 3.99, with a mean of 3.56 and the coefficient of variation of 3.2% (Table 3). These values correspond to a moderately warm climate (typical of lowlands) with a slight shift towards a moderately cold climate in some microhabitats. The most often repeated value (as many as 93 times) was 3.5 and it decided about the size of the first of the two bars in the diagram (Figure 2). The median was only slightly higher than the mode. It should be stressed that among the 200 species identified in the study area as many as 123 were represented by the value 3.5 (the range of occurrence 3-4). The most abundant among them were *Alopecurus pratensis*, *Festuca pratensis*, *F. rubra*, *Glyceria fluitans*, *Lolium perenne*, *Phleum pratense*, *Poa palustris*, *P. pratensis*

Table 3

Statistical characteristics for temperature value (*T*)

Specification	Mean	Range of variation	Coefficient of variation (%)
Entire research area	3.56	3.48-3.99	3.2
Location – Popielno	3.55 <sup>A*</sup>	3.48-3.99	2.9
– Wierzba	3.66 <sup>B</sup>	3.50-3.95	4.7
Soil type – organic	3.57 <sup>a</sup>	3.50-3.95	3.6
– mineral	3.55 <sup>a</sup>	3.48-3.99	2.9
Land use type – pasture	3.54 <sup>A</sup>	3.48-3.99	2.4
– meadow	3.60 <sup>B</sup>	3.50-3.95	4.0
Plant community – Mol-Arr	3.54 <sup>A</sup>	3.48-3.99	2.7
– Phragm.	3.63 <sup>B</sup>	3.50-3.95	4.4

\* explanations as in Table 2

and *Ranunculus acris*. When the relevés were grouped according to location, it was found that the indicator values for temperature were higher in Wierzba than in Popielno – the difference was highly statistically significant. This difference could result from the cooling effect of Lake Śniardwy on the fields located in Popielno. Wierzba is situated on a narrow inlet between Lake Beldany and Lake Mikołajskie. Higher indicator values for temperature were recorded on meadows, compared with pastures, and in communities of the class *Phragmitetea*, compared with those of the class *Molinio-Arrhenetheretea* (statistically significant differences). No such differences were observed when the relevés were classified based on soil type (mineral, organic).

The indicator values for continentality were the least differentiated of all. Among 198 species almost all were found to be neutral with respect to continentality. The indicator value for continentality was other than 3 for two taxa only, which had no impact on means for particular groups of relevés. Therefore, the indicator values for continentality are not presented in tabular and graphic form in this paper.

The mean values for soil moisture content varied from 2.83 to 5.89 and showed the highest variation of all habitat indicators – 18.1% (Table 4). The median, mode and histogram for soil moisture content indicate the domination of slightly humid habitats in the examined area (53% of relevés oscillated

Table 4  
Statistical characteristics for soil moisture value (W)

Specification	Mean	Range of variation	Coefficient of variation (%)
Entire research area	3.62	2.83-5.89	18.1
Location – Popielno	3.58 <sup>A*</sup>	2.83-5.89	17.9
– Wierzba	4.09 <sup>B</sup>	2.99-4.91	16.2
Soil type – organic	4.24 <sup>A</sup>	3.31-5.89	12.3
– mineral	3.18 <sup>B</sup>	2.83-4.40	8.5
Land use type – pasture	3.43 <sup>A</sup>	2.83-5.89	17.9
– meadow	4.19 <sup>B</sup>	3.13-4.94	10.5
Plant community – Mol-Arr	3.41 <sup>a</sup>	2.83-5.89	14.8
– Phragm.	4.52 <sup>B</sup>	3.92-5.75	9.4

\* explanations as in Table 2

around the value of 3 – Figure 2). Such moisture conditions were preferred by 59 of 200 species, including the abundant and frequent *Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis* and *Taraxacum officinale*. The ranges of variation of the above indicator values in particular groups were relatively wide, but differences between means were distinct and statistically significant. The mean indicator value for soil moisture

content was 4.09 in Wierzba and 3.58 in Popielno. Habitats on organic soils were much more humid than those on mineral soils (by over 1). Considerable differences were also observed in humidity conditions with regard to land use type: meadows occupied more humid habitats (mean value of soil moisture content – 4.19) while pastures drier ones, as indicated by the average, although the range of variation in soil moisture on pastures was wide and included also wet, almost aquatic, habitats. An analysis of the types of communities formed depending on soil moisture content showed that phytocenoses of the class *Phragmitetea* preferred humid, wet and aquatic habitats (mean value – 4.52). Communities of the class *Molinio-Arrhenetheretea* were generally formed on slightly humid soils, but also on soils with a higher moisture content (upper limit of the variation range – 5.89).

As regards trophic determined by a phytoindication method, the soils in the research area can be generally classified as fertile and nutrient-abundant, which is confirmed by the values of statistics and a histogram (Table 5, Figure 2). Only 3% of relevés fell within the 3.32-3.50 range with respect to this indicator, which corresponds to moderately nutrient-poor habitats. *Rumex obtusifolius* was reported from these habitats. There were no statistically significant differences in soil fertility and nutrient abundance between the groups distinguished based on the adopted, independent criteria. The coefficients of variation did not exceed 4% in the entire area and in particular groups.

Table 5

Statistical characteristics for soil trophic value (*Tr*)

Specification	Mean	Range of variation	Coefficient of variation (%)
Entire research area	3.89	3.32-4.20	3.4
Location – Popielno	3.90 <sup>a*</sup>	3.32-4.20	3.5
– Wierzba	3.88 <sup>a</sup>	3.64-4.02	2.8
Soil type – organic	3.90 <sup>a</sup>	3.49-4.20	2.7
– mineral	3.89 <sup>a</sup>	3.32-4.16	3.9
Land use type – pasture	3.89 <sup>a</sup>	3.32-4.20	3.8
– meadow	3.89 <sup>a</sup>	3.49-4.05	2.8
Plant community – Mol-Arr	3.89 <sup>a</sup>	3.32-4.16	3.6
– Phragm.	3.92 <sup>a</sup>	3.67-4.20	2.8

\* explanations as in Table 2

An analysis of soil acidity in grassland habitats located in the research area revealed that the majority of soils were neutral. The variation range of R was 3.56-4.79 (Table 6), and the indicator value oscillated around 4 for 98% of relevés (Figure 2). Soil acidity was at the same level in both villages, regardless of soil origin. The reaction of habitats, remaining within this

relatively narrow range, was of no importance for the newly-formed communities of the classes *Phragmitetea* and *Molinio-Arrhenetheretea*. The significance of differences was confirmed only in the case of land use type – higher indicator values (lower acidity) were recorded on pastures, which may be related to organic fertilization from natural sources, including animal feces.

Table 6

Statistical characteristics for soil acidity value (*R*)

Specification	Mean	Range of variation	Coefficient of variation (%)
Entire research area	4.16	3.56-4.79	3.8
Location – Popielno	4.16 <sup>a*</sup>	3.56-4.79	3.9
– Wierzba	4.13 <sup>a</sup>	4.01-4.31	2.2
Soil type – organic	4.17 <sup>a</sup>	3.56-4.79	4.7
– mineral	4.16 <sup>a</sup>	3.68-4.49	2.9
Land use type – pasture	4.17 <sup>a</sup>	3.68-4.79	3.3
– meadow	4.11 <sup>b</sup>	3.56-4.57	4.7
Plant community – Mol-Arr	4.16 <sup>a</sup>	3.68-4.49	2.7
– Phragm.	4.15 <sup>a</sup>	3.56-4.79	6.6

\* explanations as in Table 2

Table 7

Statistical characteristics for soil granulometric value (*D*)

Specification	Mean	Range of variation	Coefficient of variation (%)
Entire research area	4.18	3.13-4.92	5.5
Location – Popielno	4.17 <sup>a*</sup>	3.13-4.92	5.4
– Wierzba	4.26 <sup>a</sup>	3.80-4.85	7.5
Soil type – organic	4.31 <sup>A</sup>	3.13-4.92	6.6
– mineral	4.08 <sup>B</sup>	3.74-4.54	2.7
Land use type – pasture	4.11 <sup>A</sup>	3.13-4.64	4.0
– meadow	4.35 <sup>B</sup>	3.88-4.92	6.8
Plant community – Mol-Arr	4.11 <sup>A</sup>	3.74-4.64	3.3
– Phragm.	4.44 <sup>B</sup>	3.13-4.92	8.0

\* explanations as in Table 2

The indicator values for soil granulometric composition ranged from 3.13 to 4.92, at variation of 5.5% (Table 7). The values of mean (4.18), median (4.11), mode (4.00) and histogram testify to the domination of sandy loam and silt deposits (Figure 2). These habitats not only satisfied the requirements of sown species, but were also typical of 118 taxa (of 200 identified in the study area). Thus, it is obvious that under so favorable conditions these species were quite abundant. The indicator value oscillated around 4 (3.5-4.5) for as many as 91%

of relevés. There were no statistical differences between the mean indicator values recorded at two localities, whereas highly significant differences were found in groups established on the basis of other criteria. Higher values of *D* were noted on organic soils and on meadows. Communities of the class *Phragmitetea* were formed under conditions defined by higher values of this habitat indicator.

Grassland vegetation on the Popielno Peninsula is indicative of the domination of mineral and humus-rich soils in the examined area. The indicator values for organic matter content varied from 1.90 to 2.95 (Table 8), not exceeding 2.5 in the case of as many as 87% relevés (Figure 2). The mode and median for the entire sample were 2 and 2.084, respectively. A statistical analysis confirmed differences between the groups formed in accordance with all four criteria, which reached 0.26 to 0.38. The values of *H* recorded in Wierzba were found to be significantly higher than those noted in Popielno. Certainly, there was a significant difference between organic and mineral soils with regard to organic matter content, to the advantage of the former. The method applied in the study suggested also a higher organic matter content of soils under hay meadows dominated by plants of the class *Phragmitetea*.

Table 8

Statistical characteristics for organic matter content value (*H*)

Specification	Mean	Range of variation	Coefficient of variation (%)
Entire research area	2.18	1.90-2.95	10.9
Location – Popielno	2.16 <sup>A*</sup>	1.90-2.94	10.2
– Wierzba	2.42 <sup>B</sup>	1.96-2.95	12.3
Soil type – organic	2.35 <sup>A</sup>	1.98-2.95	12.1
– mineral	2.06 <sup>B</sup>	1.90-2.37	3.8
Land use type – pasture	2.10 <sup>A</sup>	1.90-2.94	8.5
– meadow	2.40 <sup>B</sup>	1.96-2.95	10.8
Plant community – Mol-Arr	2.11 <sup>A</sup>	1.90-2.94	7.0
– Phragm.	2.49 <sup>B</sup>	2.00-2.95	12.1

\* explanations as in Table 2

Table 9 presents the coefficients of simple correlation expressing a linear relationship between habitat phytoindicators within specified ranges. It should be emphasized that if the indicators showed a linear dependence, it was usually a highly significant correlation ( $p \leq 0.001$ ). An analysis of relationships between indicator values for climate conditions and the remaining ones revealed that continentality was not related to any other habitat indicator. The indicator values for light decreased along with an increase in the indicator



values for temperature, soil moisture content, soil granulometric composition and organic matter content. Among indicator values related to edaphic conditions, no correlation was found between acidity and other parameters. Warmer habitats occupied humus-rich soils. Soils rich in organic matter and soils with a higher proportion of fine fractions (silt and clay) were more humid, and the latter were also more abundant in nutrients. There was a positive correlation between soil texture and organic matter content. It should be stressed that these relationships were recorded within relatively narrow intervals of the analyzed indicators; in wider ranges of variation they could be quite different.

Table 9

Matrix of coefficients of simple correlation between indicator values for habitats

	<i>L</i>	<i>T</i>	<i>K</i>	<i>W</i>	<i>Tr</i>	<i>R</i>	<i>D</i>
<i>T</i>	-0.32***	–	–	–	–	–	–
<i>K</i>	-0.06	-0.12	–	–	–	–	–
<i>W</i>	-0.28***	0.79	0.08	–	–	–	–
<i>Tr</i>	-0.07	0.05	0.05	0.11	–	–	–
<i>R</i>	-0.03	0.012	0.00	0.04	0.18	–	–
<i>D</i>	-0.29***	0.011	0.07	0.63***	0.23**	-0.16	–
<i>H</i>	-0.44***	0.30***	0.08	0.76***	0.05	-0.23	0.56***

\* significant at  $p \leq 0.05$ . \*\* significant at  $p \leq 0.01$ . \*\*\* significant at  $p \leq 0.001$

## Discussion

Phytoindication methods of habitat assessment have been successfully employed for a long period of time both in Poland (BOROWIEC 1972) and abroad (TER BRAAK, GREMMEN 1987). International professional literature and reference data show that lists of plant species including mean indicator values may replace expensive measurements (ERSTEN et al. 1998, SCHAFFERS, SYKORA 2000, ENGLISH, KARRER 2001, DIEKMANN 2003, EWALD 2003). Polish researchers share the opinion that this method, compared to chemical analyses, is both easier to apply and more reliable, since it is based upon one of the main biocenotic principles referring to the unity of biocenosis and biotope. In Poland phytoindication methods are most often used in order to evaluate habitats for the purposes of agriculture, with weed communities serving as indicators of environmental quality (BOROWIEC et al. 1989, HOŁDYŃSKI 1989, STUPNICKA-RODZYŃKIEWICZ et al. 1986, AFFEK-STARCZEWSKA, SKRZYCZYŃSKA 2003, KAPELUSZNY, HALINIARZ 2003, ŁABZA et al. 2003). As pointed out by BOROWIEC (2003), today the applicability of this method may be limited due to intensive chemical protection of crops as well as other treatments and cultivation

measures. According to ROLA *et al.* (2003), plants cannot be reliable indicators in habitats that are under strong human pressure resulting from agricultural practices. However, these authors believe that phytoindication may be successfully applied in natural and semi-natural communities, where the scale of human interference with the natural environment is small, as well as on conventional extensive farms.

Grassland vegetation in Popielno and Wierzba prefers moderate light and temperature conditions (warm to a moderately cold climate) as well as nutrient-abundant and neutral soils, and tolerates a relatively wide range of soil moisture content. TRĄBA *et al.* (2004) conducted a study on unmanaged meadows in the San river valley and found that all plant communities that formed there were moderately photophilous, but able to grow under varied humidity, soil reaction and trophy conditions. Similar relationships were observed by BARABASZ-KRASNY (2002) on the Przemyśl Foothills (Pogórze Przemyskie). However, it seems pointless to compare these results with the numerical data obtained in the present study since the latter are of local character only and are limited to a specified, small area. Thus, it would be more advisable to compare the procedures employed as well as the possibility of their further application or substitution.

In Poland the calculated mean indicators values are usually compared with the actual results of soil analyses. The obtained data are compiled as habitat grids providing a simplified representation of certain ecological niches (WÓJCIK 1983, AFFEK-STARCZEWSKA, SKRZYCZYŃSKA 2003, CORNWELL, GRUBB 2003). In this study an attempt was made to analyze these data statistically. An interesting approach to this problem was proposed by ROY *et al.* (2000). DZWONKO (2001) and BENNIE *et al.* (2006) emphasized the significance of phytoindication methods for the assessment of time-related changes in climate and edaphic conditions. Modern remote sensing techniques use the reflectance of plant communities, calibrated in accordance with the Ellenberg indicator values, to generate visual maps of habitat conditions (SCHMIDTLEIN 2005). These techniques enable to cover extensive areas, but the obtained results should be verified during on-site investigations of plant communities. The opportunities offered by new technologies motivate researchers to employ phytoindication methods on a larger scale, with regard to both space and time.

The problem whether species abundance (cover) should be taken into consideration during habitat assessment by phytoindication methods is often discussed in Poland. In his work concerning bioindication methodology, including the method developed by Ellenberg, WÓJCIK (1983) focused on the occurrence of indicator species, but took no notice of their rank in the community. Some other authors also follow this procedure (HOŁDYŃSKI 1989). BOROWIEC *et al.* (1989) demonstrated that information on species abundance allows to

obtain a more detailed picture of habitat conditions. The authors of the present study agree with this opinion. Species cover was also considered by other authors (e.g. STUPNICKA-RODZYŃKIEWICZ et al. 1986, KAPELUSZNY, JĘDRUSZCZAK 1994). AFFEK-STARCZEWSKA and SKRZYCYŃSKA (2003) studied various soil complexes and types and found no statistically significant differences between mean ecological indicator values calculated taking species cover into account or not. This suggests that in the case of larger areas the results of calculations based exclusively on species occurrence are sufficient and reliable. However, the above authors claim that information on species abundance may be very useful for a precise description of habitat conditions in small areas.

The majority of Polish works on phytoindication refer to Ellenberg indicator values. In other countries these values are calibrated so as to adjust them to local conditions (ERSTEN et al. 1998, LAWSSON, MARK 2000), which was postulated by Ellenberg himself (ELLENBERG et al. 1991). Although KOZŁOWSKA (1991) demonstrated that the use of a 10-point scale proposed by ELLENBERG (1979) and a 5-point scale established by ZARZYCKI (1984) provides comparable results, the authors of this study recommend to use the latter, especially its revised version (ZARZYCKI et al. 2002), while evaluating habitats in Poland, since it better corresponds to local climate and edaphic conditions.

## **Conclusions**

1. Grassland vegetation in Popielno and Wierzba prefers moderate light and temperature conditions. On a 5-point scale, the values of light and temperature reached 3.47 to 4.65 and 3.48 to 3.99, respectively.

2. The soils in the area were found to be fertile, nutrient-abundant (trophy of 3.32 to 4.20) and neutral (acidity of 3.56 to 4.79).

3. Soil moisture content in the examined habitats ranged from 2.83 to 5.89 on a 6-point scale.

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## OCCURRENCE OF STEM BASE DISEASES OF FOUR CEREAL SPECIES GROWN IN LONG-TERM MONOCULTURES

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**Key words:** stem-base diseases, winter wheat, winter rye, spring barley, oats, long-term monoculture, cultivars, chemical protection.

### Abstract

The sanitary state of stem bases of four cereal species (winter wheat, winter rye, spring barley and oats) was studied in the years 1999-2004 within the framework of a long-term field experiment established in 1967 in Balcyny near Olsztyn. The first experimental factor was crop sequence, i.e. cereal growing in six-field crop rotation and 32-37-year monoculture, the second experimental factor was growing two cultivars of each cereal species, and the third one – chemical protection. The predominant disease was fusarium foot rot (*Fusarium* spp.). Eyespot (*Tapesia yellundae*) occurred at lower intensity, and cases of take-all (*Gaeumannomyces graminis*) and sharp eyespot (*Rhizoctonia cerealis*) were incidental. The intensity of fusarium foot rot and take-all was higher in long-term monoculture, whereas the incidence of eyespot and sharp eyespot was not affected by crop sequence. The main causative agents of stem-base diseases were fungi of the genus *Fusarium* (mostly *F. culmorum*), and in spring barley also *Bipolaris sorokiniana*. The cultivars tested in the study showed differentiated resistance to the above diseases. Those more resistant to fungi of the genus *Fusarium* were more frequently attacked by *Tapesia yellundae*. Fungicides inhibited the progress of stem base diseases.

## WYSTĘPOWANIE CHOROÓB PODSUSZKOWYCH CZTERECH GATUNKÓW ZBÓŻ UPRAWIANYCH W WIELOLETNICH MONOKULTURACH

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**Słowa kluczowe:** choroby podstawy źdźbła, pszenica ozima, żyto ozime, jęczmień jary, owies, wieloletnia monokultura, odmiany, ochrona chemiczna.

### Abstrakt

W latach 1999-2004 przeprowadzono badania zdrowotności podstawy źdźbeł 4 gat. zbóż (pszenica ozima, żyto ozime, jęczmień jary i owies). Doświadczenie było realizowane w układzie statycznym w Bałcynach koło Olsztyna, założono je w 1967 r. Pierwszym jego czynnikiem była uprawa ww. zbóż w prawidłowym sześciopolewym płodozmianie i w 32-37-letniej monokulturze, drugim – uprawa dwóch odmian każdego gatunku, a trzecim – stosowanie ochrony chemicznej. Chorobą dominującą na podstawach źdźbeł była fuzaryjna zgorzel podstawy źdźbła (*Fusarium* spp.), w mniejszym nasileniu wystąpiła łamliwość źdźbła zbóż (*Tapesia yallundae*), a incydentalnie zgorzel podstawy źdźbła (*Gaeumannomyces graminis*) i ostra plamistość oczkowa (*Rhizoctonia cerealis*). Fuzaryjna zgorzel podstawy źdźbła i zgorzel podstawy źdźbła występowały w większym nasileniu w wieloletniej monokulturze, natomiast nasilenie łamliwości źdźbła zbóż i ostrej plamistości oczkowej nie zależało od sposobu następstwa roślin. Głównymi sprawcami chorób podsuszkowych były grzyby z rodzaju *Fusarium* (przede wszystkim *F. culmorum*), a u jęczmienia jarego również *Bipolaris sorokiniana*. Odmiany cechowała zróżnicowana odporność na choroby podsuszkowe, przy czym te, które były odporniejsze na grzyby z rodzaju *Fusarium*, silniej atakowała *Tapesia yallundae*. Fungicydy ograniczyły nasilenie chorób podsuszkowych.

### Introduction

Proper crop rotation performs numerous regulatory functions with respect to harmful microorganisms. Organic matter from crop residues assures biological balance between soil microorganisms. A temporary lack of hosts contributes to the extinction of pathogens, or at least reduces the size of their population so that they are no longer dangerous to plants (ADAMIAK 1992). In Poland the proportion of cereals in the cropping system has already exceeded 75%, so they are usually grown in short-term monocultures. However, this regime is accompanied by numerous negative phenomena, including yield decrease, caused among other by changes in the qualitative and quantitative composition of soil microorganisms resulting from the homogeneity of crop residues (ADAMIAK 1992, KUROWSKI 2002). The compensation of pathogenic fungi and the disappearance of saprophytic fungi (antagonists

of plant pathogens) can be observed on decomposing crop residues (CURL 1963, KUROWSKI 2002, TRUSZKOWSKA et al. 1986). Stem base diseases are considered the most dangerous among all diseases caused by pathogenic fungi associated with the soil environment (BOJARCZUK et al. 1991, KUROWSKI 2002).

The aim of the present study was to determine the intensity of the stem base diseases of four cereal species grown in long-term monocultures, and to estimate the possibility of reducing the negative effects of monoculture on sanitary state of cereals by sowing cultivars with increased disease resistance and applying chemical protection with fungicides.

## Material and Methods

The study was conducted in the years 1999-2004 on trial plots of the Department of Agricultural Systems, University of Warmia and Mazury in Olsztyn, located on gray-brown podsolic soil of a very good rye complex, in Balcyny near Olsztyn. A long-term three-factor experiment was established in 1967 in a randomized split-plot design in three replications. The following experimental factors were considered:

### I. Crop sequence:

– cereal growing in two six-course crop rotations:

1. sugar beet – maize – **spring barley** – pea – winter rape – **winter wheat**;
2. potato – **oats** – fiber flax – **winter rye** – faba bean – winter triticale;

– growing of winter wheat, winter rye, spring barley and oats in 32-37-year monoculture.

### II. Cereal cultivars (two of each species):

winter wheat – Elena, Korweta; winter rye – Marder, Warko; spring barley – Orthega, Rasbet; oats – Bajka, Jawor.

### III. Chemical protection of cereals over the growing season:

*C* – control (no protection); *H* – herbicide treatment; *H+F* – herbicide and fungicide treatment.

Winter cereals were treated with the herbicide Cougar 600 SC (diflufenican + izoproturon) immediately after sowing (BBCH 00-01), and fungicide Alert 357 SC (triazole + benzimidazole) at the beginning of stem elongation (BBCH 30-31), and Amistar 250 EC (strobilurine) at the ear emergence stage (BBCH 51-53). Weed infestation in spring barley was controlled with Chisel 75 WG (tifensulfuron metile + chlorosulfuron), and in oats – with Chwastox Turbo 340 SL (MCPA + dicamba) in 1999-2001 and Mustang 306 SE (florasulam + 2.4 D) in 2002-2004. In both spring cereals disease intensity was reduced with Amistar 250 EC (strobilurine) at the end of stem elongation stage (BBCH 37-39). All plant protective agents were applied in accordance with the relevant recommendations.



The seeds were dressed with Baytan Universal 19.5 WS. Mineral fertilizers were applied in accordance with the requirements of particular cereal species, as well as the relevant fertilizing instructions. The rates and dates of cereal sowing were consistent with the relevant standards and norms.

Phytopathological observations were carried out at the dough stage (BBCH 85) on 25 stems collected from each plot. Stem-base diseases were classified according to the Ponchet scale modified by MACKIEWICZ and DRATH (1972), and results are presented as injury index (Ii%), calculated by the Mc Kinney formula. In order to determine the causative agents of stem base diseases, 30 mm segments with centrally located spots were cut out of stem bases. The fungi colonizing them were isolated by the REINECKE and FEHRMANN method (1979). Every year from each combination 30 inoculum were tested.

## Results

Eyespot (*Tapesia yallundae*) and fusarium foot rot (*Fusarium* spp.) occurred at high intensity in winter wheat, whereas take-all (*Gaeumannomyces graminis*) and sharp eyespot (*Rhizoctonia cerealis*) were sporadic only – Table 1. Crop sequence (monoculture and crop rotation) had no effect on eyespot incidence. The susceptibility of winter wheat cultivars to the pathogen was differentiated. Fungicide protection during the growing season, positively affected the health condition of plants. Fusarium foot rot was the predominant disease on stem bases of winter wheat. The intensity of this disease was considerably higher in monoculture than in crop rotation. The cultivars tested in the study showed differentiated resistance to fungi of the genus *Fusarium*. The application of the herbicide Cougar 600 SC increased disease intensity. Take-all attacked mainly winter wheat plants grown in monoculture, whereas crop sequence had no significant effect on the development of sharp eyespot. Both diseases were efficiently controlled by the fungicides used in the experiment.

The incidence of eyespot (*Tapesia yallundae*) and fusarium foot rot (*Fusarium* spp.) were high in winter rye, whereas cases of sharp eyespot (*Rhizoctonia cerealis*) were sporadic – Table 2. Only the intensity of fusarium foot rot was higher in long-term monoculture than in crop rotation. The cultivars tested in the study showed differentiated resistance to stem-base diseases. The application of the herbicide Cougar 600 SC increased the prevalence of fusarium foot rot. All stem-base disease of winter rye were successfully suppressed by fungicides.

Table 1

Intensity of stem base diseases of winter wheat in 1999-2004 (injury index in %)

Disease Pathogen	Plant protection	Crop rotation		Monoculture		LSD ( $p = 0.05$ )
		Elena	Korweta	Elena	Korweta	
Eyespot ( <i>Tapesia yallundae</i> )	C	21.2	27.5	25.0	27.7	I – n.s.; II – 1.72; III – 1.38; IxII – n.s.; IxIII – 1.95; IIxIII – n.s.; IxIIxIII – n.s.
	H	25.5	32.0	18.8	26.7	
	H+F	18.3	21.8	18.0	23.8	
	Mean	21.7	27.1	20.6	26.1	
Fusarium foot rot ( <i>Fusarium</i> spp.)	C	65.3	66.0	74.8	73.3	I – 2.04; II – 0.59; III – 0.99; IxII – 0.84; IxIII – n.s.; IIxIII – 1.40; IxIIxIII – n.s.
	H	73.8	67.3	83.3	75.7	
	H+F	68.8	65.3	78.8	73.7	
	Mean	69.3	66.2	79.0	74.2	
Take-all ( <i>Gaeumannomyces graminis</i> )	C	0.0	0.5	3.0	3.0	I – 0.32; II – n.s.; III – 0.23; IxII – n.s.; IxIII – 0.32; IIxIII – 0.32; IxIIxIII – n.s.
	H	0.2	0.0	3.2	2.3	
	H+F	0.3	0.0	1.2	1.2	
	Mean	0.2	0.2	2.5	2.2	
Sharp eyespot ( <i>Rhizoctonia cerealis</i> )	C	0.5	1.2	0.5	0.8	I – n.s.; II – 0.08; III – 0.14; IxII – n.s.; IxIII – 0.20; IIxIII – 0.20; IxIIxIII – 0.29
	H	0.7	0.0	1.2	0.0	
	H+F	0.5	0.0	0.0	0.0	
	Mean	0.6	0.4	0.6	0.3	

n.s. – not significant differences

C – control (no protection)

H – herbicide treatment

H+F – herbicide and fungicide treatment

I – crop sequence

II – cultivars

III – chemical protection

Table 2

Intensity of stem base diseases of winter rye in 1999-2004 (injury index in %)

Disease Pathogen	Plant protection	Crop rotation		Monoculture		LSD ( $p = 0.05$ )
		Marder	Warko	Marder	Warko	
Eyespot ( <i>Tapesia yallundae</i> )	C	30.2	33.8	26.3	32.8	I – n.s.; II – 1.13; III – 0.76; IxII – 1.60; IxIII – 1.10; IIxIII – 1.10; IxIIxIII – 1.56
	H	28.2	22.5	23.2	29.7	
	H+F	27.8	26.3	20.5	27.7	
	Mean	28.7	27.5	23.3	30.1	
Fusarium foot rot ( <i>Fusarium</i> spp.)	C	60.2	49.2	61.0	60.5	I – 1.76; II – 1.29; III – 1.32; IxII – 1.83; IxIII – n.s.; IIxIII – 1.87; IxIIxIII – n.s.
	H	59.5	58.3	64.7	65.5	
	H+F	53.5	48.8	57.0	59.7	
	Mean	57.7	52.1	60.9	61.9	
Sharp eyespot ( <i>Rhizoctonia cerealis</i> )	C	1.2	1.7	2.5	0.7	I – n.s.; II – 0.28; III – 0.28; IxII – 0.40; IxIII – n.s.; IIxIII – n.s.; IxIIxIII – 0.55
	H	1.0	1.5	2.0	1.3	
	H+F	0.7	0.3	0.7	0.3	
	Mean	1.0	1.2	1.7	0.8	

Explanations as in Table 1

Eyespot (*Tapesia yallundae*) and foot rot (*Fusarium* spp., *Bipolaris sorokiniana*) occurred in spring barley – Table 3. Only the intensity of foot rot depended on the experimental factors. It was higher in monoculture than in crop rotation, and the fungicide Amistar 250 EC reduced the infestation rate. The susceptibility of cultivars to this disease was differentiated.

Eyespot (*Tapesia yallundae*) and fusarium foot rot (*Fusarium* spp.) were recorded in oats – Table 4. Eyespot developed to a low degree only, and plant protective agents enabled to further reduce its intensity (except cultivar Jawor in crop rotation). Fusarium foot rot occurred at various intensity on cultivars using to the study, and Amistar 250 EC reduced the infestation rate.

Table 3  
Intensity of stem base diseases of spring barley in 1999-2004 (injury index in %)

Disease Pathogen	Plant protection	Crop rotation		Monoculture		LSD ( $p = 0.05$ )
		Orthega	Rasbet	Orthega	Rasbet	
Eyespot ( <i>Tapesia yallundae</i> )	C	6.5	5.5	10.2	9.0	I – n.s.; II – n.s.; III – n.s.; IxII – n.s.; IxIII – 0.92; IIxIII – 0.92; IxIxIII – n.s.
	H	8.7	8.5	8.7	8.0	
	H+F	8.0	8.0	8.3	9.7	
	Mean	7.7	7.3	9.1	8.9	
Foot rot ( <i>Fusarium</i> spp., <i>Bipolaris sorokiniana</i> )	C	57.8	64.2	62.8	65.3	I – 2.43; II – 0.91; III – 1.70; IxII – 1.29; IxIII – n.s.; IIxIII – 2.41; IxIIxIII – n.s.
	H	55.2	61.0	62.3	64.7	
	H+F	54.8	58.5	60.5	55.5	
	Mean	55.9	61.2	61.9	61.8	

Explanations as in Table 1

Table 4  
Intensity of stem base diseases of oat in 1999-2004 (injury index in %)

Disease Pathogen	Plant protection	Crop rotation		Monoculture		LSD ( $p = 0.05$ )
		Bajka	Jawor	Bajka	Jawor	
Eyespot ( <i>Tapesia yallundae</i> )	C	3.2	1.8	4.2	4.5	I – n.s.; II – n.s.; III – 0.32; IxII – n.s.; IxIII – 0.46; IIxIII – 0.46; IxIIxIII – 0.65
	H	2.3	3.0	2.3	2.7	
	H+F	2.3	3.2	3.2	2.8	
	Mean	2.6	2.7	3.2	3.3	
Fusarium foot rot ( <i>Fusarium</i> spp.)	C	39.5	33.2	37.2	39.5	I – n.s.; II – 0.56; III – 0.91; IxII – 0.80; IxIII – 1.29; IIxIII – 1.29; IxIIxIII – 1.82
	H	39.8	35.0	39.3	31.7	
	H+F	29.5	22.8	28.3	26.5	
	Mean	36.3	30.3	34.9	32.6	

Explanations as in Table 1

A total of 1421 fungal colonies were isolated from infested stem bases of cereals (Table 5). More colonies, including fungi pathogenic to cereals, were isolated from monoculture than in crop rotation. Stem-base diseases of winter

wheat were caused primarily by fungi of the genus *Fusarium* (45.3% of all isolates), especially *F. culmorum* (21.7%). Relatively numerous were also *Tapesia yallundae* (11.4%) and – in monoculture – *Gaeumannomyces graminis* (8.1%). Fungi of the genus *Fusarium* were isolated most frequently from infected stem bases of winter rye (42.4%). The dominant species was *F. culmorum* (26.7% isolates). Other species present in great numbers were *Microdochium nivale* (9.5%) and *Rhizoctonia cerealis* (7.1%). Fungi of the genus *Fusarium* were also isolated most often from attacked stem bases of spring barley (45.0% of isolates). The dominant species was again *F. culmorum* (23.5% of isolates), accompanied by *Bipolaris sorokiniana* (22.4%). In the case of oats, fungi of the genus *Fusarium* accounted for 53.9% of isolates, of which 39.8% were *F. culmorum* isolates. Another frequently occurring species was *Aureobasidium pullulans* (19.3% of isolates).

Table 5  
Fungi isolated from infested stem bases of cereals in 1999-2004 (number of isolates)

Fungus species	Winter wheat		Rye		Spring barley		Oat		Total	
	CR	M	CR	M	CR	M	CR	M	CR	M
<i>Acremonium strictum</i> W. Gams	3		8	1	3	2	9	6	23	9
<i>Alternaria alternata</i> (Fr.) Keissler	9	11	5	5	13	11	17	16	44	43
<i>Aureobasidium pullulans</i> (de Bary) Arnaud	10	21	8	9	7	15	27	43	52	88
<i>Bipolaris sorokiniana</i> (Sacc.) Shoem.	1	2		1	33	46			34	49
<i>Epicoccum purpurascens</i> Ehreb. et Schlecht.	5		2		1	2			8	2
<i>Fusarium avenaceum</i> (Corda ex Fr.) Sacc.	17	17	15	13	17	18	9	6	58	54
<i>Fusarium culmorum</i> (W. G. Sm.) Sacc.	32	48	41	49	38	45	65	79	176	221
<i>Fusarium equiseti</i> (Corda) Sacc.	8	9		8	5		2	4	15	21
<i>Fusarium oxysporum</i> Schlecht.	9	5	3	3	7	8	3	3	22	19
<i>Fusarium poae</i> (Peck) Wollenw.	10	12	4	7	9	12	11	13	34	44
<i>Gaeumannomyces graminis</i> (Sacc.) Arx et Olivier	7	23		5					7	28
<i>Microdochium nivale</i> (Fr.) Samuels et Hallett	12	11	17	15					29	26
<i>Mortierella alpina</i> Peyronel			8			3			8	3
<i>Penicillium</i> spp.	4		9	15	5				18	15
<i>Rhizoctonia cerealis</i> van der Hoeven	4	6	9	15	2		2	4	17	25
<i>Rhizopus nigricans</i> Ehr.	7		3			3			10	3
<i>Tapesia yallundae</i> Wallwork	22	20	4	3	7	8			33	31
<i>Trichoderma harzianum</i> Rifai		2	5		4				9	2
<i>Trichoderma koningii</i> Oudemans	3			8	5				8	8
Non-sporulating fungi	11	8	21	18	9	15	23	20	64	61
Sum	174	195	162	175	165	188	168	194	669	752
	369		337		353		362		1421	

CR – crop rotation

M – monoculture

## Discussion

The diseases whose intensity was higher in cereals grown in monoculture were: fusarium foot rot (*Fusarium* spp.), take-all (*Gaeumannomyces graminis*) and foot rot (*Fusarium* spp. and *Bipolaris sorokiniana*) occurring in spring barley. Fusarium foot rot was the predominant disease of stem bases of the four cereal species examined in the study. The main causative agent of this disease was *Fusarium culmorum*, which accounted for 27.9% of all colonies. The other species of the genus *Fusarium* (*F. avenaceum*, *F. equiseti*, *F. oxysporum* and *F. poae*) were less frequent and constituted 18.8% of all isolates. Apart from fungi of the genus *Fusarium*, also *Bipolaris sorokiniana* was isolated quite commonly from infested stem bases of spring barley (22.4% of isolates). The intensity of foot rot caused by fungi of the genus *Fusarium* was higher in monoculture than in crop rotation, and more significant differences were observed in winter cereals. Take-all (*Gaeumannomyces graminis*) was also recorded, at low intensity, on stem bases of winter wheat, especially in monoculture. Similar results, concerning higher intensity of the above diseases in cereals grown in monoculture, were previously obtained by COLBACH & HUET (1995), and KUROWSKI (2002). Many authors consider fungi of the genus *Fusarium* to be responsible for the incidence of stem base diseases over the entire growing season (BOJARCZUK et al. 1991, KUROWSKI 2002, ŁACICOWA et al. 1987, PARRY 1970).

The other stem-base diseases, i.e. eyespot (*Tapesia yallundae*) and sharp eyespot (*Rhizoctonia cerealis*), followed a similar course of development both in monoculture and crop rotation. Eyespot (*Tapesia yallundae*) symptoms were observed in all four cereal species, but the pathogen was not isolated from infested stem bases of oats. It accounted for 4.5% of all isolates. Some authors (COLBACH, HUET 1995, KUROWSKI 2002) obtained similar results, but others reported higher intensity of eyespot in monoculture (MACKIEWICZ, DRATH 1972). Sharp eyespot (*Rhizoctonia cerealis*) occurred in winter cereals only, and its incidence was generally low, but single colonies of the pathogen were isolated from all cereal species (3.0% of all colonies).

It seems that small differences in stem base diseases intensity between monoculture and crop rotation resulted from the so called "decline effect", i.e. gradually diminishing disadvantageous effects of total crop rotation rejection in older monocultures. This phenomenon, described for the first time by KÖNNECKE (1972) and then confirmed by, among other, COLBACH, HUET (1995), KUROWSKI (2002), TRUSZKOWSKA et al. (1986) is related to the state of a new balance between soil microorganisms.

The cultivars tested in the study showed differentiated tolerance to stem-base diseases. Those more resistant to fungi of the genus *Fusarium* were more

frequently attacked by *Tapesia yellundae*. It seems that due to the complex species composition of pathogens causing stem-base diseases it may be very difficult to breed cereal cultivars resistant to the majority of pathogens occurring on stem bases (BOJARCZUK et al. 1991, KUROWSKI 2002, ŁACICOWA et al. 1987, MIELKE 1995).

The application of the herbicide Cougar 600 SC to winter cereals immediately after sowing increased the intensity of fusarium foot rot, and fungicides (Alert 357 SC + Amistar 250 EC in winter cereals, and Amistar 250 EC in spring cereals) inhibited the development of stem-base disease, but their fungistatic effect was insufficient. Similar results were reported by other authors (BOCKUS 1983, COVENTRY et al. 1989, KUROWSKI, ADAMIAK 2001).

## Conclusions

1. The infection rates of Fusarium foot rot (*Fusarium* spp.) and take-all (*Gaeumannomyces graminis*) in all cereals, as well as of foot rot (*Fusarium* spp. and *Bipolaris sorokiniana*) in spring barley, were higher in monoculture than in crop rotation.

2. Crop sequence (monoculture and crop rotation) had no effect on the infection rate of eyespot (*Tapesia yellundae*) and sharp eyespot (*Rhizoctonia cerealis*).

3. Small differences in stem base disease intensity between monoculture and crop rotation resulted from the decline effect that took place in older monocultures.

4. More colonies of fungi considered pathogenic to cereals were isolated from monoculture than from crop rotation.

5. Fungi of the genus *Fusarium* were isolated most often from attacked stem bases of cereals.

6. The cultivars showed differentiated tolerance to stem-base diseases; those more resistant to fungi of the genus *Fusarium* were more frequently attacked by *Tapesia yellundae*.

7. The application of the herbicide Cougar 600 SC to winter cereals increased the intensity of fusarium foot rot, and fungicides inhibited the development of stem-base diseases, but their fungistatic effect was insufficient.

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## SELECTED THERMAL INDICATORS IN NORTHEASTERN POLAND DURING THE SECOND HALF OF THE XX CENTURY

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Key words: anomaly warm and cold days, thermal stimuli, northeastern Poland.

### Abstract

Thermal conditions of northeastern Poland were characterized on the basis of day average, maximum and minimum temperatures for the years 1951-2000. Anomaly warm and anomaly cold days were determined considering the value of deviations from the average value. To consider a given day an anomaly the deviation had to be equal or higher than  $m \pm 2\sigma$ . The stimulating influence of thermal conditions was determined on the basis of the value of day air temperature amplitude and day-to-day variability of average day temperature ( $T_i$ ), determining the frequency of occurrence of individual classes of thermal stimuli. Generally, the average month number of days with anomaly values of  $T_i$  during the entire period studied was relatively small. They occurred most frequently in winter when anomaly days with negative deviation dominated. Days with positive  $T_i$  deviation occurred mainly during the summer. Days with  $T_i \geq m+3\sigma$  and  $T_i \leq m-3\sigma$  were not found for any of the stations. The values of day air temperature amplitude showed that during the summer period the stimuli defined as sharp (day amplitude values of  $\geq 12^\circ\text{C}$ , in average 40%) while during the winter period the inert stimuli (amplitude values of  $< 4^\circ\text{C}$ ) occurred most often. On the basis of day-to-day variability of day average air temperature the stimulating effect of thermal conditions during all month was mostly classified as inert, from 80-85% during the winter to 90-95% during the summer.

### WYBRANE WSKAŹNIKI TERMICZNE W POLSCE PÓŁNOCNO-WSCHODNIEJ W DRUGIEJ POŁOWIE XX WIEKU

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Słowa kluczowe: dni anomalnie ciepłe i chłodne, bodźce termiczne, Polska północno-wschodnia.



## A b s t r a k t

Na podstawie wartości dobowych temperatur średnich, maksymalnych i minimalnych za wielolecie 1951-2000 scharakteryzowano warunki termiczne północno-wschodniej Polski. Wyznaczono dni anomalnie ciepłe i anomalnie chłodne, biorąc pod uwagę wielkość odchylenia od wartości średniej. Dany dzień uznano za anomalny, gdy odchylenie było równe lub większe od  $m \pm 2 \sigma$ . Bódczość warunków termicznych określono na podstawie wartości amplitudy dobowej temperatury powietrza i zmienności międzydobowej średniej temperatury dobowej ( $T_i$ ), wyznaczając częstość występowania poszczególnych klas bodźców termicznych. Na ogół średnia miesięczna liczba dni z anomalnymi wartościami  $T_i$  w całym badanym wieloleciu była stosunkowo niewielka. Najczęściej występowały one zimą, kiedy dominowały dni anomalne o odchyleniu ujemnym. Dni z  $T_i$  o odchyleniu dodatnim występowały głównie latem. Nie stwierdzono w ogóle dni z  $T_i \geq m + 3 \sigma$  oraz  $T_i \leq m - 3 \sigma$  na żadnej ze stacji. Wielkości amplitudy dobowej temperatury powietrza pokazały, że w okresie letnim najczęściej notowano bodźce określane jako ostre (wartość amplitudy dobowej  $\geq 12^\circ\text{C}$ , średnio 40%), natomiast w okresie zimowym – bodźce obojętne (wartość amplitudy  $< 4^\circ\text{C}$ ). Na podstawie międzydobowej zmienności średniej dobowej temperatury powietrza bódczość warunków termicznych we wszystkich miesiącach najczęściej klasyfikowano jako obojętną, od 80-85% w okresie zimowym do 90-95% w okresie letnim.

## Introduction

Among many meteorological elements air temperature has the most important influence on the possibility of rest. Its rough characteristic within a given area can be, among others, presented using thermal indicators. Complete assessment of thermal conditions, on the other hand, is possible, among others through analysis of thermal balance of man (BŁAŻEJCZYK 2004).

Determining thermal anomalies is very difficult. Depending on the needs either already small deviations from the long-term values or only critically large values can be considered anomalies. Out of the necessity to develop relatively uniform patterns it was decided that application of standard deviation (of at least  $m \pm 2\sigma$ ) or the extreme values of percentiles (90/10 percentile) was appropriate. Those measures allow interpreting the collected data as concerns thermal anomalies when we consider that ca. 2/3 of observations are within the range of  $m + 1\sigma$  or  $90\% \geq T \geq 10\%$  (KOSIBA 1974, JONES et al. 2002).

Important deviations from standard are observed mainly during the summer and winter. The intermediate seasons are characterized by large thermal diversity close to both the warm and the cold period. The literature is dominated by descriptions of weather situations considered “extreme” on the basis of currently available data sets. However, with passage of time the number of data increases and the criterion of values considered anomalies can be narrowed. As the limits of the set are expanded something that once was an anomaly today can well be within norm (JAKUBCZAK 1970, KOSIBA 1974, JONES et al. 2001, PRZYBYLAK 2002).

The study aimed at determining the level of thermal arduousness of climate in northeastern Poland.

## Materials and Methods

The meteorological data used in this study originated from 10 meteorological stations distributed relatively evenly across the area of northeastern Poland belonging to the observation network of the Institute of Meteorology and Water Management (IMGW) in Warsaw. The study encompassed a relatively long term covering the last 50 years of the 20<sup>th</sup> century. The following air temperature parameters: day average temperature ( $T_i$ ), day maximum temperature ( $T_{max}$ ) and day minimum temperature ( $T_{min}$ ) were the basic source data. With extreme temperatures available, the day air temperature amplitude ( $A_d$ ) was computed as the difference between  $T_{max}$  and  $T_{min}$ . Days anomaly warm and cold were determined considering the values of deviations from the long-term day average value (1951-2000). A day was considered an anomaly when that deviation calculated for each day of the year was equal to or larger than  $m \pm 2\sigma$ .

Additionally thermal conditions were assessed from the point of view of recreation on the basis of indicators related to variability of air temperature (BŁAŻEJCZYK 2004). For this purpose the stimulating effect indicator determined on the basis of day-to-day variability of average day air temperature ( $dT_i$ ) was used assuming the following dependences between  $dT_i$  values and the intensity of thermal stimuli:  $dT_i$  (°C)  $\leq 2$  – inert thermal stimuli;  $2 < dT_i \leq 4$  – perceptible stimuli;  $4 < dT_i \leq 6$  – strong stimuli and above 6 – sharp stimuli with irritating influence (BAJBAKOVA 1963, after BŁAŻEJCZYK 2004).

The value of day temperature amplitude that reflects the day thermal contrasts was another indicator for assessment of stimulating effects of thermal conditions. The following intensity of thermal stimuli were assigned to individual air temperature amplitude values ( $dTA$ ):  $dTA < 4^\circ\text{C}$  – inert stimuli; from 4 to less than 8 – weakly perceptible; from 8 to less than 12 – strongly perceptible and from  $12^\circ\text{C}$  – sharp stimuli (BŁAŻEJCZYK 2004).

The stimulating effect of thermal conditions in the region was defined by determining the frequency of occurrence of individual thermal stimuli classes.

## Results and Discussion

The day average values of  $T_i$ ,  $T_{max}$  and  $T_{min}$  in northeastern Poland were at the level of  $7.05^\circ\text{C}$ ,  $11.21^\circ\text{C}$  and  $2.93^\circ\text{C}$  respectively (Table 1). As concerns the month average number of days with anomaly values of  $T_i$  during the entire period covered it was relatively small. For the deviation of  $m + 2\sigma$  it was 2.75 days and for  $m - 2\sigma$  it was 3.3 days per month (Figure 1). During the entire studied period anomalies occurred more frequently during the cold year half,

in total 404 days with 328 days only during the warm year half. The results obtained are in clear opposition to the results by KUZIEWSKA (1976), who indicated more frequent appearance of anomaly days during the warm year half. Additionally the author identified them with heat waves that must satisfy the criterion of continuity while in case of anomaly days every case of temperature anomaly higher or lower than the norm is important. Nevertheless, analyzing the data for 25 years of 1951-1975 it was established that the number of anomaly days during the warm year half was actually higher by 3 days. As a consequence, the larger number of anomaly days during the cold year half during the years 1951-2000 resulted mainly from the increase in number of negative anomaly days during the months from October through January during the years 1976-2000. That increase was large enough to compensate for a 2-fold decrease in number of such days in February and 3-fold decrease in March. Additionally, during the summer year half the number of days with  $T_i \leq m-2\sigma$  decreased during almost every month.

Table 1  
Basic statistics of  $T_i$ ,  $T_{max}$ ,  $T_{min}$  in northeastern Poland during the years 1951-2000

	$\bar{x}$	$\sigma$	10%	90%
$T_i$	7.05	8.53	-3.98	17.78
$T_{max}$	11.2	9.72	-1.19	23.82
$T_{min}$	2.9	7.72	-7.21	12.26

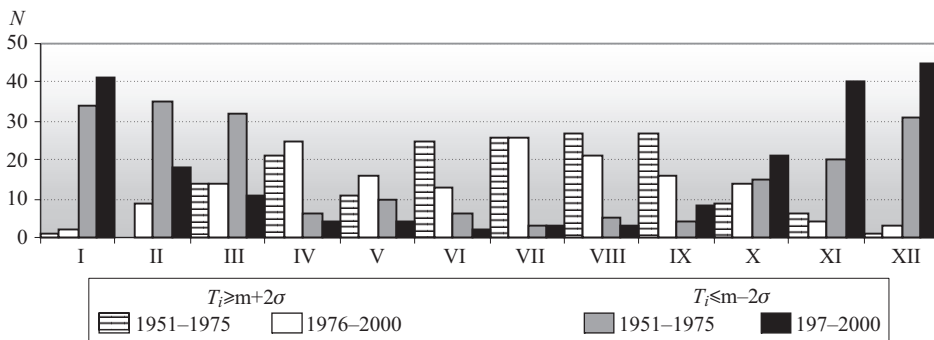


Fig. 1. Month average number of anomaly days ( $N$ ) in northeastern Poland during the periods of 1951-1975 and 1976-2000

Days of  $T_i$  with positive deviation occurred mainly during the summer with the maximum in July (1.4 days), and, as can be seen in Figure 1, the number of such days during the years 1951-2000 was smaller by almost 1/4 than

the number of days with negative deviation of  $T_i$ . Analysis of neighboring 25-year periods allowed determining an increase in number of days with  $T_i \geq m + 2\sigma$  in almost all months during the cold season (except November). On the other hand for the warm year half a decrease in the number of days of  $T_i$  with positive deviation was recorded for June, August and September.

It was generally established that warm anomaly days occurred more frequently during the summer season and days anomaly cold during the winter season similarly as had been found for the northern hemisphere by JONES et al. (2002). The months during which days of positive and negative anomaly occurred so incidentally that long-term averages reached values close to zero were December and January as well as July respectively.

During the analyses computations were also completed aiming at determining the number of days with  $T_i \geq m + 3\sigma$  and  $T_i \leq m - 3\sigma$ , however, during the period of 50 years covered no such days were recorded. During the years 1951-2000 in total ca. 700 days with  $T_i$  values above and below standard were observed with the deviations of  $m \pm 2\sigma$  from long-term averages. As concerns the spatial distribution, the number of days with  $T_i \geq m + 2\sigma$  decreased from northwest towards southeast within the range of from ca. 380 days in the vicinity of Elbląg to ca. 310 days along the line Suwałki, the Biebrza River and Białystok (Figure 2a). The distribution of isolines reflecting the number of days with  $T_i \leq m - 2\sigma$  was more meridional and the values of that measurement increased from ca. 350 days in the vicinity of Elbląg to over 440 in the vicinity of Białystok (Figure 2b). As a consequence, the difference between the western and eastern extreme positions in number of anomaly days oscillated within 100 days throughout the entire 50-year period and in the west was lower by the average of 2 days per year.

The differences in the number of anomaly days for individual stations computed for the neighboring 25-year periods for the years 1951-2000 showed large spatial variability of the analyzed characteristic. In case of anomaly hot days their number increased in the western part of the covered northeastern area of Poland (by 10 days in Elbląg, 50 in Toruń and over 90 in Mława, and decreased by 10 in Białystok and Suwałki and over 20 in Myszyniec) (Figure 2c). As concerns days anomaly cold the situation was the opposite. Their number increased in the east of the area covered. The increase was slightly smaller because it was ca. 40 days. On the other hand the decrease in the number of days anomaly cold in the west was definitely larger and exceeded 50 in Toruń and Mława (Figure 2d). Time trend of the number of positive anomaly days increased in all the region (0.021 days/year), however the number of negative anomaly days decreased in period 1951-2000 (-0.014 days/year) – Table 2.

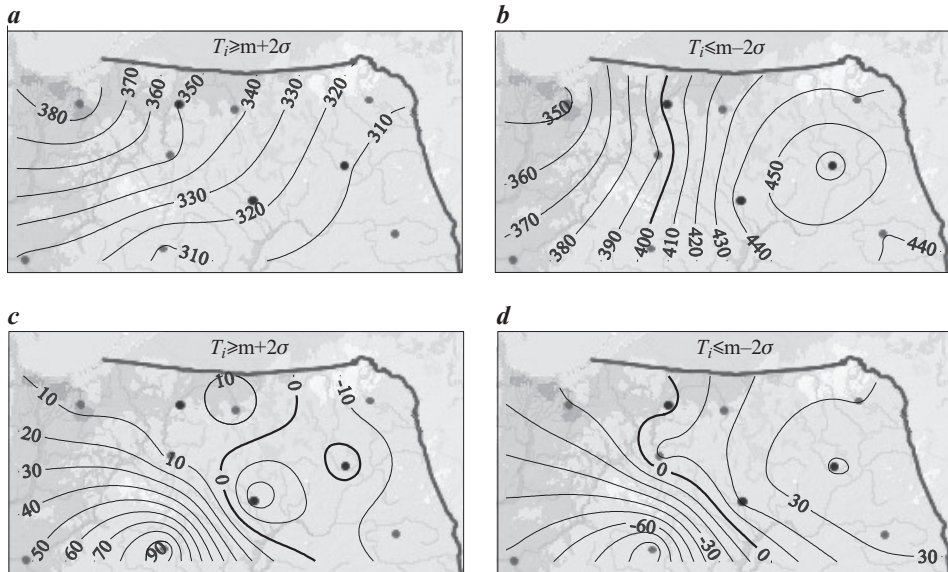


Fig. 2. Number of anomaly warm (a) and cold (b) days in northeastern Poland during the years 1951-2000 and the differences in those numbers (c and d respectively) between the periods of 1976-2000 and 1951-1975

Table 2  
Basic statistics of  $T_i$ ,  $T_{max}$ ,  $T_{min}$  in northeastern Poland during the years 1951-2000

Parameters	$a$	$\bar{x}$	$\sigma$	$R$	$R^2$	$F$	$p$
$T_i \geq m + 2\sigma$	0.021	6.620	4.624	0.067	0.005	0.219	0.642
$T_i \leq m - 2\sigma$	-0.014	8.020	6.561	0.032	0.001	0.050	0.825

The variability of day average air temperature is one of the simplest indicators allowing the thermal stimulating influence. Situations involving large day-to-day changes in air temperature are arduous from the point of view of the comfort of people resting. During the analyzed fifty-year period in the covered region of northeastern Poland the cases defined as inert were most frequently recorded (Table 3). They represented from 60 to almost 80% of the recorded thermal stimuli. Thermal stimuli defined as perceptible appeared in the region with the average frequency of 24%. The least frequent occurrence was recorded in case of sharp stimuli with irritating effects that is those with day-to-day variability of temperature exceeding 6°C. During the summer period the frequency of such situations was just 1%. January was characterized by the largest share of this type of stimulating effects (5%).

Table 3

Frequency (%) of appearance of thermal stimuli determined on the basis of day-to-day variability in day average air temperature in northeastern Poland during the years 1951-2000

Thermal impulse	Months											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Years 1951-1975												
Neutral	61	63	69	65	62	64	72	76	70	70	70	64
Sensible	25	25	23	28	30	29	23	19	25	25	24	27
Considerable	10	7	7	4	6	6	4	3	4	4	5	6
Sharp	5	4	1	2	1	1	1	1	1	1	1	3
Years 1976-2000												
Neutral	62	66	72	66	62	69	79	81	76	68	68	62
Sensible	25	24	23	27	30	27	17	18	20	25	24	25
Considerable	9	7	5	6	6	3	2	1	3	5	6	9
Sharp	4	3	1	2	2	1	1	0	1	1	2	4
Years 1951-2000												
Neutral	61	65	70	65	62	67	76	79	73	69	69	63
Sensible	25	24	23	27	30	28	20	18	23	25	24	26
Considerable	9	7	6	5	6	5	3	2	4	5	6	7
Sharp	5	3	1	2	2	1	1	1	1	1	1	3

Analysis of the frequency of occurrence in the region of specific thermal stimuli determined on the basis of the values of day amplitude (Table 4) showed that the largest percentage share of inert and weakly perceptible stimuli was recorded from January to March and from October to December. Their percentage share during the entire fifty-year period ranged from 28 to 45% for inert stimuli and from 54 to 51% for weakly perceptible stimuli. Very similar values of frequencies of occurrence for those stimuli were also found in the separate 25-year periods. During the summer half year, an increase in the share of strongly perceptible and sharp stimuli was recorded. The highest frequency of occurrence of sharp stimuli (day amplitude value of  $\geq 12^{\circ}\text{C}$ ) was recorded from May through August that is during the highest density of tourist traffic. In average 40% of days during those months were determined to have sharp stimulating effect. Only a slightly lower frequency of occurrence was determined for strongly perceptible stimuli. The share of days in that class was from 36% in May to 43% in July. The same setup of values was also observed in the individual 25-year periods. The values of day air temperature amplitude during the period covered did not allow identification of days with inert stimulating influence during the months of June and July.

Table 4  
Frequency (%) of appearance of thermal stimuli determined on the basis of day air temperature amplitude in northeastern Poland during the years 1951-2000

Thermal impulse	Months											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Years 1951-1975												
Neutral	34	28	11	5	1	0	0	1	2	10	39	45
Faintly sensible	46	45	47	31	19	16	19	20	28	46	53	45
Strongly sensible	18	21	27	35	37	37	42	39	37	32	8	9
Sharp	2	7	14	29	42	46	39	41	33	13	0	1
Years 1976-2000												
Neutral	40	28	14	3	1	0	1	1	4	10	40	45
Faintly sensible	43	48	47	32	16	23	22	20	39	50	50	44
Strongly sensible	13	20	29	34	34	38	43	40	35	29	9	10
Sharp	3	5	10	32	48	39	35	40	23	11	0	1
Years 1951-2000												
Neutral	37	28	12	4	1	0	0	1	3	10	40	45
Faintly sensible	45	46	47	32	18	20	20	20	33	48	51	44
Strongly sensible	16	20	28	34	36	37	43	39	36	30	9	9
Sharp	3	6	12	31	45	43	37	40	28	12	0	1

As a consequence the theses on a large influence of geographic location in this part of Poland on development of mainly negative anomalies are fully justified. Additionally, specific local conditions of each meteorological station modify the general trends encountered in the entire region covered.

## Conclusions

1. Appearance of anomaly cold and anomaly warm days in northeastern Poland is so rare during the year that it in no way decreases the tourism and recreation attractiveness of that region.

2. The spatial distribution of anomaly days clearly divides northeastern Poland into the warmer part in the west and southwest of the region and colder part in the east and northeast of the region.

3. The stimulating effects of thermal condition in the region of north-eastern Poland defined on the basis of day-to-day variability of day temperature showed that thermal stimuli defined as inert were recorded most

frequently, which is a positive aspect of the thermal characteristic of the region from the point of view of suitability of thermal conditions for recreation.

4. Analysis of the values of day air temperature amplitude showed that the values of that indicator during the summer period were in most cases determined to be sharp or strongly perceptible while during the cold year half as inert or weakly perceptible.

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## PERFORMANCE TESTS OF COLD-BLOODED STALLIONS FROM NORTH-EASTERN POLAND AS AFFECTED BY OWNERSHIP SECTOR

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Key words: performance tests, cold-blooded stallions, breeding sectors.

### Abstract

The history of draught performance testing in Poland dates back to the XIXth century, yet already since 1999 cold-blooded stallions have been undertaking the tests in two stages – at the age of 2.5-3.5 years (preliminary test) and at the age of 4-6 years (the exact test). Literature concerning the draught performance of cold-blooded stallions is rather outdated, therefore, it cannot be referred to in the contemporary system of conducting performance tests. Thus, this study was aimed at analysing the results of the performance tests of cold blooded stallions from north-eastern Poland.

The study covered a population of cold-blooded stallions subjected to performance tests in the years of 1999-2005 in north-eastern Poland. The analysed field of stud horses was divided into two groups in terms of ownership sector (private stallions and state stallions from Stallion Depot in Kętrzyn). A detailed analysis concerned the results of field draught tests, divided into *preliminary test* and *exact test*, taking into account the effect of sire breeding sector (state vs. private) on the results of field draught tests.

In summarizing the results of field draught tests, it may be concluded that the selection of stallions carried out in that system yields good results, and the progeny of the sires examined have a good chance of inheriting draught abilities. The results of the preliminary performance tests indicate good preparation for tests of the stallions of both ownership sectors, whereas the results of the exact test are more diversified and point to better preparation of the stud horses originating from the Stallion Depot Kętrzyn. The study demonstrated the effect of the ownership sector of a stallion on the results of performance tests. In both sectors analysed, stallions after private sires scored better results in the preliminary tests and performed far worse in the exact tests.

## ANALIZA WYNIKÓW PRÓB DZIELNOŚCI OGIERÓW ZIMNOKRWISTYCH Z PÓŁNOCNO-WSCHODNIEJ POLSKI Z UWZGLĘDNIENIEM SEKTORA WŁASNOŚCI REPRODUKTORA

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Słowa kluczowe: próby dzielności, ogiery zimnokrwiste, sektor hodowli.

### Abstrakt

Historia zaprzęgowych prób dzielności w Polsce sięga XIX w., jednak dopiero od 1999 r. ogiery zimnokrwiste przechodzą je dwuetapowo – w wieku 2,5-3,5 lat (próba wstępna) oraz 4-6 lat (próba właściwa). Literatura dotycząca ich użytkowości zaprzęgowej jest więc mało aktualna. Celem pracy była analiza wyników prób dzielności polskich ogierów zimnokrwistych z północno-wschodniej Polski.

Próby przeprowadzono w latach 1999-2005 w północno-wschodniej Polsce. Analizowaną stawkę reproduktorów podzielono na dwie grupy, uwzględniając sektor ich własności (ogiery prywatne lub państwowe ze SO Kętrzyn). Szczegółowa analiza dotyczyła wyników polowych prób zaprzęgowych (z podziałem na **próbę wstępną i właściwą**) z uwzględnieniem wpływu, jaki ma na nie sektor hodowli ogiera-ojca (prywatny, państwowy).

Selekcja ogierów prowadzona na podstawie polowych prób zaprzęgowych daje dobre rezultaty, a potomstwo badanych reproduktorów ma duże szanse odziedziczenia zdolności pociągowych. Wyniki wstępnych prób dzielności świadczą o dobrym przygotowaniu do niej ogierów z obydwu sektorów własności, natomiast wyniki prób właściwych są bardziej zróżnicowane i świadczą o lepszym przygotowaniu reproduktorów w Stadzie Ogierów Kętrzyn. Wykazano wpływ sektora własności ogiera-ojca na wyniki prób dzielności. Rozpłodniki po ojcach prywatnych w obu analizowanych sektorach zdawały lepiej próby wstępne, a dużo gorzej próby właściwe.

## Introduction

The history of draught performance dates back to the XIXth century in England when owners of the best horses organized shows and fairs to market the best animals. The shows consisted of harnessing horses to growing oaks, and the winner was the farmer whose horse withstood the highest tension for the longest period of time while trying to move the tree. In turn, the first to measure the average pulling capacity was the inventor of the steam engine – James Watt (1736-1819) (BRZESKI 1958).

In Poland, the first tests for draught horses were initiated by Lieutenant A. Krauz who constructed a device for measuring the towing strength of artillery and camp horses of the Polish Army in the years 1828-1829. Later, further interest in the performance tests of horses in a relay was observed in the inter-war period (KAPROŃ 1995).

Stallions are expected to exhibit high ergogenic capacity and balanced nervous system since draught abilities are determined genetically and are inherited by progeny (KAPROŃ 1981). Hence, performance tests are aimed at selecting horses to transfer their valuable performance traits and mild character, composed temperament and effective motion in all gaits to their progeny (KAPROŃ 1994).

Literature concerning the draught performance of cold-blooded stallions is rather outdated, thus it cannot be referred to in the contemporary system of conducting performance tests. This study was therefore aimed at analysing the results of the performance tests of cold-blooded stallions in north-eastern Poland.

## Material and Methods

The study covered a population of cold-blooded stallions subjected to performance tests in the years of 1999-2005 in north-eastern Poland. The analysed field of stud horses was divided into two groups in terms of ownership sector:

- 1) 56 stallions proven for breeding on the area of the Warmia and Mazury Horse Breeders Association (W-MZHK) – private stallions,
- 2) 31 stallions incorporated into a Stallion Depot (state stallions).

The study included only those stud horses that in the analysed period were subjected to complete performance evaluation (preliminary test and exact test). To this end, the collated data was based on years in which the stud horses were proven, which was tantamount to passing the preliminary test. After a period of 2-4 years, those stallions were subjected to the exact test.

At collecting research materials, use was made of resources of the Warmia and Mazury Horse Breeders Association in Olsztyn, Provincial Association of Horse Breeders in Białystok, Kuiavia-Pomerania Horse Breeders Association in Bydgoska, The “Nowe Jankowice” Horse Stud and Stallion Depot in Kętrzyn (*Akta... a,b,c,d,e*). The basic source of analytical data were stallion cards, protocols of performance tests and central, Internet data base of the Polish Horse Breeders Association.

A detailed analysis was carried out for results of field draught tests divided into *preliminary test* (for stallions aged 2.5-3.5 years) and *exact test* (for stallions aged 4-6 years), taking into account the effect of sire breeding sector (private vs. state) on the results of filed draught tests.

The analysis of the results of performance tests included scores obtained by the stallions from each group analysed (private and state) that were subjected to tests in the following areas:

1. private stallions underwent performance tests in the area of the Warmia and Mazury Horse Breeders Association in Olsztyn;

2. state stallions originating from District Horse Breeders Associations from various regions of Poland underwent preliminary tests' once proven they were incorporated into the Stallion Depot Kętrzyn and therein were subjected to an exact performance test.

The field draught tests were held according to the *Horse Breeding Programme for the Cold-blooded Polish Horse* (2006) elaborated by the Polish Horse Breeders Association. The programme assumes that before proving, each stud horse should pass a field draught test.

Following the guidelines of the *Horse Breeding Programme...* (2006) **the preliminary test** is held only in a walk and is divided into three short stages, with three ca. 15-sec intervals. The traits evaluated in the test include: horse will for pulling a cart at its gradual loading, horse behaviour at moving with a light cart, and once thrust bolsters have been removed. In the draught test for stallions aged 2.5-3.5 years (preliminary test) no peak strain is required from horses. The result of the test is a mean of horse behaviour at breaking a horse for harness and at harnessing to a cart, docility during the test and will for pulling, and is evaluated on a scale of 0 to 5 points. Disqualification occurs only in the case of heavy usage of the whip by a coachman or when the stallion refuses to pull a cart with one thrust bolster under and is nervous over the entire period of the test.

Adult stallions, aged 4-6 years, are subjected to obligatory, field draught tests, referred to as **the exact test** performed according to the following scheme:

1. Speed test in a trot for a distance of 1 km;
2. Speed test in a walk with a load equal to 200% of horse body weight for a distance of 1 km;
3. Pulling strength and will test with the use of thrust bolsters.

In the exact draught test, each element, namely: test in a trot, in a walk and the strength test, are evaluated on a scale of 0 to 7 points. The total result of the performance test is the sum of results from all three stages, hence the maximum number of points to be achieved is 21. According to the guidelines of the *Horse Breeding Programme...* (2006), disqualification of a stallion occurs in the case of: heavy use of a whip by a coachman at any stage of the test, horse failure to succeed in even one part of the test, i.e. in speed tests – exceeding the maximum time stipulated, and in the strength test – refusal to pull a cart with two thrust bolsters under.

The obtained results are presented in figures and tables.

In order to determine the differences between detailed results of performance tests undertaken by the stallions examined, a statistical analysis

of results was carried out by means of Duncan's test in single-factor non-orthogonal systems, using Statistica software (Statsoft).

## **Results and Discussion**

### **Analysis of results of performance tests of cold-blooded stallions as affected by ownership sector**

Control of the performance value of horses is of key significance in the selection process. Once carried out systematically and following established, objective methods, it can accurately determine the selection response (SAPUŁA 1988).

#### **Private stallions**

Performance tests for stallions aged 2.5-3.5 belonging to private breeders, referred to as preliminary tests, were carried out in the area of the Warmia and Mazury Horse Breeders Association in the years 1999-2003 (Table 1). The private stallions passed the preliminary test with an average good result (4.06 points). In the years examined, an upward tendency was observed in the results of that performance test, which may indicate the greater engagement of owners in the preparation of stud horses for this test. Only in year 2003, as compared to the previous years, did the stallions pass the test with a slightly worse score, however, the number of horses subjected to the test in 2002 was low (only 5 horses). The statistical analysis of results demonstrated significant and highly significant differences between results of the preliminary tests from particular years (Table 1).

The exact performance tests, being the final stage of the selection process, determined the further exploitation of a stallion or its culling from breeding. Out of all elements of the performance test, the private stallions achieved the worst mean number of points for time of passage in trot – 2.89 (Table 1). Nevertheless, it is the walk that is the gait determining the ergogenic capacity of each horse. Simultaneously, it is the most difficult gait in training, especially in stallions (SAPUŁA 1988). In the reported study, however, this element of the performance test was evaluated slightly better than the trot – 3.29 points.

Stallions of the Sokólska breed investigated by SAPUŁA (1988) achieved the lowest mean score for walk, as compared to the other elements of the test. As early as the 1960s NOZDRYN-PŁOTNICKI (1966) had suggested paying greater attention to a more effective walk in breeding practice and selection. The results of the current study may, thus, indicate that breeders have complied with those recommendations.

Table 1  
Results of a field draught test (preliminary and exact tests) of private stallions

Analyzed stallions		Preliminary test		Exact test				
Year of proving	Number of stallions (no.)	Point scale: 0-5 <sup>1</sup>		Point scale 0-7			Point scale: 0-21 <sup>2</sup>	
		Final result		Trot	Walk	Pulling will	Penal points	Final result (sum of points)
		$\bar{x}$ (pts.)	Verbal note					
1999	9	3.85 <sup>A</sup>	good	2.89	3.33	7.00 <sup>A</sup>	0.22 <sup>A</sup>	13.00
2000	13	3.92 <sup>A</sup>	good	3.08	3.23	6.62 <sup>A</sup>	0.46 <sup>a</sup>	12.38
2001	17	3.99 <sup>A</sup>	good	2.53	2.53 <sup>a</sup>	6.88 <sup>A</sup>	0.06 <sup>A</sup>	11.88
2002	5	4.83 <sup>Bb</sup>	v. good	3.60	4.20 <sup>b</sup>	6.60 <sup>A</sup>	1.20 <sup>Bb</sup>	13.20
2003	12	4.16 <sup>c</sup>	good	2.92	4.00 <sup>b</sup>	5.58 <sup>B</sup>	0.42 <sup>a</sup>	12.08
Total	56	4.06	good	2.89	3.29	6.54	0.36	12.34

$\bar{x}$  – arithmetic mean,  
*A, B, a, b* – values in column denoted with various letter within the same trait differ statistically, capital letters denote significance at a level of  $\alpha = 0.01$ , small letters – at a level of  $\alpha = 0.05$

<sup>1</sup> Points: Verbal note:  
5 – v. good  
4 – good  
3 – satisfactory  
below 3 – disqualification

<sup>2</sup> Points: Verbal note:  
18-21 – outstanding  
14-17 – v. good  
9-13 – good  
3-8 – satisfactory  
below 3 – disqualification

The basic element of the draught performance test is an evaluation of pulling strength and will (SAPUŁA 1988). In the element of the test, the private stallions performed very well, achieving a total mean score of 6.54 points. This implies greater emphasis that the private breeders put on this element when preparing stallions for the test, and simultaneously, a neglect of the correctness of trot and walk gaits (Table 1). The pulling strength is, however, determined by a variety of factors – the most significant of which is still training. In turn, according to KAPROŃ (1980b), the type of gear also affects the results of pulling strength and will test, especially in draught stallions that demonstrated greater pulling will in their everyday working gear. The same author showed that the maximum pulling strength depended also on the body weight of stallions subjected to the test (KAPROŃ 1980a).

The mean number of points scored by the private stallions was 12.34, which meant a good final result of the exact test. The best mean final score was reported for stallions proven in the year 2002 (13.20 points), although they achieved the highest number of penal points (1.20 points). However, this group consisted of only 5 stallions (Table 1).

The statistical analysis demonstrated significant differences in the scores for the walk and in penal points between particular years of the study and highly statistically significant differences in pulling will. Both the trot and the final result of the performance test of private stallions did not differ significantly in the analysed period of time (Table 1).

In summary, it may be concluded that in the group of private stallions, the results of the preliminary tests indicate the increased efforts of the owners in the preparation of horses. Yet, it may have only been a single occurrence and a lack of systematic training affected the emergence of problems with the re-introduction of the stud horses to work, which was indicated by unimpressive achievements in the final results of the exact tests.

### **State stallions**

A rather different situation was observed in the group of state stallions incorporated into the Kętrzyn Stallion Depot in the examined years, although the mean result of the preliminary test of this group was very similar to that of the private stallions and reached 4.32 points, which could also be evaluated as a good result (Table 2).

The statistical analysis carried out over the experimental years did not demonstrate differences between the results of the preliminary test in this group of stallions (Table 2).

In assessing the particular stages of the exact test, it may be noted that the state stallions achieved better scores than the private ones. In analysing

Table 2  
Results of a field draught test (preliminary and exact tests) of state stallions

Analyzed stallions		Preliminary test		Exact test					
Year of proving	Number of stallions (no.)	Point scale: 0-5*		Point scale 0-7			Point scale: 0-21**		
		Final result		Trot	Walk	Pulling will	Penal points	Final result (sum of points)	
		$\bar{x}$ (pts.)	Verbal note						$\bar{x}$ (pts.)
1999	6	3.94	good	5.00	2.50 <sup>Aa</sup>	5.00 <sup>a</sup>	0.00	12.50 <sup>a</sup>	good
2000	5	4.50 <sup>A</sup>	v. good	4.20	3.60 <sup>b</sup>	5.40	0.00	13.20	good +
2001	6	4.33	good	4.50	4.00 <sup>Bb</sup>	6.00	0.00	14.50	v. good
2002	9	4.22	good	4.78	4.20	6.67 <sup>b</sup>	6.56 <sup>b</sup>	15.00 <sup>b</sup>	v. good
2003	5	4.73	v. good	4.40	3.80 <sup>Bb</sup>	6.40 <sup>b</sup>	0.00	14.60	v. good
Total	31	4.32	good	4.61	3.52	5.91	0.00	14.06	v. good

$\bar{x}$  – arithmetic mean,  
*A, B, a, b* – values in column denoted with various letter within the same trait differ statistically, capital letters denote significance at a level of  $\alpha = 0.01$ ,  
\*, \*\* – Point scale and verbal notes as in Table 1



particular phases of the exact test, it was noted that the state stallions obtained 4.61 points for the trot which, when compared with the private stud horses, might indicate their better preparation for work in relay. In the case of the walk, they achieved a far better mean result of 3.52 points.

In this group of stallions, only the results of the pulling strength and will were worse (5.91 points) than the scores achieved in this element by the private stud horses (6.54).

In summarizing all elements of the exact draught test in the years 1999-2005, it is clear that the horses from the Stallion Depot Kętrzyn obtained a final result of 14.06 points, which gave them a very good score and substantially surpassed the final result achieved by the private stallions (12.34 points).

Undoubtedly, the great advantage of the state stallions was the fact that in the years in which they were under evaluation, none of the horses received penal points in any element of the test.

The statistical analysis carried out on this group of stallions found highly statistically significant differences between the numbers of points scored for walk, whereas pulling will and the final result appeared to differ statistically between particular experimental years (Table 2).

The high scores obtained in the exact tests indicate the effective preparation of this group of stallions through systematic training and higher workload compared to the stallions of the private sector, as well as to the better psychological balance of the state stud horses.

In comparing the mean results of the preliminary and exact tests between private and state stallions from north-eastern Poland, no significant differences were found in the results of the preliminary test (Figure 1).

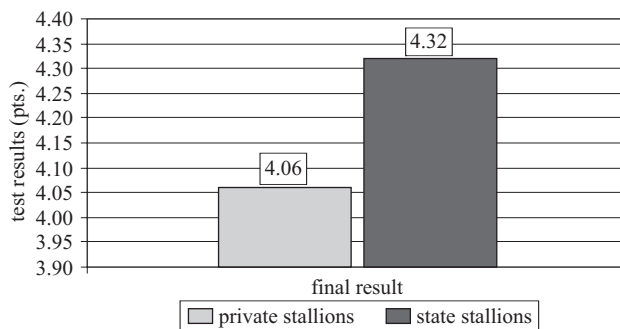


Fig. 1. Results of the field draught test of cold-blooded stallions aged 2.5-3.5 (preliminary test) from both ownership sectors

In turn, when analysing the results of the exact test, it was noted that stud horses from the Stallion Depot covered the distance in a trot and a walk faster than the stallions from the private sector. Also, in the final result of the exact test, the state stallions achieved a higher number of points (14.06). In contrast, the private stud horses were characterized by better will in pulling a cart (Figure 2).

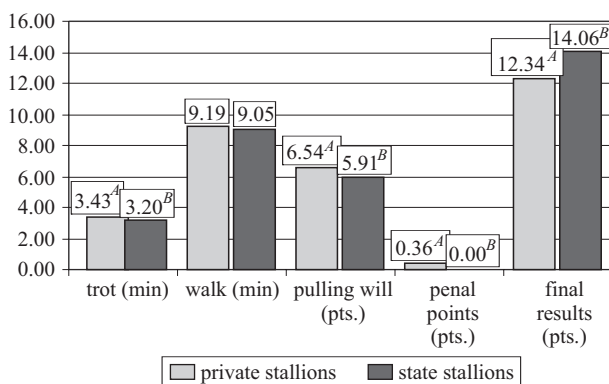


Fig. 2. Results of a field draught test of cold-blooded stallions aged 4-6 years (exact test) from both ownership sectors

The statistical analysis found highly significant differences between the results obtained for the trot, pulling will, penal points, and the final score (Figure 2).

It was observed that the mean time of pass at a walk obtained in the reported study by private (9.19) and state (9.05) stallions was worse than that achieved by cold-blooded stallions in performance tests as described by other authors: NOZDRYN-PŁOTNICKI (1966) – 10.11, SASIMOWSKI (1975) – 9.53, BUDZYŃSKI (1971) – 9.59, and SAPUŁA (1988) – 9.53.

Therefore, an improvement may be noticed in speed and motion efficiency of cold-blooded stallions subjected to performance tests in the area of the Warmia and Mazury Horse Breeders Association in the years 1999-2005 as compared to the analysis of the motion parameters of the stallions investigated in the works of the above-mentioned authors.

## **Effect of sire on the results of the performance test of cold-blooded stallions**

This analysis was aimed at determining genetic predispositions of the horses examined as affected by the breeding sector of their sires.

### **Private stallions**

In this ownership sector, tests of draught performance were carried out for 27 stud horses after state sires and 29 after private sires (Table 3).

The stallions whose sires originated from the state breeding were observed to pass the preliminary test with worse scores than those after private sires, and the difference was statistically significant. Only stallions after state sires undertaking the preliminary test in the year 2003 passed with slightly better results than those after private sires.

An opposite situation was noted in this group of stallions for the final results of the exact test which were remarkably higher in the analysed years for the progeny of state sires, as compared to the stallions after sires from private breeding (Table 3). Only in the last year did the sons of private sires pass the exact test with better scores than the stud horses after state sires.

In general, however, the mean results of the exact tests of stallions after state sires were higher than those of the stud horses whose sires originated from the private sector, however, the differences were not statistically significant (Table 3).

### **State stallions**

A very different situation was observed in the group of stallions bred in the examined years in the Kętrzyn Stallion Depot (Table 4). Out of the 31 stallions subjected to the performance test, only 10 originated after sires from the private sector.

In general, the mean results of the preliminary tests of the state stallions were slightly higher for the horses after private sires than for those after sires from the state breeding sector. The differences were, however, not statistically different (Table 4).

As with the population of the private stud horses, the results of the field draught test of stallions bred in the analysed years in the Stallion Depot Kętrzyn and subjected to the exact performance test were higher in sons of state sires both in the successive years analysed and, generally, in the whole

Table 3  
Effect of sire on results of a field draught test of private stallions

Analyzed stallions			Result of preliminary test*				Result of exact test**			
Year of proving	No. of stallions after sires from		after state sires		after private sires		after state sires		after private sires	
	state sector	private sector	$\bar{x}$ (pts.)	verbal note	$\bar{x}$ (pts.)	verbal note	$\bar{x}$ (pts.)	verbal note	$\bar{x}$ (pts.)	verbal note
1999	7	2	3.71	good	4.33	good	13.57	good +	11.00	good
2000	4	9	3.50	good	4.11	good	14.00	v. good	11.67	good
2001	9	8	3.68	good	4.33	good	11.89	good	11.88	good
2002	4	1	4.79	v. good	5.00	v. good	13.25	good +	13.00	good
2003	3	9	4.11	good	4.18	good	11.33	good	12.33	good
Total	27	29	3.87 <sup>a</sup>	good	4.24 <sup>b</sup>	good	12.78	good	11.93	good

$\bar{x}$  – arithmetic mean  
 $a, b$  – values in rows denoted with various letters differ significantly, small letters denote significance at a level of  $\alpha = 0.05$   
\*, \*\* – Point scales and verbal notes as in Table 1

Table 4  
Effect of sire on results of a field draught test of state stallions

Analyzed stallions			Result of preliminary test*				Result of exact test**			
Year of proving	No. of stallions after sires from		after state sires		after private sires		after state sires		after private sires	
	state sector	private sector	$\bar{x}$ (pts.)	verbal note	$\bar{x}$ (pts.)	verbal note	$\bar{x}$ (pts.)	verbal note	$\bar{x}$ (pts.)	verbal note
1999	6	—	3.94	good	—	—	12.50	good+	—	—
2000	4	1	4.38	good	5.00	good	13.20	v. good	10.00	good
2001	5	1	4.53	good	3.33	good	15.40	good	10.00	good
2002	2	7	4.00	v. good	4.29	v. good	15.50	good+	14.86	good
2003	4	1	4.67	good	5.00	good	14.75	good	14.00	good
Total	21	10	4.31	good	4.33	good	14.19	v. good	13.80	good+

$\bar{x}$  – arithmetic mean

Lack of significant differences

\*, \*\* – Point scales and verbal notes as in Table 1

population of state stallions examined, however the differences were not statistically significant (Table 4).

The analysis of the effect of sire on the results of performance tests of private and state stallions demonstrated that the stud horses originating from sires from the private sector did well on the preliminary test, but were worse on the exact test. Thus, in the exact tests, the best scores were obtained by the sons of state sires. This may indicate the greater ergogenic capacity of the elite state stallions and their transfer to progeny as well as to the neglect of potential capacities in the private sires, which did not allow them to demonstrate their pulling capacity.

PIETRZAK et al. (1991) in analysing the effect of breeding sector of the sire in various breeds, demonstrated that in the population of cold-blooded stallions originating from the private sector, skittish behaviour during the breaking-in of young horses occurred much more frequently. In a continuation of these studies, KAPROŃ et al. (1991a) showed that during draught performance, stallions after state and private sires behaved similarly and did not pose any serious problems. Only in the case of a few stallions after private sires was it impossible to harness them with other horses. In another study carried out on that population of stallions (KAPROŃ et al. 1991b), it was demonstrated that the sons of private sires posed more problems in draught usage after a periodical brake than those after state sires, which was confirmed in the reported study.

## **Conclusions**

1. In summarizing the results of field draught tests, it may be concluded that the selection of stallions carried out in the system yielded good results, and the progeny of the stud horses examined have a good chance of inheriting draught abilities. This would enable the achievement of a high selection response and, consequently, further improvement of the population of cold-blooded horses in Poland.

2. Results of the preliminary performance tests indicate good preparation for tests of the stallions of both ownership sectors, whereas the results of the exact test are more diversified and point to better preparation of the stud horses originating from the Kętrzyn Stallion Depot. It also results from the fact that the Depot purchases the best stallions with the highest draught potential.

3. The study demonstrated the effect of the ownership sector of sire on the results of the performance tests of the stallions. In both sectors analysed,

stallions after private sires scored better results in the preliminary tests and performed far worse in the exact tests.

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## SEMEN QUALITY PARAMETERS AND CONTENT OF SELECTED MINERALS IN BOAR BLOOD AND SEMINAL PLASMA\*

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**Key words:** boars, blood plasma, seminal plasma, Ca, P, Mg, Cu, ejaculate volume, sperm concentration, sperm motility.

### Abstract

The blood from 23 breeding boars was taken to analyse of minerals in the blood plasma 4 times in the course of 5 months. The mean Ca, P, Mg and Cu concentrations were 2.24 mmol l<sup>-1</sup>, 1.95 mmol l<sup>-1</sup>, 0.71 mmol l<sup>-1</sup> and 34.49 µmol l<sup>-1</sup>, respectively; the Ca : P ratio was 1.18. During the entire experimental period the average sperm concentration, ejaculate volume and sperm motility of all the boars was 0.43 · 10<sup>6</sup> mm<sup>-3</sup>, 303.20 cm<sup>3</sup> and 89.00%, respectively. The average total number of sperm (TNS) and the total number of motile sperm (TNMS) in the ejaculate was 130.49 · 10<sup>9</sup> and 116.12 · 10<sup>9</sup>, respectively. A statistically highly significant negative correlation ( $P < 0.01$ ) were detected between the concentration of P in the blood plasma and the ejaculate volume ( $r_p = -0.40$ ), TNS ( $r_p = -0.34$ ) and TNMS ( $r_p = -0.33$ ). There was significant positive correlation between the Ca:P ratio and the ejaculate volume  $r_p = 0.28$  ( $P < 0.01$ ), TNS  $r_p = 0.22$  ( $P < 0.05$ ) and TNMS  $r_p = 0.22$  and  $r_p = 0.21$  ( $P < 0.05$ ). The mean concentrations of Ca, P, Mg and Cu in the boar seminal plasma were 0.38 mmol l<sup>-1</sup>, 1.22 mmol l<sup>-1</sup>, 3.03 mmol l<sup>-1</sup> and 9.51 µmol l<sup>-1</sup>, respectively; the Ca:P ratio was 0.34. The mostly negative correlations were detected between the sperm parameters and concentrations of Mg in the semen plasma – from  $r_p = -0.52$  ( $P < 0.05$ ) to  $r_p = 0.12$ . There is possible, to state that concentration of monitored minerals in blood and seminal plasma influence qualitative sperm parameters. Optimizing of their concentration and proportional ratio could lead to positive influencing of sperm qualitative parameters.



**WSKAŹNIKI JAKOŚCIOWE NASIENIA ORAZ ZAWARTOŚĆ WYBRANYCH ZWIĄZKÓW MINERALNYCH W KRWI I PLAZMIE NASIENIA KNURÓW****Ladislav Máchal, Martin Hošek, Zuzana Peslarová, Ivo Krivánek**Uniwersytet Roniczy i Leśny im. Mendla w Brnie  
Republika Czeska**Słowa kluczowe:** knury, plazma krwi, plazma nasienia, Ca, P, Mg, Cu, objętość ejakulatu, koncentracja plemników, ruchliwość plemników.**Abstrakt**

Krew od 23 knurów hodowlanych pobrano 4-krotnie w okresie 5 miesięcy w celu oznaczenia zawartości związków mineralnych w plazmie. Średnie stężenia Ca, P, Mg i Cu wynosiły odpowiednio: 2,24 mmol l<sup>-1</sup>, 1,95 mmol l<sup>-1</sup>, 0,71 mmol l<sup>-1</sup> i 34,49 μmol l<sup>-1</sup>; stosunek Ca:P wynosił 1,18. W ciągu całego badanego okresu średnia koncentracja plemników, objętość ejakulatu oraz ruchliwość plemników miały wartość odpowiednio: 0,43 · 10<sup>6</sup> mm<sup>-3</sup>, 303,20 cm<sup>3</sup> i 89,00%. Średnia ogólna liczba plemników (TNS) i liczba plemników ruchliwych (TNMS) w ejakulacie wyniosły odpowiednio: 130,49 · 10<sup>9</sup> i 116,12 · 10<sup>9</sup>. Wykazano statystycznie wysoko istotną ujemną korelację ( $P < 0,01$ ) pomiędzy stężeniem P w plazmie krwi i objętością ejakulatu ( $r_p = -0,40$ ), TNS ( $r_p = -0,34$ ) i TNMS ( $r_p = -0,33$ ). Stwierdzono także istotną dodatnią korelację pomiędzy stosunkiem Ca:P a objętością ejakulatu  $r_p = 0,28$  ( $P < 0,01$ ), TNS  $r_p = 0,22$  ( $P < 0,05$ ) i TNMS  $r_p = 0,21$  ( $P < 0,05$ ). Średnie stężenia Ca, P, Mg i Cu w plazmie nasienia knurów wyniosły odpowiednio: 0,38 mmol l<sup>-1</sup>, 1,22 mmol l<sup>-1</sup>, 3,03 mmol l<sup>-1</sup> i 9,51 μmol l<sup>-1</sup>; a stosunek Ca:P – 0,34. W większości wyznaczono ujemne korelacje pomiędzy parametrami nasienia a stężeniem Mg w plazmie nasienia: od  $r_p = -0,52$  ( $P < 0,05$ ) do  $r_p = 0,12$ . Można zatem sugerować, że stężenia badanych związków mineralnych w krwi i plazmie nasienia wpływają na jakościowe parametry nasienia. Optymalizacja ich stężenia i właściwych proporcji może wpłynąć pozytywnie na jego wyznaczniki jakościowe.

**Introduction**

The reproductive ability of the boar is strongly affected by a number of factors and inputs, starting with organisational and technical factors and ending with health factors. Monitoring the quality and quantity of the ejaculate of boars used in IA stations leads to the conclusion that the quality of the ejaculate of one and the same breeding boar changes in time.

Spermatogenesis as well as production of whole semen is under endocrine regulation, especially by gonadotropins (LH, FSH) responsible for stimulation of steroidogenesis (androgens in Leydig cells; estrogens in Sertoli's cells) as well as for testicular production of regulatory proteins e.g. androgen binding protein (ABP), inhibin, activin. But there is a number of exogenic and endogenic factors influencing qualitative parameters of semen. VĚŽNÍK et al. (2004) classes also inorganic ions among the endogenic factors which influence motility of sperm. A number of authors pointed out the role of minerals in the sperm – WONG et al. (2001) mentioned that minerals

are essential in spermatogenesis and fertility. SORESENSEN et al. (1999) introduced the highlight of the effect of Ca in seminal plasma. They found a statistically significant difference in sperm motility between ejaculates with high and low Ca content. DRAGILEVA et al. (1999) stressed the role of Ca in the process of capacitation. JOCKENHOVEL et al. (1990) discovered a statistically significant positive correlation between the concentration of Cu in the semen and progressive motility of sperms ( $r = 0.23$ ). LEONHARD-MAREK (2000) and HUANG et al. (2000) pointed out that Cu affects sperm motility.

The control of Ca quantity is managed rather accurately. The level of Ca in the body fluids is controled hormonally (1.25-dihydroxycholecalciferol, parathyroide hormone, thyrocalcitonin). The Ca adopted from the feed is absorbed within the range 30-80%. Unlike resorption of Ca, resorption of P is linearly dependent to food intake (CANONG 1999). The Ca is bound to proteins in the blood plasma or possibly bound in the complexes with phosphate, hydrogencarbonate, citrate or in the form of free ions. Regrading mineral supplements, Ca is in most cases bound in the form of  $\text{CaCO}_3$ . Another form (phytate form) reduces useability of Ca in the organism. Increasing concentration of roughage in the feeding mixture leads to decrease of both organic and inorganic nutrients including Ca.

Authors differ in their estimations of the average values of quantitative and qualitative parameters of the boar ejaculate. GAMČÍK et al. (1992) reported that the average volume of the boar ejaculate was 250 to 300 ml, ranging between 80 and 900 ml. The average motility of boar sperm ranged between 60% and 90%, MÁCHAL et al. (1997) discovered that the average motility was 78.0%. GAMČÍK et al. (1992) reported that the concentration of sperm in the ejaculate of boars of different breeds ranged between 220 and  $520 \cdot 10^3 \text{ mm}^{-3}$ , MÁCHAL et al. (1997) reported the average concentration of sperm in the boar ejaculates as  $581 \cdot 10^3 \text{ mm}^{-3}$ .

The same way as the values of the qualitative and quantitative indicators of boar sperm change, so does the concentration of minerals in the blood plasma. Evaluations of the concentration of the respective parameters of the blood plasma of boars are based on the range of normal values, which were presented, for instance by VRZGULA et al. (1990) for pigs (concentration of Ca  $2.20 - 3.00 \text{ mmol l}^{-1}$ , P  $1.60 - 3.00 \text{ mmol l}^{-1}$ , Mg  $0.78 - 1.20 \text{ g l}^{-1}$  and Cu  $24.00 - 42.00 \text{ } \mu\text{mol l}^{-1}$ ), or on average normal values as reported by JELÍNEK et al. (2003); concentrations of Ca, P, Mg and Cu were  $2.50 \text{ mmol l}^{-1}$ ,  $2.3 \text{ mmol l}^{-1}$ ,  $1.23 \text{ g l}^{-1}$  and  $30.00 \text{ } \mu\text{mol l}^{-1}$ , respectively. These values are only informative and they may slightly differ due to the action of various factors and they affect the qualitative and quantitative parameters of the sperm (MÁCHAL et al. 2002).

The objective of the present study was to determine the dynamics of concentrations of Ca, P, Mg and Cu and the Ca : P ratio in the blood and

seminal plasma of boars and to find out how they are correlated with the quality parameters of their ejaculate.

## Materials and Methods

Investigations into the dynamics of correlations between the concentration of Ca, P, Mg and Cu in the blood and seminal plasma of breeding boars and the sperm concentration, sperm motility and ejaculate volume involved 23 Large White, Landrace, Belgian Landrace and Hampshire breeding boars, and crosses with Pietrain, used for ejaculate collection at the insemination station. The boars were fed a uniform feed mixture of 3.8 kg / animal / day. The mixture contained (%) seeds of barley (30), wheat (30), oats (20), wheat bran (6), SBM (10) and an addition of amino acids (2.5) and minerals (1.5).

The boars were monitored for 5 months, from February to June 2003. The ejaculates were collected on a regular basis twice a week and the following parameters were determined: sperm motility, sperm concentration, ejaculate volume, total number of sperm in the ejaculate (TNS) and total number of motile sperm in the ejaculate (TNMS). After dilution of the native ejaculate with a physiological solution (1:1) under a temperature of  $38 \pm 1^\circ\text{C}$  the percentage of sperm motility (progressive movement forward) was assessed by a subjective method. The sperm concentration was assessed hemocytometrically in Bürker's cellules.

In 5-week intervals the boar blood was sampled for analyses of the concentration of Ca, P, Mg and Cu in the plasma. Venous blood was taken from the *vena jugularis externa* always in the morning between 8 and 11 a.m. Heparin was used as the anti-coagulation agent. The concentrations of selected minerals in the blood plasma (Ca, P, Mg and Cu) were assessed within 24 h by photometry using Bio – LA kits (firm Pliva). In June 2003 the ejaculates were collected shortly before blood sampling in order to determine the actual sperm motility and the concentration and volume of the ejaculate. The ejaculates were then centrifuged ( $2.500 \text{ rev} \cdot \text{min}^{-1}$  for 15 min) to separate the seminal plasma. Ca, P, Mg and Cu concentrations were determined in seminal plasma by colorimetric and paint assay procedures.

The results were processed using mathematical and statistical methods by VENČIKOV and VENČIKOV (1977). All the results presented in the tables are mean values (with standard deviation). The results were calculated statistically according to Student's *t*-test; the relationship between the concentration of minerals in the blood plasma and concentration of minerals in the seminal plasma and their correlation with the concentration, sperm motility and volume of ejaculate were assessed by phenotypic coefficients of correlation.

## Results and Discussion

Table 1 presents the mean concentrations of Ca, P, Mg and Cu, and the Ca : P ratio in blood and seminal plasma and the average values of the qualitative parameters of the ejaculate of the breeding boars, including the dynamics of these values for the entire experimental period.

The mean concentration of Ca in the boar blood plasma over the whole experimental period was 2.24 mmol l<sup>-1</sup> and in the individual months it ranged from 2.19 mmol l<sup>-1</sup> to 2.29 mmol l<sup>-1</sup>. The mean P concentration in the blood plasma was 1.95 mmol l<sup>-1</sup> and in the individual months it ranged from 1.84 mmol l<sup>-1</sup> to 2.07 mmol l<sup>-1</sup>. The Ca : P ratio was 1.18 and in the individual months it ranged from 1.12 to 1.24.

The mean Mg concentration in the blood plasma during the whole experimental period was 0.71 mmol l<sup>-1</sup>, ranging from 0.64 mmol l<sup>-1</sup> to 0.76 mmol l<sup>-1</sup> in the individual months. The mean Cu concentration in blood plasma was 34.49 µmol l<sup>-1</sup>, ranging from 32.75 µmol l<sup>-1</sup> to 38.95 µmol l<sup>-1</sup> in the individual months. The mean concentrations of minerals in the blood plasma of boars over the whole experimental period were balanced and no statistically significant differences were detected between the individual months.

The mean concentration of Ca in the blood plasma of the boars was in the lower range of values reported by VRZGULA *et al.* (1990) and was lower than the mean value reported by JELÍNEK *et al.* (2003). The mean Mg concentration in the blood plasma was lower than values reported by VRZGULA *et al.* (1990) and JELÍNEK *et al.* (2003). The mean values of P and Cu were consistent with data of the above authors.

The deficiency of Ca can be induced dietetically as well as by the hormones insufficiency (parathyroid hormone, vitamine D) – LÜLLMANN *et al.* (2004), the deficiency of P and Mg usually occurs along with insufficient supply from feed. Both Ca and P are usually resorbed in the small intestine. The resorption of Ca is mainly influenced by sufficient acidity of the intestine fill, good concentration of vitamine D, the content of phosphates and oxalates in the food, the amount of parathyroid hormone, and by disturbed lipids treatment in the intestine. The excess of Ca<sup>2+</sup> and Mg<sup>2+</sup> ions in the feed ration creates non-resorbable phosphates in the intestine. Also P is resorbed in the form of phosphates.

Over the entire experimental period the average volume of the ejaculate of all the boars was 303.20 cm<sup>3</sup>, sperm concentration 0.43 · 10<sup>6</sup> mm<sup>-3</sup> and sperm motility 89.0%. The average total number of sperms in the ejaculate was 130.49 · 10<sup>9</sup> and the total number of motile sperms in the ejaculate (of crucial importance in terms of the number of insemination doses) was 116.12 · 10<sup>9</sup>. As for the quality parameters of the ejaculate, no significant differences

Table 1  
Average values of selected minerals of blood and seminal plasma, qualitative parameters of the ejaculate of breeding boars in the individual months and the significance of differences among them (*t*-test from February to June of 2003)

Month	<i>n</i>	Statistical measure	Average concentrations of the minerals					<i>n</i>	Average semen quality				
			Ca	P	Ca : P	Mg	Cu		Ejaculate volume	Concentra- tion of sperms	Sperm motility	Total number of sperms	Total number of motile sperms
			mmol l <sup>-1</sup>	mmol l <sup>-1</sup>	mmol l <sup>-1</sup>	μmol l <sup>-1</sup>		cm <sup>3</sup>	mm <sup>3</sup> · 10 <sup>6</sup>	%	· 10 <sup>9</sup>	· 10 <sup>9</sup>	
Blood February	23	$\bar{x}$	2.24	1.84	1.24	0.76	36.82	184	293.85	0.46	88.9	132.87	118.02
		<i>s<sub>x</sub></i>	0.29	0.27	0.23	0.31	18.72		56.10	0.12	2.3	38.08	33.71
March	23	$\bar{x}$	2.29	2.07	1.12	0.66	38.95	184	301.53	0.45	89.0	136.76	121.63
		<i>s<sub>x</sub></i>	0.11	0.23	0.11	0.04	9.18		64.06	0.10	1.7	41.60	37.16
April	23	$\bar{x}$	2.19	1.84	1.23	0.64	32.75	184	291.58	0.48	88.9	138.48	122.92
		<i>s<sub>x</sub></i>	0.15	0.31	0.24	0.05	3.51		53.58	0.11	1.9	39.21	34.23
June	23	$\bar{x}$	2.23	2.05	1.14	0.76	33.74	184	328.24	0.34	89.4	112.32	100.58
		<i>s<sub>x</sub></i>	0.18	0.40	0.30	0.10	3.86		81.70	0.07	2.4	32.97	30.11
Total	92	$\bar{x}$	2.24	1.95	1.18	0.71	34.49	736	303.20	0.43	89.0	130.49	116.12
		<i>s<sub>x</sub></i>	0.20	0.33	0.24	0.18	4.64		65.89	0.12	2.1	39.53	35.08
Semen June	23	$\bar{x}$	0.38	1.22	0.34	3.03	9.51	23	326.75	0.36	89.2	117.97	105.11
		<i>s<sub>x</sub></i>	0.39	0.22	0.40	0.09	6.66		81.70	0.07	2.4	32.97	30.11

between the average parameters of ejaculate quality in the individual months were detected.

The lowest average ejaculate volume (291.58 ml) from the boars was obtained in April 2003 and the highest in June 2003 (328.24 ml). The lowest average concentration of sperm in the ejaculate was detected in June 2003 ( $0.34 \cdot 10^6 \text{ mm}^{-3}$ ), as well as the total number of sperms ( $112.32 \cdot 10^9$ ) and total number of motile sperms in the ejaculate ( $100.58 \cdot 10^9$ ). The highest average concentration of sperm in the ejaculate was detected in April 2003 ( $0.48 \cdot 10^6 \text{ mm}^{-3}$ ), as well as the total number of sperms ( $138.48 \cdot 10^9$ ) and total number of motile sperms in the ejaculate ( $122.92 \cdot 10^9$ ).

The average ejaculate volumes were consistent with values reported by GAMČÍK et al. (1992). Sperm motility was higher than the data of MÁCHAL et al. (1997). The average concentration of sperm in the boar semen was consistent with the concentration reported by GAMČÍK et al. (1992). However they reported a lower average concentration of boar sperm than MÁCHAL et al. (1997).

Table 2 shows the dynamics of phenotype correlations among the individual criteria (concentration of minerals – Ca, P, Mg and Cu, and the Ca : P ratio in the blood plasma of boars and qualitative and quantitative parameters of the ejaculate) over the entire experimental period. A statistically highly significant ( $P < 0.01$ ) negative correlation for the entire experimental period was discovered between the concentration of P in the blood plasma and volume of the ejaculate ( $r_p = -0.40$ ), between the concentration of P in the blood plasma and total number of sperms ( $r_p = -0.34$ ), and between the P concentration in the blood plasma and the total number of motile sperms ( $r_p = -0.33$ ). Figure 1 presents the average concentrations of P in the blood plasma of boars and qualitative parameters of the boar ejaculate in the individual months. Negative correlations were discovered between the concentration of P in the boar blood plasma and ejaculate volume, i.e. between ( $r_p = -0.67 - P < 0.01$  and  $r_p = -0.33$ ), correlations between the concentration of P in the blood plasma and total number of sperms were negative and ranged between ( $r_p = -0.50 - P < 0.05$  and  $r_p = -0.19$ ), correlations between the concentration of P in the boar blood plasma and the total number of motile sperms were negative and ranged between ( $r_p = -0.51 - P < 0.05$  and  $r_p = -0.17$ ). A statistically highly significant positive correlation ( $P < 0.01$ ) for the entire experimental period was detected between the Ca:P ratio in the blood plasma of the boars and the ejaculate volume ( $r_p = 0.28$ ). For the entire period there was a statistically significant ( $P < 0.05$ ) positive correlation ( $r_p = 0.22$ ) between the Ca : P ratio in the boar blood plasma and the total number of sperms. A statistically significant close correlation ( $P < 0.05$ ) was monitored between the Ca : P ratio in the blood plasma and the total number of motile sperms ( $r_p = 0.21$ ). Figure 2 illustrates

Table 2  
Phenotype correlations between concentrations of selected minerals of the blood plasma and qualitative and quantitative parameters of the ejaculate of breeding boars during the experimental period (from February to June of 2003)

Month	Indices	n	Blood plasma					Seminal plasma		
			Ca	P	Ca:P	Mg	Cu	Semen volume	Concentration of the sperms	Motility of the sperms
February	Ejaculate volume	23	-0.31	-0.33	0.03	-0.14	0.12	-0.19		
	Sperm concentration	23	0.00	0.02	-0.05	0.13	0.38	0.15	-0.23	
	Sperm motility	23	-0.35	0.20	-0.38	-0.16	-0.18	0.60**	0.64**	
	Total number of motile sperms	23	-0.15	-0.19	0.01	0.11	0.38	0.62**	0.62**	0.01
March	Ejaculate volume	23	-0.24	-0.47*	0.34	0.01	-0.06	0.24		
	Sperm concentration	23	0.30	0.18	-0.01	0.00	-0.09	0.00	-0.07	
	Sperm motility	23	-0.52**	-0.28	0.00	-0.03	0.21	0.82**	0.74**	
	Total number of motile sperms	23	0.01	-0.24	0.26	0.04	-0.16	0.74**	0.74**	0.02
April	Ejaculate volume	23	0.01	-0.42	0.37	-0.12	-0.01	-0.01		
	Sperm concentration	23	0.39	-0.13	0.23	-0.14	-0.21	-0.01	-0.25	
	Sperm motility	23	-0.06	0.44*	-0.38	0.06	-0.08	-0.18	0.78**	
	Total number of motile sperms	23	0.31	-0.38	0.42*	-0.13	-0.13	0.61**	0.77**	-0.26
June	Ejaculate volume	23	0.31	-0.36	0.40	-0.13	-0.14	0.62**		
	Sperm concentration	23	-0.30	-0.67**	0.52*	-0.12	-0.14	-0.13	0.15	
	Sperm motility	23	-0.19	0.03	-0.17	-0.21	-0.46*	0.10	0.60**	
	Total number of motile sperms	23	-0.39	-0.50*	0.27	-0.22	-0.39	0.73**	0.57**	0.24
Total	Ejaculate volume	92	-0.20	-0.40**	0.28**	-0.05	0.07	-0.11	-0.16	
	Sperm concentration	92	0.06	-0.10	0.06	-0.04	0.08	0.06	0.71**	
	Sperm motility	92	-0.17	0.06	-0.14	-0.17	-0.08	0.59**	0.70**	
	Total number of motile sperms	92	-0.07	-0.34**	0.22*	-0.03	0.10	0.60**		-0.02

\* -  $P < 0.05$ ; \*\* -  $P < 0.01$

the average Ca : P ratio in the blood plasma and the qualitative parameters of the ejaculate in the individual months. Correlations between the Ca : P ratio in the blood plasma and the ejaculate volume of the boars were positive and ranged from ( $r_p = 0.03$  to  $r_p = 0.52$ ).

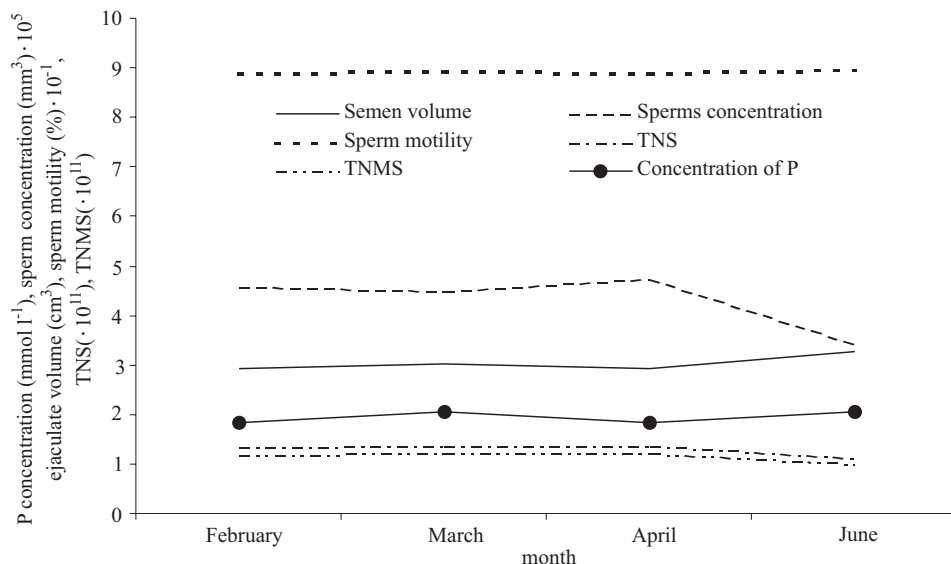


Fig. 1. The dynamics of the blood P and the qualitative indicators in the ejaculates of the boars (from February to June of 2003)

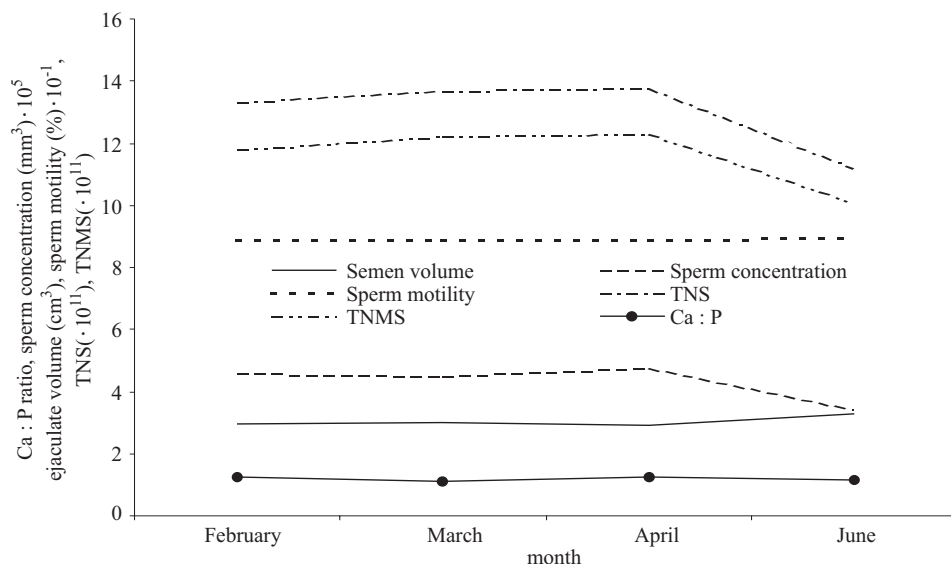


Fig. 2. The dynamics of the blood Ca : P ratio and the qualitative indicators in the sperm of the boars (from February to June of 2003)



In the seminal plasma of the boars the mean Ca and P concentrations were 0.38 mmol l<sup>-1</sup> and 1.22 mmol l<sup>-1</sup>, respectively, and the Ca : P ratio was 0.34 (Table 1). The mean Mg and Cu concentrations in the seminal plasma of the boars were 3.03 mmol l<sup>-1</sup> and 9.51 µmol l<sup>-1</sup>, respectively.

The mean concentrations of Ca, P and Cu in the blood plasma of the boars were higher than in seminal plasma (Ca – 2.23 mmol l<sup>-1</sup> vs. 0.38 mmol l<sup>-1</sup>, P – 1.95 mmol l<sup>-1</sup> vs. 1.22 mmol l<sup>-1</sup>, Cu – 33.74 µmol l<sup>-1</sup> vs. 9.51 µmol l<sup>-1</sup>, respectively). In contrast, the concentration of Mg was lower than in the seminal plasma (0.76 mmol l<sup>-1</sup> vs. 3.03 mmol l<sup>-1</sup>, respectively).

Table 3 presents the correlations between the concentrations of Ca, P, Mg, Cu and the Ca : P ratio in the seminal plasma of the boars and the qualitative and quantitative parameters of the ejaculate. With the exception of motility, the phenotype correlations between the concentration of Ca in the seminal plasma of the boars and the qualitative and quantitative parameters of the sperm were positive and ranged between  $r_p = -0.20$  and  $r_p = 0.19$ ; a similar correlation was discovered between the concentration of Cu and the qualitative and quantitative parameters of the boar sperm ( $r_p = -0.20$  to  $r_p = 0.31$ ). Negative correlations ranging between  $r_p = -0.22$  and  $r_p = -0.08$  were assessed between the concentration of P in the seminal plasma and all the qualitative and quantitative parameters of the ejaculate. Phenotype correlations between the Ca : P ratio in seminal plasma and the individual qualitative and quantitative parameters of the sperm were mostly negative and ranged between  $r_p = -0.36$  and  $r_p = 0.19$ . The correlation between the concentration of Mg in the seminal plasma and parameters of the sperm were mostly negative, i.e. between  $r_p = -0.52$  and  $r_p = 0.12$ ; correlations  $r_p = -0.52$  and  $r_p = -0.42$  were statistically significant ( $P < 0.05$ ). In accordance with data presented in VĚŽNÍK et al (2004), the influence of selected inorganic ions on sperm motility was confirmed. However, statistically evidential influence of Cu concentration in seminal plasma on sperm motility was not confirmed. Neither was finding from JOCKENHOVEL et al. (1990) on positive correlation between the concentration of Cu in the semen and progressive motility of sperms ( $r_p = -0.36$ ).

Table 3  
Phenotype correlations between concentrations of selected minerals in the seminal plasma and qualitative and quantitative parameters of the ejaculate of breeding boars

Month	Indices	n	Seminal plasma				
			Ca	P	Ca:P	Mg	Cu
April 2003	Ejaculate volume	23	0.09	-0.18	-0.23	0.12	0.31
	Sperm concentration	23	0.16	-0.08	-0.27	-0.39	0.02
	Sperm motility	23	-0.20	-0.12	0.19	-0.52*	-0.20
	Total number of sperms	23	0.19	-0.21	-0.36	-0.36	0.21
	Total number of motile sperms	23	0.15	-0.22	-0.35	-0.42*	0.19

\* –  $P < 0.05$

Although there is an amount of generally accepted recommendations concerning element presence in the standard dietary supplements, following fact holds. The use of these supplements does not have to a priori lead, regarding physiological disposition of the organism and chemical formula of the supplement, to desired saturation of the organism. Thus monitoring of their level in the blood plasma is considered to be the most accurate reflection of the real supply of the organism.

We can say that the important role of selected minerals in the blood and seminal plasma of boars in correlation with motility, sperm concentration and sperm volume was confirmed. A higher concentration of P in the blood plasma resulted in a negative correlation, especially with the ejaculate volume and total numbers of sperms in the ejaculate. Likewise, the higher level of P in the seminal plasma was negatively correlated with all the parameters of ejaculate quality. A negative correlation between sperm quality and concentration of Mg both in the blood and seminal plasma was discovered when the concentration of Mg was higher. A higher Ca : P ratio in the blood plasma of the boars was positively correlated particularly with the ejaculate volume.

The above facts show that the total number of sperm in the semen is negatively correlated with higher concentrations of P and Mg in the seminal plasma of the boars.

There is possible, in accordance with authors, to state that concentration of monitored minerals in blood and seminal plasma influence qualitative sperm parameters. Optimizing of their concentration and proportional ratio could lead to positive influencing of sperm qualitative parameters.

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## **PHYSICOCHEMICAL PROPERTIES OF COLOSTRUM AND MILK FROM ANGUS AND BLACK-AND-WHITE COWS DURING THE FIRST TEN DAYS AFTER CALVING**

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**Key words:** cattle, Black-and-White breed, Angus breed, colostrum composition, milk composition.

### **A b s t r a c t**

Changes in the composition and properties of colostrum and milk from Angus and Black-and-White cows were described in successive milking runs on the first ten days after calving. The first test milking took place directly after calving, and successive milking runs – every 6 hours on the first day, every 8 hours on the second and third day and twice on the fourth day. The collected samples were marked for the basic composition and density of milk, its active acidity, thermostability and coagulability. In comparison with Black-and-White cows, colostrum and milk from Angus cows on the first ten days after calving were characterized by a lower fat and lactose content and a higher protein content. The technological properties of colostrum and milk from both breeds were less differentiated and remained within the reference range. The colostrum from cows of both breeds, obtained in the first twelve hours after calving, was characterized by very good composition and quality. A high quality of colostrum was maintained in the Angus breed during the first five days after calving. The obtained results indicate that long-term selection for increased yield caused significant changes in the composition of colostrum and milk.

## **CECHY FIZYKOCHEMICZNE SIARY I MLEKA KRÓW RASY ANGUS I CZARNO-BIAŁEJ W PIERWSZYCH DZIESIĘCIU DOBACH PO WYCIELENIU**

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**Słowa kluczowe:** bydło, rasa cb, rasa angus, skład siary, skład mleka.

### Abstrakt

Określono zmiany składu oraz właściwości siary i mleka krów rasy angus i czarno-białej w kolejnych dojach w pierwszych dziesięciu dobach po wycieleniu. Pierwszy próbny dój wykonano bezpośrednio po wycieleniu, kolejne w pierwszej dobie co 6 godzin, w drugiej i trzeciej dobie co 8 godzin, a od czwartej doby dwa razy w ciągu dnia. W pobranych próbach oznaczono podstawowy skład mleka oraz gęstość, kwasowość czynną, termostabilność i krzepliwość. Siara i mleko krów rasy angus w porównaniu z rasą czarno-białą w pierwszych dziesięciu dobach po wycieleniu charakteryzowały się niższą zawartością tłuszczu i laktozy oraz wyższą białka. Technologiczne właściwości siary i mleka obu ras były mniej zróżnicowane i nie odbiegały od wartości fizjologicznych. Siara krów obu ras pozyskana w pierwszych dwunastu godzinach po wycieleniu charakteryzowała się bardzo dobrym składem i jakością. Dobra jakość siary utrzymywała się u rasy angus przez pierwsze pięć dób po wycieleniu. Uzyskane wyniki wskazują, że prowadzona od wielu lat selekcja w celu zwiększenia wydajności krów, spowodowała znaczne zmiany w składzie ich siary i mleka.

## Introduction

The high-yielding dairy cow population in Poland and many other countries is the product of a deliberate breeding effort which took place throughout decades to increase milk yield and fat content. This goal has been achieved at the expense of a significant reduction in protein concentration of milk. The issue of protein content in milk has become a topic of interest only in recent years (JANKOWSKA, SAWA 2004, KRZYŻEWSKI et al. 1997, SAWA et al. 2004).

Contrary to dairy cows, beef cows have never been selected for increased milk production, which is why the composition of their milk has not been deliberately modified through breeding work. The majority of available literature on the breeding of beef cattle focuses on meat performance traits and analyzes the factors determining those traits. There is relatively limited information on the composition and physicochemical properties of colostrum and milk, in particular on the first days of lactation.

The colostrum yield varies widely. The first milking after calving produces from several dozen milliliters to 15 liters of colostrum (PRITCHETT et al. 1991). Beef cows produce significantly less colostrum than dairy cows, but its quantities are sufficient to feed the calves. A higher content of colostrum components is usually reported in beef cows and breeds with a lower milk yield.

The purpose of this study was to determine changes in the composition and properties of colostrum and milk from Angus cows and Black-and-White cows in successive milking runs on the first ten days after calving.

## Materials and Methods

The study covered four Angus cows and four Black-and-White cows after second calving in October and November. A month before the planned

parturition, beef cows were transported from the home farm to a dairy farm for acclimatization. From the 30<sup>th</sup> day before calving to the third day after parturition, all animals were kept in tie stalls on litter. In the farm, cows were fed a TMR. During the dry period, cows were fed ad libitum a diet composed of: maize silage – 15 kg, grass haylage – 7 kg (enriched with 2 kg of concentrate a week before calving); and after calving: maize silage – 25 kg, grass haylage – 12 kg and concentrate – 2 kg.

Test milking runs were performed to determine changes in the composition of colostrum and milk. The first portion of colostrum was obtained directly after calving (in the first hour). Successive milking runs took place four times on the first day (every 6 hours) and three times on the second and third day (every 8 hours). Milking runs were performed twice a day from day 4 to day 10. Directly after milking runs in the cowshed, colostrum density was determined with a lactodensimeter graduated for 18°C, and active acidity (pH) and temperature were measured with the use of a Piccolo Plus pH-meter with a HF-1295 electrode. Prior to laboratory analyses, successive samples were cold-stored for around 4 hours (+ 4°C). The collected colostrum samples were marked for: thermostability – by titration of colostrum and milk with 96% ethanol, coagulability – by measuring the time of casein curd formation following the application of a 1% rennet solution (PIJANOWSKI 1984). The percentage content of total protein, fat, lactose and dry matter was measured with the use of a Combi Foss 6200 apparatus.

The results were processed statistically with the use of STATISTICA 6.0 software as part of a one-factorial analysis of variance in an orthogonal design. Mean values and coefficients of variation were calculated. The significance of differences was estimated with the use of Duncan's test.

## Results and Discussion

In the first 32 hours (6 milking runs), the average colostrum yield from Angus cows reached 8.8 kg. The total quantity of colostrum obtained within three days reached 21.4 kg, and from Black-and-White cows – 30.5 kg and 77.9 of colostrum respectively. DEGEN and YOUNG (1980) demonstrated that the daily milk yield of beef cows was 7.1 +/- 1.9 kg.

The fat content of colostrum was much higher in Black-and-White cows than in Angus cows. In view of the high variation of this trait, those differences were not statistically significant in every milking run. The fat content of colostrum from Black-and-White cows was high, and varied in successive milking runs: from 5.98% (in the first 24 hours) to 8.31% (48 hours). Similar variations were observed in colostrum from Angus cows, ranging from 2.44%

(72 hours) to 5.93% (6 hours). In KOVACS' study (1997), the fat content of colostrum from Red Angus cows in the first three hours after calving reached 8.22%. According to ZACHWIEJA et al. (1997), the fat content of colostrum obtained after parturition from crossbred cows with a varied gene proportion of beef breeds was similar to that reported in Angus cows at 5.48%. The wide variation in the obtained results is confirmed by other authors whose findings indicate fat content of 3.71% to 6.59% in colostrum from Black-and-White cows (SZULC et al. 1989, SZULC et al. 1991).

Similar changes in the protein content of colostrum from cows of both breeds were observed. The differences in the total protein content between both breeds in various milking runs were much below the differences in fat content, and did not exceed 1.5 percentage points. The highest protein content of colostrum from Angus and Black-and-White cows was recorded in the first milking run (more than 20%). A significant reduction in protein content was observed in successive milking runs, and the highest drop was noted in the first four milking runs after calving. Similarly high changes in the protein content of colostrum from successive milking runs postpartum were indicated by SZULC et al. (1989). In KOVACS' study (1997), the total protein content of colostrum from Red Angus cows obtained three hours after calving reached 20.19%. Subject to the specific nature of the conducted study, the authors indicate a very wide and differentiated range of the protein content of colostrum from Black-and-White cows, i.e. between 9.36% and 17.70% (PREŚ et al. 1995, ZACHWIEJA 1991). According to the findings of WRÓŃSKI et al. (1999), the total protein content of colostrum was higher in Angus cows than in Black-and-White cows.

The lactose content of colostrum from the analyzed breed groups increased consistently. The quantity of lactose in colostrum from Angus cows directly after calving amounted to 4.79%; a slightly lower level of lactose was found in colostrum from Black-and-White cows at 3.85%. The observed differences were statistically non-significant.

As of the fifth milking run, a higher lactose content was reported in colostrum from Black-and-White cows. The results obtained in respect of the lactose content of colostrum from Black-and-White cows in successive milking runs were similar to the findings of other authors (PREŚ et al. 1995, SIEBERS et al. 1985, SZULC et al. 1989, SZULC et al. 1991, ZACHWIEJA 1991).

Changes in the dry matter content of colostrum in successive eleven milking runs were mostly the product of changes in the protein and fat content of colostrum. The obtained results are backed by research findings of other authors (PREŚ et al. 1995, SZULC et al. 1989, SZULC et al. 1991, ZACHWIEJA et al. 1997).

Density measurement is a simple method of determining the immunological value of colostrum (Figure 1) (FLEENOR, STOTT 1980). The specific gravity

of colostrum above 1.047 g/ml is indicative of high colostrum quality, between 1.037 g/ml and 1.045 g/ml – of average quality, and below 1.035 g/ml – of poor quality. The highest density was recorded in colostrum from the first milking postpartum: 1.060 g/ml – in colostrum from Angus cows and 1.051 g/ml – in colostrum from Black-and-White cows, which is indicative of high colostrum quality. Similar results were reported by SZULC et al. (1989) in respect of colostrum from Black-and-White cows from the first milking after calving. A gradual decrease in the density of colostrum from cows of both breeds was observed in successive milking runs. Colostrum from Black-and-White cows in the fifth milking run (24 hours) and successive milking runs differed from milk only insignificantly. A slower decrease in the density of colostrum from Angus cows was observed in successive milking runs, reaching values above 1.034 g/ml between 32 and 72 hours.

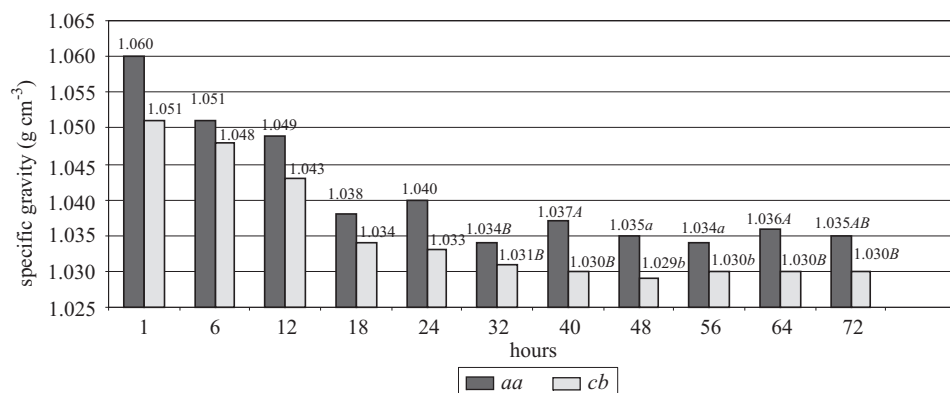


Fig. 1. Specific gravity of colostrums during the first 72 hours after calving  
Mean values marked with different letters are significantly different: capital letters  $P \leq 0.01$ ; small letters  $P \leq 0.05$

A prolonged and varied coagulation time was reported for the analyzed samples of fresh colostrum from successive milking runs after calving (Figure 2). The longest coagulation time was observed in respect of colostrum from Angus cows in the first three successive milking runs postpartum. In successive milking runs, coagulation time was shorter with significant variations between milking runs. It should be noted that the coagulation time of colostrum from Black-and-White breed was most uniform in all milking runs. It reached around 60 minutes in the first three milking runs, then gradually decreased to around 45 and 32 minutes (in the fourth and fifth run), and did not exceed 50 minutes in successive runs. ZACHWIEJA et al. (2002)



Table 1  
Changes in the composition of colostrum from Angus and Black-and-White cows in the first 72 hours after calving

Hours after calving		Colostrum (kg)		Content in colostrum (%)							
				fat		total protein		lactose		dry matter	
		aa	cb	aa	cb	aa	cb	aa	cb	aa	cb
1		2	3	4	5	6	7	8	9	10	11
1	<i>x</i>	2.1 <sup>A</sup>	9.2 <sup>B</sup>	5.30	8.20	22.19	20.91	4.78	3.85	32.35	31.73
	<i>v</i>	50.88	44.45	47.90	28.51	41.17	52.16	28.36	57.40	27.64	42.34
6	<i>x</i>	0.6	3.0	5.93 <sup>a</sup>	10.48 <sup>b</sup>	17.87	18.39	4.79	3.98	28.84	31.99
	<i>v</i>	67.71	28.41	43.92	24.05	42.88	36.13	28.98	45.20	27.82	33.46
12	<i>x</i>	0.5	4.3	4.41	7.57	13.47	14.13	5.17	6.73	23.75	25.77
	<i>v</i>	84.94	105.28	36.54	77.70	37.76	47.20	35.23	60.45	26.03	39.90
18	<i>x</i>	1.8	2.6	4.42	6.16	10.23	9.87	5.17	5.02	20.67	21.85
	<i>v</i>	147.18	50.48	77.70	52.02	46.70	40.95	47.34	29.57	46.76	38.37
24	<i>x</i>	2.30	6.0	5.03	5.97	9.10	7.92	5.31	5.99	20.64	20.92
	<i>v</i>	122.1	46.65	31.61	32.52	31.44	36.87	13.71	38.63	45.14	33.98
32	<i>x</i>	1.4 <sup>A</sup>	5.6 <sup>B</sup>	5.33	7.29	6.65	6.74	5.20	6.07	18.11	21.34
	<i>v</i>	85.80	20.72	55.78	36.84	65.36	30.84	35.08	37.65	37.98	33.99
40	<i>x</i>	0.9	6.1	3.71	7.20	5.97	6.58	5.81	5.85	16.61	20.91
	<i>v</i>	50.47	44.98	49.77	42.02	67.77	33.68	36.44	39.24	31.70	34.00
48	<i>x</i>	2.9	6.8	3.48	8.31	6.41	6.27	5.76	6.05	17.15	22.06
	<i>v</i>	131.83	41.30	48.13	20.00	35.82	19.60	41.88	39.94	49.71	28.30
56	<i>x</i>	1.4 <sup>A</sup>	7.5 <sup>B</sup>	4.29	8.16	6.27	6.35	5.38	6.02	17.31	21.82
	<i>v</i>	54.40	28.96	48.94	28.30	19.60	38.00	11.55	39.57	37.51	33.13
64	<i>x</i>	1.8	9.3	2.63 <sup>a</sup>	6.69 <sup>b</sup>	6.67	6.32	5.72	6.21	20.49	15.69
	<i>v</i>	34.97	34.53	64.71	22.30	32.57	38.54	27.78	38.42	31.73	26.45
72	<i>x</i>	2.1 <sup>a</sup>	10.0 <sup>b</sup>	2.44 <sup>a</sup>	6.76 <sup>b</sup>	6.44	6.07	5.58	6.12	15.69	20.24
	<i>v</i>	39.25	46.09	32.98	49.64	25.22	36.54	31.17	36.76	26.45	33.14

The values within a row marked with different letters are significantly different: capital letters  $P \leq 0.01$ ; small letters  $P \leq 0.05$

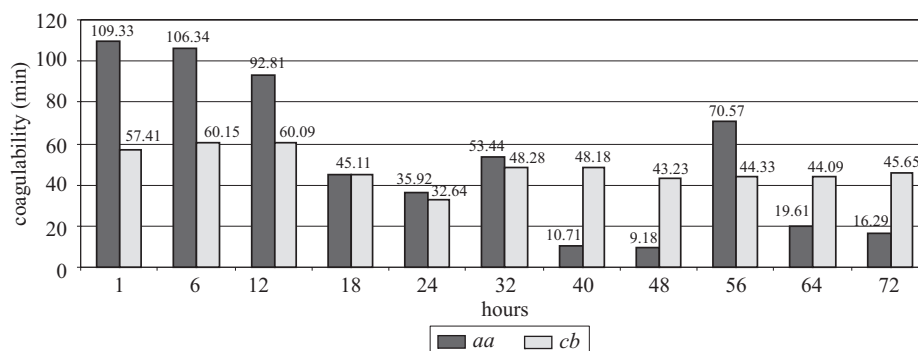


Fig. 2. Coagulability of colostrums during the first 72 hours after calving

reported a similar time of casein curd formation in colostrum from Black-and-White cows. According to CRUYWAGEN (1990), a decrease in the formation of casein curd in colostrum fed to calves lowers the availability and absorptive effectiveness of colostrum immunoglobulins. Yet in view of the findings of ZACHWIEJA et. al (2002) and BERNATOWICZ and REKLEWSKA (2003), a higher level of colostrum coagulation does not have an adverse effect on the availability of colostrum immunoglobulins in calves.

The thermostability level in fresh colostrum in successive milking runs after calving gradually increased (Figure 3). Colostrum from Black-and-White cows obtained in the first milking was titrated with 2.0 ml of alcohol, and from Angus cows – with 1.40 ml of alcohol. The quantity of alcohol applied in the titration of colostrum from Angus cows increased to 3 ml by the eighth milking run, a decrease was observed in the subsequent two runs and a repeated increase to 3 ml was reported in the eleventh milking run. Until the sixth milk round, colostrum from Black-and-White cows was characterized by higher thermostability than colostrum from beef cattle. As of the seventh milking round, a similar quantity of alcohol was required for the titration of colostrum from Angus cows and Black-and-White cows, ranging from 2 ml to above 3 ml. The low thermostability of colostrum from the first milking runs is related to a higher content of whey proteins ( ZACHWIEJA et al. 2002).

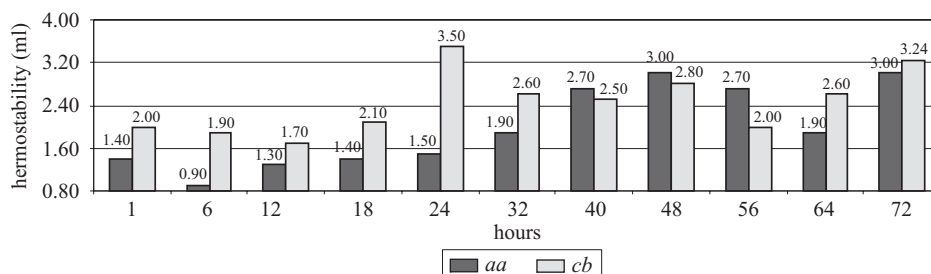


Fig. 3. Thermostability of colostrums during the first 72 hours after calving

The lowest acidity was determined in colostrum from Black-and-White cows in the first and second milking run at pH 6.2; higher acidity was observed in Angus cows at pH 6.3 (Figure 4). In successive milking runs, there were no significant differences in the level of acidity of colostrum from cows of both breeds, which remained in the range of pH 6.4 – 6.6. Colostrum was slightly acidic and its acidity varied between milking runs (from pH 6.2 to pH 6.6) within the range of values reported by other authors (CZAPLIKA

et al. 2002, WRÓŃSKI et al. 1999). A substantial increase in the milk yield of Black-and-White cows was observed between the fourth and the tenth day after calving (Figure 2). In this period, the daily yield of Angus cows ranged from 5.9 kg (day 4) to 9.7 kg (day 7) without a clear growth trend on the following days. A significant decrease in the fat content of colostrum from Black-and-White cows was reported in successive milking runs until the seventh day. Fat content was stable on the following days, reaching more than 5%. The fat content of colostrum from Angus cows varied significantly. In each milking round, the average fat content was half that reported for the Black-and-White breed.

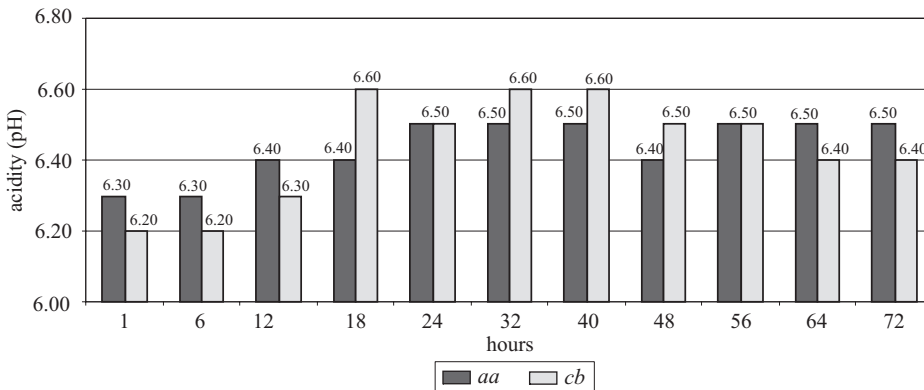


Fig. 4. Acidity of colostrums during the first 72 hours after calving

The content of total protein decreased significantly on the fourth day after calving. Protein content was stable in the subsequent milking runs. It ranged from 4.01% to 4.66% in Angus cows and was by approx. 0.8% lower in every milking run of the Black-and-White breed. The findings of other authors (CZAPLICKA et al. 2002, KAMIENIECKI et al. 1998, SABLİK et al. 2003) point to a similar decrease in the protein content of milk on successive lactation days.

The lactose content of colostrum from cows of both breeds continued to decrease on the fourth and fifth day postpartum. In successive milking runs, there were no significant differences in the level of lactose in both analyzed groups – lactose content was higher in Black-and-White cows (by around 4.5%), and ranged from 3.49% to 4.29% in milk from Angus cows. The change in dry matter content in successive milking runs resulted from changes in major milk components. In each milking run, the dry matter content of milk from Black-and-White cows was higher than in milk from Angus cows.

The specific gravity of colostrum from Angus cows on the fourth and fifth day after calving was high, reaching 1.034 – 1.05 g/ml, and a significant drop to 1.025 g/ml was observed in successive runs (until the morning run on day 7) – Figure 5. A subsequent increase was reported. The specific gravity of milk from Black-and-White cows was stable at a relatively low level, ranging from 1.026 to 1.028 g/ml. The above values are similar to the results reported for the Black-and-White breed in related literature, and are consistent with the Polish collective milk standard (*Mleko surowe. PN-A-86002 1999*).

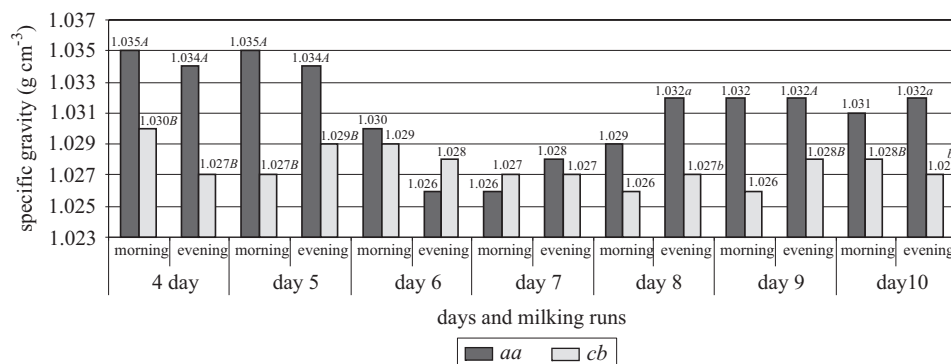


Fig. 5. Specific gravity of colostrum from 4 to 10 days after calving  
Mean values marked with different letters are significantly different: capital letters  $P \leq 0.01$ ; small letters  $P \leq 0.05$

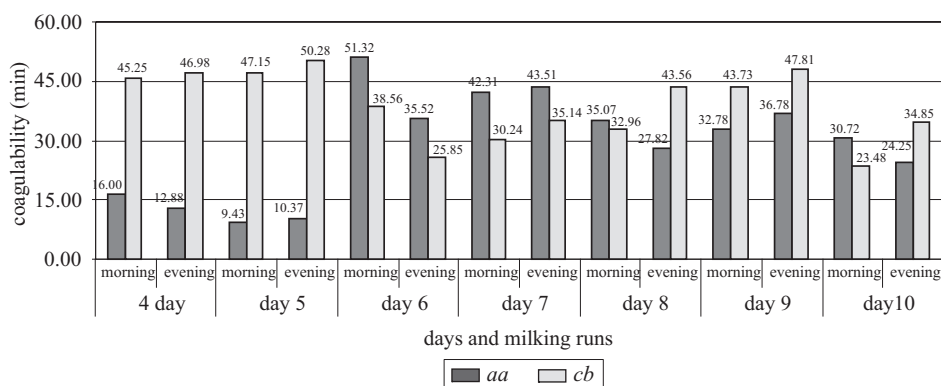


Fig. 6. Coagulability of colostrum from 4 to 10 days after calving

The coagulation time of milk and colostrum from Black-and-White cows was similar as of the fourth day postpartum, and significant fluctuations were observed in respect of the Angus breed (Figure 6). The protein coagulation

time specified by other authors covered a wide range from 2.45 min to 69.8 min (CZAPLICKA et al. 2002, WROŃSKI et al. 1999). FERTIG and FELEŃCZAK (2003) claim that milk with a short coagulation time is characterized by higher processing suitability.

In own research, the ethanol stability of bovine milk ranged from 3.2 ml (Angus) to 4.3 ml (Black-and-White) and was similar to that indicated by CZAPLICKA et al. (2002) and WROŃSKI et al. (1999) – Figure 7.

Table 2  
Changes in the composition of milk from Angus and Black-and-White cows between day 4 and day 10 after calving

Days after calving		Milk (kg)		Content in milk (%)							
				fat		total protein		lactose		dry matter	
		aa	cb	aa	cb	aa	cb	aa	cb	aa	cb
1		2	3	4	5	6	7	8	9	10	11
Day 4 morning	x	3.3 <sup>A</sup>	8.1 <sup>B</sup>	4.30 <sup>a</sup>	8.44 <sup>b</sup>	5.66	5.72	5.58	5.81	16.28	21.59
	v	54.86	20.08	57.39	14.44	14.72	39.82	30.09	39.16	21.11	25.76
evening	x	2.6 <sup>a</sup>	9.2 <sup>b</sup>	3.63	6.84	4.88	4.74	4.47	5.09	13.87	17.81
	v	35.54	39.89	79.36	3.39	8.45	21.33	6.59	24.99	24.28	12.57
Day 5 morning	x	4.4	9.9	2.99 <sup>a</sup>	7.32 <sup>b</sup>	4.64 <sup>a</sup>	3.72 <sup>b</sup>	4.41	5.33	13.06	16.24
	v	73.90	50.22	81.11	20.66	8.93	10.36	5.88	39.13	15.82	10.25
evening	x	2.8 <sup>A</sup>	12.3 <sup>B</sup>	2.49 <sup>A</sup>	6.90 <sup>B</sup>	4.52	3.65	4.32	4.42	12.23 <sup>A</sup>	15.84 <sup>B</sup>
	v	33.0	13.64	63.83	15.46	14.14	11.81	9.41	5.33	9.57	8.03
Day 6 morning	x	3.8 <sup>A</sup>	13.0 <sup>B</sup>	2.36	6.50	4.31	3.69	4.02	4.48	12.60	15.52
	v	58.05	15.49	111.8	34.71	7.74	11.86	13.71	3.24	40.68	14.10
evening	x	4.9 <sup>a</sup>	13.7 <sup>b</sup>	2.64	6.80	4.48 <sup>A</sup>	3.66 <sup>B</sup>	4.06	4.46	16.22	15.76
	v	93.02	12.34	43.83	28.44	4.52	9.97	8.66	3.60	21.85	12.28
Day 7 morning	x	4.9 <sup>A</sup>	13.1 <sup>B</sup>	2.85	5.86	4.05	3.88	3.99 <sup>a</sup>	4.56 <sup>b</sup>	14.28	15.13
	v	89.07	16.65	71.32	26.63	13.42	9.30	21.09	3.40	35.28	12.02
evening	x	4.8 <sup>A</sup>	13.0 <sup>A</sup>	2.86 <sup>a</sup>	5.68 <sup>b</sup>	4.66 <sup>a</sup>	3.86 <sup>b</sup>	3.96	4.43	13.56	14.83
	v	87.39	19.76	15.54	5.56	47.98	24.94	14.49	4.88	15.46	11.72
Day 8 morning	x	2.6 <sup>A</sup>	13.6 <sup>B</sup>	2.86 <sup>a</sup>	5.79 <sup>b</sup>	4.01	3.79	3.93	4.25	11.55 <sup>a</sup>	14.72 <sup>b</sup>
	v	52.45	17.57	24.93	21.61	19.04	8.69	7.66	16.41	5.74	6.85
evening	x	3.2 <sup>A</sup>	12.6 <sup>B</sup>	2.93 <sup>A</sup>	6.05 <sup>B</sup>	4.34 <sup>a</sup>	3.52 <sup>b</sup>	4.29	4.41	12.33 <sup>a</sup>	14.86 <sup>b</sup>
	v	55.47	33.00	40.41	5.56	11.13	7.28	8.77	11.42	13.01	2.41
Day 9 morning	x	4.0 <sup>A</sup>	13.1 <sup>B</sup>	2.84	5.11	4.23 <sup>a</sup>	3.47 <sup>b</sup>	4.25	4.53	13.14	13.97
	v	13.02	21.50	98.43	20.14	6.42	13.80	7.16	5.42	31.83	6.97
evening	x	3.6 <sup>A</sup>	13.7 <sup>B</sup>	2.93	5.38	4.29 <sup>A</sup>	3.45 <sup>B</sup>	4.25	4.56	12.18	14.26
	v	34.76	21.56	70.06	10.93	9.31	5.53	10.19	4.87	19.16	5.09
Day 10 morning	x	3.5 <sup>A</sup>	14.0 <sup>B</sup>	2.83	4.84	4.13 <sup>A</sup>	3.53 <sup>B</sup>	4.05	4.59	11.70	13.81
	v	36.47	9.97	61.06	24.55	6.90	5.28	10.82	4.31	20.87	9.18
evening	x	3.2 <sup>A</sup>	14.2 <sup>B</sup>	2.59 <sup>a</sup>	5.35 <sup>b</sup>	4.28 <sup>A</sup>	3.52 <sup>B</sup>	4.19	4.56	11.79	14.28
	v	38.86	14.76	55.31	17.93	5.21	8.81	10.76	4.50	17.91	6.45

The values within a row marked with different letters are significantly different: capital letters  $P \leq 0.01$ ; small letters  $P \leq 0.05$

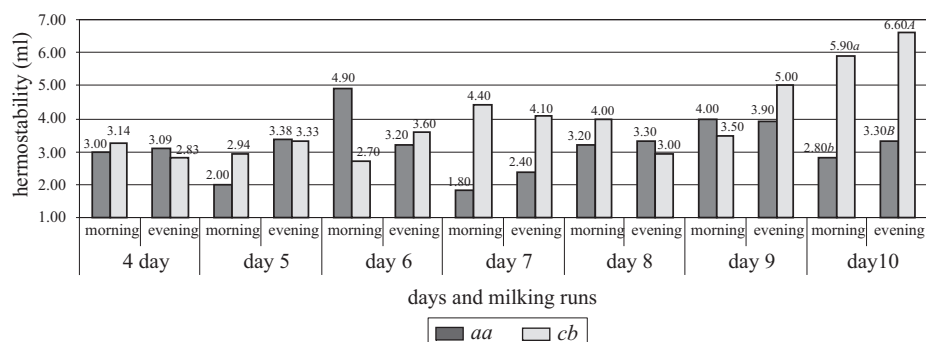


Fig. 7. Thermostability of colostrum from 4 to 10 days after calving  
Mean values marked with different letters are significantly different: capital letters  $P \leq 0.01$ ; small letters  $P \leq 0.05$

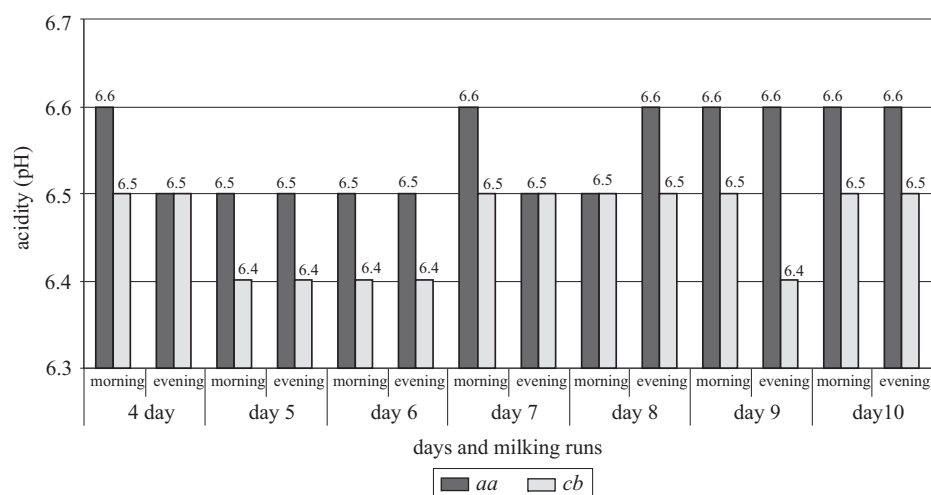


Fig. 8. Acidity of colostrum from 4 to 10 days after calving

The analyzed milk samples were characterized by active acidity which fulfils milk purchasing criteria (Figure 8). It was similar in both breeds and ranged from pH 6.4 to pH 6.6.

To conclude, it was observed that on the first ten days after calving, colostrum and milk from Angus cows were characterized by a lower fat and lactose content and a higher protein content in comparison with the Black-and-White breed. The technological properties of milk and colostrum from both breeds were less differentiated. Colostrum from cows of both breeds obtained in the first twelve hours postpartum was marked by a high content of protein

and whey proteins which were indicative of high colostrum quality. The high quality of colostrum from cows of the Angus breed was maintained until the fifth day after calving. It may be assumed that the biological quality of colostrum and milk from Angus cows was higher than from Black-and-White cows, as indicated by the high specific gravity and high total protein content. The obtained results show that long-term selection for increased yield resulted in significant changes in the composition of bovine colostrum and milk.

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## BACTERIAL POLLUTION OF AIR IN HEALTH RESORT CIECHOCINEK

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**Key words:** microbiological air pollution, air microflora, bacteria, actinomycetes, Ciechocinek health resort.

### Abstract

The microbial community of the air in the Ciechocinek resort was analyzed. Measurement sites were located in the surroundings of inhalators (graduation towers and the "Grzyb" fountain), in the recreation areas (promenade, parks), and urban areas. Microbiological analyses included enumeration of mesophilic bacteria, actinomycetes, mannitol-positive staphylococci, and hemolytic bacteria. The analyses were conducted in accordance with Polish Standard PN89/Z-04111/02. It was concluded that the abundance of bacteria was affected by meteorological conditions (temperature) and the intensity of tourism. The lowest numbers of the investigated types of bacteria were found around the open inhalators.

## MIKROBIOLOGICZNE ZANIECZYSZCZENIE POWIETRZA W UZDROWISKU CIECHOCINEK

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**Słowa kluczowe:** mikrobiologiczne zanieczyszczenia powietrza, mikroflora powietrza, bakterie, promieniowce, uzdrowisko Ciechocinek.

### Abstract

Badania mikroflory powietrza prowadzono na terenie uzdrowiska Ciechocinek. Stanowiska badawcze zlokalizowano w otoczeniu otwartych inhalatoriów (tężnie i fontanna „Grzyb”), na terenach uzdrowiskowych (deptak, parki) oraz typowo miejskich. Badania mikrobiologiczne obej-

mowały oznaczenie liczebności bakterii mezofilnych, promieniowców, gronkowców manitolododatnich i bakterii hemolizujących. Przeprowadzono je zgodnie z Polską Normą PN89/Z-04111/02. Stwierdzono, że na liczebność bakterii w powietrzu wpływ miały warunki meteorologiczne (temperatura) oraz intensywność ruchu turystycznego. Najmniejszą liczbę badanych grup bakterii stwierdzono w otoczeniu otwartych inhalatoriów.

## Introduction

Ciechocinek is the largest Polish lowland health resort and is visited by ca. 70 thousand people annually. Rich deposits of brine and favorable climatic conditions are among the curative assets of Ciechocinek. The Ciechocinek spas offer treatment for cardiovascular diseases, orthopedic injuries, arthritis, diseases of the nervous system, and women's diseases. Because the health-resort has open inhalators (graduation towers and "Grzyb"), patients with respiratory system diseases also find relief here. Chronic pharyngitis, laryngitis, tracheitis, otitis, and bronchitis among other respiratory problems are treated in the resort (LEBIEDZIEWICZ 2001).

A unique microclimate prevails in the zone surrounding the graduation towers. Graduation towers are the second link in the salt production process. The tower has a wooden structure, and brine flows down through blackthorn twigs situated between the frame ribs. Salt crystallizes as a result of water evaporation from the brine. Micro-crystals of salt settle on pillars of the frame or are carried by the wind and fall further away. The majority of the drops and salt crystals were found within 10 m of the graduation tower (KORZENIOWSKI 1997, TŁOCZEK 2000). The curative aerosol produced by the graduation towers is rich in particles of iodine, bromine, and ozone, and is also antibacterial. While spending time around the graduation tower, one can inhale the curative aerosol in a natural environment (LEBIEDZIEWICZ 2001).

Due to the scenic surroundings and diverse flora, the entire town functions as a health resort. The microbiological purity of the air, not only around the graduation towers but also in the entire town, is essential for the patients. Therefore, the purpose of this study was to examine the microbiological pollution of the air in Ciechocinek, paying particular attention to the presence of bacteria indicating air pollution with bioaerosols that originated from respiratory tracts (mannitol-positive staphylococci and hemolytic bacteria).

## Materials and Methods

### Object of the study

The town of Ciechocinek is located in Kujawy, on the left bank of the Vistula river, 25 kilometers from Toruń (52°52' N, 18°47' E). The Ciechocinek area is characterized by exceptionally high annual mean temperature (ca. +8°C) and is among the warmest regions in the lowland belt. The region is also characterized by low air humidity and unusually low precipitation; its annual total rarely exceeds 500 mm (LEBIEDZIEWICZ 2001).

Ten measurement sites were established in the Ciechocinek area (Figure 1).

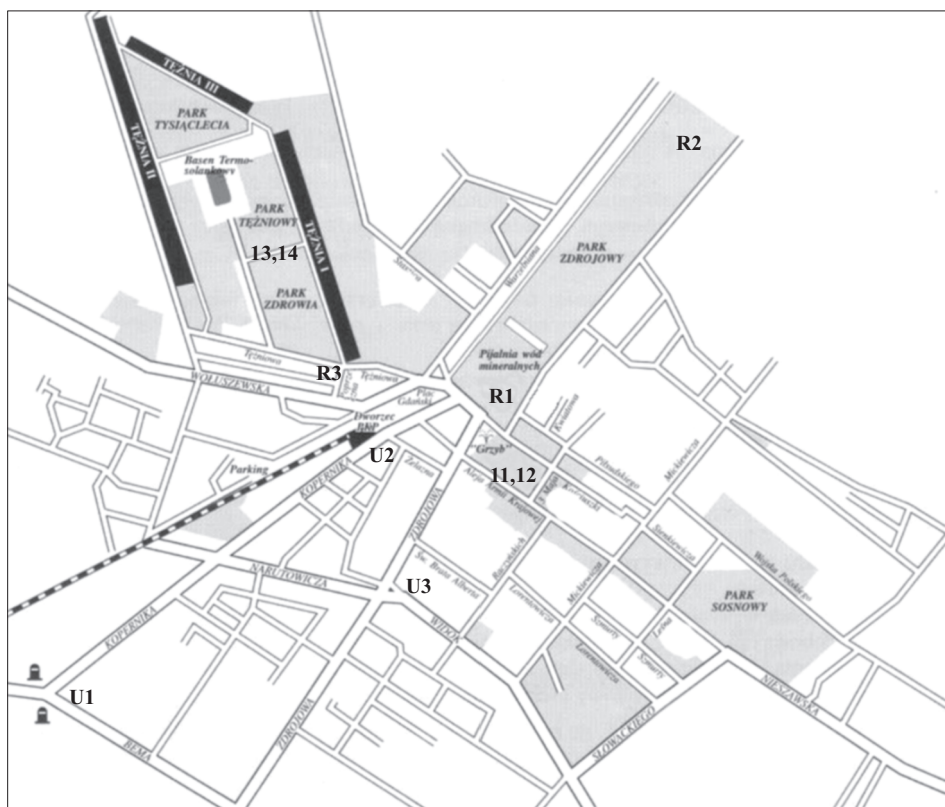


Fig. 1. The map of measurement sites in Ciechocinek health resort

Four stations were located within the zone affected by the natural inhalators: 1 m (I1) and 10 m (I2) from the “Grzyb” fountain (brine source no. 11), 1 m from graduation tower no I, on the side of the brine flow (I3), and 1 m from graduation tower no. I, on the side without the brine flow (I4). Stations located within the recreation area were situated in the Park Zdrojowy in front of Pijalnia Wód (Pump Room) (R1), by the restaurant “Oaza” (R2), and on the promenade by Tężniowa street (R3). Stations located in the town were situated at the entrance to Ciechocinek from the Toruń side, next to the intersection of Kopernik and Bem streets (U1), on Kopernik street, across from the railway station (U2), and on Widok street (U3).

### Sample collection

The air samples were collected monthly according to the Polish Standard (*Ochrona czystości powietrza*. PN-89/Z-04008/08) 1.3 m above the ground level between May, 2005 and April, 2006. The samples were obtained by the impaction method in the microbial airsampler Merck Mas-100. The air flow velocity equalled 11 m s<sup>-1</sup>. This enabled detection of particles larger than 1 µm, which was important for translocation of microorganisms.

The following meteorological parameters were measured during the sample collection: air temperature, relative humidity, and wind velocity. These measurements were carried out using a Nielsen-Kellerman anemometer, Kestrel 3500 (Table 1).

Table 1  
Meteorological parameters during the sample collection

	Temperature (°C)	Humidity (%)	Wind speed (m s <sup>-1</sup> )
12.05.2005	11.6	66.3	0.7
14.06.2005	20.7	59.5	1.1
04.07.2005	27.3	51.2	1.2
05.08.2005	24.9	52.2	1.1
28.09.2005	20.4	64.8	1.0
21.10.2005	12.6	60.7	1.2
25.11.2005	1.7	73.0	1.3
07.12.2005	1.5	79.3	0.7
19.01.2006	-6.8	74.6	1.9
27.02.2006	-2.8	72.6	3.3
23.03.2006	7.7	57.9	0.5
10.04.2006	10.2	54.8	0.9

## Microbiological analyses

The microbiological analyses included enumeration of:

a) mesophilic bacteria – according to the Polish Standard (*Ochrona czystości powietrza...* PN 89/Z-04111/02), bacteria were cultured on nutrient agar for 48 h at 37°C;

b) actinomycetes – cultured on Pochon medium (*Ochrona czystości powietrza...* PN 89/Z-04111/02) for 5 days at 26°C

c) mannitol-positive staphylococci – according to Polish Standard (*Ochrona czystości powietrza...* PN 89/Z-04111/02) on Chapman substrate; the microorganisms were cultured for 48 h at 37°C;

d) hemolytic bacteria – according to the Polish Standard (*Ochrona czystości powietrza...* PN 89/Z-04111/02); bacteria were cultured on agar with blood for 48 h at 37°C.

All results were converted to the number of cfu in 1 m<sup>3</sup> of air. The results were analyzed in STATISTICA 6.0.

## Results

The abundance of mesophilic bacteria in Ciechocinek ranged from 0 cfu m<sup>-3</sup> in the recreation area in February to 1300 cfu m<sup>-3</sup> in the town area in June. The highest numbers of mesophilic bacteria in the entire investigated area were observed between August and October, and the lowest – in January and February (Figure 2). The lowest abundances of mesophilic bacteria were observed July, when air temperature was high and the humidity was at the low level (Figure 2, Table 1).

The highest abundances of actinomycetes occurred in the air from July to November (Figure 3), with the highest concentration (27 cfu m<sup>-3</sup>) observed in August in the recreation area. The abundance of actinomycetes in specific areas of Ciechocinek varied considerably through time and was probably affected by local and temporary meteorological conditions, in particular gusts of wind.

At all research stations, the greatest abundances of mannitol-positive staphylococci were observed in October. In the stations located in the recreation area of Ciechocinek, the mean abundance of these organisms reached 140 cfu m<sup>-3</sup> (Figure 4). In contrast, in January, no staphylococci specimens were found in the air at any of the stations. Furthermore, staphylococci bacteria were absent from the area influenced by inhalators in February and March.

On average over the entire study period, the authors observed higher numbers of  $\beta$ -hemolytic (0-62 cfu m<sup>-3</sup>) than  $\alpha$ -hemolytic bacteria (0-23 cfu m<sup>-3</sup>) (Figure 5 a, b). Hemolytic bacteria were much more numerous in the air

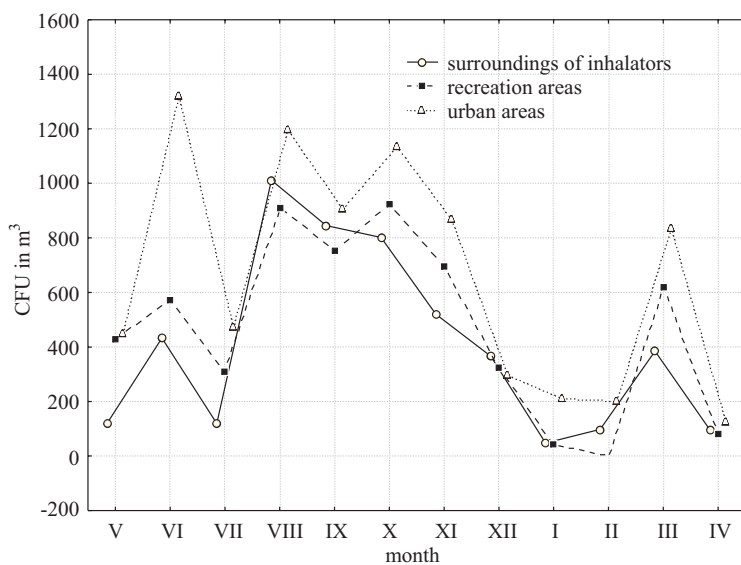


Fig. 2. Number of mesophilic bacteria in air of Ciechocinek

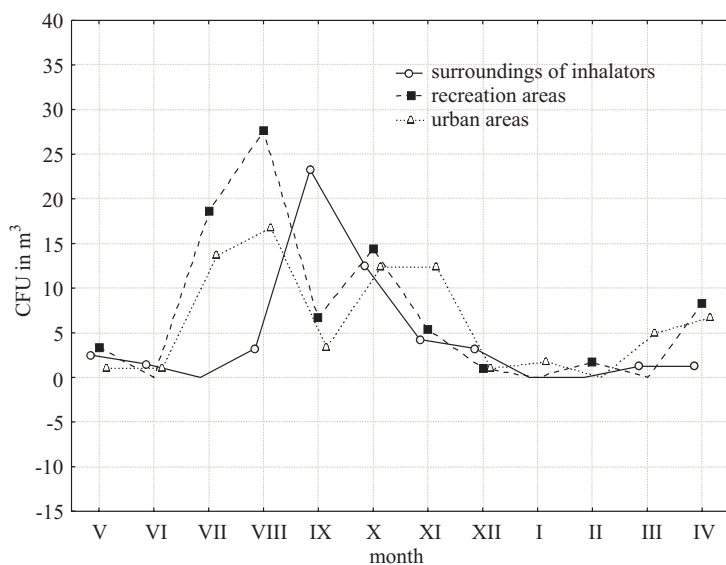


Fig. 3. Number of actinomycetes in air of Ciechocinek

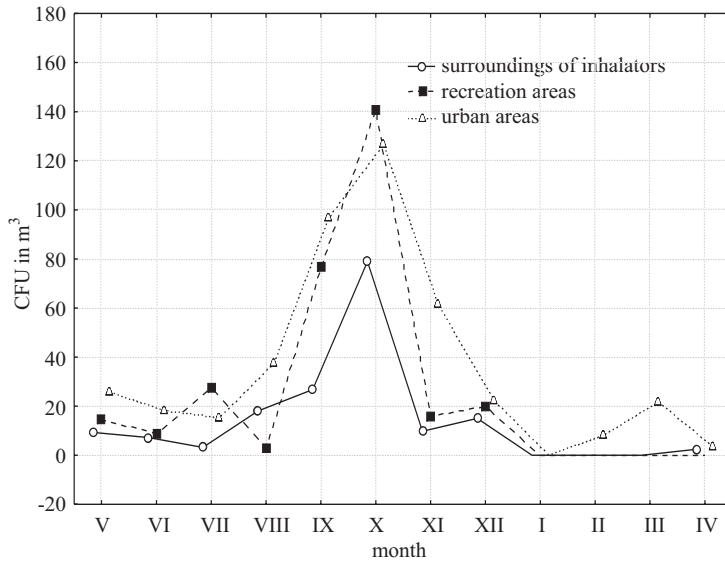


Fig. 4. Number of mannitol-positive staphylococci in air of Ciechocinek

in spring and summer months. An unusually high abundance of  $\beta$ -hemolytic staphylococci was observed in the urban area during April, 61 cfu m<sup>-3</sup>.

Based on the two way analysis of variance, it is evident that the location of research stations in specific sections of Ciechocinek and the sample collection period had a statistically significant impact ( $p < 0.05$ ) on the abundance of bacteria in the air (Table 2). The actinomycetes were an exception; no significant differences were observed in their abundances in specific sections

Table 2  
2-way ANOVA test comparing the influence of the sample collection period (month) and the location of research stations in specific sections of Ciechocinek on the numbers of bacteria in air

Microorganisms	Factor	df	Variation	F ratio	P – level
Mesophilic bacteria	month	11*	1168933*	160.73*	0.000000*
	location	2*	741580*	101.97*	0.000000*
Actinomycetes	month	11*	295.40*	44.51*	0.000000*
	location	2	86.98	13.11	0.061073
Mannitol-positive staphylococci	month	11*	11012.89*	213.18*	0.000000*
	location	2*	5093.00*	98.59*	0.000000*
$\alpha$ -hemolytic bacteria	month	11*	270.75*	12.87*	0.000000*
	location	2*	136.56*	6.49*	0.002387*
$\beta$ -hemolytic bacteria	month	11*	654.18*	36.63*	0.000000*
	location	2*	625.78*	6.45*	0.002268*

\* statistically significant impact ( $p < 0.05$ )

of the resort. The highest abundances of mesophilic bacteria, mannitol-positive staphylococci, and hemolytic bacteria were observed at stations located in the urban area of Ciechocinek, with the lowest, around the open inhalators. Only in the case of actinomycetes was the highest annual mean abundance observed at the research stations located in the recreation area, that is, primarily in the parks (Table 3).

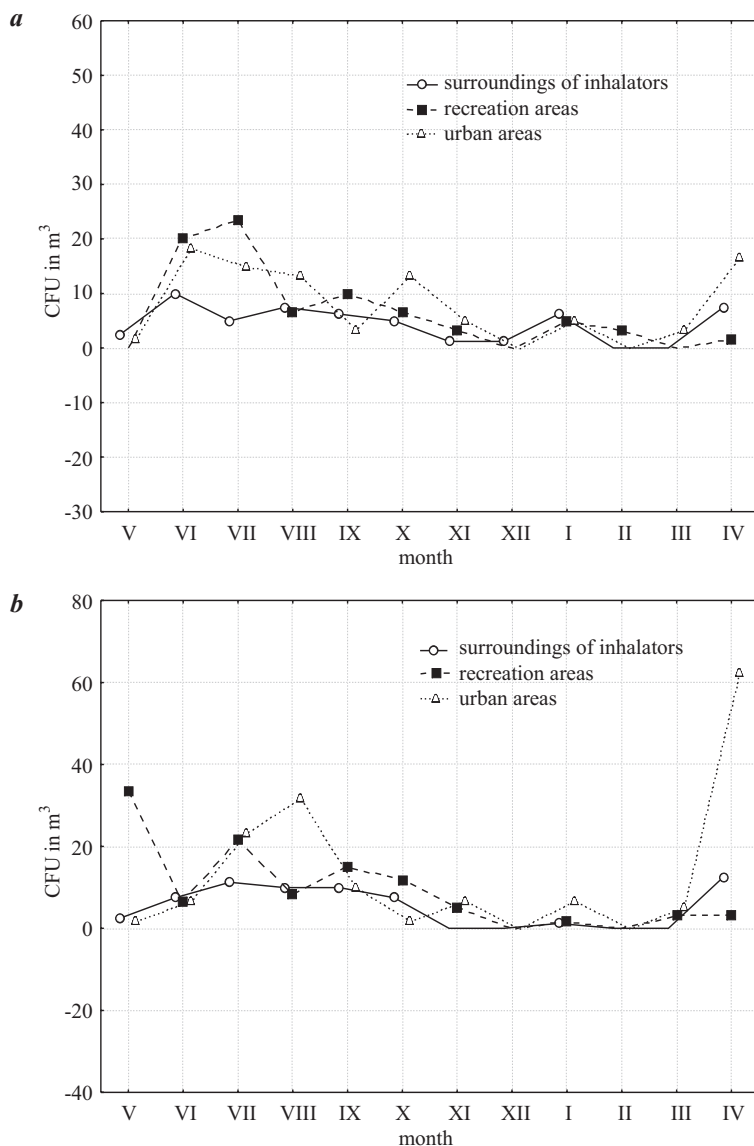


Fig. 5. Number of hemolytic bacteria in air of Ciechocinek: *a* –  $\alpha$ -hemolytic bacteria, *b* –  $\beta$ -hemolytic bacteria



Table 3  
Number of microorganisms (cfu m<sup>-3</sup>) in specific sections of Ciechocinek – average ( $\pm$ SD) from the entire study period

Microorganisms	The surroundings of inhalators	The recreation areas	The urban areas
Mesophilic bacteria	403.6 ( $\pm$ 331.4)	471.2 ( $\pm$ 321.4)	667.4 ( $\pm$ 420.2)
Actinomycetes	4.4 ( $\pm$ 6.9)	7.3 ( $\pm$ 8.7)	6.2 ( $\pm$ 6.3)
Mannitol-positive staphylococci	14.2 ( $\pm$ 21.7)	25.6 ( $\pm$ 41.3)	36.4 ( $\pm$ 38.9)
$\alpha$ -hemolytic bacteria	4.4 ( $\pm$ 4.6)	6.6 ( $\pm$ 8.6)	7.9 ( $\pm$ 7.7)
$\beta$ -hemolytic bacteria	5.2 ( $\pm$ 5.9)	9.1 ( $\pm$ 10.3)	12.9 ( $\pm$ 18.1)

Furthermore, it was found that the abundances of various bacterial groups and the air temperature were positively correlated. The correlation coefficient  $r$  ranged from 0.22 for mannitol-positive staphylococci to 0.48 for mesophilic and  $\beta$ -hemolytic bacteria; these values were statistically significant at  $p < 0.05$ .

## Discussion

According to the classification of air quality prepared by Inspekcja Ochrony Środowiska (Environmental Protection Agency), the spa areas are must be evaluated separately (*Ocena jakości powietrza...* 2004). In such areas, air quality is only assessed with respect to dustiness and chemical parameters, primarily the concentration of SO<sub>2</sub>, NO<sub>2</sub>, and CO (*Program monitoringu...* 2004). However, in addition to chemical pollution, organic pollutants, including potentially pathogenic microorganisms (viruses, bacteria, fungal hyphae and spores), are present in the air in the form of bio-aerosols. Many researchers suppose that microbiological evaluation of air quality is fully justified from an epidemiological and economical point of view (KRZYSZTOFIK 1992, KAŻMIERCZUK et al. 2004, KOŁWZAN et al. 2005). Nevertheless, in health resort areas, microbiological investigations are not conducted and are not considered important in evaluating air quality.

Publications regarding microbiological air pollution in urban areas are scarce. The majority of the published data concerns municipal facilities: sewage treatment plants and dumps (BUTAREWICZ 1999, FRĄCZEK et al. 2004, KAŻMIERCZUK et al. 2004). As a result, it is difficult to compare the obtained results.

However, the results presented above demonstrate that due to the curative character of the town the air in Ciechocinek (even in the urbanized sections of the town) can be considered clean in comparison to the air in large and densely populated urban areas. The abundance of mesophilic bacteria in the

Ciechocinek spa did not exceed 1300 cfu m<sup>-3</sup>. FILIPIAK et al. (2004) and BUGAJNY et al. (2005) report results of a similar study conducted in Poznań with abundances an order of magnitude higher: in summer, the abundance of mesophilic bacteria reached 13000 cfu m<sup>-3</sup>.

The abundance of actinomycetes in specific areas of Ciechocinek varied considerably in time and was affected by local, transient meteorological conditions, gusts of wind in particular. The highest annual mean abundance of actinomycetes was observed at stations located in the spa areas, that is, primarily in parks, where the air can be easily polluted by soil particles as a result of wind gusts.

In considering the presence of actinomycetes in the air, it should be emphasized that KAŻMIERCZUK et al. (2004) found these bacteria to be common organisms that occur in atmospheric aerosols and cannot be treated as a valuable indicator of the sanitary air quality. Furthermore, MARCINKOWSKA et al. (2004) concluded that species of actinomycetes that were isolated from the air are not hazardous from an epidemiological point of view, however, allergenic species could be among them.

In the Ciechocinek air,  $\beta$ -hemolytic bacteria were higher in abundance than  $\alpha$ -hemolytic bacteria. According to PN89/Z-04111/02, the presence of the  $\beta$ - and  $\alpha$ -hemolytic bacteria indicates that the air is polluted with particles that originate from respiratory tracts of humans and animals. KARWOWSKA (2003) demonstrated through experiments on air in closed spaces that abundance of these bacteria is closely linked to the number of people, who constitute a potential source hemolytic bacterial emission. As a result, the highest abundances of hemolytic bacteria, which indicate significant air pollution levels, were observed in the peak of the tourist season in Ciechocinek, that is, in July and August (oral information – Przedsiębiorstwo Uzdrowisko Ciechocinek S.A.).

The analysis of variance demonstrated that the location of sample collection has a statistically significant impact on the abundance of the majority of investigated bacterial groups in the air. The concentration of microorganisms in the air is probably affected by aerosol produced in the open inhalator areas. The presence of brine aerosol particles accelerates the pollutant sedimentation including cells of microorganisms present in the air. Furthermore, the aerosol produced in a graduation tower is antibacterial due to the presence of particles of iodine and bromine, among others (BURKACKA-ŁAUKAJ-TYS, ZWOLSKA 1973, TŁOCZEK 2000). Therefore, the lowest concentrations of all investigated groups of bacteria were most often observed at the stations located in the vicinity of the open inhalators.

In interpretation of the above results, one should take into account the temporary and stochastic character of airborne microflora, which

is affected by physical and chemical properties of the air and topographic and microclimatic conditions of the investigated area (COX 1989). Therefore, the presented results reflect momentary and approximate values that correspond to the microbiological pollution that occurs at a given moment in a specific area.

## Conclusions

The microbiological air quality is beneficial for curative character of Ciechocinek. Aerosol produced in open inhalators has a positive effect on the microbiological condition of the air: in areas within their operation the concentration of the researched microorganisms was lower than in other sections of the resort. The abundance of microorganisms in the resort air was affected by meteorological conditions, particularly temperature. The level of air pollution with bacteria that indicate the presence of bio-aerosols from respiratory tracts was influenced by the increased numbers of tourists, which was also partially affected by the weather conditions.

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## VARIABILITY IN PARTICULATE MATTER CONCENTRATIONS VERSUS PRECIPITATION IN POMERANIA REGION

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Key words: suspended dust, totals and days with precipitation, regression analysis.

### Abstract

The study has been aimed at determining variability in suspended particulate matter PM10 and TSP and its relation with the precipitation sums and precipitation frequency, expressed by means of the number of days with 24 hr precipitation of a diverse amount. Systematic readings from daily (24hours) measurements of suspended particulate matter concentrations recorded at 8 State Environmental Monitoring stations in Pomerania in years 1993-2002, as well as daily total precipitation data recorded in 8 IMGW meteorological stations, the latter located closely to the immision sites, were applied in the study. The principal analysis was carried out for PM10 dust, i.e. suspended dust of the aerodynamic grains diameter of up to 10  $\mu\text{m}$  and TSP dust i.e. totally suspended dust measured by the gravimetric or automatic method. Between 1993 and 2002 the PM10 concentration was on average by 20 to 40% lower than TSP concentration, whereas its variability tended to be twice as big. Average daily concentration varied typically from 15 to 35  $\mu\text{m}^{-3}$ , and from 45 to 60  $\mu\text{m}^{-3}$ , for PM10 and TSP, respectively. Precipitation, in terms of total precipitation value and the number of days when precipitation occurred, were found not to impact TSP concentration in terms of statistical significance. Nevertheless, its contribution to reducing PM10 concentration was significant. Such a role of precipitation was particularly pronounced when set against the number of days with precipitation not lower than 0.5 mm, especially in winter, and in August and January – as far as the monthly approach is concerned. Further research into precipitation air quality improving role with regards to factors disregarded in the present study such as precipitation duration and intensity characteristics, would certainly be required.

## ZMIENNOŚĆ STĘŻENIA PYŁU ZAWIESZONEGO NA POMORZU W ZALEŻNOŚCI OD OPADÓW ATMOSFERYCZNYCH

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**Słowa kluczowe:** pył zawieszony, sumy i dni z opadem, analiza regresji.

### Abstrakt

Celem pracy było określenie zmienności stężenia pyłu zawieszonego PM10 i TSP w zależności od sum opadów oraz częstości ich występowania, wyrażonej liczbą dni z opadem dobowym różnej wielkości. Wykorzystano dobowe wyniki pomiarów stężenia pyłu zawieszonego z 8 stacji pomiarowych Państwowego Monitoringu Środowiska z terenu Pomorza z lat 1993-2002 oraz dobowe sumy opadów atmosferycznych z 8 stacji meteorologicznych IMGW, położonych najbliższej stacji imisyjnych. Zasadniczą analizę przeprowadzono dla pyłu PM10 (pyłu zawieszonego o średnicy aerodynamicznej ziaren do 10  $\mu\text{m}$ ) oraz pyłu TSP (pyłu zawieszonego ogółem, mierzonego metodą wagową lub automatyczną). W latach 1993-2002 stężenie pyłu PM10 było przeciętnie 20-40% mniejsze niż pyłu TSP, ale wykazywało ponad dwukrotnie większą zmienność. Średnie dobowe stężenie pyłu PM10 wynosiło przeważnie 15-35  $\mu\text{m}^{-3}$ , natomiast pyłu TSP – 45-60  $\mu\text{m}^{-3}$ . Warunki opadowe opisane sumami opadów oraz liczbą dni z opadem nie miały statystycznie istotnego wpływu na stężenie pyłu TSP, natomiast przyczyniały się istotnie do zmniejszenia stężenia pyłu PM10. Najlepszy opis wymywającej roli opadów uzyskano za pomocą liczby dni z opadem dobowym co najmniej 0,5 mm, szczególnie podczas kalendarzowej zimy, a w ujęciu miesięcznym – w sierpniu i w styczniu. Do pełniejszej oceny wymywającej roli opadów atmosferycznych, oprócz sum opadów oraz liczby dni z różnej wielkości opadami dobowymi, należałoby uwzględnić również charakterystykę czasu trwania i natężenia opadów.

## Introduction

Particulate matter emission in Pomerania region is relatively low when compared to more industrialised regions of Poland, and it contributes to 16% of the country emission (*Rocznik Statystyczny*. 2002). The level of industrial air pollutants, in particular the ones of combustion related origin, has been found progressively reduced for the past few years. A slight reduction in particulate matter emission due to mobile sources (CZARNECKA, KALBARCZYK 2004) has been also observed. Lower particulate matter emission into the atmosphere, as well as highly reduced air pollutants from professional power sources, have contributed to lowering particulates immission. However, despite improved air quality, suspended particulates matter toxic impact has been growing, particularly in big cities. Particulate matter has an adverse effect not only on human health, but also on the vegetation, soil, water, buildings and technical facilities (JUDA-REZLER 2000, MAJEWSKI 2005). Adverse effects of air pollutants on the atmosphere are determined not only by the emission size, but also by

the current weather conditions, which though normally tend to disperse pollutants, may as well increase the air pollution level, or even lead to the occurrence of more aggressive chemicals (GONÇALVES et al. 2005). It was confirmed that particulates can travel over a distance of thousands of kilometres (QUARG 1996, RYALL et al. 2002, RODRIGUEZ et al. 2004). Among the weather factors such as the wind direction and force, air humidity, precipitation, air temperature and intense solar radiation (VAN DER WAL, JANSSEN 2000, ELMINIR 2005, TURAHOĞLU et al. 2005) had been determined as the major ones affecting particulates concentrations. Precipitation occurrence is an important atmosphere clearing process, in which precipitation particles collide with particulate matter particles and wash them down. It is understandable that for a mechanical process of clearing the air, long lasting, moderate or intense precipitation play the vital role, and their positive effect on air quality is prolonged. High TSP concentration are observed mainly during days when no precipitation occurs, in high pressure weather conditions (BLAŽEK et al. 1999, WALCZEWSKI 2000, YANG 2002, TURAHOĞLU et al. 2005).

The study has been aimed at determining variability in suspended particulate matter PM<sub>10</sub> and TSP and its relation with the precipitation sums and precipitation frequency, expressed by means of the number of days with 24 hr precipitation of a diverse amount.

## Materials and Methods

Systematic readings from daily (24 hours) measurements of suspended particulates concentrations recorded at 8 measurements sites of the Main Inspectorate for Environmental Protection, recorded for State Environmental Monitoring purposes, over Pomerania, in years 1993-2002, were analysed, as well as daily total precipitation data recorded in 8 IMGW meteorological stations, the latter located closely to the immission sites. The studied immission material was uniform neither with regards to the type of particulates under measurement, nor applied measurement methodology. Measurements comprised both TSP concentration measured with either a weight or automatic method, as well as BS determined with a reflectometry, and suspended particulate concentration with separation for particulates size lower than 10 µm (PM<sub>10</sub>), performed with a weight reference method or with automatic measuring devices (SKOTAK et al. 2002). Unfortunately, in the database collected over a decade, BS measurement results contributed to the major part of it (nearly 58%). Though since 1998 such results are incorporated into the Environmental Protection Inspection (IOŚ) reports over the elementary network, they significantly deviate from the weight and automatic measurements

(SKOTAK et al. 2002). Therefore, for the present study only the results on TSP and PM10 were taken into consideration, with separate analysis performed for each group. The TSP results were based on measurements from Gdynia and Gdańsk, and the sample consisted of 2943 elements, whereas PM10 measurements were mainly from Szczecin and Piła, and for years 2001-2002 Bydgoszcz, Gdańsk and Gdynia measurements were also included and they comprised a data set of 5562 elements. All the measurement stations in this study were located in urban areas, where suspended particulate matter concentrations nearly double the values for rural areas (SKOTAK et al. 2002).

The allowable dose for TSP, as provided in the *Rozporządzenie Ministra Ochrony Środowiska, Zasobów Naturalnych i Leśnictwa z dnia 28.04.1998 r. (Dz. U. No 55 it. 355)* is equal to  $150 \mu\text{g m}^{-3}$  and  $75 \mu\text{g m}^{-3}$  for daily and annual concentrations, respectively, whereas for PM10 it provides respectively  $125 \mu\text{g m}^{-3}$  and  $50 \mu\text{g m}^{-3}$ . Nevertheless, according to *Rozporządzenie Ministra Środowiska z dnia 5.12.2002. (Dz. U. 2003, No 1 it. 12)* since 2003 only PM10 concentrations are covered by allowable dose. 1 hour dose is equal to  $280 \mu\text{g m}^{-3}$ , while annual to  $40 \mu\text{g m}^{-3}$ .

The impact of precipitation on the suspended particulate matter was estimated by means of a simple and multiplied regression with the significance level of  $\alpha = 0.05$ . Variability over years for suspended particulates matter was determined by means of a random variability factor (V, in %), which is the average to standard deviation ratio.

## Results and Discussion

For years 1993-2002 average seasonal concentration for PM10 usually varied between 25 and 30%, whereas a monthly average concentration (Figure 1) was by 20 to 40% lower than TSP value. The highest concentrations of suspended particulate matter, recorded for the calendar winter months (December till February), reached  $37 \mu\text{g m}^{-3}$  PM10 and  $50 \mu\text{g m}^{-3}$  TSP, whereas the lowest values were observed in summer (June – August) and were equal to  $26 \mu\text{g m}^{-3}$  and  $39 \mu\text{g m}^{-3}$ , respectively. The pattern tend to coincide with the seasonal pattern of combustion process in the municipal sector and power supplies. Slightly higher PM10 concentrations was observed in spring, and for TSP in autumn. The highest concentrations for PM10 and TSP were recorded in December and January, respectively, though readings of the same level were observed also in March and October (Figure 1). The lowest average concentrations for both types of particulates were recorded in June and July, while for TSP in August. Only in December PM10 concentration level nearly reached concentration for TSP.



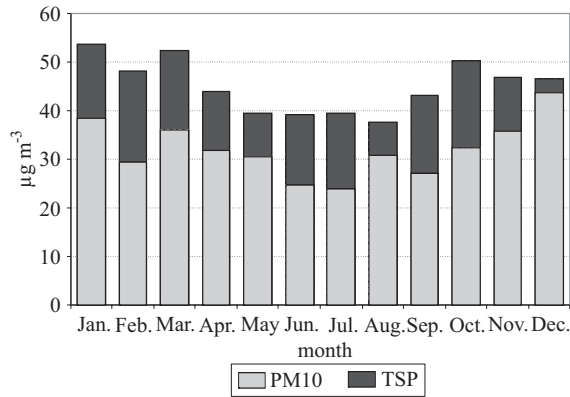


Fig. 1. Average monthly (suspended particulate matter) concentration for PM10 and TSP. Years 1993-2002

Variability of the monthly average concentrations for PM10 was generally twice as high as the total suspended particulate concentration (Figure 2), though in May and in September it tripled. Random variability coefficients for PM10 concentrations tended to vary from 35 to 65%, while for TSP they tended to fall within 20-30% range. In addition, it was found that while differences in concentration variability for TSP for all the months in general reached about 10%, random coefficients for PM10 concentrations showed contrasting values for the consecutive months of the warm season and in November, though over December – March periods the coefficients were relatively steady.

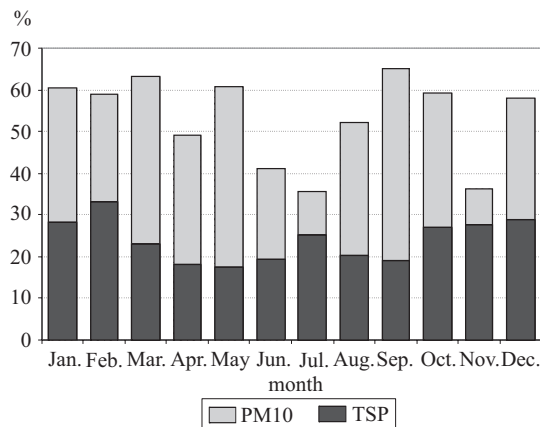
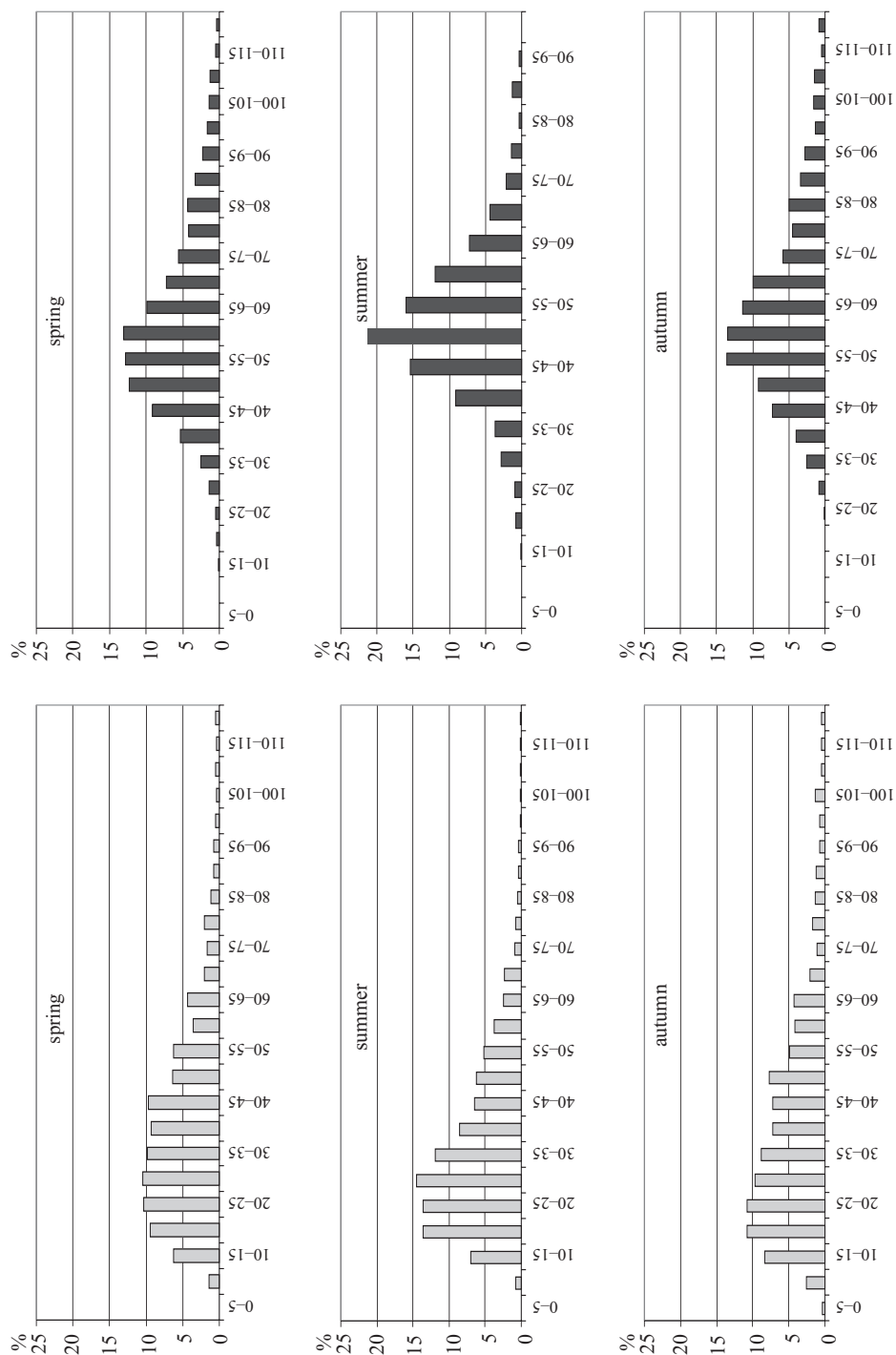


Fig. 2. Variability coefficient for (suspended particulate matter) concentration PM10 and TSP. Years 1993-2002



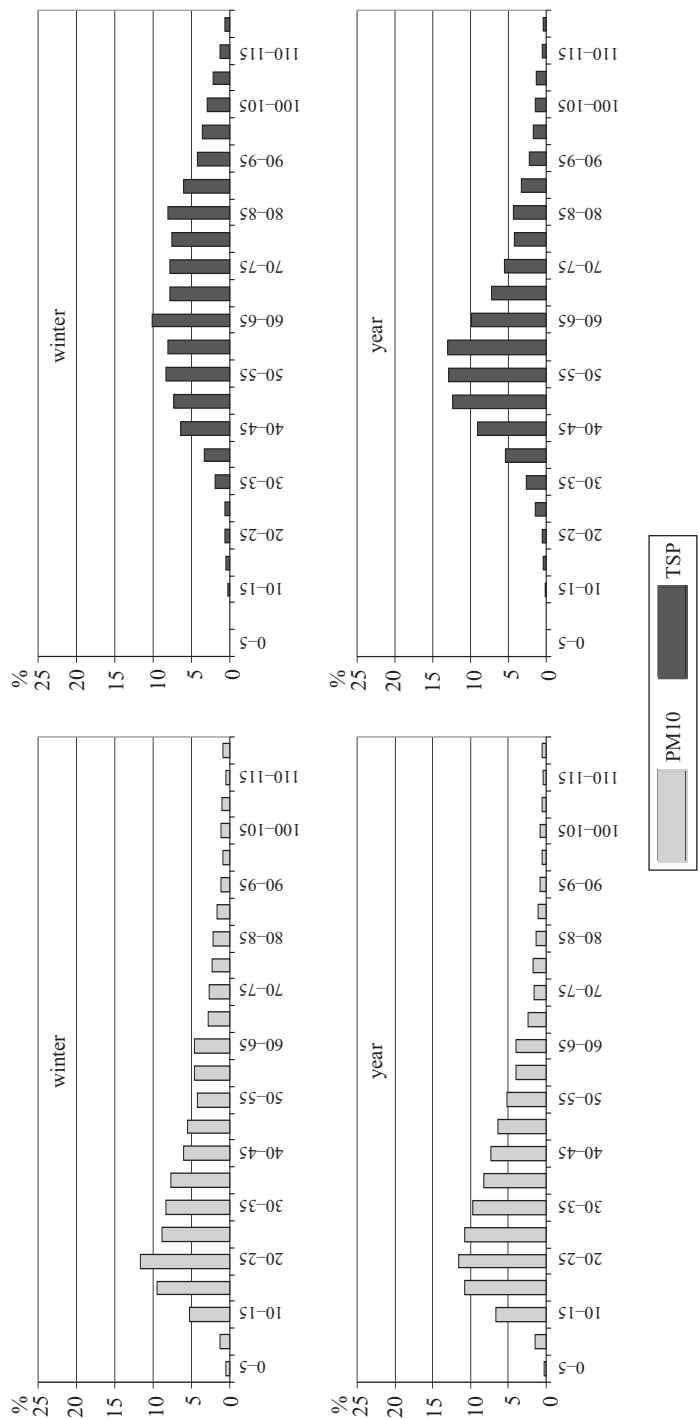


Fig. 3. Occurrence frequency (%) for daily (suspended particulate matter) concentration for PM10 and TSP within the applied ranges. Years 1993-2002

The occurrence frequency distribution patterns for the concerned classes of daily concentration were found to be different for specific types of the suspended particulates, which is shown in Figure 3. Average daily concentrations of PM10 most frequently varied from 15 to 35  $\mu\text{g m}^{-3}$ . The concerned range was most frequently pronounced in summer, while for winter time the most frequent concentration range was found to be 20-25  $\mu\text{g m}^{-3}$ , though in comparison to all the other seasons, a higher concentration exceeding 70  $\mu\text{g m}^{-3}$  was observed much more frequently to occur. In spring and autumn characteristic concentration for PM10 tended to fall within a wider range than the one for winter and summer, specifically from 15 up to 45  $\mu\text{g m}^{-3}$ , reaching even 50  $\mu\text{g m}^{-3}$ . Higher average for TSP, when compared to PM10, resulted mainly from a clearly dominant concentration from the range of nearly doubled values, from 45 to 60  $\mu\text{g m}^{-3}$ . Over calendar winter months (December – February), the daily concentration for TSP tend to reach the values from 60 to 65  $\mu\text{g m}^{-3}$ , though frequency of concentration up to 85  $\mu\text{g m}^{-3}$  occurred to be still quite high. In contrast to PM10, the major contribution of one of the typical daily ranges (45-50  $\mu\text{g m}^{-3}$ ) was clearly observed over the calendar summer. Also in spring and autumn the range of daily concentration for TSP has been found to be much narrower, namely between 50 and 60  $\mu\text{g m}^{-3}$ , than for PM10.

Comparison of concentrations for both types of suspended particulate matter, averaged over days with and without precipitation, indicated this weather factor role in clearing the air from particulate pollutants, though not always it was easy to interpret (Figure 4). Concentration for both PM10 and TSP during the days with occurring precipitation were for most months lower than in days without precipitation. Nevertheless, a reversed effect was also observed, as in January and February, in particular for TSP concentration. Still, overall the year concentrations for both particulates types under consideration was smaller for days with than without precipitation, for TSP by a mere 4%, whereas for PM10 by about 19%. TURAHÖĞLU *et al.* (2005) found that increased TSP concentration occurred in days without precipitation, with coinciding low temperature, wind and pressure. According to MICZYŃSKI (1989) findings, under mountainous conditions, the highest number of cases with average PM10 concentration occurred in days with no precipitation at all, or daily precipitation level up to 10 mm. Suspended particulate concentrations higher than the standard one, occurred solely in days with no precipitation, and has been never recorded with daily precipitation exceeding 10 mm. However, it should be noted that the presented comparison provides a limited base for concluding on a air clearing role of precipitations, since another vital factor possibly distorting such an evaluation due to being disregarded in the present study, is the emission size, which is one of the rudimentary factors shaping suspended particulate emission size.

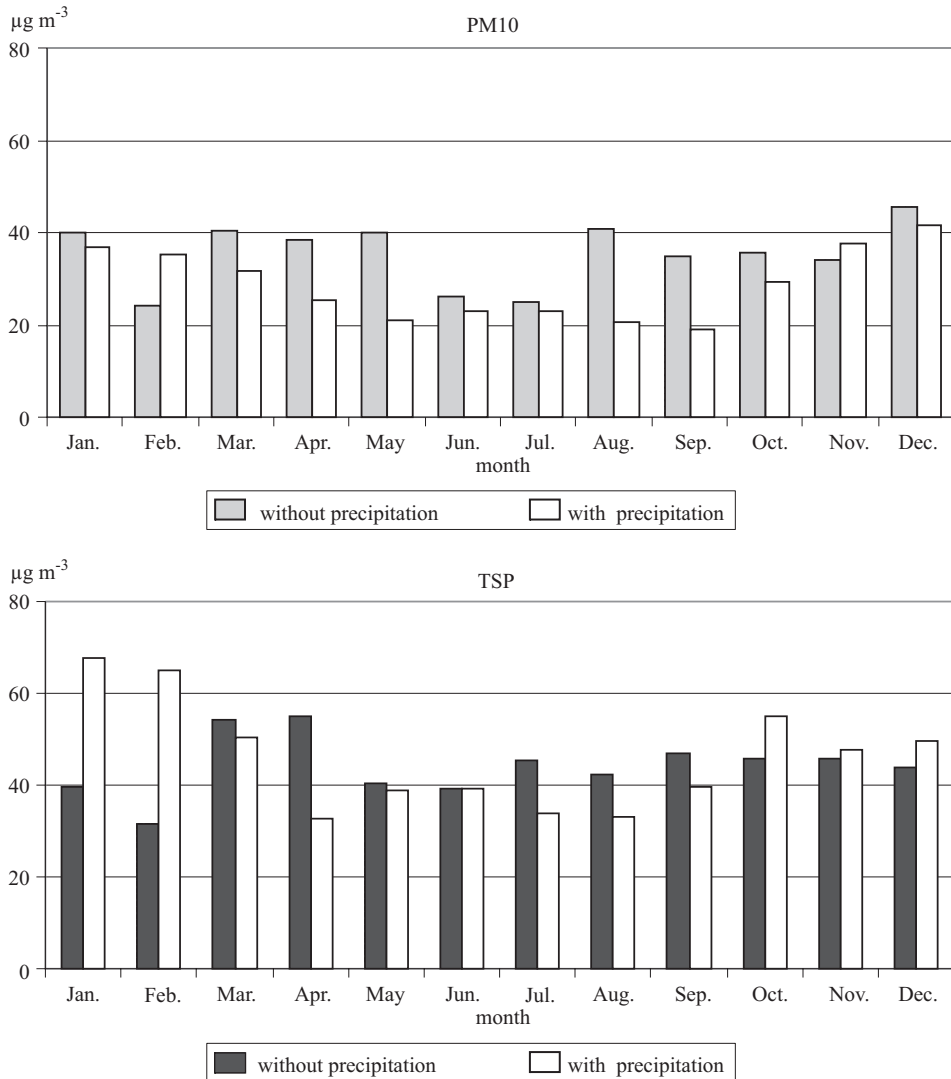


Fig. 4. Average monthly (suspended particulate matter) concentration for PM10 and TSP for days with and without precipitation. Years 1993-2002

The first approach to evaluate statistically the precipitation impact factor over suspended particulates was based on daily measurements for both variables. Correlations between suspended particulates concentration and coinciding daily total precipitations were searched, in particular with various level of daily total precipitation (of 1, 2, 3, 4, and 5 mm), as well as total precipitation over 1, 2 ... and 5 days, grouped either separately, or over periods

from 2 to 5 days preceding the concerned daily concentration of particulates. The daily approach was also aimed at assessing variability in differences in suspended particulates concentration over consecutive days of various total daily precipitation values. In all the approaches taken apart from daily precipitation analyses, analyses of particulate matter concentration, applied as a second, explanative variable, were also performed. The results from all the applied combinations were, in their major part, either statistically insignificant, or significant at  $\alpha = 0.1$ , hence a total daily precipitation proved to be an efficient indicator for demonstrating the impact of this element on neither TSP nor PM10 (particulates diameter smaller than 10  $\mu\text{m}$ ) immision. Therefore, the next stage of our research was to modify the applied earlier for months and seasons, analysis of PM10 and TSP concentration dependence on precipitation, expanding it over decade values for both variables. The analysis proved also inefficient for TSP concentration, since statistically significant results have been obtained merely for few cases, though nearly twice as small TSP base material size might have played a role in it. Whereas a significant effect of precipitation was observed in the case of PM10 suspended dust. Taking two basic features of this meteorological element into consideration, weaker relations, statistically significant only at a level of  $\alpha = 0.1$ , were obtained for the precipitation totals and much better ones for the number of days with precipitation. These results are consistent with those reported by VAN DER WAL and JANSSEN (2000).

Determinability coefficient for PM10 over the total precipitation for winter, spring and autumn were quite consistent and amounted to about 8%, whereas the weakest influence was observed for calendar summer, characterised with the highest average precipitation, coinciding with the lowest particulates concentration. In VAN DER WAL'S and JANSSEN'S research (2000) the precipitation tended to explain the change in PM10 concentration only in 5-11%. Weak correlations between TSP concentration and precipitation were also confirmed by TURAHOĞLU et al. (2005).

Determination coefficients for the dependence of the suspended particulates concentration on the number of days with daily precipitation at the minimum level of: 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mm for calendar seasons and months are illustrated in Figs. 5 and 6. All the statistically significant results showed a negative correlation for PM10 concentration on the number of days with precipitation, and the strongest influence on reducing the particulates concentration was found for the number of days with daily precipitation not lower than 0.5 mm. Daily precipitation  $\geq 0.5$  mm were most effective in clearing PM10 in winter ( $R^2$  about 26%), and the least effective in spring ( $R^2$  about 11%) (Figure 5). Determination coefficients for PM10 concentration versus daily precipitation of 0.5 mm for the periods of calendar

summer and autumn were found similar (both about 20%). A statistically significant dependence in variability for PM10 concentration, both for calendar seasons and months, were also obtained for precipitation levels  $\geq 1.0$ ,  $\geq 2.0$ ,  $\geq 3.0$ ,  $\geq 4.0$ , and  $\geq 5.0$  mm. However, whereas the changes in PM10 concentration in autumn and winter were better explainable by variability in number of days with daily precipitation  $\geq 1.0$  mm, for spring and especially summer seasons, a better description was provided by the number of days with precipitation  $\geq 2.0$  (Figure 5).

For most of the months, similarly as for seasons, the biggest determination coefficients were found usually for the number of days with daily precipitation  $\geq 0.5$  mm (Figure 6). The best explanation for PM10 concentration variability with the precipitation of that magnitude was obtained for August ( $R^2$  about 38%), which might be found particularly puzzling when set against

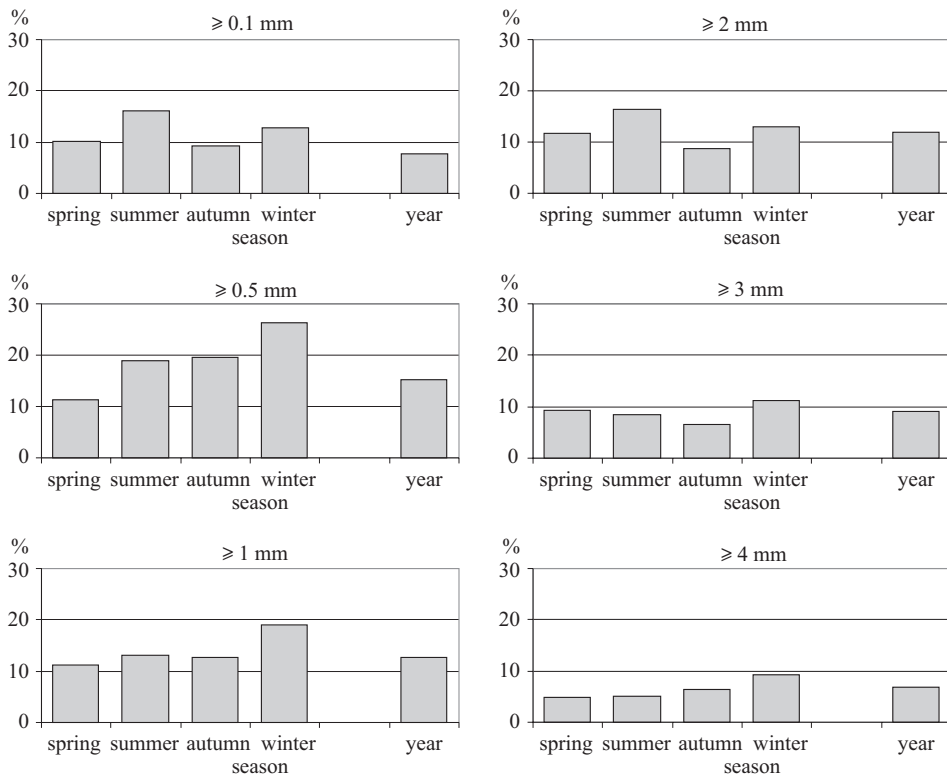


Fig. 5. Determination coefficients (%) for negative linear dependence between (suspended particulate matter) concentration for PM10 and the number of days with daily precipitation, as in seasons. Years 1993-2002

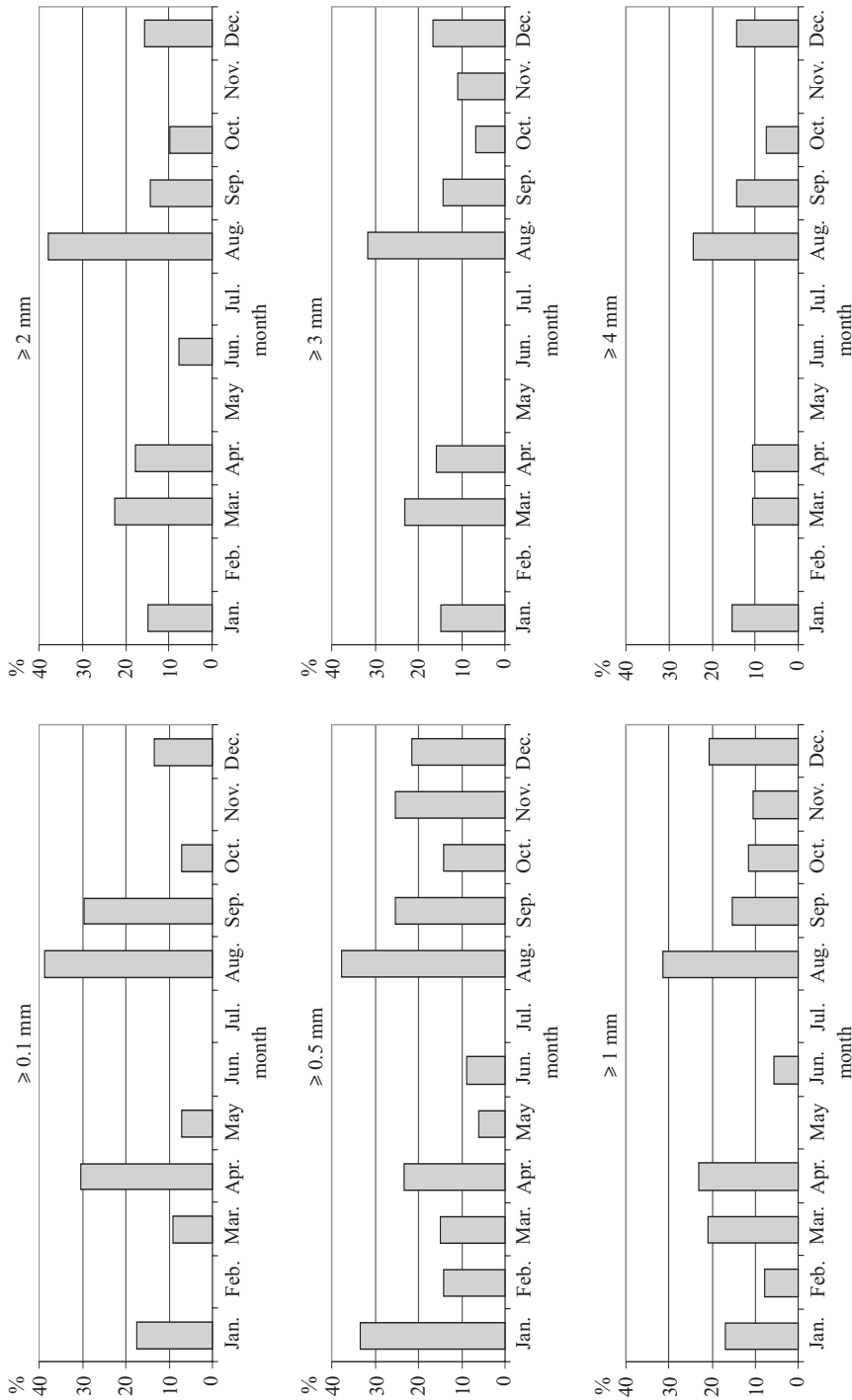


Fig. 6. Determination coefficients (%) for negative linear dependence between (suspended particulate matter) concentration for PM10 and the number of days with daily precipitation, as in months. Years 1993-2002



the insignificance of such in July. Over the considered period the number of days with precipitation  $\geq 5.0$  mm in August was only slightly higher than in July, whereas the particulates concentrations in July was higher by a mere  $5 \mu\text{g m}^{-3}$ , though the precipitation was characterised by much stronger variability (Figure 2). Still, all such considerations are difficult to be considered as explainable for such contrastive results. It might be attributable to differences in the precipitation intensity observed in July and August, but to make it explainable a separate analysis would be required. Moreover, determination coefficients for other considered in the analysis daily precipitation totals of  $\geq 0.1$ ,  $\geq 2.0$  mm, and  $\geq 3.0$  mm were also found to be much bigger in August, when compared to the remaining months of the year. Over the calendar winter, the strongest determination of daily precipitation totals over PM10 concentration was observed in January or December, depending on the considered value, while the major part of the considered precipitation levels (apart from  $\geq 0.5$  mm and  $\geq 1.0$  mm) were insignificant with reference to PM10 concentration variability in February. Such contrastingly weak results for the dependence under consideration in February, in comparison to January and March may be, however, attributed to distinctly higher than the standard monthly average temperature for February over the years 1993-2002 (MICHALSKA, KALBARCZYK 2005), which in turn might have taken effect on the lower emission rate of particulates originating from combustion, and thus finally resulting in lower PM10 emission, which can be seen in Figure 1. Significant negative correlations between air temperature and PM10 concentration for nearly all months was confirmed by ELMINIR (2005).

Unusual, when compared to the majority of the months, distribution of values for determination coefficients, was obtained in March and April. The most pronounced influence on reducing PM10 concentrations in March had daily precipitations of the level  $\geq 3.0$  mm, and only slightly smaller values, namely  $\geq 2.0$  mm, were found in February, whereas in April daily precipitation of  $\geq 0.1$  mm were most effective in reducing the concerned concentration.

The bulk of the presented results in general proved a positive role the precipitation plays in improving the air quality by reducing suspended particulate matter, whereas determination coefficients values shall be rather considered as indicative for defining statistical role of precipitation, which certainly does not impose their quantitative efficiency. Considering the fact that air clearing role of precipitation, particularly for the solid fraction of air pollutants, depends on many other precipitation properties, disregarded in our analysis, such as precipitation intensity, duration, droplet size, or dropping velocity (VAN DER WAL, JANSSEN 2000), the obtained results can be considered reliable. It shall be also noted while assessing the relations under consideration, that the data on precipitation were collected in rural environment where

the weather stations were located, quite opposite to the immision data. It has been generally known that in urban areas an increase in precipitation is observed, in particular intense precipitation occurring in the warm seasons, as well as their prolonged duration (LEWINSKA 2000). Moreover, widely known significant spatial variability of precipitation in the urban environment, due to mixed type substrates and multi type swelling and shade zones, which modify precipitation area, suggests that to improve reliability of the quantitative relations between the suspended particulates concentration and precipitation, measurement data collected at the same locations shall be provided for both variables.

Research conducted in Karwina-Ostrava and Katowice agglomerations have proven high concentration of suspended particulates to be noted under weather conditions characterised with no precipitation, coinciding with low temperature and the wind velocity lower than  $1.5 \text{ m s}^{-1}$  (BLAŽEK at al. 1999). Similarly, TURAHOĞLU at al. (2005) reported a set of conditions – such as low temperature, low wind velocity, high pressure, low precipitation and high relative humidity – as associating high TSP concentration. Hence, it seems reasonable to claim that the next stage for assessing precipitation effectiveness in clearing the air from particulate matter pollutants shall be to analyse dependencies between suspended matter concentrations and precipitation conditions, in conjunction with other weather factors. Parameters such as precipitation intensity, regularly measured at many weather stations in warm seasons, shall be definitely incorporated into such analyses.

## Conclusions

1. In years 1993-2002 the concentration of suspended particulate matter of a diameter up to  $10 \mu\text{m}$  (PM10) was in general found by 20 to 40% lower than the concentration of total suspended particulates (TSP). Nevertheless, its variability was found to have been twice bigger.

2. Average daily PM10 concentration typically fell within the range between  $15$  and  $35 \mu\text{g m}^{-3}$ , whereas TSP concentration of  $45$  up to  $60 \mu\text{g m}^{-3}$  were recorded.

3. In years 1993-2002 precipitation conditions, as provided by the total precipitation and the number of days with precipitation, were found to be statistically insignificant in terms of impacting TSP concentration levels, though they significantly contributed to reducing PM10 concentration. The number of days with precipitation was particularly effective in providing the explanation for their air quality improving role by the number of days with daily precipitation of at least  $0.5 \text{ mm}$ , especially in a calendar winter, and within the monthly approach – in August and January.

4. For a comprehensive assessment of the air quality improvement function of precipitation apart from totals of precipitation and number of days with

various daily precipitation levels, other characteristics such as precipitation duration and intensities shall be taken into account.

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**EFFECT OF THE HERBICIDE ROUNDUP 360 SL  
ON THE GENERATION TIME OF *AEROMONAS*  
*HYDROPHILA* AND *PSEUDOMONAS FLUORESCENS*  
IN LAKE WATER**

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**Key words:** bacteria, generation time, herbicide, lake water.

**A b s t r a c t**

Both strains of analysed bacteria, present in natural lake water, altered the generation time depending on the concentration of the herbicide ROUNDUP 360 SL as well as the temperature and reaction of the aqueous environment. The generation time of *Aeromonas hydrophila* ranged from 2.3 to 83.8 hours, whereas that of *Pseudomonas fluorescens* varied from 1.6 to 91.2 h. The herbicide ROUNDUP 360 SL and the environmental factors (pH and temperature) did not affect significantly the length of the generation time of *Aeromonas hydrophila*. On the other hand, the water reaction (pH) and temperature as well as the interaction of these two parameters produced a statistically significant effect on the generation time of *Pseudomonas fluorescens*.

**WPLYW HERBICYDU ROUNDUP 360 SL NA CZAS GENERACJI *AEROMONAS*  
*HYDROPHILA* I *PSEUDOMONAS FLUORESCENS* W WODZIE JEZIORNEJ**

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**Słowa kluczowe:** bakterie, czas generacji, herbicyd, woda jeziorna.

## Abstrakt

Badane szczepy bakterii występujące w naturalnej wodzie jeziornej zmieniały długość czasu generacji w zależności od użytego stężenia herbicydu ROUNDUP 360 SL oraz od temperatury i odczynu środowiska. Czas generacji *Aeromonas hydrophila* wahał się od 2,3 h do 83,8 h, a *Pseudomonas fluorescens* – od 1,6 h do 91,2 h. Herbicyd ROUNDUP 360 SL i czynniki środowiskowe (pH i temperatura) nie wpływały statystycznie istotnie na zmiany długości czasu generacji bakterii *Aeromonas hydrophila*, natomiast odczyn (pH) i temperatura środowiska oraz współdziałanie tych dwóch parametrów oddziaływały w statystycznie istotny sposób na czas generacji bakterii *Pseudomonas fluorescens*.

## Introduction

Plant protection chemicals introduced to the environment during agronomic treatments are not neutral to organisms which inhabit a given habitat, including microorganisms, which are vulnerable to even very small changes in the chemical composition of the environment. Apart from affecting the counts and biochemical characteristics of microorganisms, particularly those of bacteria, pesticides influence some important parameters which characterise bacterial populations, such as the generation time. The generation time of bacteria present in water illustrates the rate of growth of bacterial populations. Thus, it characterises productivity of aqueous ecosystems, the degree of their degradation and the course of organic matter production and destruction processes carried out by microorganisms in water ecosystems. The length of the generation time of bacteria in aqueous environments changes under the influence of various physical and chemical factors (SCHLEGEL 2000).

The two strains of bacteria tested in the present study, *Aeromonas hydrophila* and *Pseudomonas fluorescens*, belong to the genera *Aeromonas* and *Pseudomonas*, which are widespread in inland waters, including lake waters (MIKULSKI 1974, PALUCH 1973, RHEINHEIMER 1987). It has been empirically demonstrated that fluctuations in counts of *Aeromonas hydrophila* and *Pseudomonas fluorescens* bacteria in lake water are associated with the occurrence of the active substance of ROUNDUP 360 SL in water (JANKOWSKA, ZMYSŁOWSKA 2006).

The purpose of this research has been to study the effect of various concentrations of the herbicide ROUNDUP 360 SL on the generation time of the strains of *Aeromonas hydrophila* and *Pseudomonas fluorescens* in filtered lake water (void of zoo- and phytoplankton) under different water temperature and reaction conditions.

## Material and Methods

**Bacterial strains.** The strains of *Aeromonas hydrophila* and *Pseudomonas fluorescens* used for the assays were isolated from the water of Kortowskie Lake and identified according to morphological, physiological and biochemical characteristics analysed with API 20 NE assays (BioMerieux).

**Herbicide.** The preparation used for the study is a non-selective, systemic herbicide ROUNDUP 360 SL, whose active substance is glyphosate, also known as N-(phosphonomethyl)glycine. This herbicide, broadly used in Polish agriculture, is also applied to remove unwanted plants from water reservoirs. The influence of glyphosate on microorganisms depends on their species, although it is the microorganisms that play a key role in biodegradation of this chemical compound.

**Kortowskie Lake.** Water for assays was sampled from the western basin of Kortowskie Lake. The lake, which lies on the outskirts of Olsztyn, covers 89.7 ha and has an average depth of 5.9 m (the maximum depth is 17.2 m). Differences in temperatures of the lake water range from 8.4°C to 13.6°C. In the surface layer of water the amount of dissolved oxygen varies from 8.6 to 9.1 mg O<sub>2</sub> in 1 l water. The water reaction (pH) in the surface layer ranges from 7.5 to 8.4, reaching higher values during the vegetative season. The lake is eutrophic due to the constant influx of biogenic substances, mainly nitrogen and phosphorus compounds. The average concentration of phosphorus in water is 0.06-0.47 mg/l, whereas that of nitrogen is 1.05 to 3.22 mg organic N dm<sup>-3</sup> during the summer stagnation (TEODOROWICZ 1995, DUNALSKA 1999).

**Preparation of samples for assays.** Water sampled from the lake was filtered under subpressure through 0.45 µm ø 50 mm filters (Schleicher & Schuell) in order to remove zoo- and phytoplankton. Filtered water was poured to sterile glass bottles (100 cm<sup>3</sup> each). Each bottle was secured with a threaded top possessing a hole fitted with a cotton stopper. Water samples were divided into three groups, in which the water reaction was adjusted to pH: 6, 7 and 8 (± 0.2). Once this was achieved, the samples were divided into further subgroups to which the herbicide ROUNDUP 360 SL was added to obtain the following concentrations: 0.005, 0.5, 50 and 5000 µg dm<sup>-3</sup>. The control samples were free of the herbicide (0 µg dm<sup>-3</sup>). Half of the samples from each subgroup were inoculated 1 cm<sup>3</sup> 18 h bouillon culture of *Aeromonas hydrophila* and the other half were used to culture *Pseudomonas fluorescens*. All the variants of the samples were exposed for 48 h to the temperatures of 5, 10 and 20°C.

**Microbiological assays.** Counts of bacteria in the exposed lake water samples were determined with Koch's plate culture method after 2, 4, 6, 12, 24 and 48 h on mA selective medium (ARCOS et al. 1988) in the case of *Aeromonas*

*hydrophila* bacteria and on King B medium (BURBIANKA, et al. 1983) to grow *Pseudomonas fluorescens*. The cultures were incubated at 37°C for 48 h (*Aeromonas hydrophila*) and at 26°C for 72 h (*Pseudomonas fluorescens*). The results were converted into c.f.u. per 1 cm<sup>3</sup> water.

Generation times was calculated from the formula (SCHLEGEL 2000):

$$g = t \cdot \log 2 / (\log N - \log N_0),$$

where:

g – generation time in hours;

t – duration of a logarithmic phase in hours;

N<sub>0</sub> – count of bacteria at the onset of the logarithmic phase;

N – count of bacteria at the termination of the logarithmic phase.

**Statistical analysis.** The results underwent statistical processing using analysis of variance.

## Results

Figure 1 shows modifications in the generation time of *Aeromonas hydrophila* which occurred under the effect of various concentrations of ROUNDUP 360 SL in water of different pH and at different temperatures to which the samples were exposed. The generation time of these bacteria varied from 2.3 h (20°C, pH 6, herbicide concentration 5000 µg dm<sup>-3</sup>) to 83.8 h (10°C, pH6, herbicide concentration 5000 µg dm<sup>-3</sup>). At 5°C (Figure 1a) the herbicide caused small changes in the generation time of *Aeromonas hydrophila* in water which was 6 and 7 in pH. In contrast, at the water reaction of 8, the herbicide concentrations of 0.005 and 0.5 µg dm<sup>-3</sup> prolonged the generation time of *Aeromonas hydrophila* from 9 h to 6.5 and 4.6 h, respectively. At 10°C (Figure 1b), the generation time of these bacteria in water of pH 7 and 8 changed only slightly and was similar to the generation time recorded in the control sample. On the other hand, the generation time in the water of pH 6 increased considerably (from 16.8 to 83.8h) under the effect of the highest herbicide concentration (5000 µg dm<sup>-3</sup>). At 20°C (Figure 1c), the generation time of *Aeromonas hydrophila* in the water of pH 7 and 8 underwent very small changes. When the water reaction was 6, the generation time lengthened from 6.5 to 9 h at the herbicide rate of 0.5 µg dm<sup>-3</sup> but shortened from 6.5 to 2.3 h at the herbicide concentration of 5000 µg dm<sup>-3</sup>.

Figure 2 illustrates the influence of various ROUNDUP 360 SL concentrations on *Pseudomonas fluorescens* during the exposition of water samples characterised by different temperature and reaction parameters. The generation time of those bacteria varied from 1.6 h (20°C, pH 8, herbicide concentration 0.5 µg dm<sup>-3</sup>) to 91.2 h (10°C, pH 7, herbicide concentration 5000 µg dm<sup>-3</sup>).

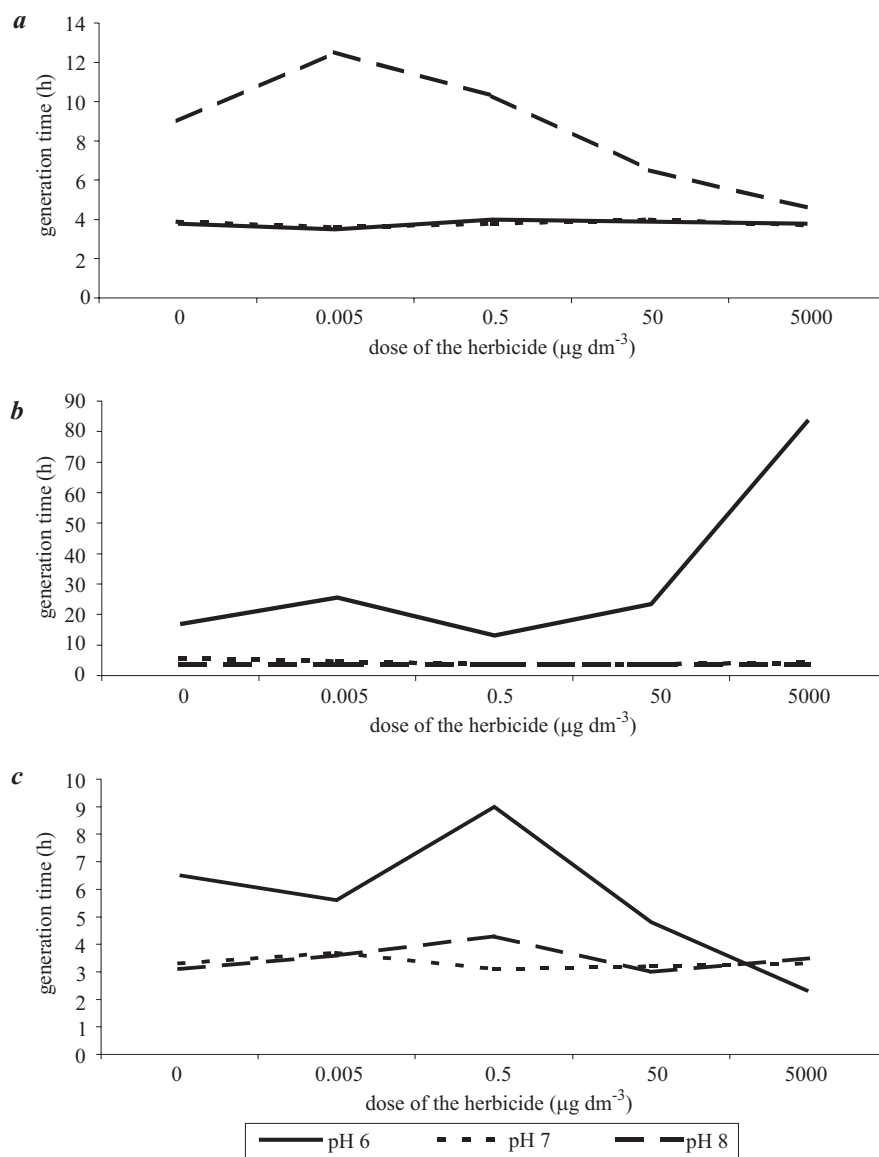


Fig. 1. Changes of the generation time of *Aeromonas hydrophila* under the influence of the herbicide ROUNDUP 360 SL in different reactions of the environment pH in filtrated lake water in temperatures: a – 5°C, b – 10°C, c – 20°C

The herbicide caused small modifications of the generation time of *Pseudomonas fluorescens* in water samples of any of the reactions tested (pH 6, 7 or 8) exposed to 5°C. Changes in counts of bacteria corresponding to their generation time at this temperature are shown in Figure 2a. In water



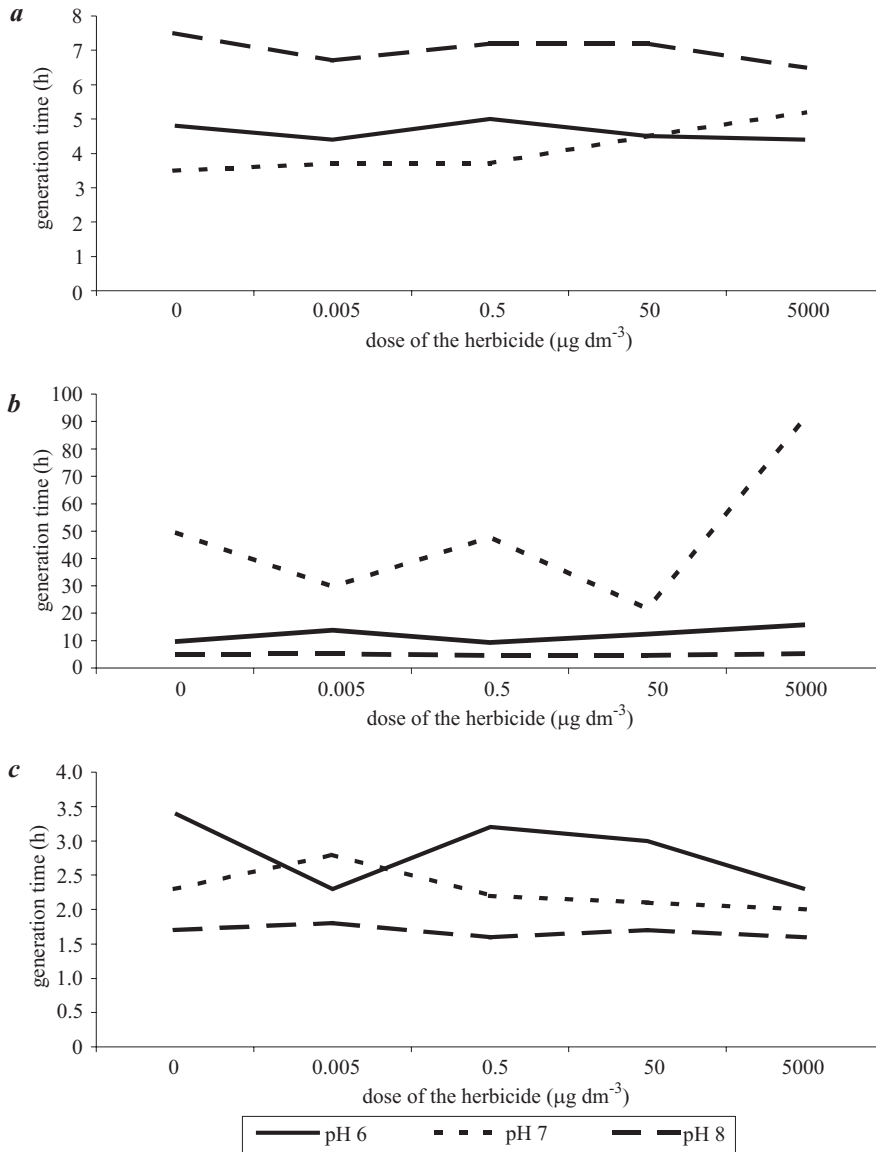


Fig. 2. Changes of the generation time of *Pseudomonas fluorescens* under the influence of the herbicide ROUNDUP 360 SL in different reactions of the environment pH in filtrated lake water in temperatures: a – 5°C, b – 10°C, c – 20°C

of pH 7 or 8 exposed to 10°C (Figure 2b) no significant changes in the generation time of those bacteria caused by different rates of ROUNDUP 360 SL were observed. However, when the pH of lake water was 6, the generation

time of *Pseudomonas fluorescens* increased from 49.4 to 91.2 h under the influence of 0.5 and 50  $\mu\text{g dm}^{-3}$  of the herbicide.

In water samples of 20°C (Figure 2c), when the pH of the aqueous environment was 8, all the four herbicide concentrations caused slight prolongation of the generation time (from 2.3 to 2.8 h). On the other hand, when the water pH was 6, the herbicide applied at 0.005  $\mu\text{g dm}^{-3}$  resulted in a shorter generation time.

Table 1 presents results of the statistical analysis, which showed that the generation time of *Pseudomonas fluorescens* was statistically significantly affected by the water pH and temperature as well as by the interaction of these two parameters. No statistically significant effect of these analysed factors on the generation time of *Aeromonas hydrophila* was demonstrated.

Table 1  
Levels of substantiality for the analysis of variation of generation times of bacteria *Aeromonas hydrophila* and *Pseudomonas fluorescens* cultured in filtrated lake water

Environmental factors	<i>Aeromonas hydrophila</i>	<i>Pseudomonas fluorescens</i>
Concentration of the herbicide	0.614785	0.371504
pH	0.026888	0.001237*
Temperature	0.041329	0.000031*
Co-operation of the concentration of the herbicide and the pH	0.540254	0.445156
Co-operation of the concentration of the herbicide and the temperature	0.422053	0.372554
Co-operation of the the pH and the temperature	0.010011	0.000102*

\* – a factor essentially influencing the change of generation times of bacteria

## Discussion

The generation time of bacteria in lake water reflects the rate of their multiplication, which indirectly conditions productivity of a water reservoir and rate of biodegradation of pollutants. The literature suggests that the generation time of bacteria depends on the conditions of a culture environment (SCHLEGEL 2000), predominantly such characteristics as pH, temperature, type and amounts of nutrient substrat, concentration of salts and original count of bacteria (CHEROUTRE-VIALETTE, LEBERT 2000, PLOTKIN, VISELLI 2000, BORGE et al. 2001, CHEROUTRE-VIALETTE, LEBERT 2002, KAMOT-SAY et al. 2002, RAZAVILAR, FAZLARA 2002). The generation time under aerobic conditions is different from that under anaerobic conditions (BELAY, RASOOLY

2002). Regarding pathogenic bacteria, their generation time depends on their activity and pathogenic strength (ERLENDSTOTTIR et al. 2001).

Changes in the generation time of microorganisms most often occur under the effect of environmental factors such as changes in temperature, nutrient substrat and various chemical compounds (JAIN et al. 2000, PERLOVA et al. 2002, REBAH et al. 2002, MICHELSEN et al. 2003). Thermal shock can prolong the generation time of bacteria to 30 hours and for some strains the resulting time could be 70 h to 21 days (LECLERCQ-PERLAT et al. 2000, PERRIN et al. 2001). Addition of microelements and complex sugars to the culture environment can shorten the generation time of bacteria; lower concentrations of nutrients lead to longer generation time (NOVIK et al. 2001, QIU-HONGDUAN et al. 2002).

The authors' own study has proved that ROUNDUP 360 SL produces a significant effect on the generation time of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. At the highest rates of the herbicide ( $5000 \mu\text{g dm}^{-3}$ ) the generation time of *Aeromonas hydrophila* exposed in filtered lake water to  $5^{\circ}\text{C}$  (water pH 8) was shortened from 9 to 4.6 h. When the water temperature was higher ( $20^{\circ}\text{C}$ ) and its reaction lower (pH 6), the generation time of these bacteria decreased from 6.5 to 2.3 h. This may suggest that the growth of *Aeromonas hydrophila* was more rapid, that is the bacteria were able to use the herbicide as a nutrient substrat. The authors' own study on the generation time of *Pseudomonas fluorescens* in filtered lake water containing the herbicide ROUNDUP 360 SL showed that it varied within a wide range of 1.6 to 91.2 h depending on a rate of the herbicide as well as the temperature and reaction of water. Longer generation time of these bacteria in filtered lake water may have been caused by the absence of zoo- and phytoplankton, which are inextricably connected with the metabolism of *Pseudomonas* bacteria (KROTON-CZARNECKA, CHRÓST 2001).

MARTIN et al. (1999), who studied degradation of the herbicide Propachlor by bacteria of the genus *Pseudomonas*, demonstrated that the generation time of these microorganisms was 3.4 h. SUBHAS and SINGH (2003) examined the generation time of *Pseudomonas aeruginosa* F10B on a medium containing Monocrotophos, an organophosphorus insecticide (vinyl phosphate), finding out that the generation time of bacteria grown on a medium with the insecticide increased from 1.29 to 2.15 h.

## Conclusions

1. ROUNDUP 360 SL affected the generation time of the strains of *Aeromonas hydrophila* and *Pseudomonas fluorescens* in filtered lake water; the actual effect depended on concentrations of the herbicide in water as well as the water temperature and reaction.

2. In filtered lake water containing different concentrations of the herbicide, the generation time of *Pseudomonas fluorescens* varied from 1.6 to 91.2 h while that of *Pseudomonas hydrophila* ranged from 2.3 to 83.8 h; the generation time was conditioned by the water temperature and reaction.

3. The herbicide ROUNDUP 360 SL added in different rates, along with the environmental conditions (pH and temperature), did not produce statistically significant effect on the modification of the generation time of *Aeromonas hydrophila*. However, the above factors affected significantly the generation time of *Pseudomonas fluorescens*.

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**EFFECTS OF TOTAL PHOSPHORUS  
AND ORTHOPHOSPHATES REMOVAL  
WITH THE METHOD OF METALS SOLUBILISATION  
ON STEEL, ALUMINIUM, AND MIXED MEDIA**

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**Key words:** phosphorus removal, metals corrosion, wastewater, anaerobic conditions.

**A b s t r a c t**

Studies were carried out of a new, unconventional method for phosphorus removal from wastewater, making use of the phenomenon of metals corrosion in wastewater. Metal ions are introduced to the solution as an effect of corrosion, instead of the up till now applied coagulation method. Likewise in the coagulation process, as a result of the occurring reactions (simplifying the mechanism more complicated in practice), sludge precipitates containing among others phosphorus. The experiments were carried out with media contain steel and aluminium. The most effective in phosphorus removal from wastewater was the uniform steel medium. The concentration of 1 mg P dm<sup>-3</sup> orthophosphates in the effluent seems very realistic and even lower values can be achieved.

**EFEKTY USUWANIA ZWIĄZKÓW FOSFORU OGÓLNEGO I ORTOFOSFORANÓW  
METODĄ ROZTWARZANIA METALI NA WYPEŁNIENIU STALOWYM, ALUMINIOWYM  
I MIESZANYM**

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**S ł o w a   k l u c z o w e:** usuwanie związków fosforu, korozja metali, ścieki, warunki beztlenowe.

## A b s t r a k t

Przeprowadzono badania nad nowym oryginalnym rozwiązaniem usuwania związków fosforu ze ścieków, wykorzystując zjawisko korozji metali w ściekach. Jony metalu wprowadzane są do roztworu na drodze korozji, zastępując stosowane do tej pory w procesie koagulacji dozowanie koagulantu. Podobnie jak w procesie koagulacji, w wyniku przebiegających reakcji, upraszczając w rzeczywistości bardziej skomplikowany mechanizm, obserwuje się wytrącanie osadów zawierających m.in. związki fosforu.

Badania przeprowadzono na wypełnieniach zawierających stal i aluminium. Najskuteczniejsze w procesach usuwania fosforu ze ścieków okazało się wypełnienie stalowe jednorodne. Osiągnięcie w odpływie stężenia ortofosforanów na poziomie poniżej 1 mg P dm<sup>-3</sup> wydaje się bardzo realne, a możliwe jest uzyskanie nawet znacznie niższych stężeń.

**Introduction**

One of the ways to reduce phosphorus amount in surface waters is to decrease its amount in treated wastewater, through application of more and more effective treatment methods.

A group of the methods comprise the physicochemical methods. The main processes occurring during phosphorus removal from wastewater with the chemical methods are chemical precipitation and sorption, running with the participation of metal ions, like iron or aluminium, present in the solution. In the conventional physicochemical methods metals are introduced in the wastewater solution by dosing relevant salts.

Analysis of the corrosion processes has indicated the possibility to apply them alternative method of metals dosing to the solution. The corrosion processes cause migration to the solution of ions that in turn may cause phosphorus compounds removal from wastewater, like in the traditional precipitation methods.

Metals corrosion is commonly regarded as a negative phenomenon. In these experiments, an attempt was made to use and present it as a phenomenon which – if controlled – may be of some benefit in wastewater treatment.

The authors shows results of his experiments into a corrosion processes in earliest article (WYSOCKA et al. 2003).

The actual conducted research was aimed to determine the impact of metal media corrosion on the effects of total phosphorus and orthophosphates removal from wastewater. They comprise the test of the realisability of the assumed goal.

The beneficial impact of iron in the form of scrap pieces, on the process of phosphorus removal from wastewater was reported also by other authors (SCHÖNBORN et al. 1997).

The authors' preliminary results (WYSOCKA 2000) can be seen as encouraging to the survey continuation in the same direction. No then, the constructed

experimental posts differed with the type of media. Steel, aluminium, and mixed media were applied, and the results compared with regard to the effects of phosphorus and orthophosphates removal.

## **Materials and Methods**

In the experiments 5 types of media were used: uniform steel, steel-aluminium with 75% steel contribution to the medium surface, steel-aluminium with 50% steel contribution to the medium surface, steel-aluminium with 25% steel contribution to the medium surface, uniform aluminium. Additionally, the experiment was run on the posts without media.

Prepared wastewater was used, as well as wastewater of natural origin.

The prepared wastewater varied by phosphorus concentration. They were prepared from beef broth or powdered milk. Wastewater prepared from powdered milk was subjected to anaerobic digestion. The natural-origin wastewater was of two types: domestic and municipal.

The single element of an experimental post comprised a glass container of 0.25 dm<sup>3</sup> volume. In this container, filled up with wastewater, medium was placed made of four metal disks (aluminium – 99% aluminium and/or steel – 48% iron), 7 cm in diameter and 0.2 cm thick. They were put on a glass rod in 1-cm distance. The whole post was protected against air penetration with a foil and placed in an incubator at 292-293 K.

Wastewater was agitated by stirring the rod with the medium once every day, until full mixing. After the planned contact time (96 hours) samples (5-10 cm<sup>3</sup> samples were taken with no sludge) were collected directly from the container.

Analyzed were: total phosphorus and orthophosphates concentration, pH, redox potential, and process temperature.

Concentrations of the examined parameters were characterized by the arithmetic averages. The hypotheses, serving to assess the differences between the individual posts, were verified on the grounds of the variance or the Student's t-test. It was assumed that the differences are statistically significant if  $p < 0.05$ .

In order to determine the impact of the medium on the effectiveness of orthophosphates removal from wastewater and to exclude the phenomenon of phosphorus compounds transformation into orthophosphates (the reduction conditions) the data analysis was carried out with reference to corrected values (Table 1, Table 2). The calculation made regarded the posts "without media" as zero sample.



Table 1  
Effects of phosphorus removal compared with wastewater after the post with no medium

Type of wastewater	Effect of total P removal in comparison with the zero sample (no medium)		Effect of orthophosphates removal in comparison with the zero sample (no medium)	
	efficiency (%)	concentr. difference (mg P dm <sup>-3</sup> )	efficiency (%)	concentr. difference (mg P dm <sup>-3</sup> )
The post with steel medium				
Domestic	69	9.6	100	11.0
Municipal	89	1.28	97	1.06
From powdered milk after anaer. digestion	97	51.8	99	46.2
From beef broth	54	0.9	91	0.9
The post with aluminum medium				
Domestic	3	0.4	-7	-0.7
Municipal	6	0.08	-10	-0.11
From powdered milk after anaer. digestion	2	1.3	2	1.2
From beef broth	4	0.1	-2	-0.0
The post with steel-aluminum medium (50% of steel contact surface and 50% of aluminium contact surface)				
Domestic	41	5.7	44	4.9
Municipal	71	1.02	87	0.95
From powdered milk after anaer. digestion	85	45.1	97	45.2
From beef broth	40	0.7	62	0.6

Table 2  
Effects of phosphorus removal compared with the wastewater after the post with no medium

Type of wastewater	Effect of orthophosphates removal in comparison with the zero sample (no medium)	
	efficiency (%)	concentr. difference (mg P dm <sup>-3</sup> )
The post with steel-aluminum medium (75% of aluminum contact surface and 25% of steel contact surface)		
Domestic	-4	-0.4
Municipal	75	0.82
From powdered milk after anaer. digestion	83	38.5
The post with steel-aluminum medium (75% of steel contact surface and 25% of aluminum contact surface)		
Domestic	94	10.3
Municipal	87	0.95
From powdered milk after anaer. digestion	98	45.9

## Results and Discussion

An attempt was made to examine the effectiveness and usefulness of metals corrosions in the processes of phosphorus removal from wastewater.

Applied were the media which contained steel and aluminium with various per cent content of those metals. Observation of the medium has allowed to conclude that in the applied wastewater conditions steel corrosion was running intensively, whereas the corrosive property of aluminium was not observed, probably due to the creation of a protective layer of aluminium oxides on the surface. This layer of oxides was not destroyed, despite the wastewater conditions and the presence of iron ions in the solution (WYSOCKA et al. 2003).

The uniform steel medium was giving the best effects of phosphorus compounds removal (Figure 1). On average, during the examinations, the steel medium revealed from 12% to 27% better effects in phosphorus compounds removal than the mixed medium containing 50% steel and 50% aluminium, and even by 48% to 92% than the aluminium medium which in such conditions was not subjected to corrosion.

Except total phosphorus concentration, examined was also content of orthophosphates (Figure 1). Concentration of this form of phosphorus after the treatment processes increased on the posts without any medium and with the aluminium medium. These concentration changes were statistically significant.

Not observed were the statistically significant differences between the wastewater treated on the aluminium medium and on the post without any medium; the aluminium medium had no direct effect on the increase of orthophosphates concentration.

The reason for the increase of orthophosphates concentration are probably the reduction conditions during the process and oxygen deficit in the wastewater. It was reported in the literature that such conditions stimulate transformations of other phosphorus forms into orthophosphate form (FALKENTOFT et al. 2000, PAK, CHANG 2000 a,b)

In the wastewater treatment by metals solubilisation the redox potential was set at the level of – 66 mV on the steel medium and 34 mV on the mixed medium. DO was not detected either. Therefore, microorganisms may participate in the processes of phosphorus compounds transformations into orthophosphates.

Only the wastewater prepared from powdered milk after anaerobic digestion revealed no similar tendency. In this wastewater concentration of orthophosphates decreased by 5% on the no-medium post and by 7% on the aluminium-medium post.

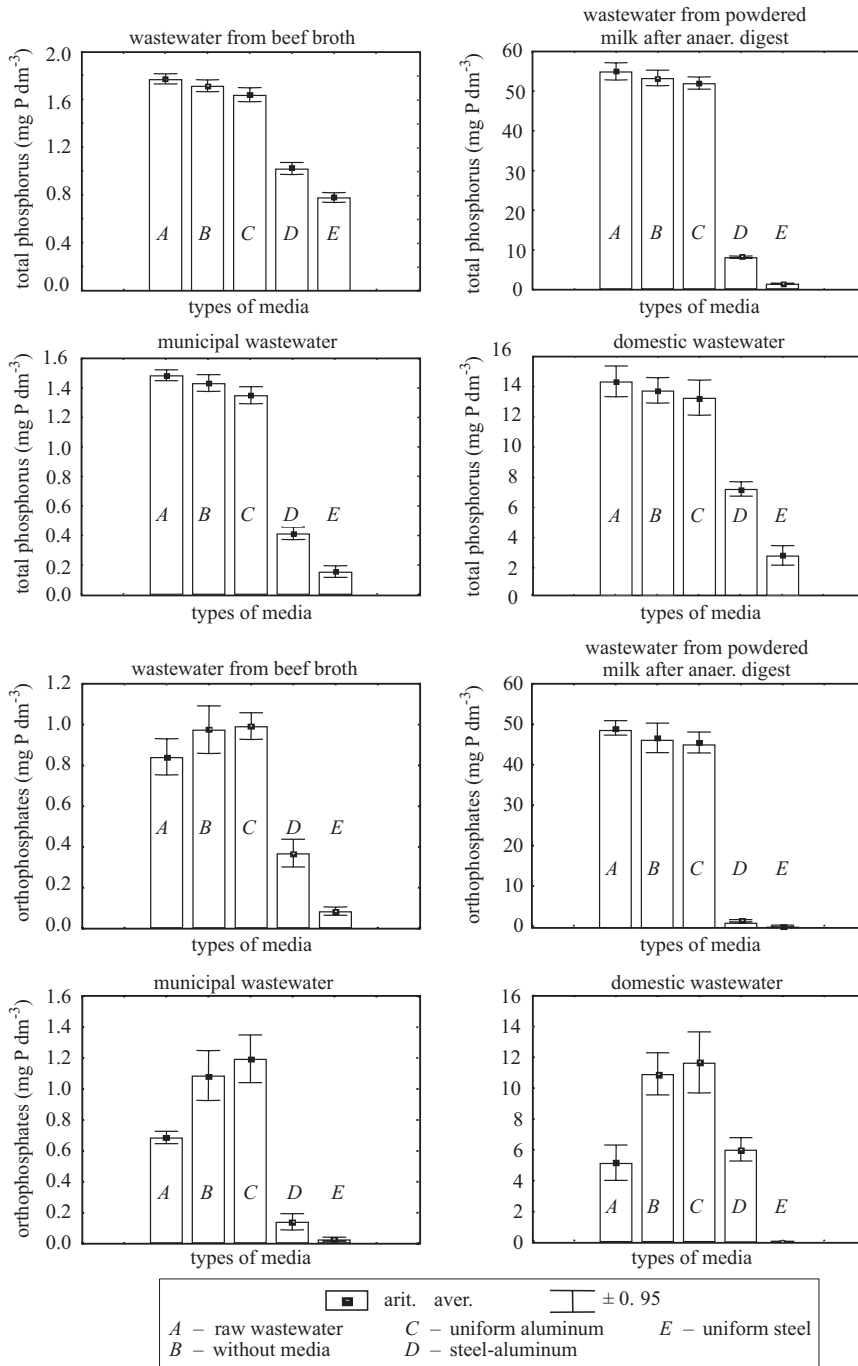


Fig 1. Effect of the medium type on the effectiveness of total phosphorus removal from wastewater

This situation can be explained with the analysis of orthophosphates content in the raw wastewater. The wastewater prepared from powdered milk after anaerobic digestion contained about 89% of phosphorus in orthophosphate form while in the other types of wastewater concentration of this phosphorus form equalled from 36% to 47%. Thus, in wastewater prepared from powdered milk after anaerobic digestion remained only 11% of phosphorus compounds that theoretically could be transformed into orthophosphates.

On the mixed medium, containing steel elements, observed was smaller increase of orthophosphates concentration, and mostly a decrease was noted.

In order to exclude the phenomenon of phosphorus compounds transformation into orthophosphates the data analysis was carried out with reference to corrected values (Table 1, Table 2).

The mixed medium containing 50% steel and 50% aluminium caused reduction of orthophosphates by 44% to 97%. While analyzing domestic wastewater it can be easily noted that in fact a 17% increase of concentration in the raw wastewater was observed. Only the correction of the data with the effects assisting the wastewater treatment on the post with no medium point at 44% reduction of the orthophosphates concentration.

The steel medium caused such high reduction of orthophosphates concentration that in any of the examined wastewater the increase of this phosphorus form concentration was observed. The obtained factual results and the corrected results are very similar and contained in the range of 90-100%.

The divergence between the effects of orthophosphates removal on the mixed medium and on the steel medium was highly variable i.e. from 2 to 116% of the value for the raw wastewater. However, it pointed out at the higher effectiveness of the steel medium, like in the case with orthophosphates.

In order to obtain the full view of the effects of orthophosphates removal on the mixed medium, additional examinations were conducted on the media with dominating content of steel or aluminium. The higher was the per cent content of steel in the medium, the better was the effect of orthophosphates removal (Figure 1). The mixed medium with steel dominance was so effective in removal of this phosphorus form that there were no significant differences noted ( $p < 0.05$ ) in the effects between this medium and the steel medium.

Observed was the effect of the type of phosphorus compounds on the results of their removal from wastewater. In all kinds of the examined wastewater higher removal efficiency regarded orthophosphates than total phosphorus. In the static system, the efficiency of orthophosphates removal varied between 90 and 99% and was always higher than the efficiency of total phosphorus removal (56-97%). The max efficiency (97%) of total phosphorus

removal was observed in the wastewater prepared from powdered milk after anaerobic digestion, containing as much as 89% orthophosphates.

The process of phosphorus removal with the metals solubilisation method is probably the share of mainly precipitation and sorption, although the activity of microorganisms should not be neglected, especially in regard to transformations of other phosphorus forms into orthophosphates, or corrosion processes (FALKENTOFT et al. 2000, PAK, CHANG, 2000 a,b, GRABIŃSKA-ŁONIEWSKA, 2000, SALVAGO et al. 1991). The difference in phosphorus compounds concentration between the liquid and solid phase is the driving force of the above mentioned processes. Not insignificant is also the effect that the pollution increase in wastewater has on the rate of corrosion processes, and thus on the amount of metal ions available in the solution. These are however only assumptions, based on the references analysis, and require farther investigation.

## Conclusion

Phosphorus removal with the method of metals solubilisation reveals positive effects only if the medium contains steel that can be easily subjected to corrosion processes. Aluminium did not corrode and thus its ions that could contribute to phosphorus removal from wastewater did not migrate to the solution.

The surveys have revealed that the most efficient removal regards phosphorus in the orthophosphate form.

The concentration of 1 mg P dm<sup>-1</sup> orthophosphates in the effluent seems very realistic and even lower values can be achieved (0.2 mg P dm<sup>-3</sup> – municipal wastewater).

Translated by MONIKA SZEWCZYK

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**COLONISATION OF THE CHANNELS  
OF MIĘDZYODRZE (NORTH-WESTERN POLAND)  
BY *SINANODONTA WOODIANA* (LEA, 1834)  
(BIVALVIA: UNIONIDAE)**

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**Key words:** Unionidae, *Sinanodonta woodiana*, alien species, invasion of molluscs.

**Abstract**

*Sinanodonta woodiana* (Lea, 1834) is an alien species in the fauna of Poland. It comes from the catchment area of the rivers Amur and Yangtze. In Poland it was found in the early 1980s in the system of heated lakes near Konin (Kujawy Lake District, C-Poland). It appeared there together with the silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1884) and big-headed carp *Aristichthys nobilis* (Richardson, 1845) introduced from Hungary. In 2003 it was found in the release channel of cooling water from the Dolna Odra power plant.

In the years 2004-2005 an inventory of the Międzyodrza (N-W Poland) channels was made in order to determine the current range of occurrence of *S. woodiana* in the Western Pomerania Region. The mussels were found at five localities below the release of the cooling water. The channels are partly frozen in winter, which indicated the adaptation abilities of this species to waters typical of temperate zone. In the outer demibranch marsupia of females the presence of glochidia was detected, which pointed to the readiness of the mussels for reproduction.

**ZASIEDLANIE KANAŁÓW MIĘDZYODRZA (POLSKA N-W) PRZEZ *SINANODONTA WOODIANA* (LEA, 1834) (BIVALVIA: UNIONIDAE)**

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**Słowa kluczowe:** Unionidae, *Sinanodonta woodiana*, gatunki obce, inwazja mięczaków.

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## A b s t a k t

*Sinanodonta woodiana* (Lea, 1834) jest gatunkiem obcym dla fauny Polski. Pochodzi z dorzecza rzek Amur i Jangcy. W Polsce stwierdzono ją na początku lat 80. w systemie podgrzanych jezior konińskich (Pojezierze Kujawskie, centralna Polska). Pojawiła się tam wraz z introdukowanymi z Węgier rybami – tołpygą białą *Hypophthalmichthys molitrix* (Valenciennes, 1884) i tołpygą pstrą *Aristichthys nobilis* (Richardson, 1845). W 2003 r. znaleziono ją w kanale zrzutowym wód pochłodniczych elektrowni Dolna Odra.

W latach 2004-2005 przeprowadzono inwentaryzację kanałów Międzyodrza (Polska północno-zachodnia) w celu określenia aktualnego zasięgu występowania *S. woodiana* na Pomorzu Zachodnim. Małże znaleziono na 5 stanowiskach poniżej zrzutu wód pochłodniczych. Kanały te należą do częściowo zamarzających w okresie zimowym, co świadczy o możliwościach adaptacyjnych tego gatunku do bytowania w wodach charakterystycznych dla klimatu umiarkowanego. W marsupiach zewnętrznych pólkrzeli samic zaobserwowano glochidia, co wskazuje na przystępowanie małży do rozrodu.

**Introduction**

Colonisation of new areas by alien species is not a rare event and it has been recently more frequent because of the more intense man's activity. The expansions are mainly related to the overcoming of geographical barriers and have a character of jump dispersal. Development of communication facilitates transportation of organisms over significant distances and transformations of habitats induce changes in the conditions of living so that species alien to a given area are able to colonise it and reproduce in it. Characterisation of the dynamics of invasion and colonisation of new areas seems particularly interesting for molluscs in view of their limited ability to change location.

It has been noted that in the inland waters in Poland the number of new invertebrates has been increasing (KASPRZAK 1977, KOLASA 1977, BIELECKI 1990, KONOPACKA 1998, WAWRZYNIAK-WYDROWSKA, GRUSZKA 2005). The molluscs alien to our fauna come mainly from Asia, for example *Lithoglyphus naticoides* (C. Pfeiffer, 1828) is a pontokaspian snail noted in Poland since 1873 (POLIŃSKI 1917). From among mussels besides *Dreissena polymorpha* (Pallas, 1771) also the presence of *Sinanodonta woodiana* (Lea, 1843) (ZDANOWSKI 1996), *Corbicula fluminea* (O. F. Müller, 1774) (DOMAGAŁA et al. 2004 b) and *Corbicula fluminalis* (O. F. Müller, 1774) (ŁABĘCKA et al. 2005) has been reported.

The coming of *Physella acuta* (Draparnaud, 1805) to Poland from the Mediterranean area has been explained by a result of development of aquaristics. In the colonised area this snail can be met in botanic garden pools, in aquaria, heated industrial reservoirs, and natural waters of the lotic and lenitic character (PIECHOCKI 1979, GLÖER, MEIER-BROOK 1998).

From the east coast of North America through British Isles *Menetus dilatatus* (Gould, 1841) has reached Poland. Numerous localities of this species



were discovered in the Konin Lakes by Berger in 1970 (BERGER, DZIĘCZKOWSKI 1977, 1979). In Germany it has been met in shallow waters beside the Mittelland-Canal in Saxony-Anhalt, in cut-off meanders and harbours of the Elbe in Saxony, Saxony-Anhalt and Brandenburg, in the mesotrophic lake Nehmitzsee in N-E Brandenburg, as well as in anthropogenic heated waters (GLÖER et al. 1998, MÜLLER et al. 2005). Also from a reservoir supplied with cooling waters (Licheńskie Lake, Kujawy Lake District, C-Poland) come the first live individuals of *Ferrissia wautieri* (Mirolli, 1960) collected by PIECHOCKI in 1983 (PIECHOCKI 1986).

The most successful in colonisation of new areas are the species of parthenogenetic mode of reproduction. In the release channels of the cooling water from the power plants Konin and Pątnów (Kujawy Lake District, C-Poland) *Melanoides tuberculatus* (O.F. Müller, 1774), representing such a mode of reproduction, was found. This snail originates from the subtropical and tropical regions of Africa, northern Australia, southern Asia and Madagascar (PIECHOCKI et al. 2003). Another newcomer representing this mode of reproduction is *Potamopyrgus antipodarum* (J.E. Gray, 1843) (KRODKIEWSKA et al. 1998). This snail has great expansion abilities as a single individual is capable of starting a new population (FALNIOWSKI 2001).

## Material and Methods

### Międzyodrze – the area of study

The Odra River has its sources in the Oderskie Mountains (Sudety Wschodnie, Czech Republic). In its lower course, in the village Widuchowa (Poland, 53°07'N; 14°24'E) the River divides into the Eastern Odra River (Regalica) and Western Odra River. The main mass of water is carried by the Eastern Odra River (nearly 76% of the water volume) (MIKULSKI, OSTAPSKA-BOJANOWICZ 1965). Between the two branches of the river there is the so-called Międzyodrze area (Figure 1). It contains a number of natural and artificial channels joining the two branches of the river and occupies about 56 km<sup>2</sup> (DYBKOWSKA-STEFEK 2000). The unique hydrological and hydraulic conditions of the area are determined by a constant and free flow of water between the Eastern and Western branches of the Odra River (DYBKOWSKA-STEFEK 1997). An important factor affecting these conditions is the supply of heated waters through the release channel of the Dolna Odra power plant. The mentioned channel of about 3.6 km in length joins the Tywa River at its mouth and together they enter the Eastern Odra River near the city of Gryfino.

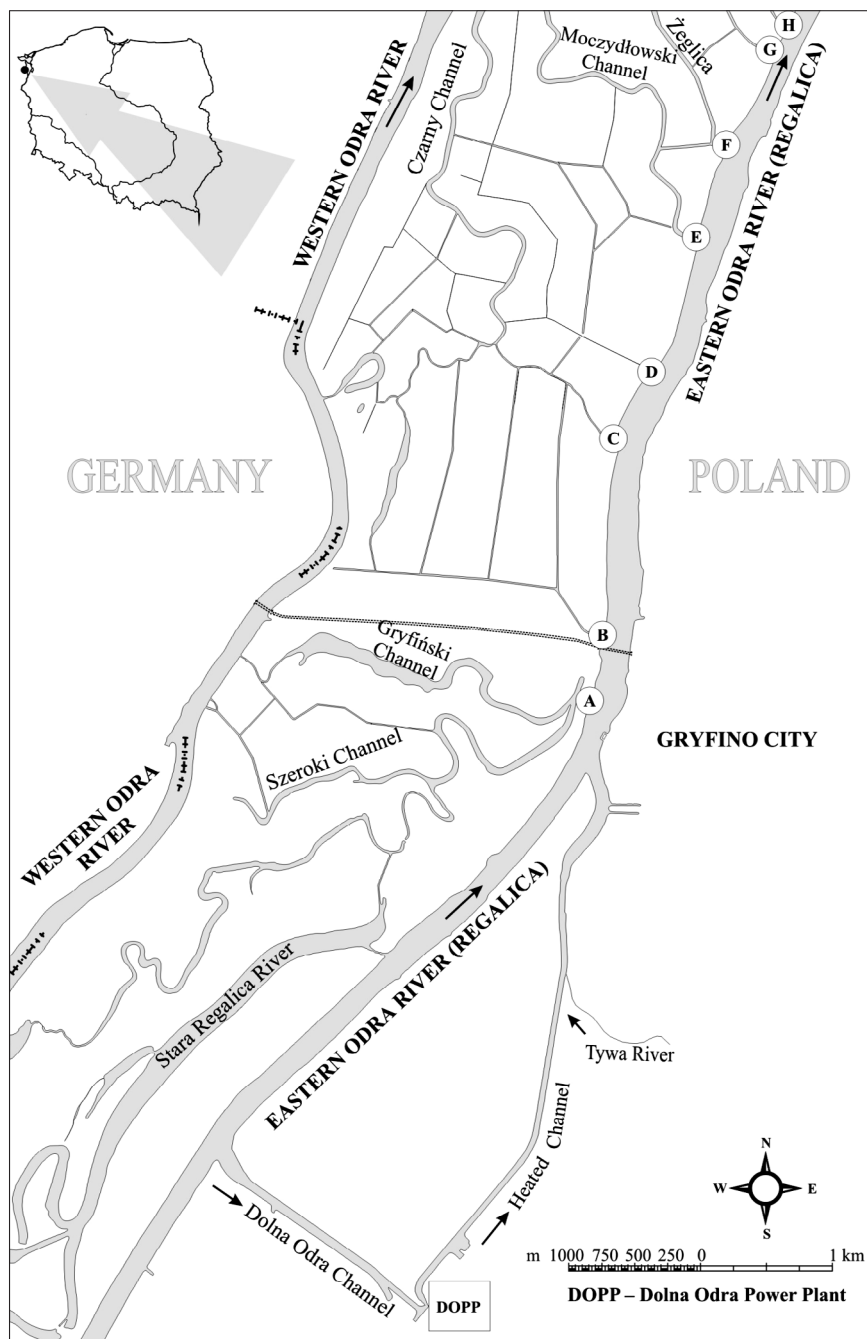


Fig. 1. Map of the Międzyodrze area showing the location (A-H) of new occurrences of *S. woodiana*.  
Heated channel – the release channel of cooling water

## Sampling and Analysis

In the years 2004-2005 an inventory of the Międzyodrze channels was made to determine the current range of occurrence of *Sinanodonta woodiana* in the area of Western Pomerania Region. The mussels were collected by free diving at the sites localised below the release of the cooling water from the Dolna Odra power plant (Figure 1, Table 1). The sites, except site D, were connected with the Eastern Odra River via always open and not working sluices.

Table 1

Location of sites of the mussel collection

Site	Location
A +	At the joint of the Gryfiński and Szeroki Channels (at the first sluice)
B +	Channel at the second sluice
C +	Channel at the third sluice
D –	The fourth channel
E +	The Moczydłowski Channel (at the fifth sluice)
F +	The Żeglarski Channel (at the sixth sluice)
G –	Channel at the seventh sluice
H –	Channel at the eighth sluice

+ sites at which *S. woodiana* was found;

– sites at which no representatives of this species were found.

The molluscs were found on the muddy bottom at the depth of 1.5-2 m. The length of their shells was measured by a vernier caliper to an accuracy of 0.01 cm. The sex of the mussels was determined on the basis of the histological slides of gonads. Paraffin sections of 5 µm thick were prepared by a rotating microtome made by Leica. They were stained progressively by Ehrlich haematoxylin and eosin, closed in a Canadian balm and watched under a light microscope Jenaval.

The physical parameters of water (temperature, oxygenation and pH) at particular sites were measured by a multifunctional meter Elmetron.

## Results

The presence of the mussels studied was checked in 8 channels of the eastern part of the Międzyodrze area from the side of the Eastern Odra River, near the city of Gryfino and below this city. All the localities were checked as to the presence of the mussel at the length of 200 m into the channel from its outlet to the Eastern Odra River. At five sites (A, B, C, E, F) individuals

of *Sinanodonta woodiana* were found. In total 20 empty shells joined by ligamentum and 16 live individuals were collected (Table 2). The greatest number of individuals were found at the localities *A* and *B*. The materials were collected in a few month systematic sampling of the channels. Site *F* was the most northward locality at which *S. woodiana* was collected and it determines the current range of this species in the Międzyodrze area. At sites *D*, *G* and *H* no representatives of this species were found.

Table 2  
List of collected empty shells and live mussels in the channels of Międzyodrze area

Site	Date of collection	Empty shells		Live mussels			Glochidia
		number	Shell length (cm) Mean $\pm$ SD range	♂	♀	Shell length (cm) Mean $\pm$ SD range	
<i>A</i>	24.04.2004			1	1	1.82	
	10.08.2004			1	1	11.02 $\pm$ 1.33 10.08-11.96	+
	6.09.2004	2	12.07 $\pm$ 1.29 11.16-12.98		1	13.31	-
	18.10.2004	2	14 $\pm$ 0.08 13.94-14.06				
	8.11.2004	3	14.48 $\pm$ 1.24 13.28-15.76				
	18.04.2005	1	11.96				
	20.05.2005	2	12.82 $\pm$ 1.22 11.96-13.68	1		13.73	
	16.06.2005	2	13.12 $\pm$ 1.27 12.22-14.02				
<i>B</i>	15.05.2004	1	13.71	1	1	13.98 $\pm$ 0.35 13.73-14.23	+
	25.04.2005	1	11.61				
	20.05.2005	4	13.2 $\pm$ 1.6 11.29-15.13	3	2	13.48 $\pm$ 3.28 9.23-17.21	+
	16.06.2005	1	13.42				
	5.09.2005	1	15.29				
	4.11.2005				1	3.82	-
<i>C</i>	13.08.2004			1		15	
<i>E</i>	20.07.2005				1	17.5	+
<i>F</i>	20.07.2005				1	14.83	-

+ in all females the marsupia were filled with glochidia;

- no glochidia in the female's outer demibranchs.

In five of the eight females glochidia were found in the outer demibranchs' marsupia (Figure 2). These five females were collected in May, July and August. Histological analysis of the mussel gonads has shown the presence of ovulated oocytes in females and developed spermatozoa in males.

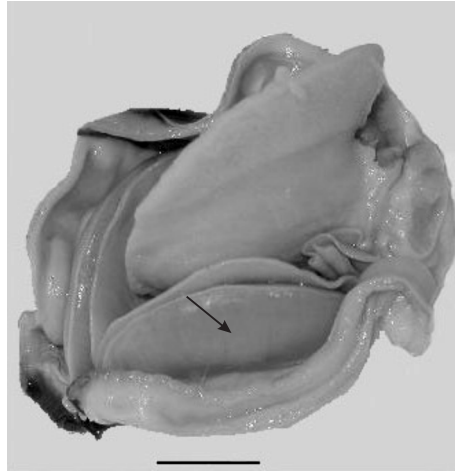


Fig. 2. Female prepared from the shell. The outer demibranchs filled with glochidia indicated by arrow. Scale bar – 1 cm

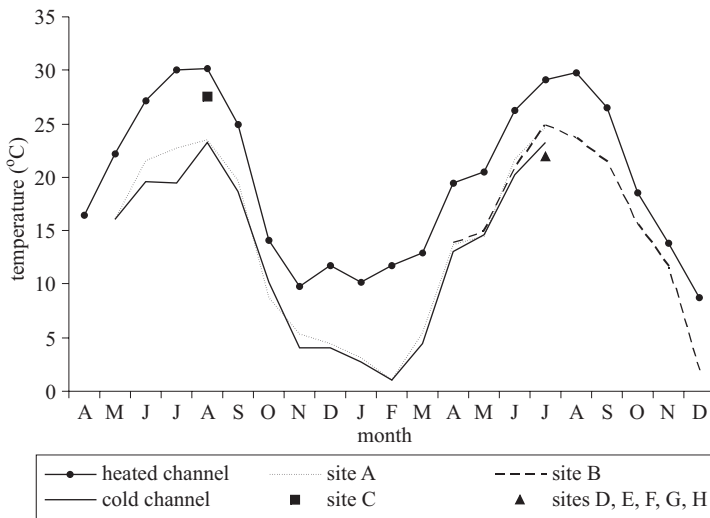


Fig. 3. Temperatures at particular sites

A living mussel of the smallest size of 3.82 cm was collected at site *B*, while the largest mussel found was a female of 17.5 cm collected at site *E* (Table 2).

The difference in the water temperature between the sites of mussel collection and the cooling water released from the power plant during a year varied from 2 to 11°C (Figure 3). Usually the content of oxygen

in water was high and reached  $8\text{--}14.9\text{ mg dm}^{-3}$ , however, in summer it decreased below  $6\text{ mg dm}^{-3}$  (sometimes even to  $4.5\text{--}5\text{ mg dm}^{-3}$ ). The oxygenation level depended on the season and not on the site location. The pH of the water varied from 7.3 to 9.2.

## Discussion

The native area of *Sinanodonta woodiana* occurrence are the Amur and Yangtze rivers. Recent interest in this species has been stimulated by its appearance as an alien species in new water reservoirs. In Europe it has been reported from Hungary, Romania, France, Italy, Slovakia and Czech Republic (PETRÓ 1984, SÁRKÁNY-KISS 1986, SÁRKÁNY-KISS, SÍRBU 2001, GIRADI, LEDOUX 1989, KOŠEL 1995, BERAN 1997, WATTERS 1999). In Central America it has been noted in Dominican and Costa Rica – in the latter it appeared in Arenal Lake most probably accompanying the introduced blue tilapia *Oreochromis aureus* (Steindachner, 1864) and Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) (KEFERL 1995, WATTERS 1999). It is present in the Philippines and in the Java and Sumatra Islands (DJAJASASMITA 1982, WATTERS 1997). Specimens *S. woodiana* were spotted in the area of the Danube Biosphere Reserve: in the Danube delta, in the Sasyk channel, for the first time in August 1999 (YURISHINETS, KORNUSHIN 2001). Subsequently its presence was established in the sand bar of the lower arm of the Wostočnoje River, however, only empty shells were found there, while live individuals occurred in the meanders of the Anankinom River. In 2002 these mussels were met over the whole area of the Danube delta (LIASHENKO et al. 2003).

In Poland *Sinanodonta woodiana* appeared in the beginning of the 1980s in the system of heated Konin lakes (Kujawy Lake District, C-Poland) accompanying the silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1884) and big-headed carp *Aristichthys nobilis* (Richardson, 1845) imported from Hungary (ZDANOWSKI 1996). They found there favourable developmental conditions, that is higher water temperatures and water turbulences, and at present the populations of these mussels have reached a position of a dominant in the periphytonic and bottom associations (AFANASJEV et al. 1997, AFANASJEV et al. 2001, KRASZEWSKI, ZDANOWSKI 2001 a, b; KRÓLAK, ZDANOWSKI 2001, SOROKA, ZDANOWSKI 2001).

In October 2003 in the release channel of cooling water from the Dolna Odra power plant (Western Pomerania Region, N-W Poland) live adults of the species and their shell tanatocenoses were found (DOMAGAŁA et al. 2003). Possible further expansion of this species into the waters characteristic of the temperate zone were mentioned even earlier (DOMAGAŁA et al. 2004 a), and

as a result of free diving collections in the Międzyodrze channels in the years 2004-05 the presence of this species was confirmed at 5 sites below the release of the heated water. The mussels were found to occur in the channels at the distance up to 200 m from the Eastern Odra River. We do not know since when exactly they have been living in the cooling water release channel and when the expansion of this species started over the Międzyodrze area. *Sinanodonta woodiana* has not been found in the channel supplying the water to the power plant, or in the channels above the cooling water release channel (ŁABĘCKA, DOMAGAŁA, unpublished data).

The mussels seem to be resistant to the unfavourable environmental conditions as they have been found at the sites in the waters partly freezing in winter. The difference in the water temperature between the cooling water channel and the sites below the cooling water release was 7-11°C in the period from December to February, so it can be assumed that the effectiveness of the Międzyodrze waters colonisation by *S. woodiana* is related to the resistance of the species to low temperatures (Figure 3). The other factors favouring the colonisation are high fertility and early age of reproductive maturity. According to DUDGEON and MORTON (1983) the mussels reproduce already in the first year of life having reached the shell length of 3-4 cm. Nevertheless, KONDO (1987) in the Asahi River (Japan) found a 2 year old female of the shell length of 4.46 cm that had just matured for reproduction. The smallest female incubating glochidia in marsupia collected in the Międzyodrze (site A) was in the shell of the length of 11.96 cm. The occurrence of females incubating larvae means that *S. woodiana* has undertaken reproduction and, after KONDO (1989) the marsupia of *S. woodiana* can contain from 12.000 to 120.000 eggs. The finding of the largest mussel of 17.5 cm at site E indicates that this species may have colonised the Moczydłowski Channel (one of the most northwards localised from among of those studied) a few years ago.

The possibility of *S. woodiana* living in waters of natural thermal regime has been already confirmed by reports of MIZERA and URBĄSKA (2003). In July 2002 in fishponds in the area of the Sierakowski Landscape Park (Wielkopolska Region, C-Poland) 5 specimens of the shell length from 17-25 cm were collected. In the autumn after emptying the fishponds some large individuals were found at the bottom. The mussels probably appeared there together with the introduction of carp fry from Gosławice near Konin (Kujawy Lake District, C-Poland) performed 5 years earlier. The developmental stage of these specimens was not determined.

The occurrence of *S. woodiana* in Poland besides the cooling water and colonisation of cooler reservoirs seems similar to the expansion of *Corbicula fluminea* in North America. For a long time it was assumed that because of *C. fluminea* sensitivity to low temperatures its range of occurrence did not

exceed 40°N. The only water reservoirs above this parallel of latitude, in which representative of this species were found were the cooling water release channels (COUNTS 1986).

According to Elton (1958) the inbred reproduction guarantees success to animals and plants attempting colonisation of new areas beyond the natural range of their occurrence. At present it does not seem that the populations of *S. woodiana* occurring in the Odra River below the release of cooling water are threatened with the inbred depression related to inbreeding and consequent homozygosity of individuals and their decreasing viability. It is expected that the populations will be supplied with the genetic material from the mussels living in the heated water of the cooling water release channel as it is supposed to have happened in the beginning. The glochidia present in their life cycle may have been introduced with the fish moving in the Międzyodrze area into particular channels of their current occurrence. They settle and survive at habitats whose water temperature is by 1-3°C higher than that typical of sites above the release of the heated water. The current range of their expansion from the outlet of the cooling water release channel to the Eastern Odra River to the Żeglarski Channel (site F) is 6 km long. It is the actual area whose waters of slightly elevated temperature they have colonised. Regardless of the current state the distribution of the species in the channels of the Międzyodrze area below the cooling water release channel and in the Eastern Odra River must be closely monitored.

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**NUMBERS AND BIOMASS OF *LUMBRICIDAE*  
ON PASTURES TREATED WITH ORGANIC  
OR MINERAL FERTILIZATION WITHIN THE AREA  
OF MAZURY LANDSCAPE PARK IN NORTHEASTERN  
POLAND**

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**Key words:** *Lumbricidae*, pasture, fertilization by compost, mineral fertilization.

**Abstract**

During the years 1997-1999, the numbers and biomass of earthworms in the soil of pastures fertilized with compost at 30 t ha<sup>-1</sup> or with mineral fertilizers at the dose corresponding with the quantity of nitrogen in compost (150 kg ha<sup>-1</sup>) situated in the area of Mazury Landscape Park in northeastern Poland was studied. The number and biomass of earthworms were significantly larger at the pasture treated with organic fertilization than at the pasture treated with mineral fertilization at ( $P < 0.05$ ); the average number was higher by 1.2 times (351-418 individuals m<sup>-2</sup> and 279-349 individuals m<sup>-2</sup>), biomass by 1.4 times (142.4-173.0 g m<sup>-2</sup> and 103.3-119.5 g m<sup>-2</sup>). In both pastures presence of three earthworm species: *Apporectodea caliginosa*, *Lumbricus terrestris*, *Apporectodea rosea* was found. *Apporectodea caliginosa* was the dominating species (77.0-93.2% of total number). The number and biomass of that species during the entire period of the experiment were significantly higher at the pasture treated by organic fertilization than at the pasture treated with mineral fertilization. On the other hand the number of *Lumbricus terrestris* possessing the share of 6.6-22.4% did not show any relation to fertilization type.

## LICZEBNOŚĆ I BIOMASA *LUMBRICIDAE* W WARUNKACH PASTWISK NAWOŻONYCH ORGANICZNIE ALBO MINERALNIE NA OBSZARZE MAZURSKIEGO PARKU KRAJOBRAZOWEGO W PÓŁNOCNO-WSCHODNIEJ POLSCE

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Słowa kluczowe: *Lumbricidae*, pastwisko, nawożenie kompostem, nawożenie mineralne.

### Abstrakt

W latach 1997-1999 badano liczebność i biomasę dżdżownic w glebie pastwisk położonych w północno-wschodniej Polsce, na obszarze Mazurskiego Parku Krajobrazowego. Pastwiska były nawożone kompostem w dawce 30 t ha<sup>-1</sup> albo mineralnie w dawce odpowiadającej ilości azotu w kompoście (150 kg ha<sup>-1</sup>). Liczebność i biomasa dżdżownic były istotnie większe na pastwisku nawożonym organicznie niż mineralnie ( $P < 0,05$ ), średnio liczebność 1,2-krotnie (351-418 os. m<sup>-2</sup> i 279-349 os. m<sup>-2</sup>), biomasa – 1,4-krotnie (142,4-173,0 g m<sup>-2</sup> i 103,3-119,5 g m<sup>-2</sup>). Na obu pastwiskach stwierdzono występowanie trzech gatunków dżdżownic: *Apporectodea caliginosa*, *Lumbricus terrestris*, *Apporectodea rosea*. Dominowała *Apporectodea caliginosa* (77,0-93,2% liczebności). Liczebność i biomasa tego gatunku przez cały okres badań były istotnie większe na pastwisku nawożonym organicznie, natomiast liczebność *Lumbricus terrestris* (6,6-22,4%) nie wykazywała większego związku z rodzajem nawożenia.

### Introduction

Many papers showed that earthworms have a favorable influence on the structure and fertility of soil and, as a consequence, productivity of crops and pastures (SHARPLEY et al. 1979, STOCKDILL 1982, TEMPLE-SMITH 1991, EDWARDS, BATER 1992, SCHEU 2004). However, the number of earthworms depends on abundance of the environment with organic matter available as nutrition, which is the main factor determining the density of population (SATCHELL 1971). Every extensive cultivation system in which organic fertilization is not applied can cause a significant decrease in the density of earthworms (EDWARDS, LOFTY 1972). Application of organic fertilizers causes an increase in the population of earthworms by from a few to by several times (SATCHELL 1955, O'CONNOR 1971, EDWARDS, LOFTY 1972, ZAJONC 1975). Mineral fertilization can influence earthworms directly through changes in the soil pH or indirectly by influencing the level of vegetable mass production (EDWARDS, LOFTY 1972). It is generally assumed that an increase in mineral fertilization, particularly with nitrogen, stimulates the development of earthworms population (HEATH 1962). However, excessively large doses of mineral

fertilizers can contribute to reduction in earthworms population (DUNGER 1964, ZAJONC 1970a,b, 1975, HONCZARENKO et al. 1971, NOWAK 1976, EDWARDS 1983). The type of fertilization applied can also influence composition of species and population structure of earthworms (KASPRZAK 1979, ANDERSEN 1980).

The influence of mineral fertilization on changes in numbers of earthworms was studied on the soils of pastures in Lithuania treating the soil with calcium at the same time (ATLAVINITE 1975). HONCZARENKO et al. (1971) in field experiments carried on pastures studied the influence of nitrogen fertilization at various doses on the number of earthworms. In some studies the density of earthworms in pastures in places with inflow of manure from feeding livestock was compared with places that were not fertilized (SEARS after FILIPEK 1963, EDWARDS, LOFTY 1972, NOWAK 1975). So far no comparative studies on influence of organic and mineral fertilization at balanced doses of nitrogen on the density of earthworms present at pastures were conducted.

The here presented study aimed at analysis of changes in density and biomass of earthworms in case of pastures treated by organic or mineral fertilization at the balanced dose of nitrogen situated within the Mazury Landscape Park in northeastern Poland. This study was a part of a wider study on the influence of organic and mineral fertilization on the natural environment of pastures (CHARKIEWICZ, CYMERMAN 1994, ADAMIAK et al. 1996, KORONA et al. 1998, KISIEL et al. 1998).

## Materials and Methods

The experiments were carried at the pasture of the Research Station of Ecological Agriculture and Preservative Animals Breeding of the Polish Academy of Science in Popielno situated in the area of the Mazury Landscape Park in northeastern Poland. The pasture was established in 1993 by sowing the pasture mix consisting of: *Lolium perenne* – 30%; *Poa pratensis* – 20%; *Festuca pratensis* – 15%; *Phleum pratense* – 4%; *Dactylis glomerata* – 3%; *Trifolium repens* – 19%; *T. hybridum*, – 3%; *T. pratense* – 3% and *Lotus corniculatus* – 3% on the area of 7.6 ha. One part of the pasture was fertilized with compost at the dose generally applied in ecological agriculture ( $30 \text{ t ha}^{-1}$  – corresponding to  $150 \text{ kg N ha}^{-1}$ ) while the other part was treated by mineral fertilization at the balanced dose equal to the quantity of nitrogen in compost. The compost was produced of bovine manure (80%) and soil (20%). Mineral fertilizers, NPK were applied at the ratio of 1: 0.5: 0.6. Fertilization was applied yearly, compost during spring and mineral fertilizers in two parts: 50% of the N dose and 100% of P and K dose during the spring and the second part

of the N dose after grazing or harvest of the first swath. In both pastures cattle was grazing in the system of quarters of 20 cows in each pasture. The place of study was a plateau set on brown soils with pH 5.9 and humus layer 26-28 cm thick. The experiments were conducted during the years 1997-1999.

Samples for examination were collected at three times during the vegetation season: in the spring, summer and autumn. Manual picking of earthworms from the soil at ten points of the pasture representing the total area of 1 m<sup>2</sup> and down to the depth of 30 cm. The composition of species, immature and mature form and wet bodyweight of the earthworms were determined. Immature individuals were assumed to be the individuals that had no saddle or hills or pubertal rolls developed.

Years 1997 and 1998 were characterized by a similar sum of atmospheric precipitations for the period from April to September (314 and 323.6 mm). In 1999, the sum of atmospheric precipitations during that period was 449.1 mm. Longer periods without precipitations in 1997 occurred during the first decade of June as well as second and third decade of August, in 1998 during the third decade of April, first – second decade of May and third decade of July while in 1999 during the third decade of July and in September (Figure 1). The average temperature for the period from April to September in 1997 and 1998 was 14.4°C, and in 1999 15.7°C. During the years 1997-1999, during the period April-September in April of all years covered and in May 1999 the temperatures were lower than 10°C.

The U-test by Mann-Whitney was applied for verification of significance of the differences in the biomass of earthworms or species of earthworms testing the hypothesis saying that the sums of ranks from tests from identified groups differ significantly. The verification of the hypotheses concerning existence of differences in numbers or species of earthworms between groups representing consecutive years of the experiments on pastures with mineral or organic fertilization was done using the  $\chi^2$  test examining the consistency of proportions in numbers of earthworms in the identified groups with the proportion of the number of samples collected.

The verification of the hypotheses concerning significance of differences in the share of immature earthworms and sexually mature earthworms depending on fertilization and collection season was done using the  $\chi^2$  test according to the procedure for testing the relation between two nominal scales (ŁOMNICKI 1995, STANISZ 1998). Numbers and biomasses of earthworms summed up obtained for one location from three samples collection periods during a year were compared between years of studies and between pastures with organic and mineral fertilization.

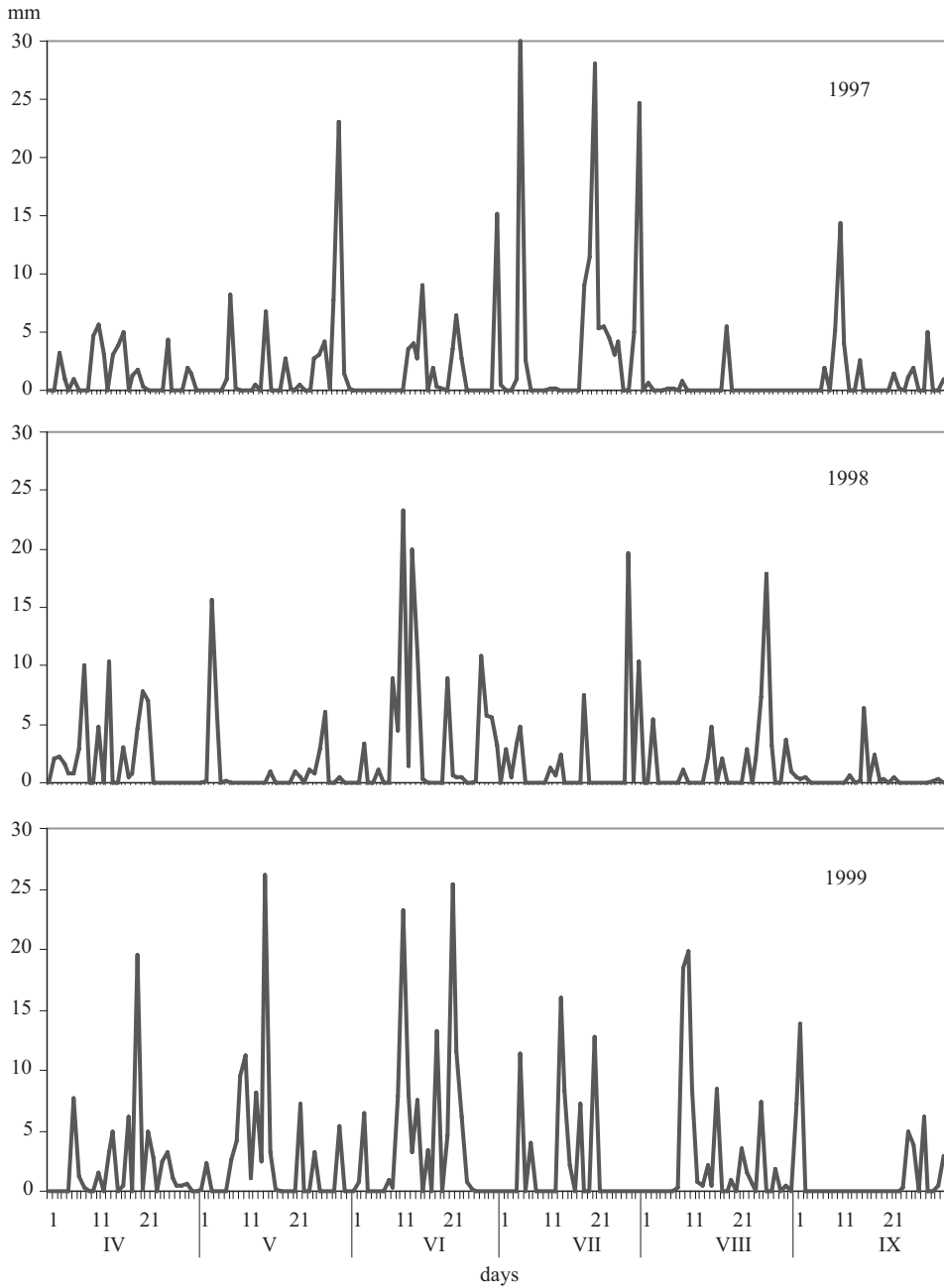


Fig. 1. Day atmospheric precipitations during the experiment period

## Results

Three species of earthworm: *Aporrectodea caliginosa* (Sav.), *Lumbricus terrestris* L. and *Aporrectodea rosea* (Sav.) were found in the soils of pastured treated with organic or mineral fertilization (Table 1). The number of earthworms in the pasture with organic fertilization during all the years of the study was significantly higher than the number of earthworms in the pasture with mineral fertilization, in average by 1.2 times higher. Similarly, the biomass of earthworms from the pasture with organic fertilization during all years of study was statistically significantly higher than from the pasture with mineral fertilization, in average by 1.4 times higher (Table 2).

Table 1  
Structure of earthworm species, total and arithmetic average number of earthworms from three collections from three periods of the vegetation season per 1 m<sup>2</sup> of pastures with mineral (min.) or organic (org.) fertilization during the experiment

Year	Fertilization type	<i>A. caliginosa</i>		<i>L. terrestris</i>		<i>A. rosea</i>		$\Sigma n$	M
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%		
1997	min. org.	972	92.7	74	7.1	2	0.2	1048	349
		1168*	93.2	83	6.6	3	0.2	1254*	418
1998	min. org.	766	85.0	134*	14.9	1	0.1	901	300
		954*	90.4	91	8.6	10	1.0	1055*	351
1999	min. org.	699	83.4	133	15.9	6	0.7	838	279
		925*	77.0	269*	22.4	7	0.6	1201*	400

\* – statistically significant values larger than ( $P < 0.05$ ) in the comparison of the numbers of earthworms from pastures treated by mineral or organic fertilization

Table 2  
Biomass of earthworm species, total and arithmetic average biomass of earthworms from three collections from three periods of the vegetation season per 1 m<sup>2</sup> of pastures with mineral (min.) or organic (org.) fertilization during the experiment

Year	Fertilization type	<i>A. caliginosa</i> (g m <sup>-2</sup> )	<i>L. terrestris</i> (g m <sup>-2</sup> )	<i>A. rosea</i> (g m <sup>-2</sup> )	$\Sigma$ (g)	M (g)
1997	min. org.	320.1	30.5	0.3	350.9	117.0
		465.0*	25.9	0.6	491.5*	163.8
1998	min. org.	259.9	49.8	0.2	309.9	103.3
		399.3*	26.1	1.8	427.2*	142.4
1999	min. org.	301.0	57.0	0.6	358.6	119.5
		412.7*	105.5*	0.8	519.0*	173.0

\* – statistically significant values larger than ( $P < 0.05$ ) in the comparison of the biomass of earthworms from pastures treated by mineral or organic fertilization



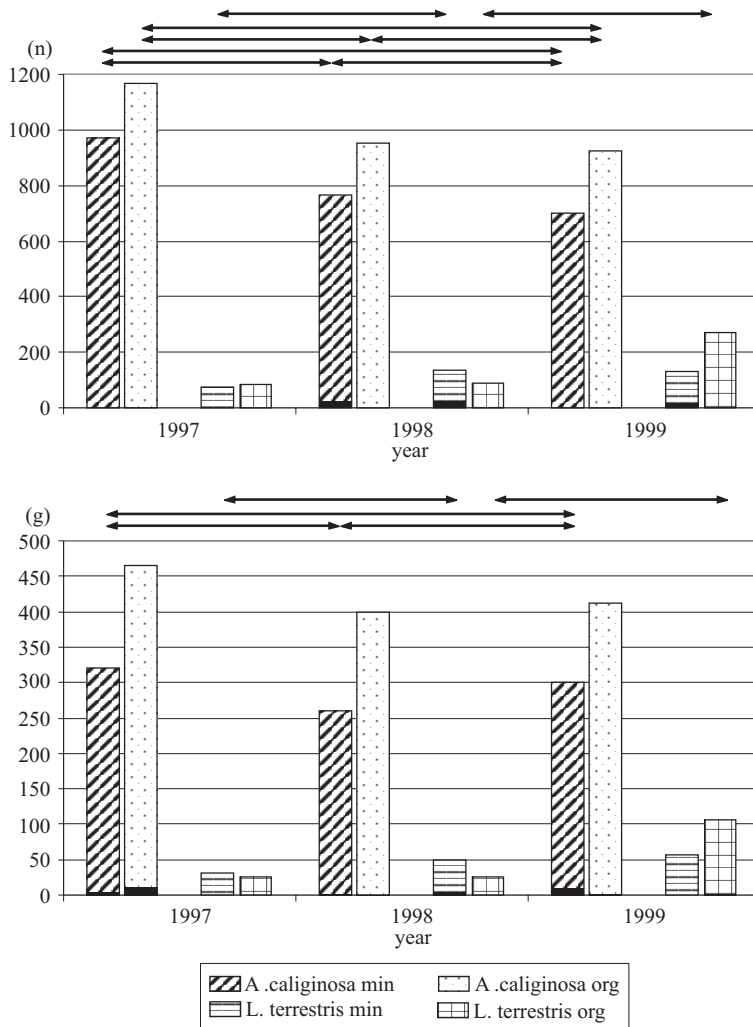


Fig. 2. Changes in total numbers and biomass of *A. caliginosa* and *L. terrestris* from three collections from three parts of the vegetation period per 1 m<sup>2</sup> of pastures treated by organic or mineral fertilization during consecutive years of the experiment. The arrows show numbers and biomasses differing significantly ( $p < 0.05$ )

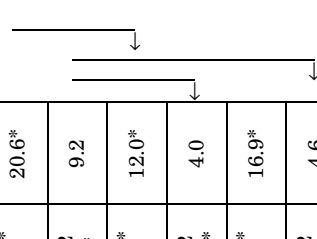
The higher numbers and biomass of earthworms from the pasture with organic fertilization are mainly the consequence of the domination of *A. caliginosa*, the number and biomass of which were significantly higher during the entire period of study at the pasture with organic fertilization. The numbers and biomasses of *L. terrestris* were not differentiated so clearly. In 1997 there were no significant differences between the pasture with organic

fertilization and the pasture with mineral fertilization. In 1998, the number of *L. terrestris* was significantly higher at the pasture with mineral fertilization but biomasses did not differ significantly. In 1999 the number and biomass of *L. terrestris* were significantly higher at the pasture with organic fertilization than at the pasture with mineral fertilization (Tables 1, 2).

In the number and biomass of *A. caliginosa* and *L. terrestris* opposite trends were found during the consecutive years of studies (Figure 2). The number and biomass of *A. caliginosa* in both pastures decreased significantly in 1998 as compared to 1997 and in 1999 the numbers and biomasses did not differ significantly from those in 1998. In 1998, in both pastures, individuals of *A. caliginosa* in the state of hibernation were observed. In analyzing the numbers and biomasses of *A. caliginosa* comprehensively for the period of from 1997 to 1999, the  $\chi^2$  and Mann-Whitney U tests showed a significant decrease in the number of *A. caliginosa* at both pastures, a decrease in biomass of *A. caliginosa* at the pasture with mineral fertilization and absence of such a trend in the biomass of *A. caliginosa* at the pasture with organic fertilization. The numbers and biomasses of *L. terrestris* increased significantly at the pasture with mineral fertilization in 1998 as compared to 1997 and did not differ significantly on the pasture with organic fertilization. In 1999, the same level of number and biomass of *L. terrestris* as in 1998 was recorded at the pasture with mineral fertilization while at the pasture with organic fertilization a significant increase in the number and biomass was recorded. Generally, during the years 1997-1999, the number and biomass of *L. terrestris* increased at both pastures.

The population of *L. terrestris* consisted mainly of immature individuals with 2.2-3.0 percent share of mature individuals in 1999 at both pastures and in 1998 at the pasture with mineral fertilization (Table 3). Mature individuals of *A. caliginosa* were present during all years of study at both pastures. A significantly higher percentage share of mature individuals was determined at the pasture with organic than at the pasture with mineral fertilization. In 1998, at both pastures a significantly lower number of immature individuals per one mature individual was observed compared to 1997. *L. terrestris* did not show diversification in percentage share of mature and immature individuals at pastures with organic or mineral fertilization and from year to year. There were also no significant differences in average biomasses of immature and mature individuals depending on the type of pasture fertilization. Differences in average biomasses were observed in *A. caliginosa*. In 1997 and 1998, the average biomasses of immature individuals were higher at the pasture with organic fertilization than at the pasture with mineral fertilization while in 1999 the average biomass of immature individuals was higher at the pasture with mineral fertilization. Average biomass of mature *A. caliginosa* individuals

Table 3  
Percentage share, descriptive statistics of bodyweight of immature (im) and mature (m) individuals and the number of immature individuals per 1 mature individual (im/m) of *A. caliginosa* and *L. terrestris* on pastures treated by mineral (min.) or organic (org.) fertilization during the experiment

Year	Fertilization type	Development stage	A. caliginosa					L. terrestris						
			n	%	im/m	d	Bodyweight		n	%	im/m	Bodyweight		
							average	SD				average	SD	
1997	min.	im	927	95.4*	20.6*		0.31	0.24	74	100.0	–	0.41	0.48	
		m	45	4.6			0.80*	0.17	0	0		–	–	
	org.	im	1054	90.2	9.2		0.36*	0.24	83	100.0	–	0.32	0.29	
		m	114	9.8*			0.73	0.15	0	0		–	–	
1998	min.	im	707	92.3*	12.0*			0.31	0.22	130	97.0	32.5	0.32	0.36
		m	59	7.7			0.72	0.17	4	3.0		1.94	1.81	
	org.	im	765	80.2	4.0			0.34*	0.24	91	100.0	–	0.29	0.27
		m	189	19.8*			0.74	0.20	0	0		–	–	–
1999	min.	im	660	94.4*	16.9*			0.41*	0.23	129	97.0	32.2	0.39	0.48
		m	39	5.6			0.70	0.18	4	3.0		1.49	0.72	–
	org.	im	760	82.2	4.6			0.38	0.26	263	97.8	43.8	0.36	0.38
		m	165	17.8*			0.74	0.16	6	2.2		1.84	1.05	–

\* – statistically significant values larger than ( $P < 0.05$ ) in the comparison of the number shares of immature and mature individuals as well as average bodyweights of earthworms from pastures treated by mineral and earthworms from pastures treated by organic fertilization during the same years of the experiment

d – statistically significant values larger than ( $P < 0.05$ ) in the comparison of the number shares of immature and mature individuals of earthworms during different years of the experiment

was significantly higher at the pasture with mineral fertilization in 1997 while in 1998 and 1999 there were no significant differences in the average biomass of mature individuals from both pastures.

## Discussion

At the studied pastures with organic or mineral fertilization presence of three earthworm species was found. Similarly low number of earthworm species (2-3) recorded at the pastures in the areas of: Mydlniki, Chełm, Biezanów in Poland (WENDORFF, BRZEZIŃSKA 1980), at the pastures of Bavaria (HINSCHBERGER, BAUER 1994) and at pastures in Hungary (ZICSI 1959). Larger numbers of earthworm species (4-6, 10) were found at the pastures in the area of Kraków and Jaworki in Poland (WENDORFF, BRZEZIŃSKA 1980) and at pastures in Denmark (HENDRISEN 1991, MATHER, CHRISTENSEN 1992).

The density of earthworms at pastures with mineral or organic fertilization in the presented study (279-349 individuals  $\text{m}^{-2}$  and 351-418 individuals  $\text{m}^{-2}$ ) as compared to the density of earthworms at pastures in the area of Kraków and Jaworki (56 to 113  $\text{m}^{-2}$ ) (WENDORFF, BRZEZIŃSKA 1980, NOWAK 1975) was three – six times higher. It was also higher than the density of earthworms at pastures of southwestern Slovakia (147 individuals  $\text{m}^{-2}$  ZAJONC, OROS 1967) and in Lithuania (100-122 individuals  $\text{m}^{-2}$  ATLAVINITE 1976). On the other hand it was compatible to the data for the pastures in Australia where in the yearly cycle the density of earthworms ranged from 269 to 740 individuals  $\text{m}^{-2}$  (BARLEY 1959). In the Netherlands, in the soil of orchards 300-500 individuals of earthworm individuals  $\text{m}^{-2}$  were recorded (RHEE, NATHAN 1961). Similarly, the biomasses of earthworms in this fertilization related study (103.3-119.5  $\text{g m}^{-2}$  and 142.4-173.0  $\text{g m}^{-2}$ ) exceeded by 4-11 times the biomasses of earthworms at pastures in the area of Kraków and Jaworki (12.6 to 32.5  $\text{g m}^{-2}$  WENDORFF, BRZEZIŃSKA 1980). Biomass of earthworms at the level of 115  $\text{g m}^{-2}$  was recorded after summer irrigation during the field experiment in the soils of Australia (SMEATON et al. 2003).

Application of fertilization with compost or mineral fertilization at the same dose of nitrogen at pastures differentiated significantly the number and biomass of earthworms at those pastures. Mainly, fertilization with compost, as compared to mineral fertilization, had a significant influence on development of the dominating species – *A. caliginosa* population. *L. terrestris* did not show such clear preference for environment conditions at the pasture with compost fertilization. ANDERSEN (1980) reported that 200  $\text{t ha}^{-1}$  of farm manure was the optimum dose for increase in number and biomass of earthworms, including *L. terrestris* and *A. caliginosa*.

The opposite trends that developed in *L. terrestris* and *A. caliginosa* during the study period (increase for *L. terrestris*, decrease for *A. caliginosa*) could be linked to different humidity preferences of *A. caliginosa* and *L. terrestris* (DAUGBJERG 1988). According to DAUGBJERG (1988), under the field conditions, in case of low soil humidity (< 16 %), mature individuals of *A. caliginosa* fall into hibernation or diapause while the activity of mature individuals of *L. terrestris* can be limited only exceptionally in case of severe drought. In 1998, at both pastures, in case of *A. caliginosa* individuals in the state of hibernation, the lowest proportions between immature and mature individuals during the study period and lower numbers at both pastures were found as compared to 1997, which matched the longer periods without atmospheric precipitations during the spring season of 1998. In case of *L. terrestris*, in 1998, the numbers of individuals were similar and higher than in 1997 while the proportions in numbers of immature to mature individuals did not differ significantly over the entire period of study. These data indicate that absence of atmospheric precipitations in 1998 limited the reproductive activity of the endogenous species *A. caliginosa*, but did not limit the population of anecic *L. terrestris* (BOUCHÉ 1977).

Absence of significant differences in the average biomass of immature and mature individuals as well as clear differences in the number and biomass of *L. terrestris* under conditions of pasture with organic fertilization as compared to the data for conditions of pasture with mineral fertilization shows that the type of applied fertilization at the doses applied at the pastures was also of no major importance for *L. terrestris*. The data indicates that at pastures with organic and mineral fertilization the availability level of food was similar for mulch feeding *L. terrestris* (DOUBLE, BROWN 1998). Differences between populations living under the conditions of the pasture with organic and pasture with mineral fertilization were shown by *A. caliginosa* – soil-feeding species (DOUBLE, BROWN 1998). During the entire study period *A. caliginosa* was characterized by larger numbers, larger biomass and larger percentage share of mature individuals at the pasture with organic fertilization than under the conditions of the pasture with mineral fertilization.

Concluding, application of fertilization with compost at 150 kg ha<sup>-1</sup> or mineral fertilization with nitrogen dose equivalent to that in the compost at pastures had different influence on the two major species of earthworm present – *A. caliginosa* and *L. terrestris*. *A. caliginosa* showed more active development at the pasture with organic fertilization than at the pasture with mineral fertilization while *L. terrestris* did not show such characteristics.

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## THE ACTIVITY OF ANTIOXIDANT ENZYMES IN SUSPENSION CULTURED TOBACCO CELLS TREATED WITH HEAVY METALS

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**Key words:** antioxidative enzymes, cell suspension, heavy metals, *Nicotiana tabacum*, oxidative burst, stress.

**Abbreviations:** CAT – catalase; GPOX – guaiacol peroxidase; SOD – superoxide dismutase; O<sub>2</sub><sup>•-</sup> – superoxide radical.

### Abstract

In this work the effect of cadmium, copper, iron and zinc on antioxidant enzymes activity in *Nicotiana tabacum* Bright Yellow 2 cells derived from suspension culture was studied. It was noticed that all of tested elements stimulated the increase in superoxide dismutases activities and initial increment, followed by the decrease in catalase activity. Zinc ions in 500 µM concentration caused continuous raise and copper ions in 10 µM caused decrease in the activities of nonspecific peroxidases. The presence of cadmium ions in 500 µM concentration in reaction mixture caused the initial decrease followed by increase in phenol peroxidases activities. Iron ions in 5 µM concentration induced the enhance of nonspecific peroxidase activity after the third hour of exposition.

## AKTYWNOŚĆ ENZYMÓW ANTYOKSYDACYJNYCH W KOMÓRKACH KULTURY ZAWIESINOWEJ TYTONIU PODDANEJ DZIAŁANIU METALI CIĘŻKICH

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**Słowa kluczowe:** enzymy antyoksydacyjne, metale ciężkie, *Nicotiana tabacum*, stres, wybuch oksydacyjny, zawiesina komórek.

**Skróty:** SOD – dysmutaza ponadtlenkowa; CAT – katalaza; GPOX – peroksydaza gwajakolowa; O<sub>2</sub><sup>•-</sup> – anionorodnik ponadtlenkowy.

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### Abstrakt

W pracy zbadano wpływ kadmu, cynku, miedzi i żelaza na aktywność enzymów antyoksydacyjnych komórek w kulturze zawiesinowej *Nicotiana tabacum* Bright Yellow 2. Odnotowano, że wszystkie testowane pierwiastki stymulowały wzrost aktywności dysmutaz ponadtlenkowych oraz początkowy wzrost, a następnie spadek aktywności katalaz. Jony cynku w stężeniu 500  $\mu\text{M}$  powodowały stały wzrost, a jony miedzi w stężeniu 10  $\mu\text{M}$  – spadek aktywności niespecyficznych peroksydaz. Obecność w mieszaninie reakcyjnej jonów kadmu w stężeniu 500  $\mu\text{M}$  skutkowała początkowym spadkiem, a następnie wzrostem aktywności peroksydaz fenolowych. Jony żelaza w stężeniu 5  $\mu\text{M}$  po trzeciej godzinie ekspozycji indukowały wzrost aktywności niespecyficznych peroksydaz.

## Introduction

For the majority of organisms it is impossible to live on Earth without the oxygen. The oxygen enables effective energy production, but in the same time, its radical forms leads to damages in cells (ARORA et al. 2002, MITTLER 2002). To avoid harmful effects caused by free radicals in organisms or to appease their consequences, plants initiate defense mechanisms involving enzymes and low mass antioxidants (ARORA et al. 2002, MITTLER 2002). From this reason, it could be assumed, that early, heavy metal-induced stress response (RAEYMAEKERS et al. 2003, ZHANG et al. 2005, ŻRÓBEK-SOKOLNIK et al. 2007), should also induce “the activation” of the enzymatic antioxidants. However, it must be remembered, that heavy metals often cause the inactivation of antioxidant enzymes (VANGRONSVELD, CLIJSTERS 1994).

In previous report we proved that cadmium, copper, iron and zinc ions enhanced hydrogen peroxide production in tobacco cell suspension culture (ŻRÓBEK-SOKOLNIK et al. 2007). Therefore, this time our main interest was to examine the effect of this metals on the activity of cellular superoxide dismutases, catalases and guaiacol peroxidases.

## Materials And Methods

### Plant Material

The tobacco (*Nicotiana tabacum* cv. Bright Yellow 2) cell suspension culture described by NAGATA et al. (1992) was used as the plant material. Cells were grown in MS medium (MURASHIGE, SKOOG 1962), supplemented with 0.2 g of  $\text{KH}_2\text{PO}_4$ , 30 g of sucrose, 0.2 mg of 2,4-dichlorophenoxyacetic acid (2,4-D), 1 mg of thiamine-HCl and 100 mg of *myo*-inositol per liter. Every seventh day 2 ml of cells in stationary-growth phase was transferred into 50 ml of fresh MS medium. The cells were grown in 300 ml Erlenmeyer flasks placed

on a rotary shaker, at 130 rpm in the dark at 27°C. For the H<sub>2</sub>O<sub>2</sub> measurements 5-day old cells after transfer were used. The cells were collected by centrifugation at  $3000 \times g$  for 90 s. The pellet of cells was washed once with fresh MS medium, repelleted by centrifugation and resuspended in MES buffer (5 mM; pH 6.7). The final cells concentration in the reaction mixtures was 20 mg of cells fresh weight per ml, and its was exposed to heavy metals for 4 hours. Every 1 hour the cells were filtrated, frozen in liquid nitrogen, and stored at -80°C.

### Determination of cells antioxidative enzymes activity

Extracts of enzymes were prepared by homogenizing the frozen cells, with cold mortar and pestle, in a cold solution containing 50 mM phosphate buffer (pH 7.8), 1 mM EDTA, 1 mM DTT and 1 mM PMSF. After then, the homogenate was centrifuged at  $1200 \times g$  for 20 minutes at 4°C and the supernatant was used for the assays.

Guaiacol peroxidase activity was determinated by monitoring guaiacol oxidation to tetraguaiacol (AMAKO *et al.* 1994). The reaction mixture (final volume 3 ml) consisted of 50 mM phosphate buffer (pH 7.0), 0.1 mM H<sub>2</sub>O<sub>2</sub>, 20 mM guaiacol, and 0.1 ml enzyme extract. The reaction was initiated by the addition of hydrogen peroxide, and monitoring the increase in absorbance at 470 nm for 3 minutes was made ( $\epsilon = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ).

Catalase activity was determinated by monitoring the disappearance of hydrogen peroxide (AEBI 1984). The reaction mixture (final volume 3 ml) consisted of 50 mM phosphate buffer (pH 7.0), 10 mM H<sub>2</sub>O<sub>2</sub>, and 0.6 ml of enzyme extract. The reaction was initiated by the addition of enzyme extract and monitoring the increase in absorbance at 240 nm for 3 minutes was made ( $\epsilon = 0,36 \text{ mM}^{-1} \text{ cm}^{-1}$ ).

Superoxide dismutase activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (BEAUCHAMP, FRIDOVICH 1971). The reaction mixture (final volume 1 ml) consisted of 50 mM carbonate buffer (pH 10.2); 0.5 mM nitroblue tetrazolium; 0.5 mM xantine; 40 mU ml<sup>-1</sup> xantine oxidase and 0.1 ml enzyme extract. The reaction was initiated by the addition of xantine oxidase and monitoring the increase in absorbance at 560 nm for 5 minutes. The measurements were made for several enzyme extracts volume, lower and higher then 0.1 ml. The relative rate of nitroblue tetrazolium reduction was calculated for all assays and the volume of SOD which gave 50% inhibition of reduction rate was pointed out.

Protein was assayed according to the method of BRADFORD (1976) using concentrated Bio-Rad micro dye binding reagent (250  $\mu$ l per 1 ml protein

solution). As a standard bovine serum albumin in concentrations ranging between 2.5 and 30  $\mu\text{g ml}^{-1}$  was used. The absorbance was measured at 595 nm.

Chemical reagents used in the studies were bought from following companies: POCh, Poland; Sigma-Aldrich, Germany; Merck, Germany; Bio-Rad, Canada. All chemicals were analytical grade.

## **Statistical Analysis**

All experiments were made at least in 4 series with 3 replications in each series. For each series the average and standard error were calculated ( $\pm$  SE). The Microsoft Excel 2003 and STATISTICA 6.0 computer programs were used.

## **Results and Discussion**

It is taken on, that superoxide dismutases are the first line of defense mechanisms against reactive oxygen species. In previous work we shown that cadmium, copper, iron and zinc induced the oxidative burst in tobacco cell suspension culture (ŻRÓBEK-SOKOLNIK et al. 2007). Therefore, we assumed that they should also stimulate the activity of SOD. Getting results confirmed this hypothesis. All tested heavy metals stimulated the increase in the activities of superoxide dismutases (Figures 1-4). However, data from literature relating to the impact of the heavy metals on antioxidant enzymes activities are heterogeneous. Stimulating effect of cadmium on SOD was presented, e.g. by OLMOS et al. (2003) and SINGH et al. (2006). GALLEGO et al. (1999) showed, that cadmium ions did not change the activities of this group of enzymes in sunflower leaves. SANDALIO et al. (2001) and ZHANG et al. (2005) confirmed that this metal causes the decrease in superoxide dismutases activities in stressed plants leaves. GALLEGO et al. (1996) proved, that copper ions stimulated, and iron and cadmium ions inhibited the activity of SOD in sunflower leaves. GWÓZDŹ et al. (1997) drawn similar conclusion from their experiments examined the influence of copper and cadmium ions on lupines roots. Basing on they own observations DEMIREVSKA-KEPOVA et al. (2004) concluded that in plants tissues, superoxide dismutases are not involved in defense mechanisms against oxidative injuries caused by copper. Data relating to iron are also various. For example, SHAINBERG et al. (2000) showed that this metal caused the increase in SOD activity in leaves, and SINHA et al. (1997) and SINHA and SAXENA (2006) showed the same phenomenon in roots but not in leaves (where the decrease of this enzymes activities was observed), in plants exposed to toxic iron levels. KAMPFENKEL et al. (1995) noticed no effect of iron on dismutases

in tobacco plants. The most homogeneous data are for zinc. Different group of scientists showed that this metal stimulated SOD activity (PRASAD *et al.* 1999, MADHAVA RAO, SRESTY 2000).

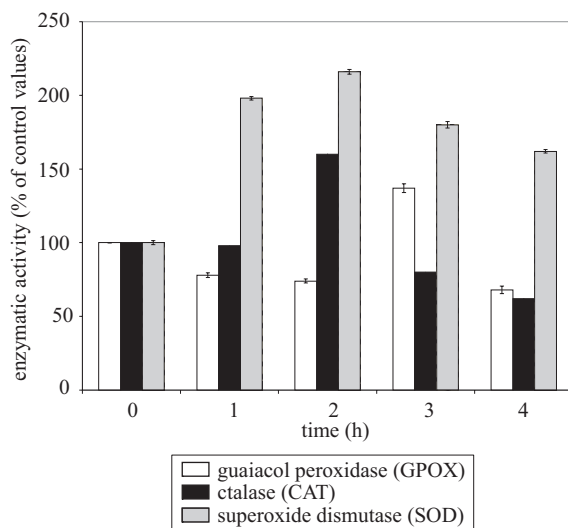


Fig. 1. The effect of 500 µM CdSO<sub>4</sub> on activity of cellular antioxidative enzymes. Values are means with standard errors

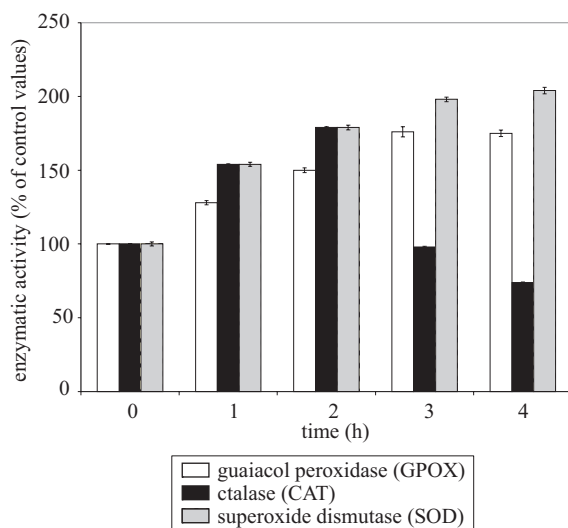


Fig. 2. The effect of 500 µM ZnSO<sub>4</sub> on activity of cellular antioxidative enzymes. Values are means with standard errors

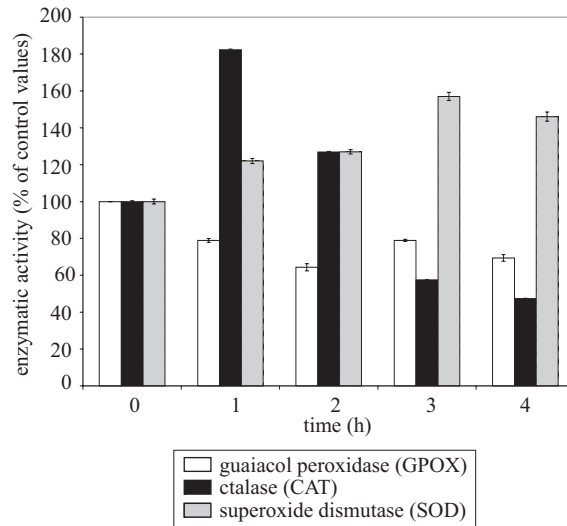


Fig. 3. The effect of 10  $\mu\text{M}$   $\text{CuSO}_4$  on activity of cellular antioxidative enzymes. Values are means with standard errors

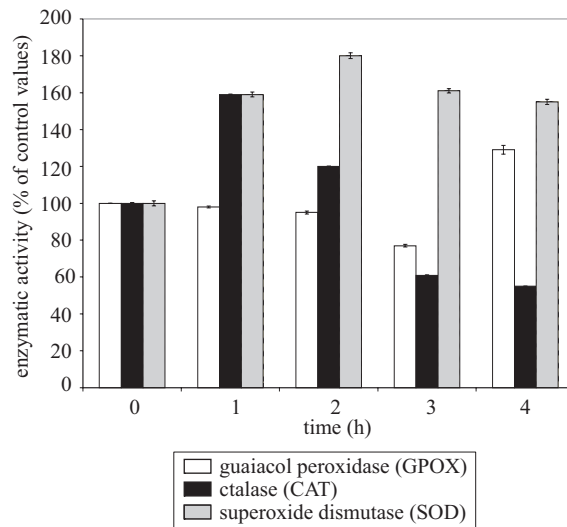


Fig. 4. The effect of 5  $\mu\text{M}$   $\text{FeSO}_4$  on activity of cellular antioxidative enzymes. Values are means with standard errors

Hydrogen peroxide, produced by superoxide dismutases, are dissolved to water by peroxidases and catalases (DAT et al. 2000). It seems, that it can be assumed, that increase in peroxidases activities is a defense response to toxic levels of heavy metals. CUYERS et al. (2002) also proposed that toxic levels

of this elements could change the activities of plants peroxidases both quantitatively and qualitatively. Nevertheless, little is known about the mechanism of peroxidase induction during plant stress response reaction (FANG, KAO 2000). In the case of tobacco cells grown in suspension culture each of used metals showed different impact on cells nonspecific peroxidases activities (GPOX) (Figures 1-4). The presence of 500  $\mu\text{M}$   $\text{CdSO}_4$  in reaction mixture gave alternately the inhibition and the stimulation of discussed enzymes (Figure 1). In second and forth hour of cells and metal co-incubation the decrease of nonspecific peroxidases activities to 70% level of control value was observed. Between second and third hour rapid stimulation of GPOX occurred. Data relating to cadmium as a stimulator of plants nonspecific peroxidases activities was shown by ZHANG et al. (2005) and SINGH et al. (2006). Another scientists proved no susceptibility of plants GPOX to this kind of metal ion (PIQUERAS et al. 1999, SANDALIO et al. 2001). Copper ions in 10  $\mu\text{M}$  concentration (Figure 3) caused about 30% decrease in guaiacol peroxidase activity in first hour of experiment, with no significant impact at subsequent time of experiment. From literature data it appears that micromolar copper concentrations caused stimulation of GPOX activity in plants exposed to heavy metal (CUYPERS et al. 2002, DEMIREVSKA-KEPOVA et al. 2004, TEWARI et al. 2006). Inhibitory effect of copper on activity of soybean membrane-located peroxidases was presented by ZANCANI et al. (1995). Basing on results from our experiments it was presumed that addition of 5  $\mu\text{M}$   $\text{FeSO}_4$  had no significant effect on cells phenol peroxidases (Figure 4). FANG and KAO (2000), on the other hand, observed the induction of peroxidase activity in rice by *de novo* enzyme biosynthesis caused by  $\text{FeSO}_4$ ,  $\text{CuSO}_4$  i  $\text{ZnSO}_4$ . They also proposed that metals (not  $\text{H}_2\text{O}_2$ ) impact on transcription level of this proteins. SATYAKAL and JAMIL (1997) and SINHA and SAXENA (2006) noticed that in conditions of iron excess the increase of nonspecific peroxidase activity occurred in plants roots section, while in leaves its decrease was observed. The continuous rise of GPOX activity in tobacco cells was provoked by zinc ions (Figure 2). Non effect of this metal on phenol peroxidases activities was demonstrated by CUYPERS et al. (2002). Another reports about  $\text{Zn}^{2+}$  ions show stimulating impact of this element on nonspecific peroxidases activities in leaves and shoots of various plants species (MADHAVA RAO, SRESTY 2000, CUYPERS et al. 2002).

All used heavy metals caused initial 50% increment, followed by decrease in catalases activities (Figures 1-4) (started between second and third hour after treating the suspension culture with metals). Literature data relating to the impact of heavy metals on this kind of enzymes are also heterogeneous. The diminution of plants catalases activities caused by the presence of cadmium ions were shown by PIQUERAS et al. (1999), DIXIT et al. (2001), SANDALIO et al. (2001) and SINGH et al. (2006). On the other hand, OLMOS et al. (2003) were not

able to measure the activities of the extracellular catalases in tobacco cell suspension culture treated with 5 mM CdSO<sub>4</sub>. Contrary to them, ZHANG et al. (2005) proved that after initial decrease in activities the increase started, and METWALLY et al. (2005) in plants stressed with cadmium noticed only the increase in catalase activity. Stimulation of catalases activities by copper ions was observed by e.g. LOMBARDI and SEBASTIANI (2005) or TEWARI et al. (2006). There are also some studies showing no effect (CHEN et al. 2000) or inhibitory effect of copper on discussed antioxidant enzymes (CHEN, KAO 1999). KAMPFENKEL et al. (1995) noticed that the presence of iron causes duplication of catalases enzymatic activities in tobacco plants without roots and growing in hydroponical conditions. VAN ASSCHE et al. (1990) proved that excess of zinc in growing medium provokes initial decrease in catalases activities (during 5 hours) and its stimulation at subsequent time (after 6 days) in hydroponics culture of bean seedlings. PRASAD et al. (1999) showed the increase of discussed enzymes in charlock shoot. On the other hand, MADHAVA RAO and SREESTY (2000) observed the decrease in catalase activities in pigeonpea seedlings treated with milimolar concentrations of zinc.

Presented results and literature data show that directions of stress responses are not always identical, because its vastly depend on plant species and examined tissue, heavy metal used and stress intensity (SCHÜTZENDÜBEL, POLLE 2002).

## **Conclusions**

On the base of outcomes it can be stated that exposure to toxic levels of heavy metals reinforce response of antioxidative systems in plants (especially superoxide dismutases). The changeability observed in catalase and guaiacol peroxidase activities suggest hydrogen peroxide generation in defense responses reactions (what was demonstrated in our previous work – ŻRÓBEK-SOKOLNIK et al. 2007), which at least in a part is produced by dismutation of superoxide. On the other hand, it can be proved that catalase and peroxidase are complementary as antioxidants. It shows, that the oxidative burst could initiate signal transduction pathway, which takes part in regulation of plants cells antioxidative response.

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**INDUCING GYNOGENETIC DEVELOPMENT  
OF IDE (*LEUCISCUS IDUS* L.) USING SEMEN  
OF OTHER FISH SPECIES**

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**Key words:** ide, dace, genome manipulation, gynogenotes.

**Abstract**

Induction of gynogenetic development of ide using semen of dace was performed. The results obtained during genetic inactivation of dace sperms showed that the most optimal time of UV irradiation dose was from 5 to 10 minutes with the optimum in minute 9 (3456 J m<sup>-2</sup>). The ide oocytes were next fertilized with genetically inactivated semen of dace and subjected several minutes later to environmental shock aiming at retaining the second polar body inside the oocyte. The obtained results were analyzed using a color marker. The highest percentage of gynogenetic ide breed (exceeding 20%) was obtained when fertilized oocytes were subjected to environmental shock lasting for 3 minutes during the 14<sup>th</sup> minute after fertilization.

**WYWOŁANIE ROZWOJU GYNOGENETYCZNEGO JAZIA (*LEUCISCUS IDUS* L.)  
Z ZASTOSOWANIEM NASIENIA INNEGO GATUNKU**

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**Słowa kluczowe:** jaź, jelec, manipulacje genomowa, gynogenoty.

## Abstrakt

Przeprowadzono indukcję rozwoju gynogenetycznego jازia z zastosowaniem nasienia jelca. Wyniki uzyskane podczas genetycznej inaktywacji plemników jelca wykazały, że najoptymalniejszy czas naświetlania promieniami UV to okres 5-10 minut z optimum w 9 minucie (dawka  $3456 \text{ J m}^{-2}$ ). Oocyty jازia zostały następnie zaplemnione inaktywowanym genetycznie nasieniem jelca i poddane kilkanaście minut później termicznemu szokowi środowiskowemu w celu zatrzymania wewnątrz nich drugiego ciałka kierunkowego. Otrzymane wyniki analizowano, wykorzystując marker barwny. Najwyższy odsetek potomstwa gynogenetycznego jازia uzyskano, gdy oocyt w 14. minucie od zaplemnienia poddano szokowi środowiskowemu trwającemu 3 minuty.

## Introduction

As a result of many years of human activity involved in human expansion and technological development a rapid decrease in populations of numerous fish species occurred. Aiming at restoring the original environment conditions we should aim at increasing the populations of endangered fish species, which can be achieved through development of stocking material production technologies for many fish species as well as biotechnological methods applied in restitution projects.

The process of gynogenesis (CHOURROUT et al. 1980), in which breed with maternal genetic material is obtained is one of the methods for obtaining fish possessing the required characteristics. That procedure is carried out in two steps. First, the genetic material contained in sperms should be destroyed in the process of irradiation with X,  $\gamma$  or UV radiation (VARADARAJ 1993). The sperms are capable then to activate the oocyte only. The oocyte stimulated to develop starts producing a haploid zygote. To achieve appropriate embryo development the oocyte must be subjected to environmental stress (KOMEN et al. 1988): thermal, chemical or pressure. There are two moments during embryo development during which the intended effect, i.e. development of diploid embryo can be achieved. The first moment occurs shortly after oocyte activation when the second directional body can be retained. By joining the cell nucleus it then restores the diploid system of the future organism. The second moment occurs later, during the first mitotic division. To be able to more accurately assess the effectiveness of genome manipulation procedures the gametes of another species or fish of different color (color markers) are used, which allows fast assessment (KUCHARCZYK 2002).

As a result of such manipulations the paternal organism has no share in transfer of genes. The fish obtained are 100 percent of maternal origin. The big advantage here is the possibility of creating recessive homozygotes and breeding lines representing high inbreed levels. As a consequence, it is possible to produce fish with better characteristics responsible for fitness, growth or resistance to parasites or diseases (KAASTRUP, HORLYCK 1987).

The study undertaken aimed at obtaining diploid, gynogenetic breed of ide using dace semen.

## Materials and Methods

### Spawners

Spawners of dace (*Leuciscus leuciscus* L.) dark (wild) in color were obtained by PZW (Polish Anglers Association) Olsztyn from natural waters while spawners of golden orfe (*Leuciscus idus* L.), i.e. yellow colored form of ide, were purchased from pond farms situated in central Poland. All manipulations with spawners and hormonal stimulation were carried out according to the methodology described by KUCHARCZYK (2002).

### Obtaining of gametes

Gametes of dace and golden orfe were obtained from the fish after application of hormonal stimulation according to the methodology described by KUCHARCZYK et al. (1999). Semen was collected from males individually into syringes. The eggs were collected into dry plastic containers. Until the time of manipulation the gametes were kept at a cool place (for maximum 60 min).

### Inactivation of the genetic material of sperms

Semen with spermatozoa motility of 70% (assessed subjectively under magnification) or higher were placed in a batch sample. The milt was diluted in 0.85% NaCl at 1:9 – that solution caused immobilization of sperms (KOMEN et al. 1988). Batches of 2.5 ml of diluted semen were transferred to Petri dishes. The thickness of semen film on dishes was around 1 mm. The UV lamp (36 W) was switched on for at least 30 minutes before initiation of irradiation. During irradiation process the sperms were automatically mixed by pendulum motion with the frequency of around 1 s. The semen exposure time to UV irradiation of 1 min was equivalent to receiving the dose of 384 J m<sup>-2</sup>. During each experiment several control groups were established:

- groups *K* – control of spawn biological quality within species (ide). The oocytes were fertilized with semen (0.05 ml per oocytes sample) that was neither diluted nor irradiated;
- groups *C* – control of biological quality of spawn fertilized with dace semen.

The oocytes were fertilized with semen (0.05 ml per oocytes sample) that was neither diluted nor irradiated;

– groups *D* – solvent quality control. Oocytes were fertilized with diluted semen (0.5 ml per oocytes sample) that was not irradiated before commencement of the experiment (groups *DP*) and after completing the irradiation (groups *DK*).

The diluted semen was irradiated for 0.5; 1; 1.5; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11 and 12 min. Next, samples of spawn were activated with irradiated semen. The fertilized spawn was incubated at 12°C in the laboratory type closed circulation equipped, among others, with temperature control possibility. The experiments were conducted in the dark to avoid photo-reactivation of the sperms' genetic material (KAASTRUP, HORLYCK 1987). The number of haploid embryos ( $1n$ ) was determined as the number of hatched deformed embryos using body color of the hatched embryos as the additional marker. In the groups where genetic inactivation of gametes occurred the breed yellow in color was obtained. The experiment was done in duplicate.

### Gynogenesis

In conducting gynogenesis the semen was diluted in 0.85% NaCl solution and then UV irradiated for 9 minutes. During each experiment a number of control groups was established, including the earlier described groups: *K*, *C* and *D*, as well as groups *I* – control of sperms genetic material inactivation effects. Oocytes were activated with diluted and irradiated semen (0.5 ml per sample of oocytes).

As oocytes were fertilized or activated with either undiluted or diluted semen, different semen volumes (0.05 and 0.5 ml respectively) were applied so that the number of sperms per 1 oocyte should be similar. In test groups, samples of around 100-150 oocytes were mixed with the diluted and irradiated semen. The time of water addition to spawn samples was designated as  $t_o$ . After 10, 12, 14, 16 and 18 minutes from the moment of activation samples of oocytes were transferred to water at  $32.5^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ . The time of spawn exposure to thermal shock was 3 and 5 minutes. Before and after the thermal shock the spawn was incubated at 12°C in a closed recirculation system. The experiments were made in duplicate.

## Statistics

The results obtained were subjected to variance analysis using the Duncan's *post hoc* test ( $P < 0.05$ ).

## Results

The relation between sperms irradiation time and spawn survival until eyed-egg-stage appearance and hatching is presented in Figure 1. The controls confirmed good quality of both male and female gametes, as the survival until hatching did not drop below 80%. Several tests ranging from 0.5 to 12 minutes were conducted to establish the optimum time of sperms irradiation. It was found out that in all tests survival rates until eyed-egg-stage appearance stage was high as from 50 to 90%. The largest number of haploid hatch (1 *N*) was obtained in case of nine minutes irradiation (dose 3456 J m<sup>-2</sup>). In that way almost 5% survival of haplonts (1 *N*) was obtained with occasional diploid individuals (2 *N*) yellow in color (spontaneous gynogenets).

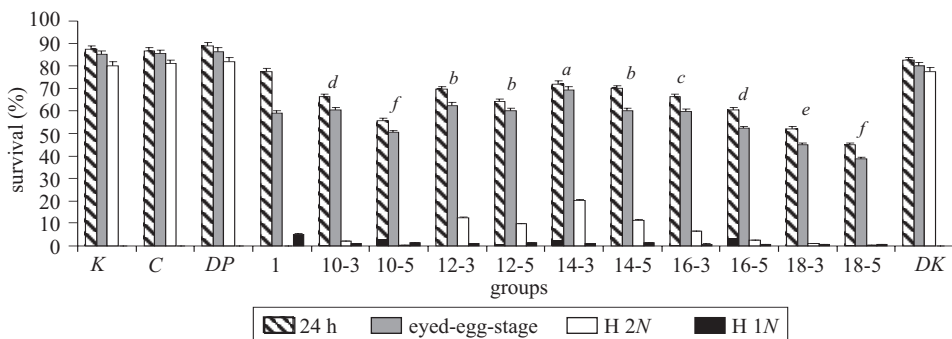
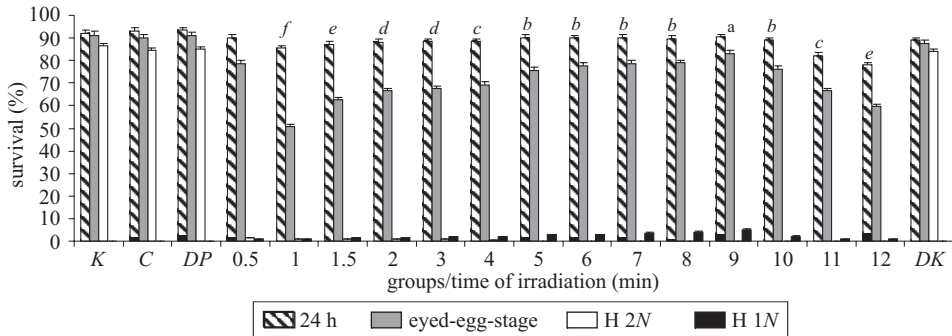


Fig. 1. The results of ide embryos survival after egg insemination using UV-irradiated dace spermatozoa. The control and treated groups are described in M & M. The treated groups marked with the same letter did not differ statistically ( $P < 0.05$ )

After establishing the optimum manipulation of sperms (inactivation of sperm genome by applying UV radiation) the optimum time parameters of the temperature shock had to be determined to double the haploid chromosomes suit of the embryo originating from the maternal organism. Figure 2 presents the survival in 10 groups subjected to thermal (warm) shock. The experiments showed that after 14 minutes from activation of oocytes, the shock of 3 minutes produced the highest survival rate of diplonts (2 *N*) reaching 20%. Occasional haploid individuals were also found in the samples.



Rys. 2. The results of gynogenetic induction of ide development. The control and treated groups are described in M & M. The treated groups marked with the same letter did not differ statistically ( $P < 0.05$ )

## Discussion

Precise determination of optimum parameters for genetic sperms inactivation and environmental shock is necessary in studies on gynogenesis. Such UV irradiation of the collected semen aiming at destruction of the genetic material positioned in the sperms is the first step. It is a relatively difficult process because the situation where the sperm is unable to activate the oocytes cannot be allowed. Induction of gynogenetic development was achieved by applying the environmental thermal shock. The goal of that step in case of gynogenesis is to obtain the double suit of chromosomes. The male participation in that process was reduced to inducing the oocyte development. The survival to the free embryo stage in the experimental groups where the oocytes were activated with UV irradiated semen was much lower than in the controls. Similar results for other fish species were obtained by STANLEY (1976), CHOURROUT et al. (1980), KOMEN et al. (1988), QUILLET et al. (1988), KOMEN et al. (1991), VARADARAJ (1993) and PACHOS et al. (2001). Very few haploid ide embryos hatched (0-5%). In majority of species haploid embryos usually die before commencement of the hatching process, during hatching or during resorption of yolk bladder (KAASTRUP, HORLYCK 1987, CHRISTENSEN, TIERSCH 1994). In case of Mozambique tilapia *Oreochromis mossambicus* (VARADARAJ 1993), northern pike *Esox lucius* (ŁUCZYŃSKI et al. 2004) and common tench *Tinca tinca* (KUCHARCZYK 2002) haploid embryos hatched. Nevertheless, in case of the majority of species (salmonids, cyprinids), on which similar studies were conducted, haploids died before commencement of the hatching process (STANLEY et al. 1975, STANLEY 1976, CHOURROUT et al. 1980, KAASTRUP, HORLYCK 1987, KOMEN et al. 1988, QUILLET et al. 1988, KUCHARCZYK 2002). Effectiveness of semen UV irradiation can be expressed as the percentage of haploid embryos

hatched (VARADARAJ 1993) or as the survival rate observed at one of easily identifiable stages of development (KOMEN et al. 1988). Comparing the data by KOMEN et al. (1988) for common carp *Cyprinus carpio* (the same diluting agent applied for inactivation of spermatozoa motility and the same dilution) and for ide, the results obtained are similar. In case of ide the highest survival rates were observed when applying the UV irradiation doses ranging from 1920 to 3840 J m<sup>-2</sup> (optimum 3456 J m<sup>-2</sup>), while in case of common carp that range was wider, from 1000 to 4000 J m<sup>-2</sup>.

During inducing gynogenetic development by application of thermal shock a very low survival rate to the free embryo stage was found. This is the consequence of overlapping by two types of manipulation (irradiation of semen and activated oocyte shock) each of them separately causing a significant decrease of survival rates as widely described (STANLEY et al. 1975, QUILLET et al. 1988, CHERFAS et al. 1990, CHERFAS et al. 1993, KUCHARCZYK 2002). In all test groups of both diploid and haploid individuals were present. This means that no experimental setup was found that would allow obtaining diploid gynogenetic breed only and exclusively. The highest percentage of gynogenets was obtained in the groups subjected to shock in minute 14 after fertilization of oocytes and the exposed to the shock for 3 minutes.

The survival distributions to free embryo stage as well as effectiveness of retaining the second directional body in the groups subjected to shock between minute 12 and 16 after activation are similar to results of gynogenesis in pike (ŁUCZYŃSKI et al. 2004). In both cases active oocytes were incubated before commencement of manipulation at 12-14°C. Despite low survival rate until free embryo stage the results obtained should be considered very well; review of gynogenesis results made by STANLEY et al. (1975) shows that in many cases of gynogenesis the survival rate reaches the level of 0.01%.

Induction of ide gynogenetic development can also find practical application in production of decorative fish line (golden orfe) for garden ponds. Retaining such characteristics as rare colors or veil-like fins can be obtained as a result of applying the gynogenesis procedure. It happens quite rarely that the results of scientific experiments could be applied in fishery practice immediately.

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## THE INTAKE OF n-3 AND n-6 POLYUNSATURATED FATTY ACIDS IN THE POLISH DIET IN RELATION TO THE INTAKE IN OTHER COUNTRIES

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**Key words:** n-3 and n-6 polyunsaturated fatty acids, the intake of nutrients.

### Abstract

The purpose of the study was to compare the intakes level of n-3 and n-6 polyunsaturated fatty acids in the diet of Poland inhabitants in relation to the intakes of these compounds in other countries. The lowest and most beneficial proportion of polyunsaturated fatty acids n-6 to n-3 is characteristic of the Japanese diet and it amounts to 4:1. The ratio of polyunsaturated fatty acids n-6 to n-3 in the Norwegian and Danish diets was similar (5:1), whereas in the Polish diet the ratio was too high (7:1), which was nutritionally unfavourable. The highest and the least favourable proportion of n-6 to n-3 fatty acids was demonstrated for the American (about 10:1) and Australian (about 8:1) diets.

### SPOŻYCIE WIELONIEASYCONYCH KWASÓW TŁUSZCZOWYCH n-3 I n-6 W POLSKIEJ DIECIE W ODNIESIENIU DO SPOŻYCIA ICH W INNYCH KRAJACH

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**Słowa kluczowe:** wielonienasycone kwasy tłuszczowe n-3 i n-6, spożycie składników odżywczych.

### Abstrakt

Celem pracy było porównanie poziomu spożycia wielonienasyconych kwasów tłuszczowych n-3 i n-6 w diecie mieszkańców Polski ze spożyciem ich w innych krajach. Najniższa i najkorzystniejsza proporcja wielonienasyconych kwasów tłuszczowych n-6 do n-3 charakteryzowała Japończyków. Wynosiła 4:1. Zbliżoną proporcję wielonienasyconych kwasów tłuszczowych n-6 do n-3 wykazano w diecie Norwegów i Duńczyków (5:1), natomiast w sposobie odżywiania się Polaków była ona zbyt wysoka (7:1), co jest niekorzystne z żywieniowego punktu widzenia. Najwyższy i najmniej korzystny stosunek kwasów n-6 do n-3 stwierdzono w diecie Amerykanów (prawie 10:1) i Australijczyków (ok. 8:1).

## Introduction

Polyunsaturated fatty acids n-3 are components of diet that have beneficial influence on health. Many studies indicate that n-3 fatty acids decrease the risk of cardiovascular diseases (arteriosclerosis, coronary heart disease, atherothrombotic endpoints), some types of cancer, inflammation and allergies (CONNOR 2000, NETTLETON 1995, SIMOPOULOS 1991). Excessive consumption of n-6 fatty acids in the diet upsets the metabolism of n-3 acids, which may interfere with the physiological balance of biologically active compounds that are synthesised from these fatty acids (SIMOPOULOS 1991, NEWTON 1996).

Alpha-linolenic acid (C18:3), docosahexaenoic acid – DHA (C22:6) and eicosapentaenoic acid – EPA (C20:5) belong to the n-3 family. Linoleic acid (C18:2) and arachidonic acid (C20:4) belong to n-6 acid group. In 1999, ISSFAL (International Society for the Study of Fatty Acids and Lipids) – where the scientists from all over the world study the influence of fatty acids on health – worked out a recommendation of the adequate intake of polyunsaturated fatty acids for adults: alpha-linolenic acid – 1% of energy (2.22 g/day), DHA and EPA – 0.3% of energy (0.65 g/day), and linoleic acid – 2% of energy (4.4 g/day). Docosahexaenoic and eicosapentaenoic acids should make up at least 0.2% of energy in the diet (0.44 g/day), while the upper limit for linoleic acid intake was established at 3% of energy (6.67 g/day) (SIMOPOULOS et al. 1999). The physiological ratio of n-6/n-3 fatty acids is about 1:1 to 4:1 (SIMOPOULOS 1996).

The aim of the study was to compare the intakes of polyunsaturated fatty acids from n-3 and n-6 family in an average diet of the inhabitants of Poland, including Warsaw, in comparison with the consumption of these fatty acids in other countries.

## Materials and Methods

The intakes of polyunsaturated fatty acids from n-3 and n-6 family was estimated based on the research results of household budgets, elaborated by the Central Statistical Office of the Polish in 2001 (LASKOWSKI 2004) as well as research data gathered using 3-day dietary records of Warsaw adult inhabitants during the period 2001/2002. On the basis of the data, the intakes of polyunsaturated fatty acids n-3 and n-6 in the diet were analysed. Polish food composition tables (KUNACHOWICZ et al. 1998) and literature data concerning the amount of isomers of unsaturated fatty acids (DANIEWSKI et al. 1998) were used in calculations. Polyunsaturated fatty acids of the n-3 family were presented as a sum of alpha-linolenic, docosahexaenoic and eicosapentaenoic acids, and n-6 as a sum of linoleic and arachidonic acids. The proportions of polyunsaturated fatty acids n-6 to n-3 were calculated as well. The results were compared with the recommendations of the International Society for the Study of Fatty Acids and Lipids of 1999 (SIMOPOULOS et al. 1999) and the recommendations of the WHO/FAO of 2003 (*Report of Joint WHO/FAO...* 2003). Our results were also compared with the literature data concerning the intake level of n-3 and n-6 polyunsaturated fatty acids in other countries.

## Results and Discussion

The contribution of fat in the diet was too high, considerably exceeding recommended values, in the diets of Poland and Denmark inhabitants, whilst in Norway it was slightly lower, yet, still exceeded values recommended by WHO/FAO (1530% of energy). The contribution of saturated fatty acids in the diets of Denmark, Poland and Norway was higher than values considered safe (according to WHO/FAO a level not exceeding 10% of diet's energy), whereas the contribution of polyunsaturated fatty acids was slightly lower or in accordance with the recommendations of WHO/FAO (6-10% of energy) – Table 1, Figure 1.

The results of the study showed that the intakes level of n-3 polyunsaturated fatty acids were similar in Warsaw, Norway, Denmark and Great Britain, whereas the Polish diet, besides Warsaw was characterised by a lower amount of these fatty acids, which was similar to the American diet. However, while analyzing the intake of particular n-3 polyunsaturated fatty acids, great differences in the structure of intake of these fatty acids in particular countries were observed.

The intake of alpha-linolenic acid was higher in Warsaw and Danish diets than in Poland, Norway, the USA and Australia (Figure 2), which was mainly

Table 1  
The intakes of fatty acids in the diets of inhabitants of different countries

Country	Dietary intake method	Fat	Fatty acids			References
			Saturated	Monoun-saturated	Polyun-saturated	
Poland, (g)	household budgets	81.5	26.3	33.4	15.0	Own research
Poland – – Warsaw, (g)	3-day dietary records	90.9	32.9	37.0	14.0	Own research
Denmark, (g)	7-d diet records	85.9	35.8	27.5	13.7	(TJONNELAND et al. 1993)
Norway, (g)	quantitative food frequency questionnaires	82.2	31.4	27.9	14.1	(JOHANSSON et al. 1998)

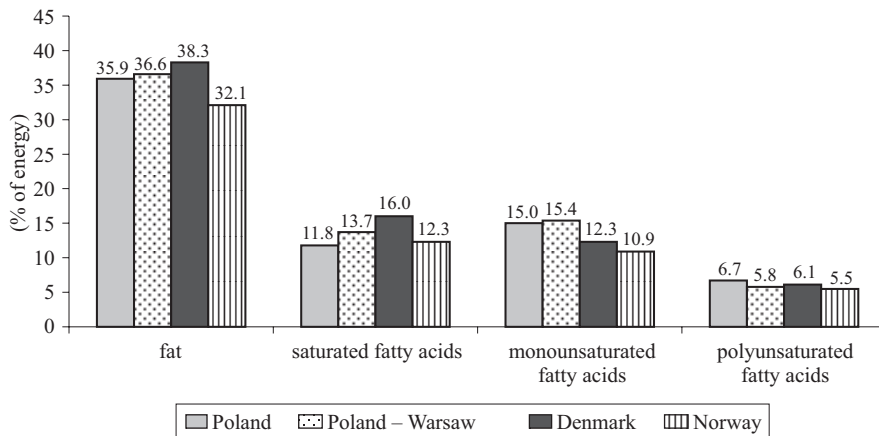


Fig. 1. The intakes of fat and fatty acids in the diets of inhabitants of different countries expressed as the percentage of diet's energy value

related to the kind and amount of plant oils in the diet. The intakes of DHA and EPA in Poland (including Warsaw), the USA and Australia was considerably lower than the intakes in Norway and Denmark (Figure 2), which resulted from a low intake of sea fish. In the Norwegian diet the consumption of these fatty acids was 0.84 g/day, yielding 0.33% of energy (JOHANSSON et al. 1998), while in the Danish diet it was 0.56 g/day – 0.25% of energy (TJONNELAND et al. 1993). DHA and EPA intakes in the USA was 0.1-0.2 g/day (KRIS-ETHERTON et al. 2000), in Australia – 0.16 g/day (MEYER et al. 2003). The consumption of DHA and EPA in the diet of the Polish amounted to 0.11 g/day (about 0.05% of energy), in Warsaw – 0.25 g/day (about 0.1% of energy). The intakes of n-6

polyunsaturated fatty acids was similar in the diets of Warsaw, Norway and Australia, whilst it was higher in the Polish, Danish and British diets (Table 2 and Figure 3), which was due to the structure and level of plant oils and margarines consumed.

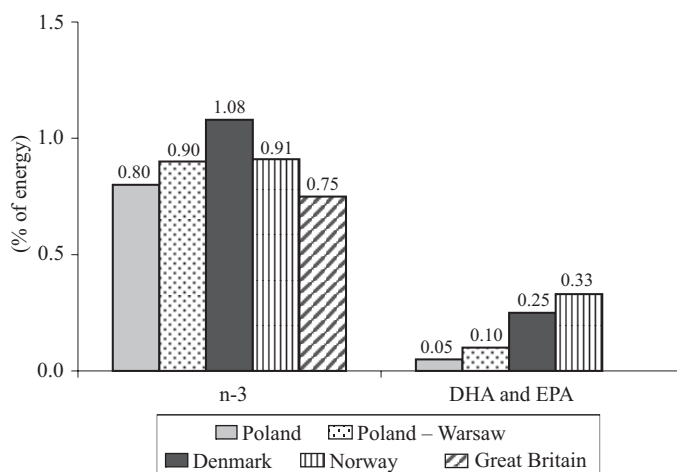


Fig. 2. The intakes of n-3 polyunsaturated fatty acids in the diets of the inhabitants of different countries expressed as the percentage of diet's energy value

Table 2  
The intakes of particular polyunsaturated fatty acids from the family of n-6 and n-3 in the diets of the inhabitants of different countries

Country	n-3		n-6		n-6/n-3	References
	Alpha-linolenic acid	EPA and DHA	Linoleic acid	Arachidonic acid		
Poland, (g)	1.64	0.11	12.14	0.15	7:1	Own research
Poland - Warsaw, (g)	1.88	0.25	9.86	0.15	5:1	Own research
Denmark, (g)	1.87	0.56	12.56	–	5:1	(TJONNELAND et al. 1993)
Norway, (g)	1.50	0.84	11.15	0.15	5:1	(JOHANSSON et al. 1998)
Australia, (g)	1.17	0.162	10.80	0.05	8:1	(MEYER et al. 2003)
The USA, (g)	1.4	0.1-0.2	15.68		10:1	(KRIS-ETHERTON et al. 2000, RAPER et al. 1992)
Great Britain, (g)	2.70		12.05		5:1	(SANDERS 2000)
Japan	–		–		4:1	(SUGANO, HIRAHARA 2000)

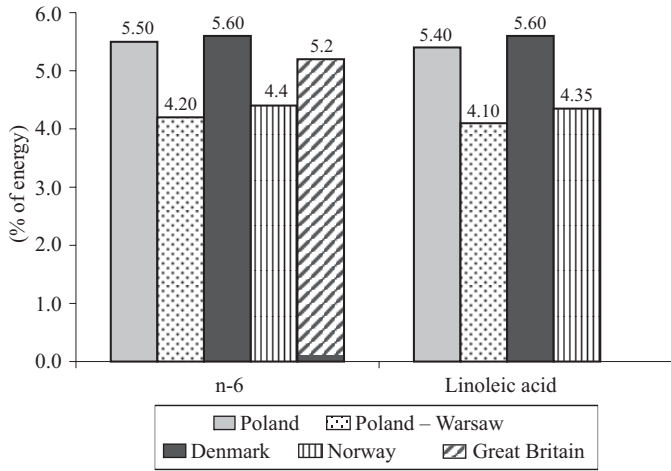


Fig. 3. The intakes of n-6 polyunsaturated fatty acids in the diets of the inhabitants of different countries expressed as the percentage of diet's energy value

The lowest and most beneficial proportion of polyunsaturated fatty acids n-6 to n-3 is characteristic of the Japanese diet, in which it amounts to 4:1 (SUGANO, HIRAHARA 2000). The ratio of polyunsaturated fatty acids n-6 to n-3 in Warsaw, Norwegian and Danish diets was close to about 5:1 (TJONNELAND et al. 1993, JOHANSSON et al. 1998), whilst in the Polish diet the ratio was too high (7:1), which was nutritionally unfavourable. The highest and the least favourable proportion of n-6 to n-3 fatty acids was demonstrated for the American diet (almost 10:1) (KRIS-ETHERTON et al. 2000) and Australian (about 8:1) (MEYER et al. 2003), whereas the physiological ratio of n-6/n-3 fatty acids ranges from 1:1 to 4:1 (SIMOPOULOS 1996).

Gender differences in the consumption of n-6 and n-3 fatty acids were observed in some studies. The intakes of polyunsaturated fatty acids from n-3 family in the diet of British women and men was lower than in Poland – Warsaw, Norway and Denmark. While analyzing the intakes of particular polyunsaturated fatty acids, it was shown that the intake of alpha-linolenic acid was higher in the diet of Polish – Warsaw and Danish men and women than in the Norwegian diet (Table 3). The inhabitants of Norway and Danish consumed higher amounts of DHA and EPA than the inhabitants of Poland – Warsaw. The consumption of these fatty acids by Norwegian women was 0.67 g/day – 0.32% of energy (JOHANSSON et al. 1998), by Danish women 0.52 g/day – 0.28% of energy (TJONNELAND et al. 1993), by Warsaw women 0.19 g/day – 0.10% of energy in the diet. Men in Norway consumed 1.00 g/day of these fatty acids – 0.33% of energy (JOHANSSON et al. 1998), the Danish – 0.59 g/day (0.22% of energy) (TJONNELAND

Table 3  
The intakes of n-6 and n-3 polyunsaturated fatty acids in the diets of women and men from different countries

Country	n-3		EPA and DHA	n-6		n-6/n-3	References
	Alpha-linolenic acid			Linoleic acid	Arachidonic acid		
Women							
Poland – Warsaw (227 women)	(g)	1.50	0.19	7.65	0.10	5:1	Own research
	(% of energy)	0.78	0.10	4.00	0.05		
	Denmark (63 women)	(g)	1.59	0.52	10.46	–	5:1
(% of energy)		0.86	0.28	5.63	–		
Norway (1627 women)	(g)	1.20	0.67	8.80	0.12	5:1	(JOHANSSON et al. 1998)
	(% of energy)	0.56	0.32	4.14	0.06		
Great Britain (1110 women)	(g)	1.41		9.9		5:1	(SANDERS 2000)
	(% of energy)	0.75		5.2			
Men							
Poland – Warsaw (182 men)	(g)	2.35	0.32	12.61	0.21	5:1	Own research
	(% of energy)	0.78	0.11	4.18	0.07		
	Denmark (23 men)	(g)	2.15	0.59	14.65	–	5:1
(% of energy)		0.82	0.22	5.57	–		
Norway (1517 men)	(g)	1.80	1.00	13.50	0.17	5:1	(JOHANSSON et al. 1998)
	(% of energy)	0.60	0.33	4.50	0.06		
Great Britain (1087 men)	(g)	2.00		14.2		5:1	(SANDERS 2000)
	(% of energy)	0.74		5.2			



et al. 1993), whilst the diet of men from Warsaw supplied 0.32 g/day, giving 0.11% of energy in the diet. The intakes of polyunsaturated fatty acids from n-6 family was lower in Polish diet – Warsaw and by Norwegian women and men than by Danish and British women (Figure 4).

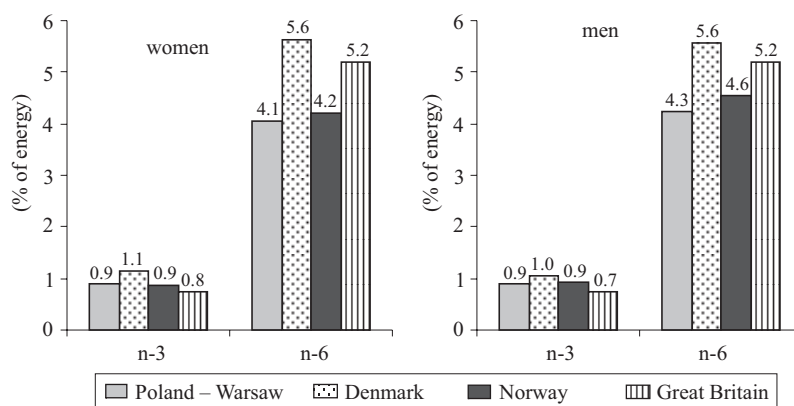


Fig. 4. The intakes of polyunsaturated fatty acids from n-3 and n-6 family by women and men from different countries expressed as the percentage of diet's energy value

The consumption of polyunsaturated fatty acids n-3 was compared to the recommendation of WHO/FAO report of 2003 at the level 1-2% of energy in the diet (*Report of Joint WHO/FAO... 2003*). It was found that only in Norway and Denmark, the contribution of n-3 acids in the diet was close to recommendation; in other countries such as Poland, Great Britain, the USA and Australia, the intakes of fatty acids from this group was too low. The contribution of n-6 acids in the diet of the investigated countries was a little lower or close to WHO/FAO recommendations, and constitute 5-8% of energy in the diet (*Report of Joint WHO/FAO... 2003*).

The comparison of alpha-linolenic acid intake from n-3 family with the recommendation of the International Society for the Study of Fatty Acids and Lipids (SIMOPOULOS et al. 1999) showed that the intake of this fatty acid in the diet of inhabitants of all countries under investigation was lower than the recommended 2.22 g/day – 1% of energy (Table 4). The consumption of longchain PUFA n3 DHA and EPA only in Norway was at the level, similar to the recommendation of ISSFAL – 0.65 g/day (0.3% of the energy in the diet) (SIMOPOULOS et al. 1999). It was found that the intakes of these fatty acids in Denmark was lower than recommendation. In countries such as Poland, the USA and Australia, the consumption of DHA and EPA was very low, consider-

ably lower than recommended values. However, the intake of linoleic acid from n-6 family was much higher than the value recommended by ISSFAL (4.44 g/day – 2% of energy) (SIMOPOULOS et al. 1999), and also several times exceeded the recommended upper limit of intake (6.67 g/day – 3% of energy).

Our study and the research conducted in the United States (KRIS-ETHERTON et al. 2000) and Japan (SUGANO, HIRAHARA 2000) show that the main sources of n-6 acids are edible fats – mainly oils, while n-3 fatty acids come mostly from edible fats as well as fish. The main source of linoleic (n-6) and alpha-linolenic (n-3) acid in the diet are plant fats – oils: rapeseed, sunflower, soybean, corn and soft margarines, arachidonic acid (n-6) – meat, eicosapentaenoic and docosahexaenoic acids (n-3) – sea fish. The main source of DHA and EPA in the diet was fish and fish products. The main source of linoleic acid (n-6) in the diet were plant fats: margarines and oils (Figure 5).

Table 4  
Adequate Intakes (AI) of polyunsaturated fatty acids for adults by ISSFAL (SIMOPOULOS et al. 1999)

Fatty acids	% of energy	g/day*
Linoleic acid	2.0	4.44
(upper limit)	3.0	6.67
Alpha-linolenic acid	1.0	2.22
DHA+EPA	0.3	0.65
DHA to be at least	0.1	0.22**
EPA to be at least	0.1	0.22

\* amount for the diet of 2000 kcal of energy

\*\* for pregnant and lactating women – 0.3

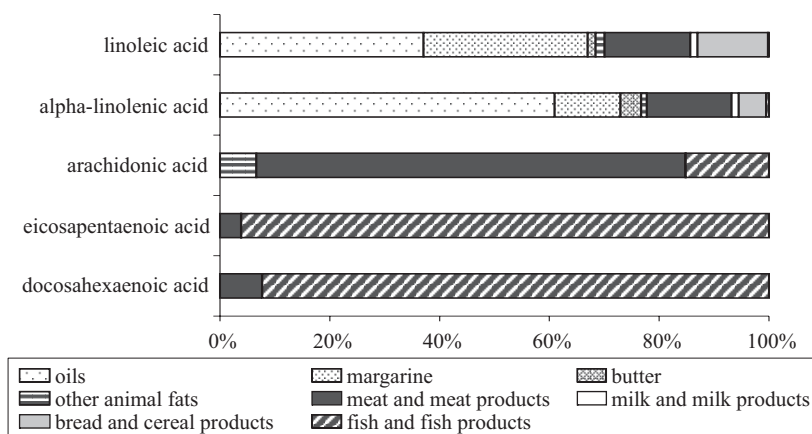


Fig. 5. The sources of polyunsaturated fatty acids in Poland

## Conclusion

The contribution of fat in the diet was too high, exceeding recommended values in the diets of Poland, Denmark and Norway inhabitants. Similar trends were observed in case of saturated fatty acids, whilst the contribution of polyunsaturated fatty acids was slightly lower or in accordance with recommendations.

The intakes of n-3 polyunsaturated fatty acids in the diet of inhabitants of all countries under investigation (except Japan) was lower than recommended values. The consumption of n-6 polyunsaturated fatty acids was much higher than the value established as recommended.

The lowest and most beneficial proportion of polyunsaturated fatty acids n-6 to n-3 is characteristic of the Japanese diet and it amounts to 4:1. The ratio of polyunsaturated fatty acids n-6 to n-3 in the Norwegian, Danish and Polish – Warsaw (with more affluent people than the rest of the country) diets was similar (5:1), whereas in the Polish diet the ratio was too high (7:1), which was nutritionally unfavourable. The highest and the least favourable proportion of n-6 to n-3 fatty acids was demonstrated for the American (about 10:1) and Australian (about 8:1) diets.

The main sources of linoleic (n-6) and alpha-linolenic (n-3) acid in the diet are plant fats – oils and margarines, arachidonic acid (n-6) – meat, DHA and EPA (n-3) – sea fish and fish products.

There is a need for increase intake of n-3 polyunsaturated acids, especially DHA and EPA, in the diet achieved by increasing the consumption of sea fish and their products. Oils included in the diet rich in linoleic acid (from n-6 family) should be replaced with oils containing large amounts of alpha-linolenic acid (from n-3 group) e.g. rapeseed oil.

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## MICROBIOLOGICAL CONTAMINATION OF VEGETABLE SALADS

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Key words: vegetable salads, microbiological quality, *Enterobacteriaceae*.

### Abstract

Vegetable salads available at retail stores, supplied by various producers, were analyzed in the study to determine the counts of aerobic mesophilic bacteria, coliforms and spore-forming *Bacillus* species, as well as the presence of *Staphylococcus aureus*, *Clostridium perfringens* and *Listeria monocytogenes*. Strains of Gram-negative rods and *Bacillus cereus* were isolated and identified. None of the salads contained *S. aureus*, *Cl. Perfringens* or *Listeria monocytogenes*. It was found that in 88% of samples the population size of aerobic mesophilic microbes was of the order of 4 to 5 log cfu g<sup>-1</sup>; higher and lower counts were recorded sporadically only. In 58% of samples the population size of coliforms and *Bacillus* species ranged from below 2.00 to 2.90 log cfu g<sup>-1</sup>, and from 2.00 to 3.95 log cfu g<sup>-1</sup> respectively. Gram-negative rods isolated from the examined salads were identified to the following genera: *Citrobacter*, *Providencia*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Serratia*, *Yersinia*, *Pseudomonas* and *Flavobacterium*. The species *Providencia retgeri*, *Citrobacter freundii* and *Enterobacter aerogenes* were the most frequent among them. A total of 84 salad samples were tested in the experiment, of which 3 were found to contain strains of *Y. enterocolitica*, and 6 – strains of *E. coli*. The presence of spore-forming *Bacillus cereus* was confirmed in 34% of salads.

### MICROBIOLOGICZNY STAN SURÓWEK WARZYWNYCH

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Słowa kluczowe: surówki warzywne, jakość mikrobiologiczna, *Enterobacteriaceae*.

## Abstrakt

Badaniom poddano 83 próbki surówek warzywnych 4 różnych producentów, dostępne w handlu detalicznym. Oznaczono liczbę bakterii mezofilnych tlenowych, pałeczek grupy coli i przetrwalników laseczek z rodzaju *Bacillus*, a także obecność *Staphylococcus aureus*, *Clostridium perfringens* i *Listeria monocytogenes*. Wyizolowano i zidentyfikowano szczepy pałeczek gramujemnych i laseczek *Bacillus cereus*. W żadnej z badanych próbek nie stwierdzono obecności *S. aureus*, *Cl. Perfringens* i *Listeria monocytogenes*. Wykazano, że 88% surówek zanieczyszczały drobnoustroje mezofilne tlenowe o liczebności 4-5 log jtk g<sup>-1</sup>, populacje mniejsze i większe stwierdzano sporadycznie. Oprócz tego w 58% próbek występowały pałeczki z grupy coli (liczebność w granicach od poniżej 2,00 do 2,90 log jtk g<sup>-1</sup>) i przetrwalniki laseczek rodzaju *Bacillus* (liczebność rzędu 2,00-3,95 log jtk g<sup>-1</sup>). Z badanych surówek wyizolowano również pałeczki gramujemne, które zaklasyfikowano do rodzajów: *Citrobacter*, *Providencia*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Serratia*, *Yersinia*, *Pseudomonas*, *Flavobacterium*. Najczęściej występowały gatunki *Providencia retgeri*, *Citrobacter freundii* i *Enterobacter aerogenes*. Z 3 próbek wyizolowano ponadto szczepy *Y. enterocolitica*, a z 6 – *E. coli*. Obecność przetrwalników *Bacillus cereus* stwierdzono w 34% surówek.

## Introduction

Vegetable salads belong to convenience, minimally processed foods of the ready-to-eat type. Minimal processing technologies are non-thermal technologies of food processing, which means that the final product is comparable with the original raw material in terms of freshness, naturalness and nutritional value (ŚWIDERSKI 2003). Vegetables are good sources of many vitamins, like vitamin C, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, folic acid, biotin, pantothenic acid and  $\beta$ -carotene – characterized by the highest provitamin A activity. Vegetables provide also dietary fiber known to aid digestion, which still increases their nutritive value.

Microbiological contamination may pose a serious problem in the production of minimally processed plant raw materials. Microbial contamination levels are the highest in root vegetables (ŁANIEWSKA-TROKENHEIM et al. 2003, ŁANIEWSKA-TROKENHEIM et al. 2002,). Saprophytic and pathogenic microflora coming from the soil, water, wastewater and air can be found on the surface of vegetables (BOROWSKA et al. 2006). Those microbial contaminants, difficult to eliminate with the use of non-thermal techniques, determine the quality of minimally processed products. The microbiological quality of minimally processed vegetable products depends on the effectiveness of methods employed to disinfect and process the raw material, as well as on proper personal hygiene and sanitation practices (BRACKETT et al. 2001, NOGUEIRA et al. 2003). Manufacturers are required to offer products considered safe for human consumption, so compliance with strict sanitation requirements is essential during the harvest and processing of raw vegetables as well as during the distribution of ready-to-eat products.

Vegetable salads available on the market satisfy the existing sanitation requirements and are characterized by good microbiological quality and a high

nutritional value, comparable with fresh vegetables (WÓJCIK-STOPCZYŃSKA 2004). Research results show that the microbiological contamination levels found in minimally processed vegetable salads do not constitute a direct threat to consumer health. However, the degree of their contamination with saprophytic microflora was found to be considerable (SAGOO et al. 2003). The rapid development of minimal processing technologies and the potential human health risk associated with the consumption of fresh, ready-to-eat foods made it necessary to establish and implement certain consumer safety regulations.

## Materials and Methods

The experimental materials consisted of vegetable salads purchased at local retail stores within the expiry period. The analysis was performed on 83 samples of vegetable salads supplied by 4 different producers, divided into 5 groups based on the number of components (mixed – multi-component and two-component) and the addition of oil, mayonnaise or yogurt.

The salads were assayed for the count of: aerobic mesophilic bacteria – nutrient agar incubated at 30°C/24-72 h, coliforms – Chromocult Coliform agar (Merck) incubated at 37°C/24 h, spore-forming *Bacillus* species – pasteurization at 80°C/15 min., medium proposed by Mossel (Merck) incubated at 37°C/24-48 h. The presence of *B. cereus* was determined in 10 g of salad following pasteurization, proliferation in broth and isolation according to Mossel. The presence of spore-forming *Clostridium* species was determined in pasteurized (80°C/15 min) 1 g samples of salad in RCM (Merck) incubated at 37°C/72 h under anaerobic conditions. In order to isolate *Clostridium perfringens* positive samples were transferred onto the surface of TSC agar and incubated at 37°C/24 h under anaerobic conditions. The presence of coagulase-positive staphylococci was determined in 1 g of salad, following proliferation in Giolitti-Cantoni broth incubated at 37°C/24-48h, and transfer onto RPF medium (Noack). The presence of strains of the genus *Enterococcus* was determined in 1 g of salad following proliferation in broth with bromocresol purple and sodium azide (Merck) and transfer onto Slanetz and Bartley agar (Merck) incubated at 37°C/24-48 h. The presence of *Listeria monocytogenes* was determined in 25 g of salad. Proliferation in Fraser medium incubated at 37°C/20 h was followed by a rapid TECRA UNIQUE test (Noack). In addition, after proliferation the cultures were transferred onto Oxford agar (Merck) and incubated at 37°C/24 h.

Strains of Gram-negative rods isolated from Chromocult Coliform agar were identified using API 20 tests (bioMerieux). The characteristic colonies of *Bacillus cereus* were isolated as described by Mossel, and confirmation tests

were carried out. Colonies suspected of belonging to *Listeria* were isolated from Oxford agar for identification.

## Results

The analysis of the microbial contamination of 84 samples of vegetable salads supplied by 4 different producers showed that none of the salads posed a direct threat to human health. None of them contained *Staphylococcus aureus*, *Clostridium perfringens* or enterococci in 1 g samples. *Listeria monocytogenes* was not found in 25 g samples of the salads, either. Five samples contained bacteria of the genus *Listeria*, but identification tests enabled to classify the isolated strains as *Listeria innocua*. For comparative purposes, the salads were divided into 5 groups differing with respect to dressing. The occurrence frequency ( $\log \text{cfu g}^{-1}$ ) of microbial contaminants categorized as aerobic mesophilic microflora, coliforms or spore-forming *Bacillus* species were determined within the groups. It was found that in 88% of samples the population size of aerobic mesophilic microbes was of the order of 4.09 to 5.97  $\log \text{cfu g}^{-1}$ , irrespective of the producer, composition or dressing added (Table 1). Lower bacterial counts, reaching several thousands per g, were observed in 3 salads only, while 7 salads representing 4 different groups contained millions of microbes per g. Mixed (multi-component) salads with oil dressing (WO) constituted the largest group among those tested in the study. In 56% of them the level of contamination with aerobic mesophilic microbes ranged from 4.09 to 4.88  $\log \text{cfu g}^{-1}$ . In the other groups of salads contamination of that order was recorded in fewer samples (31-43%). In those groups of salads the highest percentages of samples (43 to 57%) were characterized by contamination levels of the order of 5.02 to 5.97  $\log \text{cfu g}^{-1}$ . It was also found that only in the group of mixed salads with oil dressing there were no samples in which the counts of aerobic microbes would reach 6.00  $\log \text{cfu g}^{-1}$ . Contamination with coliforms was below 2.00  $\log \text{cfu g}^{-1}$  in 28% of samples. In 40% of samples it varied from 2.01 to 2.90  $\log \text{cfu g}^{-1}$ . The highest percentage of samples (42-43%) in which the population size of coliforms exceeded 3.05  $\log \text{cfu g}^{-1}$  was noted in the group of mixed salads with yogurt dressing and in salads in their own juice.

The majority of salads were composed of common cabbage or Chinese cabbage and carrots in different proportions, combined with other vegetables. Only 17 salads did not contain the above vegetables, but peppers and root vegetables such as celeriac or red beet. Those vegetables are contaminated with similar soil microbes, and their processing at plants with identical sanitation and hygiene procedures enables to obtain products with the desired microbi-



Table 1  
Microbiological contamination of vegetable salads

Kind of salad symbol	Total sample size	Aerobic mesophilic bacteria log cfu g <sup>-1</sup>	Number of samples	Coliforms log cfu g <sup>-1</sup>	Number of samples
Mixed (multi-component) salads with oil dressing – WO	23	3.88-3.92	2	< 2.00	9
		4.09-4.88	13	2.00-2.60	8
		5.02-5.86	8	3.08-3.90	4
				4.12	1
				5.00	1
Mixed (multi-component) salads with yogurt dressing – WJ	19	4.47-4.95	6	< 2.00	8
		5.02-5.80	11	2.00-2.77	3
		6.00-6.04	2	3.17-3.89	8
Mixed (multi-component) salads with mayonnaise – WM	15	4.13-4.89	6	< 2.00	3
		5.31-5.97	7	2.00-2.84	8
		6.13-6.60	2	3.04-3.86	4
Two-component and mixed (multi-component) salads in their own juice – W	14	3.71	1	< 2.00	1
		4.15-4.91	6	2.03-2.57	7
		5.19-5.83	6	3.18-3.82	6
		6.33	1		
Two-component salads with oil dressing – DO	13	4.17-4.96	5	< 2.00	3
		5.09-5.66	6	2.02-2.90	8
		6.13	2	3.05	1
				4.02	1

ological quality. The type of dressing used or the lack of dressing had a slight effect on total microbial counts.

The results of the present experiment indicate that the number of vegetables used for making the salads had no considerable impact on their microbiological contamination. Such pre-processing technological processes as scalding or chlorination allow to reduce the numbers of microbes on the surface of vegetables (BOROWSKA et al. 2006, BRACKETT et al. 2001). The additives used during salad production, including dressings, herbs and spices may cause microbiological contamination. As a result, the contamination levels were found to be comparable in all kinds of salads, regardless of the number of vegetables used to produce them.

The presence of single spore-forming *Bacillus* species in 1 g samples was recorded in 28% of salads. The frequency of their occurrence was the lowest in multi-component salads with oil dressing (WO). In 50% of two-component salads with oil dressing (DO) contamination with spore-forming *Bacillus* species reached 3.01 to 3.50 log cfu g<sup>-1</sup>. In numerous samples of salads with mayonnaise the population size of those microbes ranged from 2.01 to 2.50 log cfu g<sup>-1</sup>. In 69% of samples the counts of spore-forming bacteria

Table 2

Contamination of vegetable salads with spore-forming *Bacillus* species

Kind of salad – symbol	Total sample size	Spore-forming aerobic bacilli log cfu g <sup>-1</sup>	Number of samples	Number of samples with <i>Bacillus cereus</i>
Mixed (multi-component) salads with oil dressing – WO	23	< 2.00 2.00-2.77 3.18-3.91 4.09	11 7 4 1	7
Mixed (multi-component) salads with yogurt dressing – WJ	19	< 2.00 2.00-2.90 3.01-3.74 4.05	5 6 7 1	4
Mixed (multi-component) salads with mayonnaise – WM	15	< 2.00 2.00-2.48 3.15-3.28	2 11 2	3
Two-component and mixed (multi-component) salads in their own juice – W	14	< 2.00 2.00-2.85 3.23-3.95 4.28	3 4 6 1	6
Two-component salads with oil dressing – DO	13	< 2.00 2.12-2.30 3.01-3.61	3 2 8	1

reached  $10^2$  to  $10^3$  cfu g<sup>-1</sup> (Table 2). Higher contamination levels were noted sporadically only. The analysis of pasteurized samples did not permit the determination of the abundance of vegetative forms of members of the genus *Bacillus*. It is possible that vegetative cells of this genus were present in greater populations than spore-forming cells. This is confirmed by the fact that the counts of aerobic mesophilic microbes were by 2 to 3 log cycles higher than the counts of spores and the other bacteria determined in the study. Soil is the natural environment of spore-forming bacilli, so they are always present on vegetables. Seasonings and spices may be also a source of those bacteria. For instance, black pepper added to all tested salads could introduce bacterial spores in the amount of  $10^6$  to  $10^8$  cfu g<sup>-1</sup>, or  $10^2$  to  $10^4$  cfu g<sup>-1</sup> if sterilized by radiation (WARMIŃSKA-RADYKO et al. 2001). The analysis of broth cultures of pasteurized 10 g samples revealed the presence of *Bacillus cereus* in 21 samples (23.6% of salads). No colonies of this species were found in original cultures of pasteurized salads, which is indicative of the presence of spores of other species of this genus. Strains of *Bacillus cereus* are capable of growing at 5-6°C and may produce toxins in dairy products under such conditions. Those toxins are stable at low temperatures and remain active at 4°C (BERTHOLD et al. 2005). Depending on the activity of a given strain and individual sensitivity, *Bacillus cereus* whose population size reaches  $10^4$  to  $10^6$  cfu g<sup>-1</sup> may cause food

poisoning. Cases of alimentary intoxication have been reported in children, elderly people and people with weakened immune systems who consumed food products containing even lower numbers of *Bacillus cereus* cells. VALERO et al. (2000) demonstrated that the growth of *Bacillus cereus* was inhibited in minimally processed vegetables stored at 5-8°C, whereas temperatures higher than 12°C promoted the proliferation of those bacilli (VALERO et al. 2003). It was found that salad acidification to pH 5.0 limits cell proliferation caused by lag phase prolongation, while pH below 4.5 inhibits cell growth even at 25°C (ŚWIDERSKI 2003). Vegetable salads can be naturally preserved with such flavor additives as lemon juice, lime juice or cranberry juice. Studies on model juices of these fruits confirmed the inactivation of such pathogens as *Salmonella*, *E. coli* O157:H7 and *Listeria monocytogenes*, and the reduction in their population by 5 log cycles (ŁANIEWSKA-TROKENHEIM et al. 2002).

69 strains of Gram-negative rods isolated from salad samples were classified into the family *Enterobacteriaceae*. Based on the results of API 20E tests, 10 species belonging to 7 genera were identified (Table 3). 20 strains of Gram-negative, oxidase-positive rods were also isolated and classified into the genera *Pseudomonas* and *Alcaligenes*. The following species were most frequent among the isolated strains: *Providencia rettgeri* (20% of isolated strains), *Citrobacter freundii* (17%) and *Enterobacter aerogenes* (13.5%) (Table 3). Three species of the genus *Providencia* were recorded in the tested salads. Those rods

Table 3

Frequency of isolation of strains belonging to particular bacterial

Species	Number of strains	% of isolated strains	Kind of salad symbol
<i>Escherichia coli</i>	6	7	WO, WD, W
<i>Citrobacter freundii</i>	14	17	all
<i>Citrobacter diversus</i>	4	4.5	W
<i>Enterobacter aerogenes</i>	12	13.5	all
<i>Klebsiella ssp.</i>	3	3.4	WMJ, W
<i>Providencia rustigianii</i>	4	4.5	WO
<i>Providencia rettgeri</i>	18	20	all
<i>Providencia alcalifaciens</i>	1	1.1	WM
<i>Yersinia enterocolitica</i>	3	3.4	WMJ
<i>Serratia odorifera</i>	2	2	WO
<i>Pseudomonas vesicularis</i>	4	4.7	WO
<i>Pseudomonas alcaligenes</i>	5	5.6	WMJ, WM
<i>Pseudomonas solanacearum</i>	8	9	WO, W, DO
<i>Pseudomonas ssp.</i>	2	2	W
<i>Alcaligenes faecalis</i>	3	3.4	W

are widely distributed in nature and occur in human and animal feces. Antibiotic-resistant strains of *Providencia* often cause urinary system infections, particularly in hospitalized patients.

The salads contained also two species of the genus *Citrobacter*. This genus belongs to the coliform group and is part of the normal flora of the human digestive tract. Those rods are commonly found in human and animal feces, as well as in the soil, surface waters and food. Outside the intestines, the above bacteria are opportunistic and may cause infections of the urinary tract and respiratory system. Moreover, hospital strains of *Citrobacter* may also cause dangerous hospital infections (SZEWCZYK 2005).

*Escherichia coli* strains accounted for 7% of all isolated strains. Strains of the genus *Klebsiella* not identified to species were not numerous, either.

Strains of *Yersinia enterocolitica* were isolated from 2 mixed salads with yogurt and mayonnaise dressing. Those psychrotrophic rod-shaped bacteria are frequently isolated from cold-stored foods. They cause alimentary infections and intoxication as well as enteritis in both infants and adults (SZEWCZYK 2005). *Yersinia enterocolitica* is widely distributed in nature (soil, surface waters) and can also live in the intestines of animals.

As for the other rod-shaped bacteria of the family *Enterobacteriaceae*, 2 strains of *Serratia odorifera* were isolated from salads. This species, considered saprophytic or commensal, occurs in the soil, surface waters, on plants and in the intestines. Sometimes it causes hospital infections.

Rods of the genus *Pseudomonas* occur naturally in surface waters, tap water and in the soil. Due to their ability to grow under low temperatures, they are often the dominant components of bacterial microflora in cold-stored food products. Many strains can decompose such complex compounds as proteins, cellulose and lignins, and – in consequence – cause undesirable changes in the quality of stored foods.

All isolated strains of rod-shaped bacteria are common in the human environment and constitute part of natural intestinal microflora. If present in low numbers in food products, they do not pose a health risk to consumers. However, investigations into the antibiotic-resistance of strains isolated from foods with satisfactory microbiological quality may reveal a new threat, namely the possibility of transmitting antibiotic-resistant strains to humans through food.

## Conclusions

1. All tested vegetable salads were found to be safe for consumption and none of them posed a direct threat to human health.

2. The population size of aerobic mesophilic microbes was comparable in all salads, irrespective of the composition or dressing added, and ranged from 4.09 to 5.86 log cfu g<sup>-1</sup>.

3. The majority of isolated strains of Gram-negative rods were identified to species belonging to 7 genera of the family *Enterobacteriaceae*: *Providencia*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Yersinia* and *Serratia*. Oxidase-positive rods of the genera *Pseudomonas* and *Alcaligenes* were less numerous among the isolated strains.

4. The presence of *Bacillus cereus* in cold-stored salads may result in an increase in the counts of those bacteria as well as in the possibility of toxin production.

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## LOCAL HORMONAL REGULATIONS IN FEMALE REPRODUCTIVE ORGANS OF DOMESTIC ANIMALS

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**Key words:** local hormonal regulation, retrograde transfer, local destination transfer.

### Abstract

The discovery of local transfer of ovarian hormones (steroids, oxytocin) and uterine prostaglandins ( $\text{PGF}_{2\alpha}$  and  $\text{PGE}_2$ ) in cow, ewe and sow created a new point of view on hormonal regulation of the reproduction and thereby on the regulation of the estrous cycle of the female. It was demonstrated that retrograde transfer of hormones to the place of its secretion and destination transfer of ovarian hormones to the oviduct and uterus, and uterine prostaglandins to the ovary and oviduct is realized on the base of morphological and physiological adaptations of blood and lymphatic vessels of the mesovarium and mesometrium. These processes result in continuous local elevation of the concentration of sex hormones in blood supplying female reproductive organs by 30-70% in comparison with systemic arterial blood. Local destination and retrograde transfer of ovarian hormones and uterine prostaglandins is a way by which reproductive organs are selectively supplied with increased amount of these hormones, adequate to their needs.

### LOKALNE REGULACJE HORMONALNE W NARZĄDACH ROZRODCZYCH SAMIĆ ZWIERZĄT DOMOWYCH

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**Słowa kluczowe:** lokalne regulacje hormonalne, zwrotny transfer, lokalnie docelowy transfer.

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## A b s t r a k t

Wykrycie systemu lokalnego, zwrotnego i docelowego, transferu hormonów jajnikowych (hormony steroidowe, oksytocyna) i macicznych prostaglandyn ( $\text{PGF}_{2\alpha}$  i  $\text{PGE}_2$ ) u krowy, owcy i świni spowodowało zmianę w rozumieniu hormonalnej regulacji rozrodu, w tym regulacji cyklu rujowego samicy. Wykazano, że lokalny, zwrotny transfer hormonów do miejsca ich wytwarzania (jajnik, macica) oraz docelowy transfer hormonów jajnikowych do jajowodu i macicy, a także macicznych prostaglandyn do jajnika i jajowodu odbywa się w oparciu o przystosowania morfologiczne i fizjologiczne naczyń krwionośnych i limfatycznych krezki jajnika i krezki macicy. Wynikiem tych procesów jest m.in. lokalne podwyższenie o 30-70% stężenia hormonów płciowych we krwi zaopatrującej narządy rozrodcze samicy w stosunku do ich stężenia w obwodowej krwi tętniczej. Lokalnie docelowy i zwrotny transfer hormonów jajnikowych i macicznych prostaglandyn umożliwia wybiórcze dostarczenie do narządów rozrodczych zwiększonej ich ilości, zgodnie z zapotrzebowaniem tych narządów.

**Introduction**

Reproductive processes in females undergo a multi-level regulatory mechanism based principally on negative feedback. This regulation is supported by the following systems: nervous, endocrine, immunological and circulatory. The endocrine system plays main role in these regulations. The participation of the endocrine system is revealed by alteration in concentrations of hormones and other regulatory factors in the blood and changes in the concentrations of their receptors in tissues. Regulatory factors reach the reproductive organs with systemic blood or via a local transfer from the venous and lymphatic outflow into the arterial blood supplying these organs.

The concentration of hormones which participate in the regulation of the estrous cycle, pregnancy and parturition, measured in the peripheral blood is relatively well-known. However, measurements of hormone levels in different parts of the uterine and ovarian vasculature, pointed to the presence of the mechanisms locally regulating the estrus cycle and pregnancy in females of domestic animals (KRZYMOWSKI et al. 1981, 1981/82, 1982, 1982a, 1986, 1987, 1990, KRZYMOWSKI, STEFAŃCZYK-KRZYMOWSKA 2002, 2004, KOZIOROWSKI 1996, KOZIOROWSKI, PESTA 1989, KOZIOROWSKI et al. 1986, 1988, 1989, 1989a, STEFAŃCZYK-KRZYMOWSKA 1996, STEFAŃCZYK-KRZYMOWSKA et al. 1990, 1994, 1997, 1998, 1998a, 2002, 2004, 2006, STEFAŃCZYK-KRZYMOWSKA, KRZYMOWSKI 2002, WĄSOWSKA, STEFAŃCZYK-KRZYMOWSKA 2006). These studies provided new information and changed our point of view on the regulation of the estrous cycle in domestic animals.

**Mechanisms providing local increase of hormones concentration in female reproductive organs and its physiological consequence**

Morphological studies of GINTHER (1974, 1976, 1981), GINTHER and DEL CAMPO (1974) and DEL CAMPO and GINTHER (1974) afforded possibilities

for demonstration that uterus influenced the corpora lutea (CL) via a local humoral pathway. It was proved that an active substance produced in the uterus, identified as a  $\text{PGF}_{2\alpha}$  (LUKASZEWSKA, HANSEL 1970, MCCrackEN 1971, MCCrackEN *et al.* 1972) which outflowed with uterine venous blood could permeate to the ovary via counter current mechanism, reach the CL and initiate the luteolysis (MCCrackEN *et al.* 1972).

In cows, similarly as in pigs, ewes and females of other domestic animals, venous vessels of the mesovarium run in the close apposition of the ovarian artery (Figure 1), whose wall is thinning at the place of their direct contact (DEL CAMPO, GINTHER 1974). The majority of uterine and ovarian arteries and arterioles running in the area of the mesometrium and mesovarium in sows and cows is enmeshed by a dense net of venous vessels forming veno-venous network (KRZYMOWSKI *et al.* 1982, 1990, KOZIOROWSKI 1996, STEFAŃCZYK-KRZYMOWSKA *et al.* 2002). Moreover, DOBOSZYŃSKA *et al.* (1991) and JANKOWSKA *et al.* (2001, 2003) showed the presence of lymphatic system structures in the mesovarium and mesometrium (subovarian lymphatic plexus, precollectors and collectors vessels in mesovarium and mesometrium), which could participate in the local hormones exchange and the estrous cycle regulation (KRZYMOWSKI, STEFAŃCZYK-KRZYMOWSKA 2002, 2004, STEFAŃCZYK-KRZYMOWSKA, KRZYMOWSKI 2002).

Besides  $\text{PGF}_{2\alpha}$ , in the vasculature of the reproductive system in cows and gilts also estradiol, progesterone and testosterone concentrations increase locally by permeation from ovarian lymph and venous blood to arterial blood (KRZYMOWSKI *et al.* 1981/1982, 1982, 1982a, KOTWICA *et al.* 1982, KOZIOROWSKI, PESTA 1989, STEFAŃCZYK-KRZYMOWSKA *et al.* 2002, 2004, WĄSOWSKA, STEFAŃCZYK-KRZYMOWSKA 2006) (Figure 1). This increased supply with ovarian hormones locally influences the vascular function and at the same time produces changes in hormonal supply of the reproductive organ which consequently result in cyclic changes during the estrus cycle. The result of recent study suggests the action of local feedback between increased supply of steroid hormones to the ovary and the secretion of these hormones and involvement of activin and inhibin to this regulation in the pig (WĄSOWSKA, CHŁOPEK 2005).

Many studies were performed to explain the morphological adaptation of the mesovarian vasculature and the mechanism of  $\text{PGF}_{2\alpha}$  penetration from the uterus to the ovary (KRZYMOWSKI *et al.* 1981, 1982, 1982a 1990, KOTWICA *et al.* 1982, 1983, KOZIOROWSKI 1996, KOZIOROWSKI *et al.* 1989, STEFAŃCZYK-KRZYMOWSKA *et al.* 1994, 1997, 1998, 1998a, 2002, STEFAŃCZYK-KRZYMOWSKA, KRZYMOWSKI 2002, SCHRAMM *et al.* 1986, 1986a)

In 2002 KRZYMOWSKI and STEFAŃCZYK-KRZYMOWSKA (KRZYMOWSKI, STEFAŃCZYK-KRZYMOWSKA 2002, 2004, STEFAŃCZYK-KRZYMOWSKA, KRZYMOWSKI 2002) summarised their earlier studies and presented the following concept



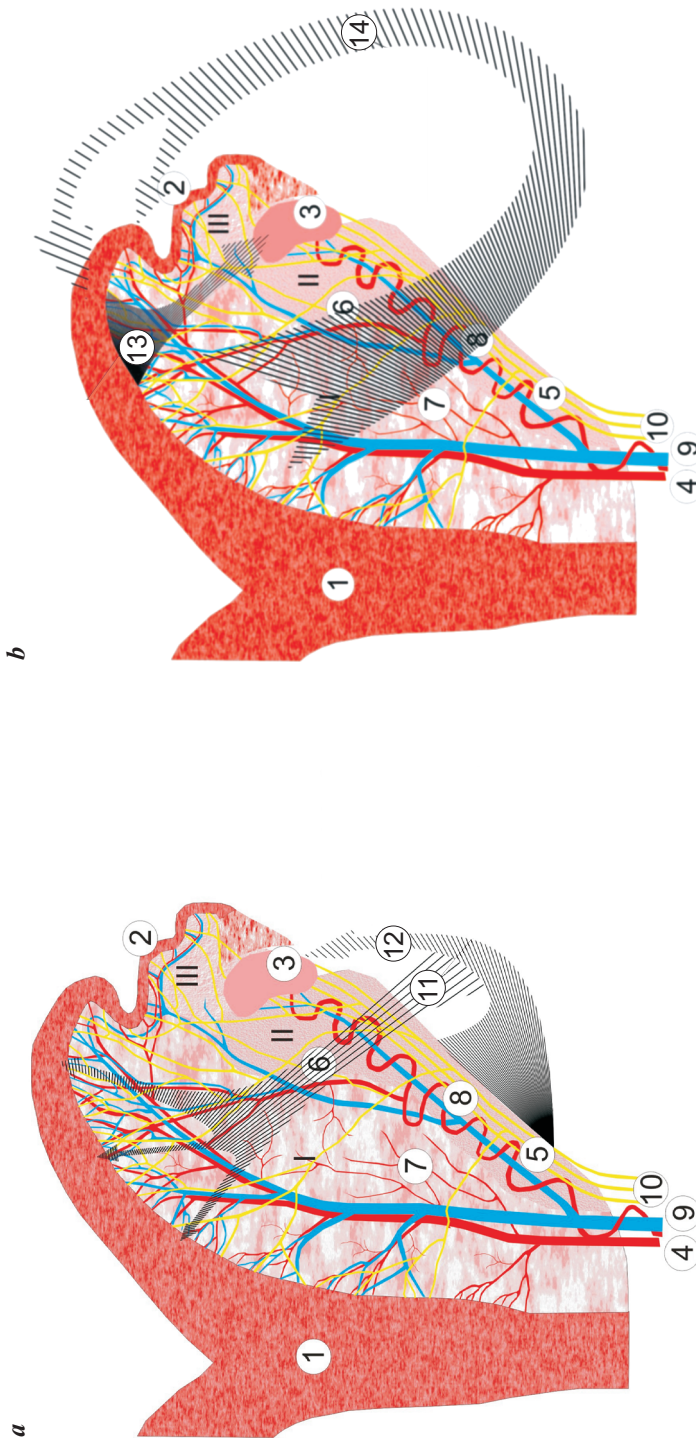


Fig. 1. Schematic diagram of the vasculature of bovine broad ligament and the retrograde and local destination transfer of ovarian hormones (a) and uterine prostaglandins (b). 1 – mesometrium, II – mesometrium, III – mesosalpinx; 1 – uterus, 2 – oviduct, 3 – ovary, 4 – uterine artery, 5 – ovarian artery, 6 – uterine branch of the ovarian artery, 7 – branches of the uterine artery supplying muscular layer of the mesometrium, 8 – ovarian vein, 9 – utero-ovarian vein, 10 – procollector and collector lymphatic vessels, 11 – destination transfer of steroids, 12 – retrograde transfer of steroids, 13 – destination transfer of prostaglandins, 14 – retrograde transfer of prostaglandins.

of local endocrine regulation of female reproductive organs function. In the mesovarium three complementary processes proceed:

- highly effective retrograde transfer of ovarian hormones, which enhances the concentration of particular steroid hormones in arterial blood supplying the ovary by 78-150% of its value in systemic blood,
- destination transfer of ovarian hormone from the mesovarium to the uterus and oviduct, which elevates local concentration of steroid hormones in arterial blood supplying the oviduct and uterus by 30-70%,
- destination transfer of  $\text{PGF}_{2\alpha}$  from uterine venous and lymphatic effluent to the ovarian artery.

All these hormonal exchanges in the mesovarium and mesometrium are performed by:

- a) indirect transfer, based on mesovarian microcirculation and the function of the veno-venous network on the surface of the ovarian and uterine artery branches and the lymphatic vessels of the mesovarium and mesometrium,
- b) direct transfer between adjoining vessels (counter current mechanism) from the ovarian venous and lymphatic effluent and from uterine venous and lymphatic effluent into the ovarian and uterine artery.

In the experiments with  $^3\text{H-PGF}_{2\alpha}$  or  $^3\text{H-PGE}_2$  it was demonstrated that prostaglandins permeated into the ovary faster and in greater amount from lymphatic than from venous vessels of the mesometrium (KOZIOROWSKI et al. 1986, KRZYMOWSKI et al. 1986, 1987, HEAP et al. 1985, 1989, STEFAŃCZYK-KRZYMOWSKA et al. 2005). Moreover, in experiment with labeled prostaglandin applied into lymphatic vessels (injection under the perimetrium) it was demonstrated that 98-99% of  $^3\text{H-PGF}_{2\alpha}$  outflowed with lymph, but only 1-2% with venous blood (KOZIOROWSKI et al. 1986). High concentration of prostaglandins in ovine uterine lymph was earlier revealed in *in vivo* condition (ABDEL RAHIM et al. 1983).

Uterine  $\text{PGF}_{2\alpha}$  is synthesized, mainly by endometrium, during estrous cycle and accumulated in reproductive organs (STEFANŃCZYK-KRZYMOWSKA 1996). The concentration of prostaglandin in arterial blood is limited by its decomposition in the lungs. After a first passage with blood through the lungs, 35% of prostaglandin remains active, while after the second and third passage only 15% is still active (DAVIS et al. 1984). However, it was demonstrated at the first time in 1986, that in pigs and cows, during the luteal phase, (KRZYMOWSKI et al. 1986, KOZIOROWSKI et al. 1986) and in pigs during early pregnancy and pseudopregnancy evoked by estradiol (KRZYMOWSKI et al. 1987)  $\text{PGF}_{2\alpha}$  is retrograde transferred to the uterine horn after its permeation in mesometrial vasculature from venous and lymphatic effluent into uterine arterial blood. Back (retrograde) transfer of  $\text{PGF}_{2\alpha}$  is especially important in regulation of the estrous cycle. During luteal phase,  $\text{PGF}_{2\alpha}$  after its local permeation into arterial

blood is involved together with high concentration progesterone of in constricting of uterine arterial vessels. By this way  $\text{PGF}_{2\alpha}$  participate in the reduction of blood supply to the uterus. Recently, retrograde transfer of  $\text{PGE}_2$  was also demonstrated (STEFAŃCZYK-KRZYMOWSKA et al. 2005, 2006).

Based on earlier research, mainly conducted on pigs, KRZYMOWSKI and STEFAŃCZYK-KRZYMOWSKA (2002, 2004, 2007) presented a new theory on the role of the changes in uterine blood supply (depending on estradiol and  $\text{PGE}_2$  or progesterone and  $\text{PGF}_{2\alpha}$  domination) in the regulation of the estrous cycle in animals. According to these theory, uterine blood supply is one of main factors involved in the regulation of the estrous cycle. On days 10-12 of estrous cycle the blood flow in the uterus decreases to 30-40% in pigs (FORD, CHRISTENSON 1979) and to 10% in sheep (ROMAN-PONCE et al. 1983) of its volume at the time of estrus and ovulation. It is well documented that a decrease in the blood flow, in response to progesterone domination and the action of the retrograde transferred  $\text{PGF}_{2\alpha}$  during the luteal phase, caused the regressive changes in endometrial cells. These changes cause a very limited supply of hormones, oxygen and other essential components, which leads to a release of endometrial cells elements with great amount of  $\text{PGF}_{2\alpha}$  to the extracellular space.  $\text{PGF}_{2\alpha}$  penetrate into small lymphatics and venous blood vessels. At the same time, the uterus begins contractions, caused mainly by hypothalamic oxytocin, and resulting in  $\text{PGF}_{2\alpha}$  excretion into lymph and venous blood leaving the uterus.

In cows and in other domestic animals  $\text{PGF}_{2\alpha}$  is released from the uterus in pulses (see review MCCracken et al. 1999). Recently it has been demonstrated that luteolysis resulted from around five release episodes of  $\text{PGF}_{2\alpha}$  (MANN, LAMING 2006). In the late luteal phase, secretion of  $\text{PGF}_{2\alpha}$  is stimulated by oxytocin originating from hypothalamus, and next this release causes oxytocin secretion from the corpus luteum via the mutual feedback (HEAP et al. 1989). Removing around 50% of oxytocin during the early luteal phase and 75% during the middle and late phase influences the length of the cycle in cows (KOTWICA, SKARZYŃSKI 1993). It was shown in experiments conducted on the isolated cow uterus with ovary and broad ligament that ovarian oxytocin could be transferred to the uterus (local destination transfer) and to the ovary (retrograde transfer) (KOZIOROWSKI et al. 1989). Similarly, local destination transfer of ovarian oxytocin to the oviduct and uterus in early pregnant gilts in *in vivo* condition (STEFAŃCZYK-KRZYMOWSKA et al. 1996) and retrograde transfer of this hormone to the ovary in ewes (SCHRAMM et al. 1986a) were revealed. Oxytocin takes part in stimulating myometrium constriction and progesterone secretion (MARES, CASIDA 1963, TAN et al. 1982). MIYAMOTO and SCHAMS (1991) managed to start progesterone secretion caused by oxytocin in the early luteal phase in *in vitro* research. It was shown that a significant oxytocin concentration enhancement was preceded by an increased progesterone level after infusing noradrenaline

into the abdominal artery (KOTWICA et al. 1991, SKARŻYŃSKI, KOTWICA 1993, JAROSZEWSKI, KOTWICA 1994). These results suggest that the luteolysis is controlled by the steroid system, but most importantly by progesterone. In the cows and sheep, progesterone applied during the early luteal phase induced an earlier luteolysis (SCHAMS et al. 1998, OTTOBRE 1980) and seems to be the main factor causing initiation of luteolysis by controlling the pathway of prostaglandin synthesis as well as  $\text{PGF}_{2\alpha}$  content in endometrium (LAFRANCE, GOFF 1985, 1988).

The development of analytical methods and particularly molecular biology techniques allows for more exact observations of changes taking place in the animal reproductive system dependent on the stage of its function. The sensitivity of cells to regulatory factors is determined by the presence of their unoccupied receptors. During the luteal phase the amount of  $\text{PGF}_{2\alpha}$  receptors in corpus luteum does not change. It was shown that the factor deciding about initiation of luteolysis is sensitization of  $\text{PGF}_{2\alpha}$  receptors enabling this process (SKARŻYŃSKI, OKUDA 1999, 2000, SKARŻYŃSKI et al. 2000, 2000a). Oxytocin, progesterone and noradrenaline desensitize luteal cells to the action of  $\text{PGF}_{2\alpha}$ , in opposite, nitrogen oxide sensitizes luteal cells to  $\text{PGF}_{2\alpha}$  action through inhibiting progesterone and oxytocin synthesis. Research during the last decade shows that tumor necrosis factors (TNF) synthesized in the corpus luteum participate in the local regulations, where  $\text{TNF}\alpha$  is a factor in causing luteolysis whilst  $\text{TNF}\gamma$  by inhibiting  $\text{TNF}\alpha$  action functions as a luteal protector during the luteal phase and pregnancy (OKUDA et al. 2002). Also insulin-like growth factors (IGF I and IGF II) are essential local regulators of ovarian functions (WEBB et al. 2002). It has to be assumed that further research in the area of the reproductive system with the use of the molecular biology techniques will allow a more exact understanding of the processes taken place in the ovaries and will allow for new forms of treatment of its dysfunctions.

## Conclusions

Local destination and retrograde transfer of ovarian hormones and uterine prostaglandins is a way by which reproductive organs are selectively supplied with increased amount of these hormones, adequate to their needs. The ovarian steroid hormones, oxytocin, uterine prostaglandins and locally secreted cytokines contribute to the regulation of cyclic function of the reproductive organs. Prominent part of this regulation concerns alteration of blood vessels function and blood supply to the ovary and uterus. Blood supply is accepted important mediator of the ovary and corpus luteum function (NISWENDER et al. 1976, WILTBANK et al. 1990, MIYAMOTO et al. 2005). The action of ovarian steroid

hormones together with uterine prostaglandins on uterine blood vessels conditions cyclic alterations, which include proliferation, secretion and rebuilding of the endometrium and are involved in the regulation of cyclic function of the female reproductive organs (KRZYMOWSKI, STEFAŃCZYK-KRZYMOWSKA 2002, 2004, 2007).

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