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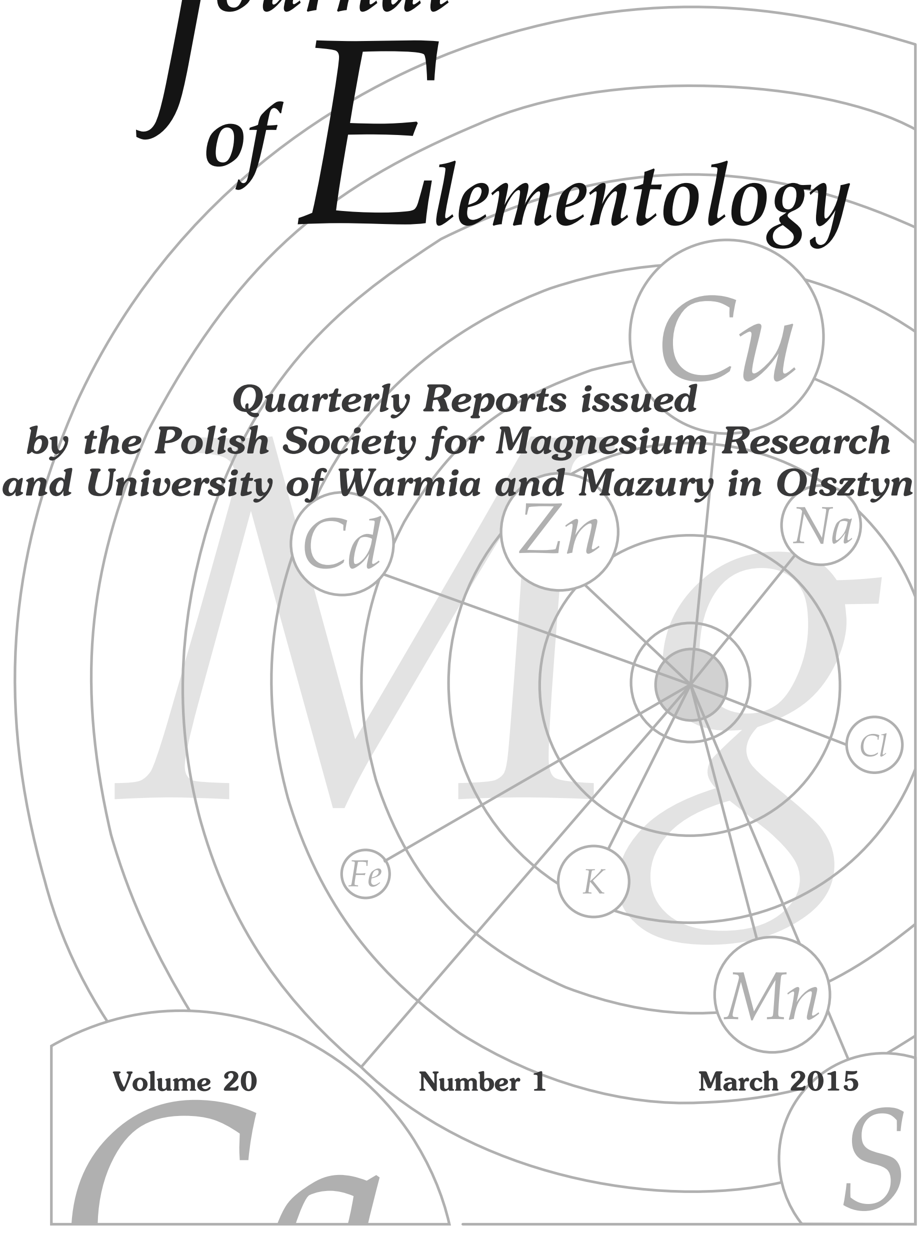

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PHYTOINDICATIVE EVALUATION OF HABITAT CONDITIONS OF SOILLESS FORMATIONS RECLAIMED WITH FLOTATION SLUDGE, SEWAGE SLUDGE AND USED MINERAL WOOL UNDER THE INFLUENCE OF THE JEZIÓRKO SULPHUR MINE*

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Abstract

The floristic observations presented below were carried out in 2011, on 5-acre plots delineated on an area of degraded soil. The soil-reclamation experiment, which began in June 2004, was set up on devastated soilless formation affected by the Jeziórko Sulfur Mine. In the experiment, the impact of different (200, 400, 800 m^3 ha^{-1}) doses of mineral wool and sewage sludge on characteristcis of the soilless formation was analyzed. The strongly acidic soilless formation (weak loamy sand) was reclaimed using flotation sludge in a dose of 100 t ha^{-1} for de-acidification, and various combinations of mineral wool and sewage sludge. The sward sown on plots pretreated as above consisted of a clover reclamation mixture with the following composition: *Festuca pratensis* – 41.2%, *Festuca rubra* – 19.2%, *Lolium perenne* – 14.7%, *Lolium multiflorum* – 12.4%, *Dactylis glomerata* – 6.5%, *Trifolium pratense* – 6%. A phytoindication method was employed to assess the impact of different reclamation methods of soilless formation on shaping the habitat conditions. This assessment took into account the following indicators: soil moisture, trophism, pH, organic matter content, resistance to salinity and to an increased content of heavy metals. The method for soilless formation reclamation was found to produce an effect on the analyzed ecological factors. The most favorable habitat conditions were recorded in soil reclaimed with mineral wool; they were worse in soil reclaimed with mineral wool and treated with NPK, but the worst parameters occurred in soil with the addition of sewage sludge and mineral wool.

Key words: ecological indicative numbers of vascular plants, reclamation, soilless formation, sulfur mine.

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INTRODUCTION

Borehole sulfur mining results in multiple transformations of the natural environment, particularly evident in soils due to the influx of chemically active minerals, poor native soil quality as well as the process of mining (Kołodziej, Słowińska-Jurkiewicz 2004, Lecyk, Brzezińska 2007, Gołda 2008). Native sulfur, sulfides and sulfur oxides emitted during the mining process are potential carriers of sulfuric acid into the soil, which inevitably leads to intense acidification and degradation, often devastation. These changes create a toxic environment for most crops, affected by an acute shortage of nutrients and increased mobility of phytotoxic elements (Motowicka-Terelak, Dudka 1991, Motowicka-Terelak 1993).

For the reclamation of degraded soils and their reconstruction on soilless formations near the Jeziórko Sulfur Mine, flotation sludge is successfully used for de-acidification, while adequate quality municipal sewage sludge is incorporated into soil to improve the resources of organic matter and nutrients (Baran et al. 2006, Krzywy et al. 2008).

Research on the application of used mineral wool for reclamation of devastated soils has been conducted for several years in that area. The results indicate strong influence of mineral wool on the formation of chemical, physicochemical and physical properties of reclaimed soils (Baran et al. 2008).

The plant cover is a reflection of inter-relationships and dependencies between the natural environment elements, especially the climate and soil properties. Changes in plant communities result from natural effects and anthropogenic factors, which vary in time and space (Roo-Zielińska 2004, Kalembasa, Malinowska 2009, Sądej, Namiotko 2010).

It is known that many plant species are good indicators of habitat conditions. Many years of research have led to the identification of specific association of many plant species with habitat factors, which is expressed in the form of ecological indicative numbers (Ellenberg et al. 1991, Zarzycki et al. 2002). This analysis may help to verify whether the technical and biological reclamation techniques implemented in degraded and devastated areas are adequate.

The aim of this study was to evaluate, using the phyto-indication method, the habitat conditions shaped on soilless formation reclaimed using flotation sludge, sewage sludge and used mineral wool from crops grown under cover, in an area under the impact of the Jeziórko Sulfur Mine.

MATERIAL AND METHODS

The study was carried out on experimental plots established in 2004 on some devastated area affected by the Jeziórko Sulfur Mine. For the reclamation of soilless formation, flotation sludge, municipal sewage sludge, used mineral wool Grodan and NPK fertilizers were applied. Strongly acidic soilless formation (weak loamy sand) was reclaimed using flotation sludge at 100 t ha^{-1} for de-acidification, and mineral wool or sewage sludge in various combinations. In the experiment, the impact of different (200, 400, 800 m^3 ha^{-1}) doses of mineral wool and sewage sludge on the features of soilless formation was analyzed. Grass with a clover reclamation mixture of the following composition: *Festuca pratensis* – 41.2%, *Festuca rubra* – 19.2%, *Lolium perenne* – 14.7%, *Lolium multiflorum* – 12.4%, *Dactylis glomerata* – 6.5%, *Trifolium pratense* – 6%, was sown on prepared plots

The paper contains results from the first year (2004) and several years afterwards (2011), in which phyto-sociological records were taken. Soil samples for laboratory analyses were collected each year in autumn.

The following analyses were performed on the collected samples: pH in 1 mol KCl dcm^{-3} by potentiometry, Corg. content by the Tiurin method modified by Simakov, Nog. by the Kjeldahl method. Eleven phyto-sociological records were taken by the method elaborated by BRAUN-BLAQUET (1964) on plots reclaimed in the various ways. The habitat conditions were determined using indicative scores given by ZARZYCKI et al. (2002). The following indicators were taken into account: W – soil moisture content (1 – very dry, 2 – dry, 3 – fresh, 4 – moist, 5 – wet, 6 – water); Tr – trophism (1 – extremely poor soils, 2 – poor, 3 – moderately poor, 4 – abundant, 5 – very abundant); R – soil acidity (1 – strongly acidic soils, 2 – acidic, 3 – moderately acidic, 4 – neutral, 5 – alkaline); H – organic matter content (1 – soils poor in humus, 2 - mineral-humic soils, 3 – soils abundant in organic matter); S – resistance to NaCl presence in soil (1 – species tolerating increased NaCl content, 2 – species grown mainly on soils with increased NaCl content); M – resistance to increased heavy metals content in soil (1 – species tolerating increased content of heavy metals, 2 – species requiring increased concentrations of heavy metals).

RESULTS

In 2011, the pH value in the surface layer of the soilless formation reclaimed with different doses of used mineral wool and fertilized with NPK ranged from 5.2 to 6.0; in the formation reclaimed by sewage sludge, sewage sludge with mineral wool or mineral wool, the pH range was within 6.3-6.9. The reclamation method also influenced Corg. content. The highest average content of organic carbon was in the soil reclaimed with sludge, sewage sludge and mineral wool (12.3-23.2 g kg^{-1}); in the other reclamation variants, the Corg. content was similar (11.2-15.6 g kg^{-1}). The reclamation method had no effect on the total nitrogen content. However, the reclaimed soil was characterized by a higher content of total nitrogen (an average of 1.6 g kg^{-1}) than the control soil (1.1 g kg^{-1}) – Table 1.

Table 1

General properties of reclaimed soil

Reclamation variants	pH w KCl		Corg		Ntot.	
			(g kg^{-1})			
	2004	2011	2004	2011	2004	2011
NPK - control	6.9	6.4	9.5	8.9	0.4	1.1
Sewage sludge - control	7.3	6.8	5.7	14.2	0.9	1.4
Sewage sludge + mineral wool 200 m^3 ha^{-1}	7.2	6.9	8.6	23.2	1.2	2.4
Sewage sludge + mineral wool 400 m^3 ha^{-1}	7.2	6.7	10.6	13.6	1.2	1.3
Sewage sludge + mineral wool 800 m^3 ha^{-1}	7.1	6.8	9.7	12.3	1.0	1.4
Mineral wool 200 m^3 ha^{-1}	7.2	6.4	7.3	11.2	0.8	1.2
Mineral wool 400 m^3 ha^{-1}	7.1	6.3	12.1	15.2	1.4	1.6
Mineral wool 800 m^3 ha^{-1}	6.9	6.5	9.5	14.6	1.1	1.4
NPK + mineral wool 200 m^3 ha^{-1}	7.1	5.9	7.0	15.6	0.8	1.6
NPK + mineral wool 400 m^3 ha^{-1}	7.2	5.2	13.6	11.8	1.6	1.1
NPK + mineral wool 800 m^3 ha^{-1}	6.8	6.0	10.1	13.0	1.4	1.4

Seven years after the onset of the experiment, in addition to the plant species introduced as part of reclamation treatments, other plants were noted, which had entered the area by spontaneous succession over the analyzed soilless formation reclaimed with sewage sludge and varied mineral wool doses. The soils reclaimed with mineral wool were grown mainly by meadow species from the *Molinio-Arrhenathertea* class and by synanthropic and lawn species, as compared to other plots.

The range of humidity indicator ranged between 2 (dry habitat) to 6 (aquatic habitats). The analyzed area was dominated by species of fresh habitats (indicator 3), which accounted for 28.9% of the studied flora (Figure 1), with an average of 9 plant species (Table 2). Slightly less common (23.7%)

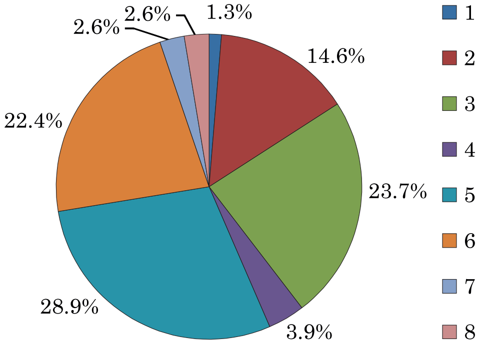

Fig. 1. Moisture content indicator:
1 – indicator 2; 2 – indicator 2-3; 3 – indicator 2-4;
4 – indicator 3; 5 – indicator 3-4; 6 - indicator 4;
7 – indicator 4-5; 8 – indicator 5-6

Table 2
Influence of reclamation method on the number of plant species according to the moisture content indicator

Reclamation variants	Moisture content indicator (W)							
	2*	2-3	2-4	3	3-4	4	4-5	5-6
NPK - control	7	8	2	9	9	2	.	1
Sewage sludge - control	4	3	1	9	2	.	.	.
Sewage sludge + mineral wool 200 m^3 ha^{-1}	1	4	2	8	2	.	1	1
Sewage sludge + mineral wool 400 m^3 ha^{-1}	1	5	1	6	3	.	.	.
Sewage sludge + mineral wool 800 m^3 ha^{-1}	.	7	1	8	3	.	.	1
Mineral wool 200 m^3 ha^{-1}	3	7	3	9	7	.	1	.
Mineral wool 400 m^3 ha^{-1}	3	7	3	11	10	.	.	.
Mineral wool 800 m^3 ha^{-1}	3	8	2	13	10	.	1.	.
NPK + mineral wool 200 m^3 ha^{-1}	3	6	3	10	8	.	.	.
NPK + mineral wool 400 m^3 ha^{-1}	2	8	3	9	6	1	.	.
NPK + mineral wool 800 m^3 ha^{-1}	1	8	2	12	6	1	.	.
Min.	1	3	1	6	2	1	1	1
Max.	7	8	3	13	10	2	1	1
Average	3	6	2	9	6	1	1	1
Coefficient of variation	64.8	27.2	39.8	20.8	51.6	-	-	-

* Key under Figure 1

were species with the indicator 2-3 (intermediate habitat between dry and fresh). A relatively high share was created by species with the indicator 3-4 (moist and fresh habitat), representing 22.4% of the analyzed vascular flora. Species with indicators 2 (dry habitat), 4 (moist habitats), and 5 (wet habitats), as well as 5-6 (wet and water habitats) represented much smaller shares: 14.6%, 2.6%, 1.3%, respectively. Over 3.9% of the total flora did not correspond to any specific moisture content indicator (range of indicators 2-4).

Our analysis of moisture indicators for the species present on the soilless formation reclaimed with the studied methods shows that the largest number of species of fresh (indicator 3) and intermediate between fresh and moist habitats (indicator 3-4) occurred on plots reclaimed using mineral wool, while fewer such plants were documented on soil reclaimed using wool with NPK (Table 2). The lowest number of such species was recorded after the application of sewage sludge and mineral wool.

The study shows that the soilless formation reclaimed with the tested methods was dominated by species of neutral habitats (indicator 4), which accounted for 39.6% of all the flora (Figure 2). Much fewer species were assigned indicators 2-3 (2.6%), 3 (6.6%), 3-4 (11.8%), 5 (2.6%), and 5-4 (15.8%). Species with a broad spectrum of adaptability to the acidity of substrate (species with indicators in the ranges 2-5 and 3-5) made up 14.5% of the total analyzed flora.

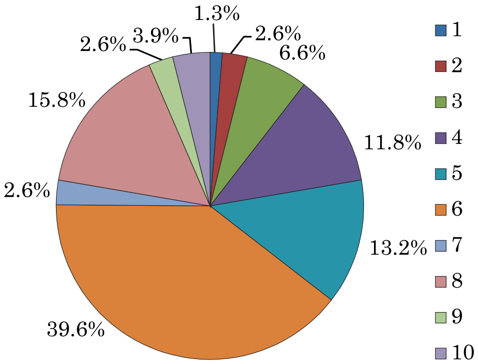

Fig. 2. The pH value indicator:
1 – indicator 2-3; 2 – indicator 3; 3 – indicator 3-4;
4 – indicator 3-5; 5 – indicator 4; 6 - indicator 2-4;
7 – indicator 4-5; 8 – indicator 5; 9 – indicator 4-5;
10 – indicator 2-5

Our analysis of individual reclamation variants indicates that the largest number of species with indicator 4 (neutral habitat) was present in the reclamation variant with mineral wool, fewer in the variant with mineral wool and NPK, and the fewest - with sewage sludge and mineral wool (Table 3). Regardless of the reclamation variant, the average number of species with indicator 4 was twelve, while those with indicator 4-5 were six on average. For these indicators, the variation coefficient was also the lowest.

Table 3

Influence of reclamation method on the number of plant species according to the acidity indicator

Reclamation variants	Acidity indicator (R)									
	2-3*	3	3-4	3-5	4	2-4	4-5	5	5-4	2-5
NPK - control	1	.	4	.	7	1	6	.	.	1
Sewage sludge - control	1	3	4	4	17	.	6	2	1	1
Sewage sludge + mineral wool 200 m^3 ha^{-1}	1	.	5	2	15	.	6	.	1	1
Sewage sludge + mineral wool 400 m^3 ha^{-1}	.	.	5	2	12	.	2	.	.	.
Sewage sludge + mineral wool 800 m^3 ha^{-1}	.	.	2	2	7	.	5	.	.	.
Mineral wool 200 m^3 ha^{-1}	.	1	2	5	15	.	5	.	1	1
Mineral wool 400 m^3 ha^{-1}	.	.	5	6	15	.	5	.	2	1
Mineral wool 800 m^3 ha^{-1}	.	.	4	6	17	1	6	.	2	1
NPK + mineral wool 200 m^3 ha^{-1}	.	.	3	6	11	1	7	.	1	1
NPK + mineral wool 400 m^3 ha^{-1}	1	1	3	5	10	.	6	.	2	1
NPK + mineral wool 800 m^3 ha^{-1}	1	1	3	4	11	1	7	.	1	.
Min.	1	1	2	2	7	1	2	2	1	1
Max.	1	3	5	6	17	1	7	2	2	1
Average	1	2	4	4	12	1	6	2	1	1
Coefficient of variation	-	66.7	30.8	40.2	29.0	-	24.7	-	37.6	-

* Key under Figure 1

Fertility of habitats was assessed according to the trophic indicator (Tr). The analysis of this indicator (Figure 3) reveals the presence of species with the full spectrum of trophic requirements within the examined vascular flora. Species with very low nutritional requirements (indicator 1-2), moderately poor habitat species (indicator 3), and abundant and fertile habitats species (indicator 4 and 5) alike grew on the studied soilless formation reclaimed with different methods. The results show the advantage of abundant habitat species, eutrophic ones (trophic indicator 4), representing 34.3% of the total flora studied. The smallest share was made up by taxa with low trophic requirements (poor and moderately poor habitats - indicator 1-2 and 2), as well as habitats intermediate between abundant and very abundant.

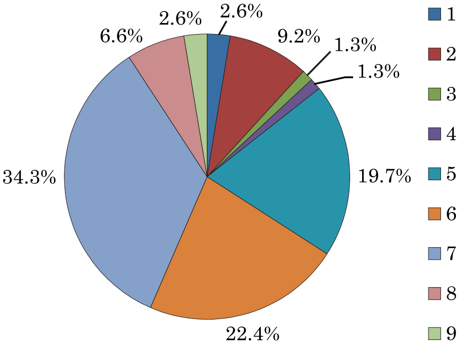

Fig. 3. Trophic indicator:
1 – indicator 1-2; 2 – indicator 2;
3 – indicator 2-3; 4 – indicator 2-4;
5 – indicator 3; 6 - indicator 3-4;
7 – indicator 4; 8 – indicator 4-5;
9 – indicator 4-3

Our analysis of trophic indicators for species grown on the soil reclaimed using the tested waste shows that the largest number of fertile habitats species (indicator 4) and intermediate between moderately poor and fertile ones (indicator 3-4) was found on plots reclaimed with mineral wool, fewer on soil reclaimed with wool + NPK, and the fewest on plots where sewage sludge as well as sewage sludge with mineral wool were applied (Table 4). The highest mean species abundance, regardless of the reclamation variant, was assigned to plants characterized by indicator 4 (10 species) and 3-4 (7 species).

The study carried out on reclaimed soilless formation indicates a significant advantage of species associated with mineral-humic soils (indicator 2). On average, they represent 76.3% (22 species) of the studied flora, regardless of the reclamation method. The contribution of species with indicator 1 (soils poor in humus) and 3 (soil abundant in organic matter) is much smaller:

Table 4

Influence of reclamation method on the number of plant species according to the trophic indicator

Reclamation variants	Trophic indicator (Tr)								
	1-2*	2	2-3	2-4	3	3-4	4	4-5	4-3
NPK - control	2	5	.	.	8	8	14	2	1
Sewage sludge - control	.	2	.	.	5	3	6	2	.
Sewage sludge + mineral wool 200 m^3 ha^{-1}	.	1	.	.	2	4	9	2	1
Sewage sludge + mineral wool 400 m^3 ha^{-1}	.	.	.	.	3	5	7	2	.
Sewage sludge + mineral wool 800 m^3 ha^{-1}	.	.	.	.	3	5	8	4	1
Mineral wool 200 m^3 ha^{-1}	1	1	.	1	5	8	10	3	1
Mineral wool 400 m^3 ha^{-1}	.	1	.	1	6	11	11	3	1
Mineral wool 800 m^3 ha^{-1}	.	2	.	.	5	10	16	3	1
NPK + mineral wool 200 m^3 ha^{-1}	.	1	.	1	5	9	10	3	1
NPK + mineral wool 400 m^3 ha^{-1}	.	2	.	1	5	10	9	2	.
NPK + mineral wool 800 m^3 ha^{-1}	.	.	1	.	6	8	11	2	1
Min.	1	1	1	1	2	3	6	2	1
Max.	2	5	1	1	8	11	16	4	1
Average	2	2	1	1	5	7	10	3	1
Coefficient of variation	47.1	72.3	-	-	34.5	36.6	28.9	27.0	-

* Key under Figure 1

2.6% and 5.3%, respectively. In some plots, the proportion of species with a broad spectrum of requirements in relation to the organic matter content (indicators 1-2 and 2-3) was apparent (Figure 4).

The analysis of the impact of different reclamation methods indicates that the largest number of species with indicator 2 (mineral-humic soils) was on the plots with mineral wool, fewer – on plots with mineral wool plus NPK, and the fewest – on plots with sewage sludge and mineral wool. On

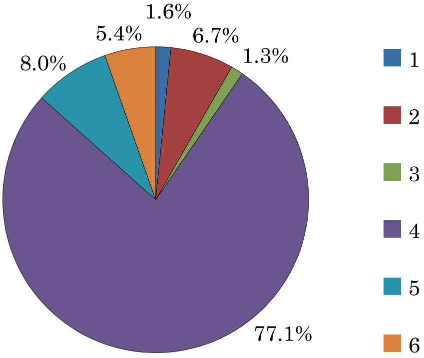

Fig. 4. Organic matter content indicator:
1 – indicator 1, 2 – indicator 1-2,
3 – indicator 1-3, 4 – indicator 2,
5 – indicator 2-3, 6 - indicator 3

the soil reclaimed using sewage sludge and mineral wool, noteworthy is the presence of species with a very wide spectrum in terms of the organic matter content demand (indicator 1-3) – Table 5.

Table 5

Influence of reclamation method on the number of plant species according to the organic matter content

Reclamation variants	Organic matter content indicator (H)					
	1*	1-2	1-3	2	2-3	3
NPK - control	.	2	1	15	.	1
Sewage sludge - control	1	5	.	28	3	3
Sewage sludge + mineral wool 200 m^3 ha^{-1}	.	.	1	22	4	3
Sewage sludge + mineral wool 400 m^3 ha^{-1}	.	1	1	15	2	1
Sewage sludge + mineral wool 800 m^3 ha^{-1}	.	1	1	12	1	1
Mineral wool 200 m^3 ha^{-1}	1	1	.	23	3	2
Mineral wool 400 m^3 ha^{-1}	.	1	.	28	3	2
Mineral wool 800 m^3 ha^{-1}	.	2	1	28	3	3
NPK + mineral wool 200 m^3 ha^{-1}	.	2	.	23	3	2
NPK + mineral wool 400 m^3 ha^{-1}	1	2	.	22	2	2
NPK + mineral wool 800 m^3 ha^{-1}	1	1	.	22	3	2
Min.	1	1	1	12	1	1
Max.	1	5	1	28	4	3
Average	1	2	1	22	3	2
Coefficient of variation	-	68.3	-	25.6	30.5	38.7

* Key under Figure 1

The analysis of the indicator of resistance to NaCl in the soil under the plot experiment shows the presence of so-called *facultative halophytes* (species tolerating an increased NaCl content) – Table 6. They represent 32.9% of the studied flora. The analysis of this indicator in the context of the type of waste used for reclamation indicates that most species exhibiting the resistance to an elevated NaCl content (indicator 1) grew on plots reclaimed with mineral wool, fewer on plots treated with mineral wool + NPK, and the fewest were found on plots with sewage sludge and mineral wool application.

Table 6

Influence of reclamation method on the number of plant species according to the resistance to NaCl content

Indicator NaCl resistance content (S)	Reclamation variants										
	NPK - control	sewage sludge	sewage sludge + mineral wool			mineral wool			mineral wool + NPK		
			200*	400	800	200	400	800	200	400	800
1	9	15	13	10	7	15	16	18	15	13	16

* 200, 400, 800 – doses of mineral wool in m^3 ha^{-1}

The study shows an increased content of heavy metals on the soilless formation reclaimed with sludge, sewage sludge and different doses of mineral wool. Elevated concentrations of heavy metals occurred in 11.8% of the examined flora. Our analysis of the resistance to an increased content of heavy metals (M) shows that the largest number of species with indicator 1 was found on soils reclaimed with mineral wool, slightly fewer on soil reclaimed with mineral wool and NPK fertilization, and the fewest - in the case of soil treated with sewage sludge and mineral wool (Table 7).

Table 7

Influence of reclamation method on the number of plant species according to the resistance to the increased heavy metals content

Indicator increased resistance to heavy metals content (M)	Reclamation variants										
	NPK - control	sewage sludge	sewage sludge + mineral wool			mineral wool			mineral wool + NPK		
			200*	400	800	200	400	800	200	400	800
1	3	3	5	2	3	6	6	6	6	5	6

* 200, 400, 800 – doses of mineral wool in m^3 ha^{-1}

DISCUSSION

The analysis of the trophic indicator for the reclaimed soilless formation has shown the presence of species with a broad spectrum of trophic requirements within the vascular flora of the examined habitats. Species with small nutritional requirements as well as ones of moderately poor and abundant, and even very abundant habitats, were documented. However, the dominant group were the species attributed to abundant (eutrophic) habitats. Research by Cabała, Jarząbek (1999) shows that a former landfill in Chorzow was dominated by species which preferred conditions typical of abundant soils. Klimko et al. (2004) indicate that the Wałbrzych dumping grounds used by the coal mines Thores and Victoria were also overgrown by species with different trophic requirements. However, the percentage of taxa with different trophic indicators was similar. According to Jędrzejko, Olszewski (2006), the largest group of plants in post-exploitation areas of four liquidated coal mines was composed of mesophilic species, whilst eutrophic and oligotrophic plants formed a small group.

The examined habitats of reclaimed soils were overgrown by plant species with different moisture requirements (from dry to wet). However, species preferring the moisture conditions typical of fresh soils were prevalent (indicator 3). Similar dependencies were observed during the research made by Cabała, Jarząbek (1999) on the former dumping grounds in Chorzow. In turn, Klimko et al. (2004) indicate that the Wałbrzych dumps were overgrown by flora with the predominance of wet habitat species (indicator 5).

Our analysis of the organic matter content indicates that species preferring conditions typical of mineral-organic soils dominated in the conditions created by the soilless formation reclamation methods submitted to our analysis. A similar dependence was suggested by CABAŁA, JARZĄBEK (1999).

In terms of the acidity indicator, the study of the reclaimed soilless formation reveals that the largest group was composed of species characteristic for neutral soils. This demonstrates that acidic degradation, as the main form of environmental degradation in the area affected by sulfur mining, has been effectively eliminated. CABAŁA, JARZĄBEK (1999) reported the presence of species preferring substrates with moderately acidic to slightly acidic pH in the former dumping grounds in Chorzow.

The study indicates the occurrence of species tolerating an increased content of NaCl and heavy metals in the soil in the examined area.

The analysis of the reclamation methods and their effects showed that the greatest diversity with respect to both the number of species and the analyzed ecological indicators was in the soilless formation reclaimed with mineral wool, lower – with mineral wool and fertilized with mineral fertilizers NPK, and the lowest – with the addition of sewage sludge and mineral wool. A much greater diversity of species in terms of the analyzed indicators was recorded in the control plot, which was fertilized with mineral NPK fertilizers.

CONCLUSIONS

1. The soilless formation devastated due to the acidic pressure and afterwards reclaimed with flotation sludge, municipal sewage sludge and waste mineral wool from crops grown under cover achieved the habitat conditions where the dominant were plant species preferring moist habitats, typical of fresh soils, with the trophism structure corresponding to the abundant soils (eutrophic), neutral pH, as well as anthropogenic soil with the organic matter content same as in mineral-humic soils.

2. Among the reclamation methods, the most favorable habitat conditions were created on the soilless formation reclaimed with mineral wool, slightly worse conditions were obtained through the application of mineral wool and NPK fertilization, while the least beneficial was the use of sewage sludge along with mineral wool.

3. A dose of mineral wool did not produce a univocal impact on ecological indicators (soil moisture content, acidity, trophism, organic matter content, resistance to salinity, and increased concentrations of heavy metals).

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EFFECT OF ZINC SUPPLEMENTATION ON THE DISTRIBUTION OF LEAD IN TISSUES OF RATS INTOXICATED BY LEAD COMPOUNDS

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Abstract

The permissible threshold level of lead in blood (Pb-B) is currently established at 5 µg dL^{-1}, but evidence suggests that it is impossible to determine the safety threshold for lead (Pb) and any exposure, especially in children, must be considered as potentially harmful. Methods used to reduce the concentration of Pb in blood (e.g. EDTA, penicillamine) are not always effective and are associated with serious side effects. One of the proposed dietary supplements in the case of exposure to Pb and low blood Pb concentrations is zinc (Zn), but the published literature on its effectiveness is limited. Therefore, the aim of this study was to clarify whether Zn supplementation may help reduce the concentration of Pb in the blood and tissues of rats, at the Pb-B level previously recognized as safe. Tests were performed on 6-8 week old male Wistar rats. Rats were divided into control and experimental groups: Group C – rats receiving drinking water *ad libitum* for 4 weeks; Group Pb – rats receiving Pb acetate 0.1% (PbAc) in drinking water *ad libitum* for 4 weeks; Group Zn – rats receiving $ZnCO_3$ 300 mg kg^{-1} diet for 4 weeks; Group Pb+Zn – rats receiving PbAc in drinking water *ad libitum* plus 300 mg $ZnCO_3$ kg^{-1} diet for 4 weeks. The applied dose of 300 mg of $ZnCO_3$ kg^{-1} diet results in a high but non-toxic Zn level. The concentrations of Pb and Zn in blood, plasma, liver and bone were determined by emission spectrometry in inductively coupled argon plasma (ICP OES). Incidental exposure of adult rats to Pb at doses resulting in the level of Pb in blood below the previously recognized as safe one caused: (i) increased Pb concentration in the bones and plasma and its reduction in the whole blood and liver (ii) simultaneous supplementation of rats exposed to Pb with a high but non-toxic dose of zinc did not result in the reduction of the Pb concentration in the blood and tissues of rats, nor did it induce any changes

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in the distribution of Pb in the examined tissues (iii) supplementation of diets with a high but non-toxic dose of Zn is not an effective method of reducing the concentration of Pb in blood at Pb-B previously recognized as safe. However, the therapy consisting of zinc supplementation to support the action of chelators could be crucial for the elimination of Pb from the body.

Key words: lead, zinc, lead tissue distribution, zinc supplementation, lead threshold level.

INTRODUCTION

Despite the implementation of many regulations governing amounts of lead (Pb) in the environment, the risk of exposure to Pb in different periods of human life remains high and the toxicity of Pb is still one of the major health problems resulting from both environmental and occupational exposure (CDC 2004, 2005, 2007, ABADIN et al. 2007, WHO 2010, HRUBÁ et al. 2012). In developed countries, the increasing awareness of harmful effects of a lead-contaminated environment on the human body has eliminated Pb from production of fuels, paints, batteries and many consumer products such as cups, plastic toys and jewelry (EP 2005, EU 2008, EP 2009, WHO 2010). However, exposure to Pb, especially in children, continues to be mainly due to contact with soil and house dust contaminated with Pb or tap water contaminated by leaching Pb pipes. There is considerable aerial Pb emission from smelters, ore mining and processing, Pb acid battery manufacturing and coal combustion activities such as electricity generation (CDC 2007, 2012). Pb exposure particularly affects children, due to behavioral factors such as frequent hand-to-mouth activities *(pica),* greater gastrointestinal absorption and an immature blood/brain barrier (LIDSKY, SCHNEIDER 2003).

The permissible threshold level of Pb in blood (Pb-B) is currently established at 5 μg dL^{-1} (CDC 2012), but evidence suggests that it is impossible to determine the safety threshold for Pb, hence any exposure, especially in children, must be considered potentially harmful (BARANOWSKA-BOSIACKA et al. 2012, 2013). It should also be noted that Pb is subject to cumulative effects, and because it does not break down nor is it biodegradable, any assessment of the environmental threat should take into account the effect of chronic exposure. The half-life of Pb in the body ranges from 30 days in the fast exchange systems (blood, soft tissue) to as much as 27 years in slow exchange systems (bone) (RIEDT et al. 2009). Constant exposure to Pb at low concentrations leads to its accumulation in the body, where it is distributed to organs (liver, kidney, lung, pancreas, spleen, brain) and accumulated in bones (GULSON et al. 2002, NASH et al. 2004, CHEŁCHOWSKA et al. 2012) – Figure 1.

At the same time. there are no effective and safe methods of detoxification. Techniques used to reduce the concentration of Pb in blood (e.g. EDTA, penicillamine) are not always effective and are associated with serious side effects. One of the proposed dietary supplements in the case of exposure to Pb and low blood Pb concentrations is zinc (Zn), but the published literature

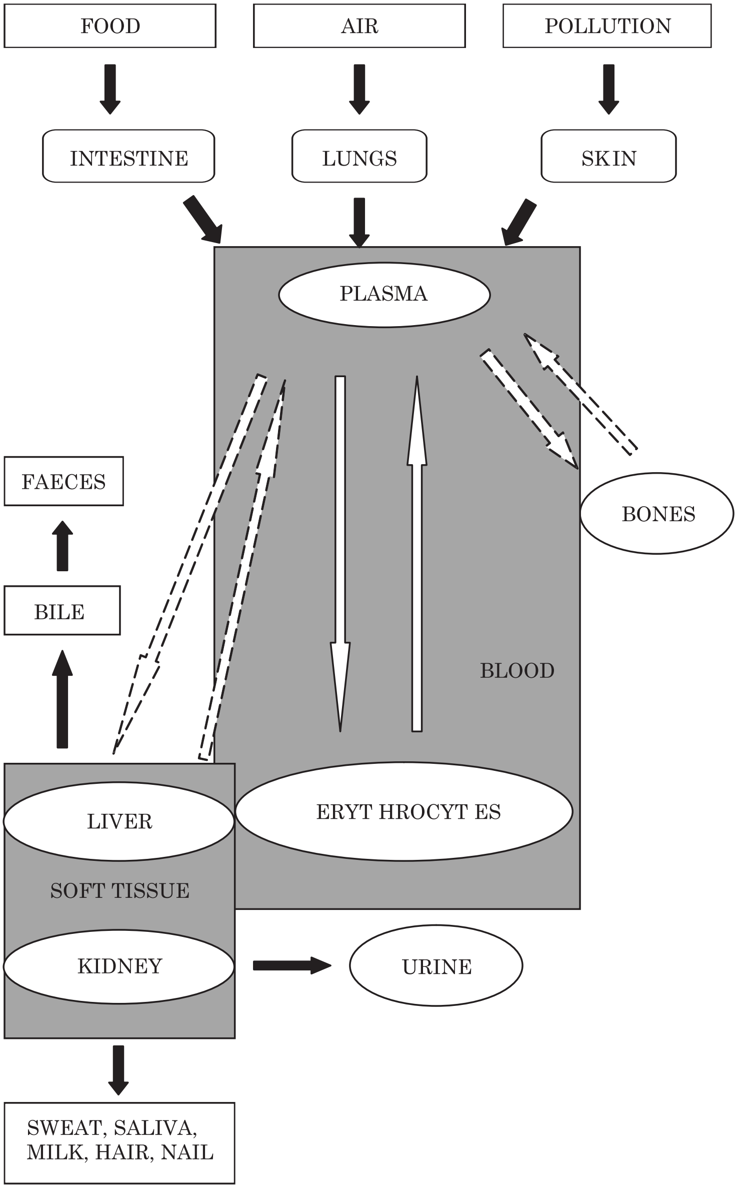


Fig. 1. Diagram illustrating the absorption, distribution and elimination of Pb from the body: bold arrows – routes of absorption, thin arrows – excretion pathways, dashed arrows – movement inside the body; the main locations of Pb in the body (on the basis of GIEL-PIETRASZUK et al. 2012)

on its effectiveness is limited. Therefore, the aim of this study was to clarify whether Zn supplementation may help reduce the concentration of Pb in the blood and tissues of rats, at the Pb-B level previously recognized as safe.

MATERIAL AND METHODS

Animals

The study was performed according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and the European Community Council Directive for Care and Use of Laboratory Animals, and was approved by a local ethics committee (The Pomeranian Medical University in Szczecin Committee on the Use and Care of Animals).

Toxicity procedure

Tests were performed on 6-8 week old male rats, inbred Wistar strain (*n* = 32). The mean body weight of rats at the start of experiment was about 105 g (21.30±SD). Clinically healthy animals were received from farms of the J. Nofer Institute of Occupational Medicine in Łódź (Poland). During the experiments, the rats were kept in cages under standard conditions. Animals were fed with Murigran feed (Agropol Motycz, Poland) and had free access to food and water. According to the manufacturer, the feed contained grains, products from cereal grains, products from genetically modified legume seeds, fish meal, by-products of oilseeds, feed fats and mineral supplements, enzymes, amino acids and a mineral-vitamin mixture. The detailed composition of the feed according to the manufacturer was (the content is given as % of the volume of feed): crude protein 23%, crude fat 3%, ash 8%, P 0.6% at 0.2%, Ca 1.1%, crude fiber 5.0% max., and dietary additives such as L-lysine (1.3%), dl-methionine (0.7%), L-threonine (0.7%), vit. A (E672) 500,000 IU g^{-1}. vit. D3 (E671) 500,000 IU g^{-1}. The manufacturer does not provide information about the content of Zn or Pb in feed (leaflet of Murigran, Agropol).

Rats were divided into four experimental groups. The study was conducted according to the following pattern:

Group C	– rats receiving drinking water ad libitum for 4 weeks (*n* = 8);
Group Pb	– rats receiving Pb acetate 0.1% (PbAc) in drinking water ad libitum for 4 weeks (*n* = 8);
Group Zn	– rats receiving $ZnCO_3$ 300 mg kg^{-1} diet for 4 weeks (*n* = 8);
Group Pb+Zn	– rats receiving PbAc in drinking water ad libitum plus 300 mg $ZnCO_3$ kg^{-1} diet for 4 weeks (*n* = 8).

The solution of PbAc was prepared daily in disposable plastic bags (hydropac, Anilab. Poland) from solid reagent directly in the desired concentration; the solution was not acidified. The volume of intaken liquids did not differ significantly between the experimental and control rats.

Previous studies (Baranowska-Bosiacka et al. 2012) have shown that the applied dose resulted in the concentration of Pb in the whole blood of animals (Pb-B) below the limit permissible in the blood in humans (<5 μg dL^{-1}). The applied dose of 300 mg of $ZnCO_3$ kg^{-1} diet caused a high but non-toxic Zn level (Jamieson et al. 2006).

After the completion of the experiment, the animals were anesthetized with pentobarbital sodium given intraperitoneally at a dose of 200 mg kg^{-1} body weight, and then exsanguinated by cutting the apex of the heart. Blood (5 mL) was collected into vacutainer tubes intended for the determination of heavy metals with a heparin containing needle (250 IU) used as anticoagulant. Organ samples were collected (liver and bones) and immediately frozen in liquid nitrogen for further analysis at -80°C.

Emission spectrometry in inductively coupled argon plasma (ICP OES) Pb, Zn determination

The concentrations of Pb and Zn in the material were determined by emission spectrometry in inductively coupled argon plasma (ICP OES) using the camera Optima 2000 DV (Perkin Elmer), after prior digestion in a microwave oven Microwave (Anton Paar).

Test portions of the liver and bone (0.6 g) were transferred to a quartz pressure vessel, to which then 5.0 mL of 65% HNO_3 (Suprapur. Merck) and 0.5 mL of 30% H_2O_2 (Suprapur. Merck) were added. After closing, the vessels with whole blood were placed in a microwave oven equipped with a temperature control system and a constant pressure in each of the quartz vessel. Mineralization parameters were: 0-5 min; a linear gradient from 100 to under 600 W, 6-10 min - 600 (const.), 11-20 min - 1000 W or less after reaching the limit value (75 MPa or 300°C), from 21 to 35 min - cooling the vessel.

Pb and Zn were determined directly in solutions prepared as explained above or diluted 100 times or 1000 times in order to obtain an optimal concentration range for ICP (of the order of several mg L^{-1}). A certified multi -element standard called Merck ICP Multielement Standard IV served as a reference. Solution standards were supplemented with the acid used for digestion added at the level found in the mineralized samples. In order to further minimize the potential of possible interference of sample into the plasma and other types of physical disorders in the plasma of argon, the analysis was performed using an internal standard, hence yttrium (Y) was introduced to the solutions of samples and standards at a concentration of 0.5 mg L^{-1} Y. All measurements of the emitted radiation intensity were performed by selecting a longer optical path of the axial (along the plasma) spectrometer. The measurement parameters: RF power generator – 1300; spray temperature – 30°C, the injection of the sample into the nebulizer – 1.5 mL min^{-1}; gas – argon 5.0, the flow of argon plasma – 15 L min^{-1} flow auxiliary argon – 0.5 L min^{-1}; the transfer of argon nebulizer – 0.8 L min^{-1}; wavelength of 206.200 nm, Zn, Pb wavelength – 220.353 nm.

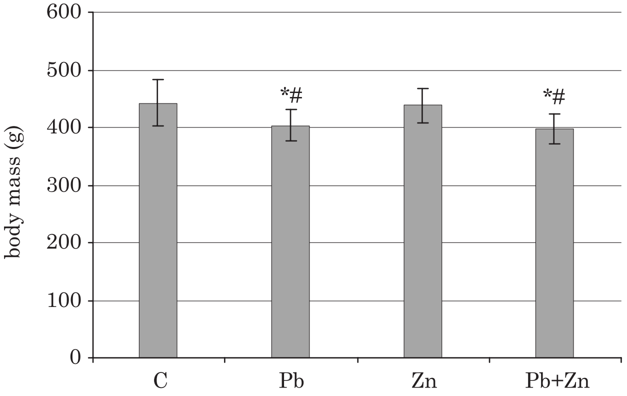
Statistical analysis

The results were analyzed statistically using Statistica 6.1 software. Arithmetical means and Standard Deviations (SD) were calculated for each of the studied parameters. The distribution of results for individual variables was obtained by the Shapiro-Wilk test. As most of the distributions deviated from a normal distribution, non-parametric tests were used for further analyses. Correlations between the parameters were examined by the Spearman's rank correlation coefficient (Rs). In order to assess differences between the studied groups, the non-parametric Mann-Whitney *U*-tests or Kruskal-Wallis tests were used, and *p*-values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Body mass

The mean body weight of rats exposed to lead (Pb group) was significantly lower compared to the control group (C group), by 9% (p = 0.049). Significantly lower than the control group (11%, *p* = 0.049) was also the body weight of rats that received Pb and were supplemented with zinc (Zn+Pb group). Also, the reduction of the body weight of rats treated with Pb compared to the group treated with Zn alone (Zn group) was statistically significant: 8% (*p* = 0.05). The 9% decrease (*p* = 0.04) observed in the group of rats that received both Pb and Zn, compared with the group supplemented with Zn (Zn group), proved to be statistically significant as well (Figure 2).



C group – rats receiving drinking water *ad libitum* for 4 weeks (*n* = 8);
Pb group – rats receiving Pb acetate 0.1% (PbAc) in the drinking water *ad libitum* for 4 weeks (*n* = 8);
Zn group – rats receiving $ZnCO_3$ 300 mg kg^{-1} diet for 4 weeks (*n* = 8);
Pb+Zn group – rats receiving PbAc in drinking water *ad libitum* plus 300 mg $ZnCO_3$ kg^{-1} diet for 4 weeks (*n* = 8);
* – difference statistically significant vs C group (control) $p < 0.05$;
# – difference statistically significant vs Zn group $p < 0.05$;

Fig. 2. Body mass of rats subjected to 4-week exposure to: Pb (Pb group), lead and zinc supplementation (Pb+Zn group), zinc supplementation (Zn group) and control group (C group)

Lead concentration in the whole blood

The average concentration of Pb in whole blood of rats in the group exposed to lead (Pb group*)* was approximately 160-times higher (significantly at p = 0.00015) than in the control group (C group). Also, the Pb concentration in the blood of rats which received Pb and Zn (Pb+Zn group) (p = 0.00015) was significantly higher than in the control (by 94%). A statistically significant increase in the Pb concentration (by 94%) also occurred in the Pb compared to Zn group (Zn group) (p = 0.00031). An increase in the concentration was also observed in the group administered lead and zinc (Zn+Pb group) compared with the one supplemented with zinc (Zn group), being about 160-fold higher (p = 0.00031) – Table 1.

Lead concentration in plasma

The average concentration of Pb in the plasma of rats exposed to lead (Pb group) was significantly higher in comparison with the control group (C group), by about 171-fold (p = 0.00031). Significantly higher, by 99% as compared to the control group, was also the concentration in plasma of rats that received the Pb and Zn (Pb+Zn group, p = 0.00031). A statistically significant increase was observed in the Pb concentration (by 99%, p = 0.00031) in the group receiving lead (Pb group) compared with the one supplemented with zinc (Zn group). An increased concentration (about 130-fold, p = 0.00031) occurred in the group receiving Pb and Zn (Zn+Pb group) compared with the one supplemented with Zn (Zn group) – Table 1.

Table 1

The concentration of Pb in the whole blood (Pb-B) and plasma (Pb-P) of rats after 4 weeks of exposure to Pb (Pb group), lead and zinc supplementation (Pb+Zn group), zinc supplementation (Zn group) and the control group (C group)

Group	**C** (n = 8)	**Zn** (n = 8)	**Pb** (n = 8)	**Pb+Zn** (n = 8)
	Pb-B (μg dL^{-1})			
	0.183±0.014	0.188±0.012	3.1317±0.333$^{***\#\#\#}$	3.270±0.395$^{***\#\#\#}$
	Pb-P (μg dL^{-1})			
	0.003±0.0007	0.003±0.0002	0.410±0.0627$^{***\#\#\#}$	0.400±0.0346$^{***\#\#\#}$

C group – rats receiving drinking water *ad libitum* for 4 weeks (n = 8);
Pb group – rats receiving Pb acetate 0.1% (PbAc) in the drinking water *ad libitum* for 4 weeks (n = 8);
Zn group – rats receiving $ZnCO_3$ 300 mg kg^{-1} diet for 4 weeks (n = 8);
Pb+Zn group – rats receiving PbAc in drinking water *ad libitum* plus 300 mg $ZnCO_3$ kg^{-1} diet for 4 weeks (n = 8).
The results are expressed as means ±standard deviation.
*** – difference statistically significant vs C group, $p < 0.001$ (Mann-Whitney test);
– difference statistically significant vs Zn group, $p < 0.001$ (Mann-Whitney test).

Lead concentration in liver

The average concentration of Pb in the liver of rats exposed to lead (Pb group) was significantly higher (by about 400 times) compared to the control group (C group), p = 0.000155. Also, the concentration of Pb in the liver of rats that received Pb and Zn (Pb+Zn group) was significantly higher than in the control (by 97%, $p = 0.00015$). A statistically significantly higher concentration of lead in the liver (96%, $p = 0.00031$) was found as well in animals treated with Pb (Pb group) compared to the group treated with Zn alone (Zn group). Over a 250-fold increase ($p = 0.00031$) was observed in the group of rats that received both lead and zinc (Zn+Pb group) versus the group supplemented with zinc (Gr Zn), which was a statistically significant difference, too (Table 2).

Table 2

The concentration of Pb in the liver and bones of rats subjected to 4-week exposure to Pb (Pb group), Pb and zinc supplementation (Pb+Zn group) zinc supplementation (Zn group) and control group Pb group (C group)

Group	**C** ($n = 8$)	**Zn** ($n = 8$)	**Pb** ($n = 8$)	**Pb+Zn** ($n = 8$)
	Pb liver (µg g^{-1} d.m.)			
	1.751±1.026	2.297±1.001	7.212±2.150$^{***\#\#\#}$	6.063±1.053$^{***\#\#\#}$
	Pb bones (µg g^{-1} d.m.)			
	0.333±0.145	0.427±0.106	28.744±18.197$^{***\#\#\#}$	27.166±8.256$^{***\#\#\#}$

Key: under Table 1.

Lead concentration in bones

The average concentration of Pb in the bones of rats exposed to lead (Pb group) was significantly higher (by about 850 times) than in the control group (C group), $p = 0.000155$. Significantly higher (by 98%, $p = 0.000155$) than the control was also the Pb concentration in the bones of rats that received Pb and were supplemented with zinc (Zn+Pb group). A statistically significant increase (by 98%) was observed in the bone Pb concentration among the group receiving Pb (Pb group) compared with the group supplemented with Zn (Zn group), $p = 0.00031$. An increase in the concentration (about 600-fold, $p = 0.0003108$) was likewise observed in the group receiving Pb and Zn (Zn+Pb group) in comparison with rats receiving Zn alone (Zn group) – Table 2.

Zinc concentration in plasma

The average concentration of Zn in the plasma of rats exposed to zinc (Zn group) was significantly higher (by 63%) than in the control group ($p = 0.00031$). Also, the concentration in plasma of rats that received Pb and Zn (Pb+Zn group) was significantly higher (42%, $p = 0.000155$) than the control (C group). A decrease in plasma Zn in the group receiving Pb (Pb group) was statistically significant compared with the group supplemented with Zn (Zn group), at 72% ($p = 0.00031$). An increase in the Zn concentration (by 81%, $p = 0.00031$) was also observed in the group receiving Pb (Pb group) versus the one receiving Pb and Zn (Zn+Pb group) – Table 3.

Zinc concentration in liver

The average concentration of Zn in the liver of rats exposed to lead (Pb group) was significantly lower, by 32% (p = 0.049), in comparison with rats supplemented only with Zn (Zn group) – Table 3.

Zinc concentration in bones

The average concentration of Zn in the plasma of rats exposed to lead (Pb group) was significantly lower (by 17%) than in the control group (C group), p = 0.0018. Also, a decrease (by 12%) in the concentration of plasma Zn in the group receiving Pb (Pb group) compared with the group supplemented with Zn (Zn group, p = 0.0029) was statistically significant. A decrease in the Zn concentration by 10% (p = 0.0028) was also observed in the group administered Pb (Pb group) compared to the group receiving Pb and Zn (Zn+Pb group) – Table 3.

Table 3

The zinc concentration in plasma (Zn-P), liver, and bone of rats after 4 weeks of exposure to Pb (Pb group), Pb and zinc supplementation (Pb+Zn group) zinc supplementation (Zn group) and control groups (C group)

Group	**C** (n = 8)	**Zn** (n = 8)	**Pb** (n = 8)	**Pb+Zn** (n = 8)
	Zn-P (mg L^{-1})			
	1.376±0.204	2.242±0.158***	1.3041±0.235$^{\#\#\#\&\&\&}$	2.358±0.160***
	Zn liver (mg kg^{-1} d.m.)			
	3.919±0.945	4.290±0.887	3.245±0.921$^{\#}$	3.191±1.354
	Zn bones (mg kg^{-1} d.m.)			
	232.722±28.952	216.748±19.787	193.163±14.636$^{***\#\&}$	213.304±13.226

Key to the groups: under Table 1
The results are expressed as means ± standard deviation.
*** – difference statistically significant vs C group; $p < 0.001$ (Mann-Whitney test);
# – difference statistically significant vs Zn group; $p < 0.05$ (Mann-Whitney test);
– difference statistically significant vs Zn group; $p < 0.001$ (Mann-Whitney test);
& – difference statistically significant vs Zn+Pb group; $p < 0.05$;
&&& – difference statistically significant vs Zn+Pb group; $p < 0.001$.

Distribution of lead in the tissues examined

In the control group (C group), almost half of the average Pb level was found in the bones of rats (49%), the remainder being almost equally divided between whole blood and liver (about1/4). An average percentage of lead in blood plasma was 0.4% (Figure 2).

In the group of rats subjected to 4-week exposure to lead (Pb group), the average percentage of lead in the bones increased compared to the control group (C group) by nearly a quarter, and was over 70%. However, Pb levels in the liver and blood were reduced by 7% and 18%, while the content in plasma significantly increased in comparison to the control group and amounted to 1% of the average amount in the tissues examined (Figure 2).

In the group of rats given Zn supplementation (Zn group), the average percentage of lead in bone was similar to the control group (C group). Distribution of Pb in the whole blood and plasma was at the same level as in the control group. There was a significant positive correlation between the concentration of Zn and Pb in the liver (Rs = +0.89) in this group (Figure 2).

In the group of rats subjected to 4-week exposure to lead and zinc (Pb+Zn group), the percentage distribution of Pb in bones was similar to the group of rats treated with lead (Pb group). The percentages of Pb in the whole blood, plasma and liver were also similar to the ones in the group of rats treated with lead, in which there was a significant negative correlation between the serum Zn concentrations and Pb content in the liver (Rs = -0.71) and bone (Rs = -0.73) – Figure 3, Table 4.

Lead

The four-week exposure of rats to 0.1% PbAc in drinking water caused an increase in the concentration of Pb in all examined tissues. The available literature informs that more than 90% of lead contained in the blood is absorbed by red blood cells, while 10% remains in the plasma, of which from 40% to 75% binds to plasma proteins, mainly albumin. The rest of Pb in plasma binds to low molecular weight ligands, while only 0.01% of the total pool of Pb is in the free state (Giel-Pietraszuk et al. 2012). In the present study, a 16-fold increase was shown in the Pb concentration in the blood as compared to the control group, 87% of Pb was found in erythrocytes, while only 13% remained in the plasma, thereby confirming the strong affinity of the metal to erythrocytes.

The bones are considered to be the main reservoir of Pb in the body – over 90% Pb has been shown to accumulate in the tissue, where it is far less metabolically active than in soft tissues. In the view of the fact that bones belong to slow changing tissues, Pb detoxification in bones takes a very long time and can last from a dozen to several dozens of years. In our study, more than a 90-fold increase in the concentration of Pb compared with the control group was observed in the bones of the tested animals, and the content of Pb in the tissue was 10 times higher than in the whole blood, thereby confirming the cumulative capacity of the tissue with regard to lead. However, according to many recent studies, even the bone lead pool, previously regarded as a deposit site for this element and also a form of its detoxification, can be mobilized in various physiological states such as pregnancy, osteoporosis and hormonal disorders (Gulson et al. 2002, Nash et al. 2004, Riedt et al. 2009). Under these conditions, lead deposited in bones may be released into the bloodstream and therefore to other tissues/organs (Giel-Pietraszuk et al. 2012).

Kulikowska et. al (1994) also observed increased concentrations of Pb in all examined tissues of rats exposed to 500 ppm of PbAc for 6 weeks. The concentration of Pb in the liver was four times higher (1.15 μg g^{-1} dry mass), and in the whole blood several times higher (62 μg dL^{-1}) than in the control

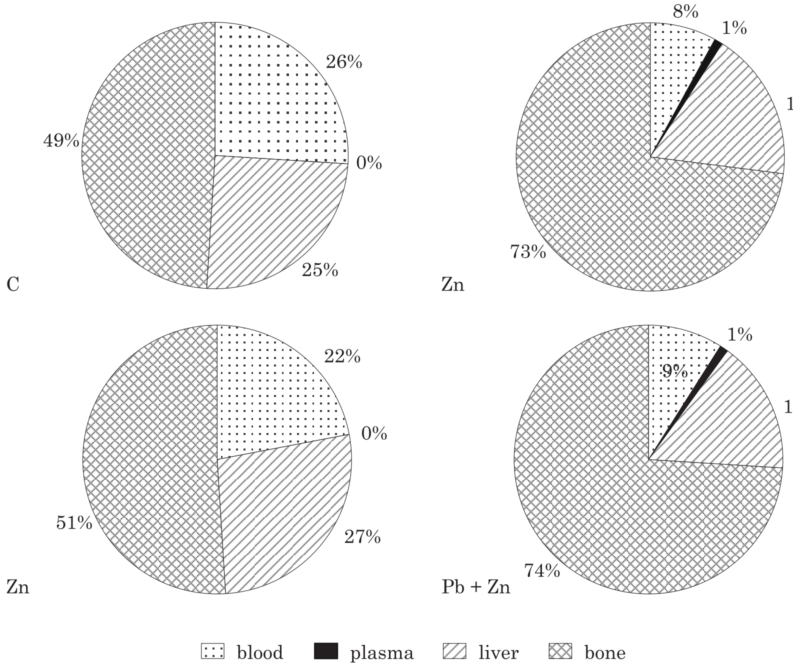


Fig. 3. Average per cent distribution of Pb in the control group (C group), rats subjected to 4-week exposure to Pb (Pb group), zinc supplementation (Zn group), lead and zinc supplementation (Pb+Zn group)

Table 4

The correlation between the concentration of zinc in blood plasma (Zn-P) and the concentration of Pb in the tissues tested

Group	Correlation	Spearman rank correlation coefficient	Significance of difference (*p*)
C	Zn-P – Pb plasma	0.19	0.6514
	Zn-P – Pb liver	-0.05	0.9108
	Zn-P – Pb bone	+0.38	0.3518
Pb	Zn-P – Pb plasma	+0.04	0.9394
	Zn-P – Pb liver	+0.25	0.5887
	Zn-P – Pb bone	-0.17	0.7016
Zn	Zn-P – Pb plasma	0.04	0.9394
	Zn-P – Pb liver	**+0.89**	**0.0068*** **
	Zn-P – Pb bone	-0.53	0.2152
Zn+Pb	Zn-P – Pb plasma	-0.35	0.4316
	Zn-P – Pb liver	**-0.71**	**0.0503***
	Zn-P – Pb bone	**-0.73**	**0.0503***

group. The lead concentration in the bones of the examined rats reached a value of more than 90-fold higher than in the control and was approximately 94 μg g^{-1} d.m.

Ademuyiwa et al. (2010) investigated the effect of various concentrations of Pb on its distribution in the blood and other organs such as the liver, kidney, spleen and heart. For 12 weeks, rats were given 200, 300 and 400 ppm Pb. In all examined tissues, there was an increase in the concentration of the toxic element. In the whole blood of rats treated successively with 200, 300 and 400 ppm Pb doses, the Pb concentration increased 6, 9 and 5 times in comparison to the control. Similarly, in the liver there was a 10-, 3.5- and 2.5-fold increase in the concentration of this element in relation to the control group. The authors concluded that an increase in the concentration of Pb in blood is proportional to the administered dose. In our study, the spleen was the only organ which showed such dose dependency.

Batra et. al (2001) studied the effect of different doses of testicular and epididymal Pb in rats treated sequentially with 10, 50 and 200 mg Pb kg^{-1}. The increase in the concentration of Pb in the testes of rats was proportional to the administered dose, but there was no such relation in the case of the epididymis, confirming the data obtained by Ademuyiwa et al. (2010).

Kozielec et. al. (1994) conducted research on children to demonstrate the relationship between the age and Pb level in hair. They examined 135 children aged 5 to 14 years. The experiment did not take into account the body weight and sex of the children. The experimental results showed that the highest average Pb content in hair appeared in the youngest group of respondents, i.e. children from 5 to 6 years old, where it averaged 2.8 μg g^{-1} d.m. and 2.68 μg g^{-1} d.m. The level of Pb in hair of older children was significantly lower. This relationship may indicate a better ability to uptake Pb by the youngest children.

Zinc

The four-week supplementation of rats with zinc carbonate at a dose of 300 mg kg^{-1} diet did not cause significant changes in the body weight compared with the control group. Also, the amount of food intake was similar to the control animals. There was a significant, 63% increase in plasma Zn compared with the control group, while in the liver and bone there was no significant increase in the concentration of Zn. A significant increase in plasma Zn indicates its actual absorption from the gastrointestinal tract. The duration of the experiment may have been insufficiently long for Zn contained in the plasma to be transferred to organs. It cannot be excluded that extension of the experimental period would have increased the concentration of Zn in all examined tissues. This argument is supported by the work of Mazurek-Machol et al. (2005), who analyzed the content of Zn in tissues in a 12-week experiment. Rats were given two different doses of Zn in water: the lower dose was 1 mg kg^{-1} while the higher one reached 3 mg kg^{-1}. Same as in the present study, the supplementation had no effect on the body weight and mobility of rats in comparison with the control group. The Zn concentration in serum was about 18% higher versus the control in both supplemented. groups The Zn concentration in urine was approximately 32% higher in the group receiving the higher dose and slightly lower in the group supplemented with the lower dose Zn. In the group treated with the higher Zn dose, there was also a significant, 24% increase in the concentration of this element in bones. In both supplemented groups, there was an increase in bone mass caused by zinc supplementation, although it was not statistically significant. It was shown that the degree of absorption of Zn was proportional to the dose.

In our study, rats treated with Zn supplementation and simultaneously receiving PbAc in drinking water had a lower concentration of Zn in plasma. This could be due to some antagonism between Zn and Pb (Pasternak et al. 2000), since both elements have been shown to possess a similar mechanism of absorption in the intestine, hence competing with each other in the process of intestinal transport and intracellular transport (Morawiec 1991). In addition, Pb also induces the inhibition of intestinal absorption of other microelements (Hornowska et al. 1996), and act antagonistically towards Zn in cells (e.g. binding by proteins). Presumably, this was the case in our study, where rats fed Pb in drinking water had a reduced Zn concentration in plasma. Slight decreases in the content of Zn during Pb administration compared with the control group were observed in blood and the liver, although the differences were not statistically significant. However, there was a significant decrease in the bone Zn concentration (about 17% compared to the control group). It can be assumed that exposure to Pb had a negative effect on the concentration of Zn in bone probably also due to an increased elimination of zinc from the body in response to the increased supply of Pb. It is also conceivable that longer exposure or a higher dose of Pb could be additionally reflected by decreased concentrations of Zn in other examined tissues.

Pasternak et al. (2000) examined the effect of different concentrations of Pb on the Zn content in the skin of rats. The animals received 100 or 300 mg L^{-1} PbAc in drinking water. After six weeks of the experiment, a decrease was observed in the concentration of Zn compared with the control group in both groups. Under exposure to a higher concentration of Pb, the Zn concentration in the skin was 43.6 μg g^{-1} of tissue, which was about 17% less than in the control group, where the concentration of Zn was 52.1 μg g^{-1} of tissue. At a Pb concentration of 100 mg L^{-1}, a 5% decrease in the concentration of Zn in the skin was also observed, although it was not statistically significant. It was found that the Pb administered in a diet lowered the concentration of Zn in the skin of rats, and this relationship was proportional to the dose of Pb. A slightly different result was obtained by Ademuyiwa et al. (2010), who investigated the relationship between the administration of various concentrations of Pb on the Zn content in the blood, heart, liver, spleen, and kidneys. The animals were divided into 4 groups, which received 200, 300 or 400 ppm Pb. Their results confirmed the negative impact of Pb on the Zn concentration in the blood, and the lowering of the blood Zn content was directly proportional to the dose, same as reported by Pasternak et al. (2000). However, such a dose-related dependence was not found in other organs, i.e. the kidney, liver and spleen.

Kozielec et al. (1994) studied the Pb content in the blood of children of different ages and its impact on the level of other microelements in hair. It was shown that 7.4% of patients had low levels of zinc in their hair. Those children also had the highest blood Pb (from 3.5 μg dL^{-1} - 8.7 μg dL^{-1}), which would confirm the mutual rivalry of both elements.

Zinc supplementation

Numerous studies have concluded that Pb affects the metabolism of many divalent elements (Skoczyńska 2006). It has been shown that both the excess and deficiency of individual elements acts on other micronutrients primarily by affecting their absorption from the gastrointestinal tract and/or cellular transport. The mechanism of this influence may be used to eliminate or reduce the absorption of Pb from the body, because it has been shown that it competes for binding of ligands to such elements as Zn, Cu or Ca (Hornowska et al. 1996). Another important factor is the aforementioned antagonism with other elements, such as Ca, Fe and Zn in absorption from the gastrointestinal tract (Pasternak et al. 2000).

Our study on rats treated for four weeks with PbAc in drinking water and rats which, in addition to PbAc, were supplemented with 300 mg kg^{-1} $ZnCO_3$ diet, showed a Zn-induced reduction in Pb in all examined tissues except blood. A decrease in the concentration of Pb was the highest in the liver (by 16%) compared to a 5% decline in the bones, while only 2% in plasma. Differences in concentrations were not statistically significant although it is possible that longer supplementation time would have resulted in a significant decrease in the concentration of Pb, as evidenced by the observed significant strong negative correlation between the concentration of Zn and Pb in the liver (Rs = -0.71, $p = 0.05$) and bone Rs = -0.73, $p = 0.05$).

In the experiment of Batra et al. (2001), the effect of 3-month zinc supplementation on the concentration of Pb was investigated in the testes and epididymis of rats treated with lead. One group of animals received 50 mg Pb kg^{-1}, and the other one was given 50 mg Pb kg^{-1} and supplemented with 1 mg Zn kg^{-1}. The concentration of Pb in the testes of rats that had received only Pb was 1586 ng g^{-1} tissue while in rats also supplemented with Zn the Pb concentration decreased by as much as 33% and was 1064 ng g^{-1} tissue. Similarly, Pb concentration in the epididymis decreased from 477 ng g^{-1} tissue to 218 ng g^{-1} tissue, a 55% decline. According to the researchers, the main cause of the protective effect of Zn was the limited accessibility of binding sites for Pb in the intestine. Kulikowska et. al. (1994) during a six-week-long experiment gave 500 ppm Pb to rats, of wihch one group received Zn concentration of 240 ppm for two weeks. There was a 30% decrease in the bone Pb content and a 50% decrease in blood Pb. In other organs such as the liver, kidney and spleen, despite Zn supplementation, Pb levels were maintained at a level similar to the group exposed to lead.

In treatment of children with elevated blood Pb levels above the currently regarded as safe (5 μg dL^{-1}), chelation therapy is recommended. As it is known, chelates are complexes in which the so-called central ion (in most cases this a divalent or trivalent metal) is bound to organic ligands. Due to the excess of Pb or the deficiency of micronutrients with a competitive activity in the body (Ca, Zn, Fe, Cu), these elements may be displaced and replaced by lead. Compounds which can prevent this in chelation therapy are EDTA (ethylene-diamine-tetraacetic acid) or DMSA (2,3-dimercaptosuccinic acid). The latter has been used in the removal of Pb from the soft tissues and bones since in the 1990s, when it was introduced in the United States in order to reduce Pb in children (SAPOTA, LIGOCKA 1996). The answer to the question whether these compounds are able to eliminate the already established effects of exposure to Pb, however, requires further research, but they certainly cause a reduction in Pb concentration in blood. A study by TANDON et al. (1994) was expected to answer to the question whether chelators with an additional methionine and Zn supplementation cope better with the removal of Pb from tissues of rats than chelators without additives. It turned out that the addition of zinc and methionine to EDTA or DMSA significantly improves the removal of Pb from tissues and excretion in the urine.

Honey-based preparation called Propolis was tested as an alternative to the previously used DMSA. SAPOTA, LIGOCKA (1996) in an experiment carried out on 4 groups of rats studied the effect of various concentrations of Propolis and DMSA on the content of Pb in tissues. Each treatment group received Pb for 6 days at a dose of 5 mg kg^{-1} per day. During the experiment, the first group was supplemented with DMSA preparation, while the other was given Propolis at a lower concentration of 15/2, and the third one received Propolis at a higher concentration of 25/3. The control group consisted of rats that received only lead. A significant decrease was shown in the concentration of Pb in rats treated with DMSA. The reduction of this element was observed in almost all examined tissues. The effect of Propolis was less spectacular, with the concentrations of Pb decreasing in almost all examined tissues (from 2-36%) *albeit* in a statistically insignificant manner. The comparison of the two Propolis concentrations showed a higher Pb reduction and better elimination of this element after the more diluted preparation had been administered.

The use of chelators such as EDTA and DMSA is the most effective method to remove Pb from cells. It should be borne in mind that both chelators have a number of side effects, for example non-selective binding to all divalent metals, including the essential ones, meaning that a long-term therapy of Pb poisoning causes acute deficiencies of other elements; thus indications for the use of chelating agents remain controversial. Chelation has eliminated the actual mortality in Pb poisoning, but there are no reports indicating that chelation is effective in relieving neurological effects of Pb poisoning. In addition, the use of chelating preparations such as EDTA or BAL requires a painful and expensive parenteral therapy with the concomitant risk of nephrotoxicity. Currently used chelating agents such as d-penicillamine (hydrocracking, Cuprimine) are effective as oral chelating agents at lower concentrations of Pb in the whole blood. The side effects, however, include allergy, nausea, haematuria, and a drop in the counts of blood platelets and white blood cells. Undoubtedly, an important role is also played by proper nutrition and satisfying the demand for vitamins, ions and minerals, especially in the most vulnerable patients such as small children. It must be remembered that the shortage of elements such as Cu, Zn, Fe and Mg increases the harmful effects of Pb (HORNOWSKA et al. 1996). Therefore, therapy consisting of Zn supplementation only or Zn supplementation supporting the action of chelators already in use may be crucial for the elimination of Pb from the body.

CONCLUSIONS

Incidental exposure of adult rats to Pb at doses resulting in the level of Pb in blood below the previously recognized as safe resulted in: (i) increased Pb in the bones and plasma and the reduction in the whole blood and liver (ii) simultaneous supplementation of rats exposed to Pb with high but non-toxic dose of zinc: – did not result in the reduction of the concentration of Pb in the blood and tissues of rats, – did not result in changes in the distribution of Pb in the tissues examined (iii) supplementation of diets with high but non-toxic dose of zinc is not an effective method for reducing the concentration of Pb in the blood at Pb-B previously recognized as safe. However, the therapy consisting of zinc supplementation to support the action of chelators could be crucial for the elimination of Pb from the body.

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EFFECT OF SULPHUR FERTILISATION ON THE CONTENT OF MACROELEMENTS AND THEIR IONIC RATIOS IN POTATO TUBERS

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Abstract

Next to nitrogen, phosphorus, potassium and magnesium, sulphur plays an important role in plant metabolism and is considered to be one of the most important nutrients. As sulphur deficit in Polish soils has been increasing in the last few decades, research has been carried out in order to evaluate the impact of different sulphur rates and forms on the content of macroelements (N, P, K, Ca, Mg, S, Na) and their ratios in the Mila cultivar potato tubers. A strict, three-year field experiment was set up on Haplic Luvisol with acid reaction (5.3 pH_{KCl}), mean richness in available forms of phosphorus, potassium and magnesium but low in sulphur. The field experiments involved the sulphur application in doses of 20 and 40 kg S kg^{-1} in the form of sulphate (VI) (K_2SO_4 and $(NH_4)_2SO_4$) as well as in elemental form (Wigor S, containing 90% of pure sulphur and 10% of bentonite). Although potatoes represent the group of crops with low sulphur requirements, the research has clearly indicated that this component has definite impact on the content of macroelements in tubers. It was found that sulphur application, irrespective of its form and rate, compared with control sample generally increased the content of nitrogen, sulphur and magnesium in potato tubers, decreasing at the same time calcium content.

The effect of the form of sulphur fertilisers on the content of macroelements was not univocal. The values of all the ratios calculated ($K^+:Mg^{+2}$, $K^+:(Ca^{+2}+Mg^{+2})$ and $(K^++Na^+):(Ca^{+2}+Mg^{+2})$), except for $K^+:Ca^{+2}$, have generally decreased after sulphur application, which indicates positive changes in the nutritive value of potato tubers.

Key words: sulphur, fertilisation, potato tubers, macroelements, ionic rations.

INTRODUCTION

Owing to its specific physiological role in plant metabolism, sulphur is considered to be one of the key nutrients for plants (Haneklaus et al. 2000,

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Sahota 2006). Its deficit decreases plant yield and often leads to deterioration of the yield quality, which is determined by the content of minerals and their ratios (Eppendorfer, Eggum 1994, Blake-Kalff et al. 2003). At the turn of the 20th and the 21st century, data on sulphur deficit in Poland (Szulc 2008) and many other countries (Stern 2005, Morris 2007) was published. Symptoms of sulphur deficiency are observed not only in the plant species with high sulphur requirements but also in those which require relatively less S, including potato (*Solanum tuberosum* L.) (Pavlista 1995, Klikocka 2004, Sharma et al. 2011). Sulphur enhances starch synthesis in tubers, is a component of proteins and many enzymes, limits the content of nitrates and reducing sugars, which in turn improves the technological value of potato (El-Fayoumy, El-Gamal 1998, Lalitha et al. 2000). It increases the resistance of this species to environmental stresses and plays an important role in protecting the plants from pests and diseases (Walker, Both 1994). Klikocka (2005) demonstrated that induced sulphur fertilization increased potato resistance to infection with *Rhizoctonia solani* fungi. In regions polluted with sulphur compounds and with decreased soil pH, smaller infestation with Colorado potato beetle (*Lepti-notarsa decemlineata*) as well as an increased potato resistance to common scab (*Streptomyces scabies*) is observed (Pavlista 1995). The content of sulphur in potato tubers is on average between 0.7-2.0 g kg^{-1} and its uptake ranges from 18 to 40 kg ha^{-1} (Klikocka 2004).

In Europe, potato tubers are the staple food in everyday diet. In the last decades, potato consumption has been growing steadily in countries where historically potatoes had hardly been grown at all, particularly in South-Eastern Asia (Sharma et al. 2011). The consumption value of tubers is defined on the basis of the content of dry weight, starch and the content of respective macroelements. Maintaining the cation-anion balance is one of the major factors governing an adequate course of plant metabolism as well as the yield size and quality.

Sulphur plays a very important role in plant physiology. Moreover, its balance in agroecosystems has been disturbed, thus research has been carried out to evaluate the effect of varied sulphur rates and forms on the content of selected macroelements and their equivalent ratios in the Mila cultivar potato tubers.

METHOD AND MATERIALS

Field experiments were performed from 2003 to 2005 at the Experimental Station of the Faculty of Agriculture of the University of Technology and Life Sciences in Bydoszcz, located at Wierzchucinek (53°26′ N, 17°79′ E). A mid-early cultivar of table potato called Mila was grown in *Haplic Luvisol* formed from till of very good rye complex. The III b soil valuation class soil was of acid reaction (pH_{KCl} – 5.3) and exhibited an average richness in the available forms of phosphorus, potassium and magnesium. The content of sulphate form (VI) $S\text{-}SO_4^{2-}$ was low (the mean of 9.4 mg kg^{-1}).

A strict fertilisation experiment was performed in 3 replicates. The experiment was set up as a single-factor experiment following the randomised block design. The area of the plot was 20 m^2, and for harvest - 16 m^2. The experimental factor involved the type of mineral fertilisation containing sulphur in the ionic or elemental form. The following fertilisation treatments were considered:

S_1 – 0 kg S ha^{-1};

S_2 – 20 kg S ha^{-1} in the form of ammonium sulphate (VI);

S_3 – 40 kg S ha^{-1} in the form of ammonium sulphate (VI);

S_4 – 20 kg S ha^{-1} in the form of potassium sulphate (VI);

S_5 – 40 kg S ha^{-1} in the form of potassium sulphate (VI);

S_6 – 20 kg S ha^{-1} in the form of Wigor S fertiliser;

S_7 – 40 kg S ha^{-1} in the form of Wigor S fertiliser.

Wigor S is a mineral fertiliser, containing 90% of elemental sulphur and 10% of bentonite.

Cultivation treatments and fertilisation were made in accordance with the agrotechnical guidelines for potato. Uniform mineral fertilisation was applied before sowing. Nitrogen was sown at the dose of 100 kg N ha^{-1} in the form of ammonium nitrate (for the treatments with ammonium sulphate (VI) balanced with the rate of nitre, considering the nitrogen introduced with sulphate). Phosphorus (25 kg P ha^{-1}) was sown in the form of triple superphosphate and potassium (120 kg K ha^{-1}) as 50% potassium salt, balancing the nutrient introduced into the treatments with potassium sulphate (VI). Prior to potato planting, FYM was applied at the dose of 30 t ha^{-1}.

For a more complete description of the weather conditions over the research period, the values of the Selyaninov hydrothermal coefficient (tab. 1) were calculated:

$$k = P/0.1\Sigma t,$$

where:

P – monthly precipitation sum (mm)

t – monthly daily air temperature sum >0°C.

In the region where the experiment was carried out precipitations are low (the annual average of about 450 mm) and demonstrated considerable variation in successive years, which has been reflected in the calculated values of the Selyaninov coefficient (Table 1). The highest temperature and the lowest precipitation were noted in the 2005 plant growing season. In all the months, except July, extremely dry or dry conditions were recorded. In 2003 and 2004, the mean values of the Sielianinow coefficient for the entire potato-growing season were higher than the long-term mean, although they varied much in respective months of the plant growing period.

Table 1

The Selyaninov hydrothermal coefficient values throughout the research period

Years	Months					Mean
	May	June	July	August	September	
2003	0.86	1.88	2.44	0.86	1.65	1.54
2004	2.29	0.64	1.33	0.94	1.82	1.40
2005	0.41	0.58	1.78	0.31	0.41	0.69
Mean 1949-2005	1.01	1.15	1.26	0.95	0.97	1.07

$K<0.4$ – extremely dry conditions, $0.4<K<0.7$ very dry, $0.7<K<1.0$ dry,
$1.0<K<1.3$ quite dry, $1.3<K<1.6$ optimal, $1.6<K<2.0$ quite moist, $2.0<K<2.5$ moist

After harvest, the following values were determined for the dry weight of potato tubers produced at the temperature of 105°C:

- the nitrogen content by the Kjeldahl method,
- phosphorus – by the colorimetric method with the use of ammonium molybdate, on a type DR-2000 colorimeter,
- magnesium – by atomic absorption spectrometry (AAS),
- calcium, potassium, sodium – by atomic emission spectrometry with the use of a flame photometer Flapho-4,
- total sulphur using the ICP OES method *(Inductively Coupled Plasma Optical Emission Spectrometry*) method mineralization in a mixture of concentrated acids: HNO_3 and $HClO_4$ (Khan 2012).

The content of macroelements was expressed in gram equivalents and the following ratios were calculated: $K^+:Mg^{+2}$, $K^+:Ca^{+2}$, $K^+:(Ca^{+2}+Mg^{+2})$ and $(K^++Na^+):(Ca^{+2}+Mg^{+2})$.

The results were statistically verified by the means of variance analysis in the split-plot design following the model compliant with the experimental design. To evaluate the significance of mean treatment differences the Tukey's range test at the probability of $P = 0.05$ was applied.

RESULTS AND DISCUSSION

The research showed that potato tubers contained most potassium (an average of 19.8 g kg^{-1}), slightly less nitrogen (13.0 g kg^{-1}) and considerably less phosphorus (3.17 g kg^{-1}), magnesium (1.71 g kg^{-1}), sodium (1.54 g kg^{-1}), total sulphur (1.26 g kg^{-1}) and calcium (1.29 g kg^{-1}) – Table 2. After sulphur application, analogously to the report by Skwierawska et al. (2008) from experiments on cabbage, onion and spring barley, a significant impact on the content of most of the macroelements in potato tubers was observed. As for nitrogen, sulphur and magnesium, after application of sulphur-containing fertilisers, generally there was a significant increase in their mean content

Table 2

Content of macroelements in potato tubers (g kg^{-1})

Years	Control	Form of fertilizer						Mean	LSD$_{0,05}$
		$(NH_4)_2SO_4$		K_2SO_4		Wigor S			
		dose of sulphur (kg S ha^{-1})							
	0	20	40	20	40	20	40		
	K_1	K_2	K_3	K_4	K_5	K_6	K_7		
The content of nitrogen									
2003	11.9	13.7	14.1	12.2	13.0	12.4	12.7	12.8	1.56
2004	12.0	11.1	12.5	12.2	12.7	12.2	11.7	12.0	0.67
2005	13.3	13.3	13.5	14.5	14.5	14.4	15.0	14.1	1.22
Mean	12.4	12.7	13.3	12.9	13.4	13.1	13.1	13.0	0.55
The content of phosphorus									
2003	3.23	3.33	3.53	3.50	3.27	3.40	3.40	3.38	0.21
2004	3.23	3.43	3.17	3.17	3.30	3.27	3.13	3.24	n.s.
2005	3.00	2.67	2.63	2.90	3.00	3.10	2.97	2.90	0.35
Mean	3.16	3.14	3.11	3.19	3.19	3.26	3.17	3.17	0.12
The content of potassium									
2003	21.0	21.7	21.3	21.0	20.7	21.0	20.4	21.0	n.s.
2004	19.5	20.5	20.3	20.5	21.0	20.8	19.5	20.3	0.78
2005	18.4	17.3	17.3	18.2	18.6	18.5	18.2	18.1	1.05
Mean	19.7	19.8	19.6	19.9	20.1	20.1	19.4	19.8	0.71
The content of calcium									
2003	1.46	1.65	1.50	1.41	1.05	1.20	1.01	1.25	0.52
2004	1.52	0.99	1.17	1.04	1.01	1.21	0.87	1.12	0.30
2005	1.90	1.43	1.37	1.33	1.40	1.40	1.40	1.46	0,39
Mean	1.63	1.36	1.35	1.26	1.15	1.27	1.09	1.29	0.19
The content of magnesium									
2003	1.43	2.02	1.49	1.66	1.86	1.62	1.75	1.69	0.236
2004	1.25	2.07	1.55	1.69	1.95	1.64	1.73	1.70	0.358
2005	1.56	2.02	1.64	1.74	1.97	1.62	1.75	1.76	0.320
Mean	1.41	2.03	1.56	1.70	1.92	1.63	1.74	1.71	0.058
The content of sodium									
2003	1.23	1.23	1.17	1.27	1.30	1.40	1.27	1.27	n.s.
2004	1.67	1.53	2.07	1.17	1.47	1.43	2.50	1.69	n.s.
2005	1.63	1.70	1.37	1.67	1.70	1.73	1.73	1.65	n.s.
Mean	1.51	1.49	1.53	1.37	1.49	1.52	1.83	1.54	n.s.
The content of total sulphur									
2003	0.83	1.31	1.28	1.14	1.19	1.18	1.36	1.19	0.078
2004	0.91	1.42	1.38	1.29	1.36	1.36	1.55	1.32	0.056
2005	0.90	1.40	1.37	1.23	1.30	1.30	1.47	1.28	0.064
Mean	0.88	1.38	1.34	1.22	1.28	1.28	1.46	1.26	0.062

over the three years of research in comparison with the control sample (K_1). Calcium was the only element on which sulphur had contrary impact.

Nitrogen was that component in potato tubers whose content was significantly modified by sulphur fertilisation. It is difficult, however, to state firmly which of the sulphur containing fertilises was most effective. The content of nitrogen in potato tubers was considerably affected by seasonal variations, thus it is difficult to define precisely, under field conditions, how significant the direct impact of sulphur was. In 2003, the significantly highest nitrogen content was recorded following the application of ammonium sulphate (VI) (K_2 and K_3); in 2004, such an effect was induced by potassium sulphate (VI) (K_5) and in 2005, it was caused by Wigor S (K_7). A more advantageous impact of elementary sulphur rather than the ionic one observed in the 2005 season (the third research year) was probably the result of the gradual release of the nutrient over time and a lower susceptibility to losses. That form requires biological oxidation and its impact depends on the activity of soil microorganisms (*Thiobacillus* sp.) (AULAKH 2003).

In general, the dose of 40 kg S ha^{-1}, as compared with that of 20 kg S ha^{-1}, stimulated a higher nitrogen content in potato tubers, although the differences were not always significant. Many authors (MCGRATH, ZHAO 1996, SUD et al. 1996, INAL et al. 2003, KACZOR, BRODOWSKA 2009, BARCZAK 2010) confirmed the positive influence of sulphur application on the plant protein synthesis and thus the nitrogen content in the yields.

The impact of sulphur fertilisation on the content of phosphorus and potassium in potato tubers was not unambiguous (Table 2). As for phosphorus, a significant increase in the content of that element, as compared with the control sample, was reported only in 2003 on plots K_3 and K_4; for potassium, it appeared on all the plots, except for K_7, in 2004. In 2005, after the application of ammonium sulphate (VI) at the doses of 20 and 40 kg S ha^{-1} (K_2 and K_3), a significant decrease was observed in the content of phosphorus and potassium in tubers in comparison with the control sample. According to SAWICKA and PSZCZÓŁKOWSKI (2004), the ionic composition of potato is genetically conditioned, despite being affected by high phenotypic variation depending on the impact of various environmental factors. Apart from fertilisation, another essential factor shaping the macroelements is the weather, especially the precipitation and temperature pattern during the vegetation period. A sufficient water supply alleviates the unfavourable impact of high temperatures. The years of field experiments were characterised by high variations in weather conditions (Table 1).

The elements whose content increased after the application of sulphur, in comparison with the control sample, were magnesium and sulphur. As for magnesium, by analogy to the results obtained by BRODOWSKA, KACZOR (2009) for wheat and cocksfoot, a higher content of the element was recorded after the ionic rather than elemental form of sulphur. The sulphur content was more enhanced by the application of ammonium sulphate(VI) as well as

Wigor S than potassium sulphate(VI). Each research year, the tubers demonstrated higher concentrations of these macroelements in response to 40 kg S ha^{-1} in the form of potassium sulphate(VI) as well as Wigor S rather than after the use of 20 kg S ha^{-1} of these fertilisers. Out of the two ammonium sulphate(VI) doses applied, the magnesium content in tubers was significantly more favourably affected by 20 kg S ha^{-1}.

The average ratio of the content of nitrogen to sulphur (N:S) in potato tubers was 10.5:1 (Table 3). In all the plants fertilised with sulphur, in comparison with the control sample, its ratio was narrowed. This change was due to a higher increase in the content of sulphur rather than nitrogen in tu-

Table 3

Expressed in gram equivalents ionic ratios of macroelements in potato tubers

Years	Control	Form of fertilizer						Mean	$LSD_{0.05}$
		$(NH_4)_2SO_4$		K_2SO_4		Wigor S			
		dose of sulphur (kg S ha^{-1})							
	0	20	40	20	40	20	40		
	K_1	K_2	K_3	K_4	K_5	K_6	K_7		
N:S									
2003	14.3	10.5	11.0	10.7	10.9	10.5	9.3	11.0	1.86
2004	13.4	7.9	9.1	9.5	9.3	9.0	7.5	9.4	2.21
2005	14.8	9.5	9.9	11.8	11.2	11.1	10.2	11.0	3.20
Mean	14.2	9.6	10.0	10.7	10.5	10.2	9.0	10.5	0.69
K:Mg									
2003	4.55	3.16	4.39	3.93	3.42	4.00	3.58	3.86	0.102
2004	5.04	2.87	4.04	3.76	3.32	3.91	3.47	3.77	0.218
2005	3.77	2.24	3.18	3.28	2.96	3.55	3.20	3.17	0.147
Mean	4.45	2.76	3.87	3.66	3.23	3.82	3.42	3.60	0.169
K:Ca									
2003	7.37	6.73	7.30	7.61	10.12	9.02	10.35	8.36	0.83
2004	6.93	10.87	8.87	10.31	10.64	8.88	11.46	9.71	0.55
2005	5.09	6.25	6.36	7.09	6.98	6.95	7.14	6.55	n.s.
Mean	6.46	7.95	7.51	8.34	9.25	8.28	9.65	8.21	0.36
K:(Mg+Ca)									
2003	2.80	2.22	2.74	2.58	2.56	2.76	2.66	2.62	0.055
2004	2.88	2.26	2.78	2.75	2.53	2.71	2.66	2.65	n.s.
2005	2.16	1.64	2.12	2.22	2.08	2.34	2.18	2.11	n.s.
Mean	2.61	2.04	2.55	2.52	2.39	2.60	2.47	2.46	0.147
(K+Na):(Mg+Ca)									
2003	3.08	2.43	3.00	2.84	2.83	3.07	2.95	2.92	0.063
2004	3.29	2.54	3.26	3.03	2.83	3.03	3.24	3.03	n.s.
2005	2.48	1.92	2.41	2.59	2.40	2.71	2.53	2.43	n.s.
Mean	2.95	2.30	2.89	2.82	2.69	2.94	2.91	2.79	0.163

bers after fertilisation. Many reports (Janzen, Bettany 1984, McGrath, Zhao 1996, Kaczor, Brodowska 2009) point to a strong interaction between nitrogen and sulphur as the elements indispensable for the synthesis of sulphur amino acids used in protein synthesis. At the same time, the N:S ratio in the indicator plant parts facilitated the determination of the sulphur fertilisation requirements in plants (Withers et al. 1995, Blake-Kalff et al. 2003, Grant et al. 2003). As for potato, according to Klikocka (2004), a typical N:S value is about 12:1. Krzywy et al. (2002) found an optimal ratio between sulphur and nitrogen in plants grown for animal feed around 15-16:1.

There was a significantly negative effect of sulphur, both in the ionic and elemental form, on the content of calcium in potato tubers. The mean differences between the sulphur non-fertilised plot (K_1) and the fertilised ones were 16.6% (K_2), 17.2% (K_3), 22.7% (K_4), 29.4% (K_5), 22.1% (K_6) and 33.1% (K_7). The negative impact of sulphur fertilisers on the content of calcium in tubers was evident, especially in 2004 and 2005, when on all the plots fertilised with sulphur there was a significant decrease in the content of that nutrient, in comparison with the control sample. A particularly high decrease in the content of calcium in tubers was the result of the application of 40 kg S a^{-1} of Wigor S containing the elemental form of sulphur (K_7).

So far, sodium has been shown as an indispensable nutrient for just a few plant species. Its physiological role is less known than that of potassium; in literature there are some studies of the impact of sulphur fertilisation on its content in yields of crops. In the current study, there was no significant impact of sulphur fertilisers on the content of sodium in potato tubers.

In general, the form of sulphur applied did not affect the content of macroelements and their ionic ratios. Sulphur in the elemental form does not dissolve in water; hence there is a lower risk of sulphur loss by leaching in light soils. On the other hand, it must undergo biological oxidation with participating *Thiobacillus* genus bacteria to sulphate form (VI), (available to plants), which makes sulphur in the elemental form act more slowly than in the ionic form. The process of that microbiological transformation depends on many factors, e.g. on the soil properties, particularly its biological activity, on the level of fertiliser sulphur fragmentation as well as on precipitation and temperature (Aulakh 2003). Their pattern in each growing season was different (Table 1), which excluded any firm statement as to which sulphur forms had the strongest impact on the content of macro elements in potato tubers.

Variations in weather conditions in respective research years caused bigger differences between the mean content of the nutrients in potato tubers in successive years than the differences due to fertiliser sulphur. Analogously to the report by Klikocka (2004), it was found that the content of macroelements in potato tubers was influenced more by the year-related hydrothermal conditions than by sulphur fertilisers. As for nitrogen, calcium and magnesium, the highest concentrations were noted in the driest year 2005, when – in all the months of the plant growing season, except for July

– the precipitation was lower then the long-term mean. With that in mind, it seems that sulphur fertilisation effectiveness in the accumulation of those elements, nitrogen in particular, including protein nitrogen, is higher in years with lower precipitation than in those plant growing seasons when the rainfall conditions are similar to the long-term mean.

Although the content of macroelements in crop yields is generally well-known, significantly less information is available on ratios between these nutrients, in particular, about the impact of sulphur fertilisation on their values. The ratios of macroelements determining the ionic equilibrium in potato tubers grown either for human consumption or animal feed can determine their nutritive value.

As a result of sulphur fertilisation, the values of K^+:Mg^{+2}, K^+:(Ca^{+2}+Mg^{+2}) and (K^++Na^+):(Mg^{+2}+Ca^{+2}) ratios generally decreased in comparison with the control sample, whereas the value of the K^+:Ca^{+2} ratio increased. These particular changes in quantitative ratios were caused by sulphur fertilisation, which in turn led to changes in the content of respective nutrients, especially magnesium and calcium, in potato tubers.

The ionic equilibrium of a plant is one of the major factors determining the quality of crop yields because an excessive intake of specific cations or anions limits the concentration of other, frequently valuable macro- and microelements. Many authors claim that a high content of potassium decreases whereas that of calcium and magnesium enhances the nutritive value of the yields of plants grown either for animal feed or for human consumption (Krzywy et al. 2002). In practice, animal feed more often has an excess rather than a deficit of potassium. What is especially undesirable is an excessively wide quantitative ratio of univalent cations to divalent ones in animal feed for ruminants, which as a result of hypomagnesemia can lead to grass tetany in cattle. Thus, the reported changes in the values of K^+:Mg^{+2}, K^+:(Ca^{+2}+Mg^{+2}) and (K^++Na^+):(Ca^{+2}+Mg^{+2}) are beneficial because they are caused by a significant increase in the amount of magnesium in potato tubers owing to sulphur fertilisation. The calcium content plays an important role in maintaining the equilibrium between univalent and divalent ions, which – like magnesium - can show an antagonistic effect towards potassium.

Drawing on the present research, it may be stated that sulphur fertilisation generally has a favourable influence on the content and the quantitative ratios of macro elements in potato tubers. However, developing final conclusions is not easy due to lack of significance of the differences.

CONCLUSIONS

1. It was found that sulphur fertilisation, irrespective of its form and dose, has generally increased the content of nitrogen, sulphur and magne-

sium in potato tubers, decreasing the content of calcium in comparison with the control sample.

2. The application of 40 kg S·ha^{-1} in the form of potassium sulphate(VI) as well as Wigor S in each of the research years stimulated a higher content of sulphur and magnesium in potato tubers than the rate of 20 kg S·ha^{-1}.

3. The values of K^+:Mg^{+2}, K^+:(Ca^{+2}+Mg^{+2}) and (K^++Na^+):(Ca^{+2}+Mg^{+2}) ratios in potato tubers following an application of sulphur generally decreased in comparison with the control sample, which is considered to be a positive tendency for the nutritive value of tubers.

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NITROGEN FRACTIONS IN TIMOTHY GRASS (*PHLEUM PRATENSE* L.) FERTILIZED WITH DIFFERENT DOSES OF MINERAL FERTILIZERS

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Abstract

The effect of mineral (NPK) fertilization on the content of nitrogen fractions in timothy grass was assessed from the results of a three-year field experiment conducted at the Elizówka Experimental Farm near Lublin, on podzolic soil developed from loess. The experiment was included 27 fertilized treatments, i.e. nine nitrogen and potassium variants tested, each in four replications. against the background of three increasing phosphorus fertilization doses. Nitrogen was applied as ammonium nitrate at a dose of 120 kg N ha^{-1} (N1) and at two-fold (N2) and three -fold (N3) higher doses. Phosphorus was applied in the form of granular triple superphosphate at a dose of 34.9 kg P ha^{-1} (P1) and at two-fold (P2) and three-fold (P3) higher doses. Potassium was used as potassium salt (47.3 %) at a dose of 83 kg K ha^{-1} (K1) and at two-fold (K2) and three-fold (K3) higher doses. After grass harvest, plant material was sampled and the content of total, protein, ammonium and nitrate nitrogen(V) was determined in duplicate. It was found that the content of the nitrogen fractions analysed in timothy grass, i.e. total, protein, mineral, ammonium and nitrate(V) nitrogen, was significantly positively correlated with the mineral fertilization, in particular, with nitrogen and phosphorus and, to a lesser extent, with potassium. The mean share of protein nitrogen in total nitrogen was 53.8%; mineral, ammonium, and nitrate nitrogen fractions constituted 4.0, 2.5, and 1.5%, respectively. Timothy grass fertilized with increasing doses of nitrogen, phosphorus, and potassium fertilizers contained the largest share of protein nitrogen in total nitrogen, did not contain excessive amounts of nitrates(V) and proved to be valuable bulk feed.

Key words: *Phleum pratense* L., nitrogen fractions, NPK fertilization.

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INTRODUCTION

Mineral fertilization exerts a strong effect on the yield and quality of crop plants. Among essential nutrients (N, P, K), nitrogen fertilization plays the key role. It has an impact on the biomass growth and plant chemical composition (Stuczyński 1969, Koter, Krauze 1978, Gąsior, Kaniuczak 1996, Havstad, Aamlid 2006, Bednarek 2011, Ashikaga et al. 2012). It also increases the content of this element in tissues and has an effect on its fractional composition (Koter, Krauze 1978, Czuba 1996, Bednarek 2005, Pelletier et al. 2007, Bednarek, Reszka 2009). Higher N doses typically lead to increased levels of total nitrogen and reduced amounts of protein nitrogen; additionally, there is a rise in the levels of mineral nitrogen, including nitrate nitrogen(V) (Nesić et al. 2008). The chemical composition of plants depends on agrotechnical practice (fertilization) and natural factors (soil, climate) (Bednarek et al. 2009). The content of nitrates in plants, particularly in forage crops, has an immediate effect on their quality (Tremblay et al. 2005, Havstad, Aamlid 2006, Pelletier et al. 2007, Ashikaga et al. 2012). In some animal organisms, deleterious effects are exerted by nitrates(V) as well as their derivatives, i.e. nitrates(III). Some authors argue that if forage is the only source of food, the content of nitrates(V) contained therein should not exceed 0.7 - 1.5 g $N\text{-}NO_3$ kg^{-1} d.w. Others claim that forage containing less than 3.5 - 4.5 g $N\text{-}NO_3$ kg^{-1} d.w. can be given to animals without harm, whereas forage with a content of 4.5 - 5.5 g $N\text{-}NO_3$ kg^{-1} d.w. sometimes causes poisoning, and animals should not be fed with forage containing > 6.5 g $N\text{-}NO_3$ kg^{-1} d.w. (Czuba 1996, Tremblay et al. 2005). A major role in nitrogen uptake and metabolism in plants is played by an optimal supply of other nutrients, including phosphorus, potassium and magnesium (Koter 1968, Mengel et al. 1976, Lee, Rudge 1986, Czuba 1996).

The aim of the study was to determine the effect of mineral (NPK) fertilization on the content of nitrogen fractions in timothy grass cultivated on podzolic soil developed from loess. Particularly, we tested the following hypothesis: the role of varied fertilization mineral doses of three major nutrients (N, P, K) on accumulation of total nitrogen, protein nitrogen, $N\text{-}NO_3$, and $N\text{-}NH_4$ in timothy grass.

MATERIAL AND METHODS

The present study is based on results of a three-year field experiment conducted at the Elizówka Experimental Farm near Lublin (2003-2005) and established on podzolic soil developed from loess. Before the experiment, the soil contained 77.2 mg P kg^{-1}, 187.0 mg K kg^{-1}, 44.0 mg Mg kg^{-1}, and 1.18% of humus, and the pH_{KCl} was 4.9. The experiment was comrpised 27 fertilized treatments, namely nine nitrogen-potassium combinations were tested against the background of three increasing phosphorus fertilization doses, each in four replications. The plot area was 50 m^2, of which 36 m^2 were harvested. Nitrogen was applied as ammonium nitrate (34% N) at a dose of 120 kg N ha^{-1} (N_1) and at two-fold (N_2) and three-fold (N_3) higher doses. Phosphorus was applied in the form of granular triple superphosphate (20.1% P) at a dose of 34.9 kg P ha^{-1} (P_1) and at two-fold (P_2) and three-fold (P_3) higher doses. Potassium was used as potassium salt (47.3 %) at a dose of 83 kg K ha^{-1} (K_1) and at two-fold (K_2) and three-fold (K_3) higher doses. The entire dose of phosphorus and half of the potassium dose were introduced into soil before sowing timothy grass var. Skrzeszowicka. In the second and third year, the fertilizers were applied after harvesting the third swath; the second dose of potassium was applied each year after the first swath. Nitrogen was added in three equal parts in early spring (immediately after the plant growth started) and after the first and second swath. Timothy grass mowed in the first swath was harvested in the third decade of May or the first decade of June; the second harvest took place in the third decade of July and the third grass harvesting was done in the third decade of September or the first decade of October. After harvest, plant material (from three swaths and three years) was sampled and the following parameters were determined in duplicate: total nitrogen with the Kjeldahl method; protein nitrogen using the Kjeldahl method with trichloroacetic acid after water extraction and protein precipitation; ammonium nitrogen using the Nessler reagent method; and nitrate nitrogen(V) with the phenoldisulphide acid method and mineral nitrogen ($N\text{-}NH_4$+$N\text{-}NO_3$). The content of nitrogen fractions determined in timothy grass was estimated using the *triple* test *cross analysis of variance with the* Tukey's confidence *semi-interval* ($p < 0.05$).

RESULTS AND DISCUSSION

Depending on the mineral fertilization applied, the content of total nitrogen in dry weight of timothy grass ranged from 19.1 to 28.1 g N kg^{-1} (Table 1). The biggest and significant impact on levels of this nitrogen form was exerted by fertilization with the increasing doses of this nutrient. Its mean content was 19.8 at a single nitrogen dose (N_1), 24.6 at a double dose (N_2), and 27.5 g N kg^{-1}d.w. at a triple dose (N_3). In comparison with the single dose, the double dose of ammonium nitrate increased the N content by 24.2%, and the three-fold higher nitrogen dose resulted in a 38.9% increase in the N content. The mean content of nitrogen was 23.4 at the single dose (P_1), 24.0 at the double dose (P_2), and 24.5 g N kg^{-1} d.w. at the triple dose (P_3) of phosphorus. The application of two-fold and three-fold higher doses of granular triple superphosphate increased the content of this nitrogen form by 2.6 % and 4.7%, respectively, in comparison with the single dose. Under

Table 1

Total nitrogen content in timothy grass (g N kg^{-1} d.w.)

Fertilization	P_1			P_2			P_3			N Average
	K_1	K_2	K_3	K_1	K_2	K_3	K_1	K_2	K_3	
N_1	19.1	19.1	19.5	19.3	21.4	18.4	19.5	20.6	21.0	19.8
N_2	24.1	24.4	23.4	23.9	25.4	24.4	26.3	23.4	25.9	24.6
N_3	27.1	27.5	26.6	27.5	28.0	27.4	27.6	28.1	28.0	27.5
PK Average	23.4	23.7	23.2	23.6	24.9	23.4	24.5	24.0	25.0	24.0
P Average	23.4			24.0			24.5			
K Average				23.8	24.2	23.8				

LSD$_{\leq 0.05}$: N, P, K – 0.2; NK, PK – 0.6; NPK – 1.2

the effect of the increasing potassium doses, the total nitrogen content in timothy grass did not exhibit vector trends and reached 23.8 at a single potassium dose (K_1), 24.2 at a double potassium dose (K_2), and 23.8 g N kg^{-1} d.w. at a triple potassium dose (K_3). In comparison to the single dose, the relative increase in the content of this form of nitrogen at the double dose was just about 1.7%. A similar effect of mineral fertilization, in particular with nitrogen, was reported by Gawęcki and Mikołajczak (1978), Dubiel et al. (1991) and Czuba (1996). Bednarek (2005) found that among the three experimental factors (N, P, K), nitrogen and phosphorus fertilization had a significant effect on the content of this nitrogen form, which was over two-fold lower in roots of *Dactylis glomerata* than in sward.

Depending on the form and dose of mineral fertilization, the content of protein nitrogen in timothy grass ranged from 10.7 ($N_1P_1K_1$) to 15.3 g N kg^{-1} d.w. ($N_3P_3K_1$) – Table 2. The application of single, double and triple N doses yielded 11.3, 12.9, and 14.4 g N kg^{-1} d.w. of this nitrogen fraction, respectively. The double nitrogen dose (relative to the single one) increased the content of protein nitrogen on average by *ca* 14.2%, while the triple dose increased this parameter by 27.4%. The increase was statistically significant.

Table 2

Protein nitrogen content in timothy grass (g N kg^{-1} d.w.)

Fertilization	P_1			P_2			P_3			N Average
	K_1	K_2	K_3	K_1	K_2	K_3	K_1	K_2	K_3	
N_1	10.7	11.2	12.1	11.9	11.8	11.4	11.4	10.6	10.7	11.3
N_2	12.6	13.2	12.8	13.0	13.1	13.1	13.3	12.4	12.5	12.9
N_3	13.7	15.0	14.4	14.7	13.7	14.5	15.3	14.2	14.0	14.4
PK Average	12.3	13.1	13.1	13.2	12.8	13.0	13.3	12.4	12.4	12.9
P Average	12.9			13.0			12.7			
K Average				13.0	12.8	12.9				

LSD$_{\leq 0.05}$: N, P, K – 0.1; NP, NK, PK – 0.2; NPK – 0.6

Phosphorus fertilization did not induce distinct changes in the content of this nitrogen fraction. After the application of a single dose of this nutrient, the mean content of protein nitrogen in the plant was 12.9 g N kg^{-1} d.w. The double and triple doses resulted in the protein nitrogen content of 13.0 and 12.7 g N kg^{-1} d.w., respectively. The double phosphorus dose raised the content of this fraction just 0.78% compared to the single dose. Potassium fertilization did not result in considerable, directional changes in the protein nitrogen content. Plants fertilized with the single dose of the element contained 13.0 g N kg^{-1} d.w., whereas those receiving the double and triple doses had 12.8 and 12.9 g N kg^{-1} d.w., respectively. The protein nitrogen content in timothy grass was not significantly correlated with the increasing phosphorus and potassium doses, possibly indicating a high content of the available forms of these compounds in the soil. Krzywy et al. (1996) reported a steady increase ranging from 17.8 (N_0) to 18.7 g N kg^{-1} d.w. (N_3) in the protein nitrogen content induced by this nutrient (N) in meadow sward. Similar patterns were found by Stuczyński et al. (1970, 1971), Stuczyńska (1973), and Wiater (1996). Krzywy et al. (1996) reported that increasing doses of nitrogen caused a relatively steady increase in the content of protein nitrogen in grass, and potassium fertilization induced insignificant differences in that content. Bednarek (2005) found that the content of this nitrogen fraction was associated with nitrogen and phosphorus fertilization and was 66% higher in sward than in roots. Wiater (1996) reported a beneficial effect of fertilization with 240 kg N ha^{-1} on the share of non-protein and protein nitrogen in pasture sward, and an increase of a dose up to 360 kg N ha^{-1} more substantially elevated the level of non-protein rather than protein nitrogen. This corresponded with the results of Koter (1973), Koter and Krawczyk (1981). Gąsior and Kaniuczak (1996) noted that the mean content of protein nitrogen in the first-swath hay was about 0.5% higher than that in hay harvested from the second regrowth. This was also reported by Stuczyński (1969).

The content of ammonium nitrogen in timothy grass depended on the form and dose of mineral fertilization and ranged from 0.42 to 0.83 g N kg^{-1} d.w. (Table 3). The nitrogen fertilization resulted in a significant increase in the

Table 3

Ammonium nitrogen content in timothy grass (g N kg^{-1} d.w.)

Fertilization	P_1			P_2			P_3			N Average
	K_1	K_2	K_3	K_1	K_2	K_3	K_1	K_2	K_3	
N_1	0.48	0.43	0.42	0.48	0.49	0.64	0.53	0.48	0.54	0.50
N_2	0.52	0.56	0.54	0.62	0.69	0.66	0.73	0.55	0.60	0.61
N_3	0.54	0.60	0.58	0.79	0.83	0.72	0.80	0.60	0.63	0.68
PK Average	0.51	0.53	0.51	0.63	0.67	0.67	0.69	0.54	0.59	0.60
P Average	0.52			0.66			0.61			
K Average				0.61	0.58	0.59				

LSD$_{\le 0.05}$: N, P, K – 0.01; NP, NK, PK – 0.03; NPK – 0.07

content of this N fraction. The application of a single ammonium nitrate dose increased the average N-NH$_4$ content to the level of 0.50 g N kg^{-1} d.w.; double and triple doses yielded an increase to 0.61 and 0.68 g N kg^{-1} d.w., respectively. Compared with the single dose, the double and triple doses produced a 22% and 36% increase, respectively, in the content of this nitrogen fraction. Fertilization with the increasing phosphorus doses significantly increased the nitrogen fraction content in timothy grass. The single, double and triple phosphorus doses increased the nitrogen content to 0.52, 0.66, and 0.61 g N kg^{-1} d.w., respectively. In comparison with the single dose, double and triple doses yielded a 26.9 % and 17.3% increase in the element content, respectively. Potassium fertilization caused a slight but significant reduction in the N-NH$_4$ content. Single, double, and triple doses of this nutrient yielded 0.61, 0.58, and 0.59 g N kg^{-1} d.w. of protein nitrogen, respectively. Compared with the single dose, double and triple doses decreased the content by *ca* 4.9 and 3.3%, respectively. Bednarek (2005) reported that only nitrogen fertilization had a significant effect on the content of this N fraction in sward, which in aerial parts of *Dactylis glomerata* was 27% higher than in roots. Similar regularities were noted by Stuczyński (1969) and Gąsior and Kaniuczak (1996), who reported a slightly higher content of this nitrogen form in hay from swath II than from swath I. Czuba (1996) found that plants are considerably less tolerant to a high content of NH$_4^+$ rather than to NO$_3^-$ ions, as toxicity symptoms appear in response to as littile as 0.2-0.4 g N-NH$_4$ kg^{-1} d.w. Potassium deficiency in plants fertilized intensively with nitrogen resulted in excessive accumulation of toxic amounts of amines and ammonium ions in tissues, which induced damage to aerial parts of plants, including plant death (Czuba 1996).

The content of nitrate nitrogen(V) in timothy grass ranged from 0.15 to 0.83 g N kg^{-1} d.w., depending on a dose and form of mineral fertilization (Table 4). Application of single, double, and triple ammonium nitrate doses yielded a content of 0.22, 0.29, and 0.56 g N kg^{-1} d.w., respectively. Compared with the single dose, the relative increase in this nitrogen form produced by the double and triple NH$_4$NO$_3$ doses reached 31.8 and as much

Table 4

Nitrate nitrogen(V) content in timothy grass (g N kg^{-1} d.w.)

Fertilization	P$_1$			P$_2$			P$_3$			N Average
	K$_1$	K$_2$	K$_3$	K$_1$	K$_2$	K$_3$	K$_1$	K$_2$	K$_3$	
N$_1$	0.16	0.16	0.15	0.23	0.48	0.23	0.23	0.18	0.20	0.22
N$_2$	0.18	0.19	0.22	0.39	0.31	0.26	0.36	0.37	0.29	0.29
N$_3$	0.39	0.27	0.54	0.64	0.76	0.68	0.66	0.48	0.65	0.56
PK Average	0.25	0.21	0.30	0.42	0.52	0.39	0.42	0.34	0.38	0.36
P Average	0.25			0.44			0.38			
K Average				0.36	0.36	0.36				

LSD$_{\leq 0.05}$: N, P, K – 0.05; NP, NK, PK – 0.09; NPK – 0.19

as 154.5%, respectively. Fertilization with single, double, and triple doses of granular triple superphosphate yielded the N-NO_3 content in timothy grass of 0.25, 0.44, and 0.38 g N kg^{-1} d.w., respectively. In comparison with the single dose, the increase resulting from the double and triple doses reached 76 and 52%, respectively. Fertilization with the increasing doses of potassium salt did not change significantly the content of this nitrogen fraction in plants. It remained on the same level of 0.36 g N kg^{-1} d.w., irrespective of a potassium dose applied. KRZYWY et al. (1996) reported a significant increase from 0.62 ($N_0P_0K_0$) to 1.43 g N kg^{-1} d.w. ($N_3P_3K_2$) in this nitrogen fraction in sward. Similarly, DUBIEL et al. (1991) found that an increase in nitrogen fertilizer doses was accompanied by an increase in N-$NO_{3,}$ which exceeded the admissible level of 2.2 g N kg^{-1} d.w. in objects fertilized with 480 kg N ha^{-1}. BEDNAREK (2005) reported that nitrogen fertilization only (and potassium fertilization in roots) exerted a significant effect on the content of this N fraction, exhibiting four-fold lower levels in *Dactylis glomerata* roots than in sward. Application of 80 kg N ha^{-1} $swath^{-1}$ did not made the content of this N fraction exceed the critical threshold (15 g NO_3 kg^{-1} d.w.), whereas after an application of 120 and 160 kg N ha^{-1} $swath^{-1}$ (in total, 600 and 1120 kg N ha^{-1} $year^{-1}$) the level of 15 g NO_3 kg^{-1} d.w. was exceeded by 19 and 47% of samples, respectively (PRINS 1983). Accumulation of nitrates in plants was associated not only with the nitrogen content in soil, but also with meteorological factors or with molybdenum, manganese, phosphorus, potassium, and magnesium fertilization (KOTER 1968, CZUBA 1996, BEDNAREK, RESZKA 2009, BEDNAREK et al. 2009). Phosphorus fertilization also enhanced the uptake of nitrate N from the environment, whereas elevated potassium fertilization lowered the percentage of nitrates(V) and decreased their share in the total nitrogen absorbed by plants (KOTER 1968). CZUBA (1996) found that plants could contain up to 15 g N-NO_3 kg^{-1} d.w., and a plant variety is to some extent important in nitrate accumulation. After application of 100 kg N ha^{-1}, different varieties of *Dactylis glomerata* contained from 0.68 to 1.30, meadow fescue from 0.74 to 1.06, and winter ryegrass from 0.56 to 1.11 g N-NO_3 kg^{-1} d.w.

In general, application of increasing doses of nitrogen fertilizers caused a very distinct and, in many cases, significant elevation of nitrate(V) levels in sward (KOTER 1968, STUCZYŃSKI et al. 1970, 1971, KOTER 1973, STUCZYŃSKA 1973, KOTER, KRAUZE 1978, KOTER, KRAWCZYK 1981, CZUBA 1996, GĄSIOR, KANIUCZAK 1996, KRZYWY et al. 1996, WIATER 1996).

The content of mineral nitrogen (the sum of ammonium nitrogen and nitrate nitrogen V) in timothy grass increased after nitrogen fertilization and reached 0.72 g N kg^{-1} d.w. (N_1); 0.90 g N kg^{-1} d.w. (N_2), and 1.24 g N kg^{-1} d.w. (N_3) – Table 5. The relative increase induced by the double and triple doses was 11.7 and 16.1%, respectively. The application of phosphorus also increased the content of this nitrogen fraction in plants: P_1 – 0.77 g N kg^{-1} d.w., P_2 – 1.10 g N kg^{-1} d.w., and P_3 – 0.99 g N kg^{-1} d.w. The relative increase was 14.3 (P_2) and 12.9% (P_3). The increasing potassium fertilization caused a slight decline in the mineral nitrogen content in timothy grass: K_1 – 0.97 g N

Table 5

Sum of ammonium nitrogen and nitrate nitrogen(V) content in timothy grass (g N kg^{-1} d.w.)

Fertilization	P_1			P_2			P_3			N Average
	K_1	K_2	K_3	K_1	K_2	K_3	K_1	K_2	K_3	
N_1	0.64	0.59	0.57	0.71	0.97	0.87	0.76	0.66	0.74	0.72
N_2	0.70	0.75	0.76	1.01	1.00	0.92	1.09	0.92	0.89	0.90
N_3	0.93	0.87	1.12	1.43	1.59	1.40	1.46	1.08	1.28	1.24
PK Average	0.76	0.74	0.81	1.05	1.19	1.06	1.11	0.88	0.97	0.96
P Average	0.77			1.10			0.99			
K Average				0.97	0.94	0.95				

LSD$_{\le 0.05}$: N, P, K – 0.06; NP, NK, PK – 0.13; NPK – 0.27

kg^{-1} d.w., K_2 – 0.94 g kg^{-1} d.w. (a 3.1% decrease in the content in comparison with K_1) and K_3 – 0.95 g N kg^{-1} d.w. (a 2,1% decrease in the content).

The percentage of protein nitrogen in total nitrogen declined from 57.1 (N_1) to 52.4% (N_2 and N_3) after nitrogen fertilization. Phosphorus fertilization also resulted in a slight decrease in the share of this N fraction: 55.1 (P_1), 54.2 (P_2), and 51.8% (P_3). Potassium fertilization had an irregular effect on the relative content of protein nitrogen: 54.6 (K_1), 52.9 (K_2), and 54.2% (K_3). Nitrogen and phosphorus fertilization induced an increase while potassium fertilization did not change significantly the content of mineral nitrogen in timothy grass: 3.9 (N_1), 3.7 (N_2), 4.5% (N_3); 3.3 (P_1), 4.6 (P_2), 4.0% (P_3); 4.1 (K_1), 3.9 (K_2), and 4.0% (K_3).

The percentage of ammonium nitrogen in total nitrogen was relatively even and did not depend on the form and dose of mineral fertilization. It reached 2.47-2.52% (nitrogen fertilization), 2.22-2.75% (phosphorus fertilization), and 2.40-2.56% (potassium fertilization). Nitrogen and, to a lesser extent, phosphorus fertilization substantially increased the proportion of nitrate nitrogen(V) in total nitrogen: 1.11 (N_1), 1.18 (N_2), 2.04% (N_3); 1.07 (P_1), 1.83 (P_2), and 1.55% (P_3). Potassium fertilization did not induce significant changes in the proportion of this nitrogen fraction in total nitrogen: 1.51 (K_1), 1.49 (K_2), and 1.51% (K_3). Regardless of forms and doses of mineral fertilizers applied in the experiment, the average share of nitrogen fractions (protein, mineral, ammonium, and nitrate) in total nitrogen content in timothy grass was 53.8% (protein nitrogen), 4.0% (mineral nitrogen), 2.5% (ammonium nitrogen) and 1.5% (nitrate nitrogenV). The percentage of nitrate nitrogen V in mineral nitrogen was 37.5%. The content of the nitrogen fractions analysed indicates that timothy grass is a valuable bulk feed.

CONCLUSIONS

1. The content of nitrogen fractions assessed in timothy grass, i.e. total, protein, mineral, ammonium, and nitrate(V) nitrogen was significantly positively correlated with mineral fertilization, mostly with nitrogen and phosphorus, and to a lesser extent with potassium.

2. The average percentage of protein nitrogen in total nitrogen was 53.8; the values for mineral, ammonium, and nitrate nitrogen were 4.0; 2.5, and 1.5%, respectively.

3. Timothy grass fertilized with increasing doses of nitrogen, phosphorus and potassium contained the highest levels of protein nitrogen in total nitrogen, did not contain excessive amounts of nitrates(V) and proved to be valuable bulk feed.

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THE SENSITIVITY OF *BRASSICA NAPUS* SSP. *OLEIFERA* TO CADMIUM (Cd) AND LEAD (Pb) CONTAMINATION AT DIFFERENT pH OF MINERAL AND ORGANIC SOILS

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Abstract

In a model pot experiment, the influence of elevated levels of cadmium and lead in soils on rapeseed (*Brassica napus* L.) growth, development, biomass (g DM pot^{-1}), cationic ratios (Cd^{2+}:-Ca^{2+}, Cd^{2+}:Mg^{2+}, Pb^{2+}:Ca^{2+}, Pb^{2+}:Mg^{2+}) and tolerance index Ti, has been examined. The tested soils were: two organic soils (acidic and neutral) and a mineral one with pH differentiated into neutral and acidic. The metals were spiked into soils in doses of 10 mg Cd kg^{-1} DM and 100 mg Pb kg^{-1} DM of soil. Under the applied doses, the sensitivity of rapeseed to Cd was higher than to Pb. The application of Cd caused visible symptoms of chlorosis on plant leaves and a statistically significant ($p < 0.01$, $p < 0.05$) decrease in aerial biomass. The influence of Pb on *B. napus* biomass yield was not significant. The soil type and its reaction differentiated the biomass of plants in the following decreasing order: mineral neutral > organic neutral > mineral acidic > organic acidic. The Cd added to soil increased the Cd^{2+}:Ca^{2+} and Cd^{2+}:Mg^{2+} ionic ratios, while Pb caused an increase in Pb^{2+}:Ca^{2+} and Pb^{2+}:Mg^{2+} ratios compared to the control. The significantly higher values for the above ions ratios were recorded in rapeseed roots rather than in shoots. The results showed a crucial role of the soil type in determining the milimolar ionic relations in *B. napus* plants. The plant was more vulnerable to chemical composition changes in contaminated treatments comparing to control when grown on mineral soils (both acidic and neutral) than on organic ones. The high Ti value proves generally low sensitivity of *B. napus* to soil Cd and Pb contamination.

Key words: *Brassica napus* biomass, sensitivity, Cd, Pb, tolerance index, ionic ratios, mineral soil, organic soil, pH.

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INTRODUCTION

Atmospheric emissions caused by mining, smelting, coal combustion and other activities such as the chemical industry, contaminate soils by transporting heavy metals like Cd, Pb, Cu, Ni, Zn and other pollutants in the air and subsequently depositing them on soils and the earth surface overgrown by plants (Medyńska-Juraszek, Kabała 2012, Adamczyk-Szabela 2013). Direct application of some fertilizers, sewage sludge, composted solid wastes or industrial residues additionally increases the load of metals in agricultural environment (Domańska 2006, Medyńska-Juraszek, Kabała 2012, Domańska et al. 2013). Cadmium and lead are non-essential elements and can be phytotoxic to plants when present in soils in elevated amounts. Their uptake and toxicity to plants depend on many factors such as plant species or variety, chemical form (speciation of metal in the soil), pH, organic matter content and presence of other ions (Das et al. 1997, Domańska 2002, 2006, Nouairi et al. 2006, Domańska et al. 2013, Wyszkowska et al. 2013). However, the safe level of Cd or Pb concentration at plants can be harmful to humans and animals.

The advantages and possibilities of using plants from *Brassicae species* (*B. juncea, B. campestris, B. napus)* in phytoremediation and phytoextraction were reported by many authors (Marchiol et al. 2004, Ghosh, Singh 2005, Gisbert et al. 2006, Grispen et al. 2006). Phytoextraction of heavy metals by *B. napus* also seems to be possible and promising (Marchiol et al. 2004), especially on moderately contaminated soils (Grispen et al. 2006). Phytoremediation studies often focus on understanding the mechanisms of plant tolerance to a particular metal and on important plant parameters like degree of accumulation of contaminants, plant growth rate, biomass production, etc. In this work, the toxicity of Cd or Pb to *B. napus* was examined on a basis of a pot experiment designed to determine if this plant is sufficiently tolerant to these metals, when grown on contaminated soils. The research was carried out on two types of organic soils and a mineral one, which differed in pH values. The growth, development and biomass of the tested plant, depending on soil type, pH and cadmium or lead addition, were determined. The experiment design also allowed us to evaluate the influence of soil type on *B. napus* chemical composition, when cultivated on Cd or Pb contaminated soil. This can indicate the usefulness of the plant for phytoremediation on particulate soil types or possibilities of cropping *B. napus* for other purposes on studied types of soils.

MATERIAL AND METHODS

Soils

The research was based on analysis of plant and soil materials gained from a pot experiment performed on two organic soil types and a mineral one with different pH. Soil material was taken from the surface layer (0-20 cm). Organic peat-bog soil developed from short peat with neutral reaction (Fibric Histosols) (*Taxonomy*... 2011) was taken from arable lands near municipal and industrial purification plant in Hajdów near Lublin (Poland). Acidic soil (Dystri-Fibric Histosol - FAO – WRB 1998) developed from high peat was taken from the surroundings of Łukcze Lake in Lublin region. The mineral soil Haplic Podzols (*Taxonomy*... 2011), with the granulometric composition of light loamy sand was taken from agricultural areas of the Lubelskie region. Its neutral reaction was obtained by adding $CaCO_3$ applied according to 2.0 hydrolytic acidity. The main properties of the soils, determined before the experiment, are listed in Table 1.

Table 1

Selected properties and concentrations of metals in soils used for experiment

Soil		pH_{KCl}	C org. (%)	Available total					Granulometric composition		
				P	K	Mg	Pb	Cd	share (%) of fraction of diameter (mm)		
				(mg kg^{-1})					1-0.1	0.1-0.2	<0.02
Mineral	acidic	4.6	1.32	82	89	42	5.49	0.10	74	18	8
	neutral	7.2									
Organic	acidic	3.6	64.0	94	141	385	20.98	0.30	not determined		
	neutral	7.2	18.7	786	183	1662	16.65	0.43	not determined		

Pot experiment

The soil material was placed in pots of 5 dm^3 volume. Pots contained 4.8 kg of organic acidic soil, 5.5 kg of organic neutral soil and 6.4 kg of mineral soil. During the experiment, mineral soil humidity was adjusted to 60% of maximum water capacity and 80% in the case of organic soils by adding water to the constant weight. Mineral fertilization in the form of mineral salts: NH_4NO_3 – 0.10 g N kg^{-1} soil, $CaHPO_4$ – 0.07 g P kg^{-1} soil and KCl – 0.15 g K kg^{-1}, was applied in all pots, dividing the NH_4NO_3 dose into two equal parts – before and after sowing. Cd and Pb as $Cd(NO_3)_2$ and $Pb(NO_3)_2$ were introduced once before sowing at doses: 10 mg Cd kg^{-1} and 100 mg Pb kg^{-1} dry mass (DM). The metals were applied into soils according to the following design:

0 – control treatment with no metal addition,

Pb = Pb(II) treatment – lead dose of 100 mg kg $^{-1}$ DM,

Cd = Cd(II) treatment – cadmium dose of 10 mg kg $^{-1}$ DM.

Spring rapeseed (*Brassica napus ssp. oleifera* L.) (20 seeds) was sown on 30 of April and plants were harvested at the flowering stage. During the growing period, the growth and development of plants were observed. After harvesting, underground biomass (roots) was rinsed with deionized water and aerial biomass (shoots) and roots were separated and weighed. The plant samples were oven-dried, after which DM of roots and shoots of *B. napus* was determined.

Chemical analyses

Collected soil samples were air-dried, crushed in porcelain mortar and sieved through 1 mm mesh. The following were determined in the prepared samples: granulometric composition (mineral soil) by means of the Cassagrande's areometric method with modifications by Prószyński, pH – potentiometry in 1 M KCl (Polish Standard 1997), content of organic matter ($C_{org.}$) – the Tiurin's method, content of available P and K using the Egner and Riehm's method, and content of available Mg using the Schachtschabel's method, all being standard methods used by Polish Agricultural Stations. The content of organic matter in organic soils was determined by annealing (Bednarek et al. 2004). The total content of Cd and Pb in soils was analyzed after digesting the soil samples in a mixture of concentrated acids HCl and HNO_3 in the ratio of 3:1 (*aqua regia*) (Polish Standard 2001, 2002). The total metal concentrations in the solution were determined by means of flame atomic absorption spectrometry (AAS).

Concentrations of Ca and Mg in plants were determined using AAS after mineralization in concentrated H_2SO_4. Cadmium and lead determinations in plant samples were achieved by applying the AAS technique after plant material digestion in concentrated H_2SO_4 with H_2O_2 addition (Ostrowska et al. 1991).

Data analysis

The experiment including 6 plots in four replicates on mineral soil, and 6 plots in four replicates on organic soils was set up using the randomised block method.

Data obtained from the experiments were statistically processed by means of variance analysis with Tukey confidence intervals. Differences were considered to be significant at $p = 0.05$ and highly significant at $p = 0.01$ level of significance.

Molar ratios of $Cd^{2+}:Ca^{2+}$, $Cd^{2+}:Mg^{2+}$, $Pb^{2+}:Ca^{2+}$, $Pb^{2+}:Mg^{2+}$ were calculated for root and shoot in *B. napus* as well as the tolerance index (Ti) of *B. napus* to Cd and Pb, which specifies the degree of inhibition of plant growth in contaminated soil. Ti = biomass of plants on treated (Cd or Pb) soil (g DM pot^{-1})/ biomass of plants on untreated (0 - control) soil (g DM pot^{-1}).

RESULTS

Effects of cadmium and lead on growth of plants

The growth and development of plants in particular treatments varied. The poorest emergence and uneven germination were observed on mineral acidic soil. On the mineral soil, de-acidified in a dose equivalent to 2.0 Hh, and on the organic ones, the emergence of rapeseed plants was simultaneous. Initially, there was also no difference in the growth of plants in the variants with metals. As the plant grew, 1.5 months after sowing, plants in the treatments on the mineral neutral soil were the highest, most developed and all started the flowering phase at the same time. In the Cd treatments on mineral acidic soil, *B. napus* leaves had yellowing edges and the generative phase was delayed. In *B. napus* plants from treatments on organic soils, chlorosis on older leaves occurred. On acidic peaty soil, the *B. napus* leaves had slightly purple colour, which indicates P deficiency. Much lower shoots grown on the organic acidic soil, when comparing with the other soils, suggest also the shortage of other elements. Lead did not affect the overall growth of the plant. The symptom of cadmium toxicity was the chlorosis of leaves.

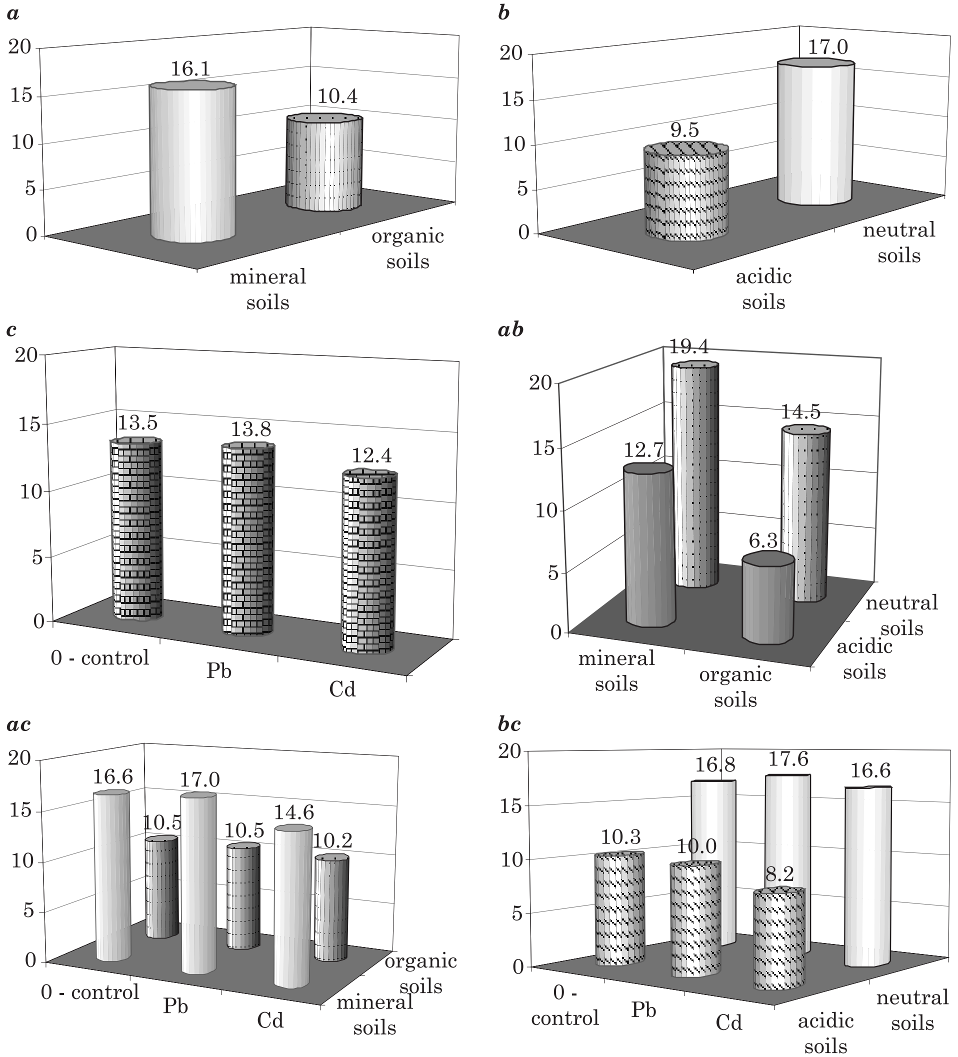
Effects of cadmium and lead on biomass of plants

The experimental factors (type of soil, pH, Cd and Pb addition) significantly influenced the rapeseed aerial (shoots) biomass (g DM pot^{-1}) – Figure 1. Highly significant interactions between these factors were also recorded. Much higher average biomass of plants was obtained on mineral (16.1 g pot^{-1}) than on organic (10.4 g pot^{-1}) soils. There was also statistically significant higher shoot biomass obtained from neutral soils (17.0 g pot^{-1}) than from acidic ones (9.5 g pot^{-1}). The soil type and pH significantly affected the changes of above sequence of obtained plant's biomass as follows: mineral neutral > organic neutral > mineral acidic > organic acidic.

In plants grown on mineral acidic soil, Cd caused a significant decrease (10.2 g pot^{-1}) in rapeseed biomass, compared with the control (14.6 g pot^{-1}), while on mineral neutral soil, there was a considerable increase in the plant biomass (20.7 g pot^{-1}) under the influence of Pb, as compared to the control (18.6 g pot^{-1}). With regard to the control, a significant DM decrease also occurred in Cd and Pb treatments on neutral organic soil.

Considering the mean values, a statistically significant fall of *B. napus* DM (12.4 g pot^{-1}) in comparison with the control (13.5 g pot^{-1}) was observed in the Cd treatment.

The amount of underground (roots) DM was significantly dependent on the type of soil and its pH (Figure 2). More DM of roots was produced on mineral (3.2 g pot^{-1}) than on organic soil (2.4 g pot^{-1}). On mineral neutral soil, the growth of rapeseed roots was better than on mineral acidic one, which



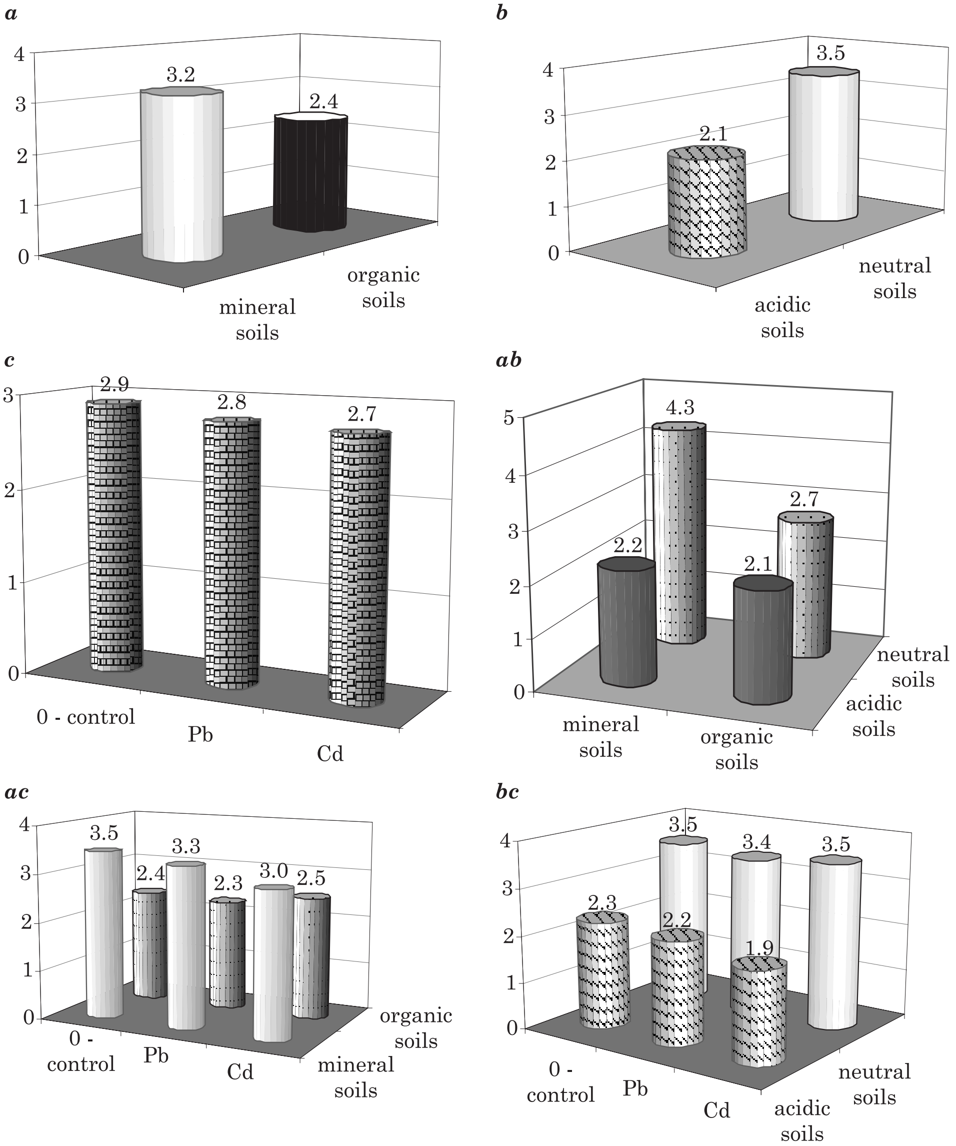
LSD $p = 0.05$*; $p = 0.01$** (*a* – soil, *b* – soil reaction, *c* – metal); *a* 0.45**, *b* 0.45**, *c* 0.67**, *ab* 0.85**, *ac* 1.16**, *bc* 1.16**

Fig. 1. Mean aerial biomass of *Brassica napus* (g DM pot^{-1})

resulted in an almost 2-fold increase in biomass, as compared to the acidic soil. The metals did not influence remarkably the biomass of rape roots.

Tolerance index (Ti)

The tolerance index Ti is fundamental to the evaluation of the plant's sensitivity towards toxic metals (Table 2). The lowest Ti value (0.7) for rapeseed (shoots and roots) was recorded on mineral acidic soil contaminated



LSD p = 0.05*; p = 0.01**, *a* 0.25**, *b* 0.25**, *ab* 0.48**

Fig. 2. Mean underground biomass of *Brassica napus* (g DM pot^{-1})

with Cd. The index value Ti = 0.9 implicating a slightly reduced tolerance of plants was also reported for mineral soil in the Pb treatment and in treatments with the addition of Cd (shoots) and Pb (roots) on neutral organic soil. In the remaining treatments, Ti ranged from 1.0 to 1.1. High Ti values for most of the contaminated treatments demonstrate high tolerance of rapeseed to cadmium and lead.

Table 2

Tolerance of *Brassica napus* to Cd and Pb in dependence on soil pH, metal addition and the content of organic matter

Object			Ti*	
Soil	pH	metal	shoots	roots
Mineral	acidic	0	1.0	1.0
		Pb	0.9	0.9
		Cd	0.7	0.7
	neutral	0	1.0	1.0
		Pb	1.1	1.0
		Cd	1.0	1.0
Organic	acidic	0	1.0	1.0
		Pb	1.1	1.0
		Cd	1.1	1.1
	neutral	0	1.0	1.0
		Pb	1.0	0.9
		Cd	0.9	1.1

* Ti = biomass of plants on treated (Cd or Pb) soil (g DM pot^{-1})/biomass of plants on untreated (0 - control) soil (g DM pot^{-1}).

Cationic ratios

An effect of Cd and Pb soil contamination on the cationic ratios (Cd^{2+}:Ca^{2+}, Cd^{2+}:Mg^{2+}, Pb^{2+}:Ca^{2+}, Pb^{2+}:Mg^{2+}) in *B. napus* plants was observed (Table 3). The ratio values of Cd^{2+} to Ca^{2+} in analyzed plant shoots and roots varied from 0.01 to 13.41. The highest ratios were recorded in plants from Cd contaminated treatments on mineral soil, which were within the range from 1.34 in plant shoots from neutral soil to 13.41 in plant roots from acidic soil. The Cd^{2+}:Ca^{2+} ratios in plants from Cd-uncontaminated soils (both mineral and organic) did not exceed 0.08. The results indicate that in plants growing on organic soils, the Cd^{2+}:Ca^{2+} ratio values were the same or much lower than in analogous treatments on mineral soil and even in treatments contaminated by Cd, in which it did not exceed 0.63. The ratio of Cd^{2+} to Ca^{2+} in plant roots was usually higher than in the aerial parts of plants. There was no effect of Pb addition on Cd^{2+}:Ca^{2+} ratios in *B. napus* shoots comparing to the control treatments, but slight differentiation occurred in roots.

Mg and Ca have many chemical similarities. Therefore, as in the case of Cd^{2+}:Ca^{2+}, the highest Cd^{2+}:Mg^{2+} ratios were found in plants from Cd-contaminated treatments, especially on mineral acidic soil (16.64 – shoots and 21.43 – roots). The *B. napus* roots absorbed more Cd than shoots and showed the highest values of Cd^{2+}:Mg^{2+}. There was hardly any effect of Pb addition to soil on the Cd^{2+}:Mg^{2+} ratio in *B. napus*, in comparison to the control.

Table 3

Relationship between shoot and root divalent cation ratios of *Brassica napus* in response to cadmium or lead contamination

Soil	Treatment		Shoots				Roots			
	pH	metal	Pb^{2+}: Ca^{2+}	Pb^{2+}: Mg^{2+}	Cd^{2+}: Ca^{2+}	Cd^{2+}: Mg^{2+}	Pb^{2+}: Ca^{2+}	Pb^{2+}: Mg^{2+}	Cd^{2+}: Ca^{2+}	Cd^{2+}: Mg^{2+}
Mineral	acidic	0	0.03	0.11	0.03	0.10	0.30	0.53	0.04	0.08
		Pb	0.22	0.78	0.03	0.12	11.47	19.70	0.08	0.15
		Cd	0.02	0.07	4.33	16.64	0.49	0.78	13.41	21.43
	neutral	0	0.01	0.03	0.01	0.04	0.33	0.52	0.06	0.09
		Pb	0.08	0.29	0.01	0.03	4.61	8.94	0.06	0.11
		Cd	0.01	0.06	1.34	5.32	0.11	0.20	9.46	16.82
Organic	acidic	0	0.01	0.02	0.01	0.02	0.26	0.58	0.02	0.05
		Pb	0.03	0.04	0.01	0.02	0.91	1.94	0.01	0.03
		Cd	0.01	0.02	0.16	0.27	0.27	0.58	0.41	0.87
	neutral	0	0.01	0.05	0.01	0.02	0.32	0.17	0.06	0.03
		Pb	0.02	0.07	0.01	0.02	0.98	0.56	0.05	0.03
		Cd	0.01	0.05	0.04	0.15	0.17	0.11	0.63	0.43

The results show that the soil type played a crucial role in determining the milimolar ratios between ionic ratios of Pb^{2+}:Ca^{2+} and Pb^{2+}:Mg^{2+} in *B. napus* (Table 3). In plants from Pb treatments on organic soils, the Pb^{2+}:Ca^{2+} and Pb^{2+}:Mg^{2+} ratios were much lower than on mineral soil, and often close to the control value. In *B. napus* shoots from Cd contaminated organic soils, the Pb^{2+}:Ca^{2+} and Pb^{2+}:Mg^{2+} ratios were the same as in the control treatment.

The concentration of Pb significantly affected the ionic relations in *B. napus* roots for all Pb-contaminated treatments. A particularly high increase in the Pb^{2+}:Ca^{2+} and Pb^{2+}:Mg^{2+} ratios occurred in *B. napus* roots from mineral acidic soil: 11.47 and 19.70, respectively (Table 3). The Cd added to neutral soils decreased the Pb^{2+}:Ca^{2+} and Pb^{2+}:Mg^{2+} ratios in *B. napus* roots, but when added to acidic soils, the heavy metal most often slightly increased these values as compared to the control.

DISCUSSION

In recent years, a number of studies have examined the ability of *Brassica* species to take up heavy metals from contaminated soils, growth media or hydroponic culture (Salt et al. 1995, Grispen et al. 2006, Meyers et al. 2008, Selvam, Woon-Chung 2009, Qiu et al. 2011), and indicated the potential of plants in absorbing and translocating metals to the aerial parts (Selvam, Woon-Chung 2009). Gambuś (1997) reported lower sensitivity of rapeseed than other plants to Cd, 15 mg Cu, 12 Ni, 90 mg Pb and 60 mg Zn, when applied jointly into the soil.

The soil type and its pH seem to be basic factors in evaluating the usefulness of *B. napus* plant for phytoremediation purposes. In the present study, the soil type and pH significantly differentiated the biomass yield in the following decreasing order: mineral neutral > organic neutral > mineral acidic > organic acidic. This sequence proves high sensitivity of *B. napus* to soil acidity and shows the importance of soil type in controlling the plants growth.

The soil pH is one of the most important factors affecting the metal mobility and availability to plants. It was found that on mineral acidic soil (Cd treatment), the generative phase was delayed and leaves had yellowing edges. Chlorosis resulting from excess Cd can be due to iron deficiency caused by the interaction of the metals with foliar iron (Das et al. 1997). According to Selvam, Woon-Chung (2009), *B. napus* plants cultivated on fine loamy soil did not show any toxicity symptoms up to 50 mg Cd kg^{-1} DM.

On acidic peaty soil, the *B. napus* leaves had slightly purple colour, which may indicate lower availability of P (P immobilization processes) for plants in the conditions of high activity of H^+. P deficiency symptoms can be also a result of P and Cd interactions within the plant (Das et al. 1997). Qiu et al. (2011) reported an important role of P in the Cd uptake and translocation via the processes involved in the bonding Cd to the cell wall fraction and forming the Cd-phosphate complexes.

The highest sensitivity of *B. napus* to soil Cd contamination resulting in biomass depletion was found in the treatment on acidic mineral soil. It was probably the result of nutrient imbalances caused by higher Cd uptake from acidic soil. As recorded by Selvam and Woon-Chung (2009), differences in *B. napus* shoots dry weights were significant only at 50 mg Cd kg^{-1} soil treatment. The significant *B. napus* roots reduction was observed at 6 mg Cd kg^{-1} soil treatment in *B. napus – B. parachinensis* co-cropping system.

In the Pb treatment on mineral neutral soil, a statistically significant increase of biomass, when compared to the control, was recorded. At an elevated soil pH value, the solubility of Pb in soil decreases (Domańska 2006, Badora 2012) and limits the availability of the metal to plants.

The usefulness of the tolerance coefficient (Ti) in an assessment of plants' vulnerability to soil contamination with heavy metals was underlined by Pikuła and Stępień (2007). In our studies, Ti was in the range of 0.7-1.1, which proves that *B. napus* was generally tolerant to applied doses of cadmium and lead.

The results show that the soil type played a crucial role in determining the milimolar ratios between studied ions (Cd^{2+}:Ca^{2+}, Cd^{2+}:Mg^{2+}, Pb^{2+}:Ca^{2+}, Pb^{2+}:Mg^{2+}) in *B. napus*. The highest values were recorded for plants from the Cd treatments on mineral soil – (Cd^{2+}:Mg^{2+}) up to 21.42 in roots.

A large increase in the Pb^{2+}:Ca^{2+} and Pb^{2+}:Mg^{2+} ratios occurred in *B. napus* roots from mineral acidic soil. It was probably the result of higher Pb mobility in acidic soil. BADORA (2012) reported a significant increase of Pb mobility observed at pH below 5. Significantly higher values of Cd^{2+}:Ca^{2+}, Cd^{2+}:Mg^{2+}, Pb^{2+}:Ca^{2+}, Pb^{2+}:Mg^{2+} ratios were found in *B. napus* roots rather than in shoots. Most of Pb taken up by a plant accumulates in roots (GAMBUŚ 1997), hence roots are the first barrier to the Pb translocation to shoots. Those properties were also reflected in the Pb^{2+}:Ca^{2+}, Pb^{2+}:Mg^{2+} ionic ratios in roots, which were higher by 1 or even 2 orders of magnitude than in shoots, respectively. Cadmium, as more mobile element than lead, was easily absorbed and translocated to shoots. *B. napus* was less susceptible to mineral composition changes when grown on contaminated organic soil rather than on mineral one. The research on Cd and Pb fractionation in organic soil prove that organic compounds may affect the binding Pb and Cd in organic fraction (DOMAŃSKA et al. 2013) and limit the metal uptake. The studied mmol ratios in *B. napus* plants from organic soils were low (below 0.30 in shoots) and according to obtained data, this obviously excludes this plant from Cd and Pb phytoextraction purposes, when cropping on that types of soils at given levels of Cd and Pb contamination.

CONCLUSIONS

1. The highest sensitivity of *B. napus* to soil Cd contamination (reduction of DM from shoots, poor growth and delayed generative development) was observed in the treatment on acidic mineral soil.

2. On mineral soil under Cd and Pb stress, *B. napus* produces higher biomass than on organic soil, but its mineral composition (ionic ratios) distinctly changes as compared to the control, while the chemical composition of plants from contaminated organic soils changed slightly.

3. The high Ti values (except from mineral acidic soil) prove that *B. napus* was generally tolerant to metals.

4. The soil type played a crucial role in determining the milimolar ratios of the examined ratios of ions: Pb^{2+}:Ca^{2+} and Pb^{2+}:Mg^{2+} in *B. napus*. Large changes in the mineral composition were observed in plants from mineral soils: typically an increase in ratios, as compared to the control. In plants from organic soils, the changes were small. This indicates the protective role of soil organic substance on *B. napus* chemical composition and excludes the plant species from Cd, and Pb phytoextraction purposes on that type of soil.

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INFLUENCE OF GROWTH CONDITIONS AND GRAFTING ON THE YIELD, CHEMICAL COMPOSITION AND SENSORY QUALITY OF TOMATO FRUIT IN GREENHOUSE CULTIVATION

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Abstract

The most popular and efficient growing medium in the soilless crop cultivation consists of rockwool slabs. Nowadays, researchers strive to develop other, more environmentally friendly growing media in horticultural practice. The aim of this work was to assess the effect of growth media and grafting on the yield and fruit quality of tomato in greenhouse cultivation. The tomato cultivar Admiro F_1 was used in the study. Half of the plants were grafted on the rootstock Maxifort. Tomatoes were cultivated on organic medium coconut fiber slabs and on rockwool slabs, the latter commonly used as the standard growing medium for tomato. Total yield and distribution of fruit weight classes in the total yield were investigated. The chemical quality attributes of tomato fruit, vitamin C, total soluble solids, titratable acidity, total sugars, dry matter, nitrates, phosphorus, potassium and calcium were determined. The total yield of tomato fruits was higher from plants cultivated on rockwool slabs than on coconut fiber slabs, which showed that at present rockwool is a better substrate for tomato cultivated all year round. The fruit weight distribution depended on the substrate used. Plants cultivated on rockwool slabs were characterized by higher fruit weight while those cultivated on coconut fiber slabs showed a tendency to grow smaller. There were no significant differences in the distribution of weights between fruits obtained from grafted and non-grafted plants. The chemical composition and sensory quality were mainly affected by the fruit harvest date and, to a lesser degree, by the substratum used and grafting.

Key words: transplant preparation, coconut fiber, rockwool slab, quality fruit.

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INTRODUCTION

Tomato is one of the most important horticultural crops in the world (Flores et al. 2010). The cultivated area worldwide has increased by about 23% during the last 10 years and greenhouse cultivation has become economically important (He et al. 2007, FAO 2009). The most popular and efficient growing medium in the soilless crop cultivation consists of rockwool slabs. Nowadays, researchers strive to develop other, more environmentally friendly growing media in horticultural practice New, organic substrates have been introduced in hydroponic culture in order to substitute peat, which is a non-renewable resource, and use less rockwool or perlite due to their problematic recycling (Domeno et al. 2009, Jarosz et al. 2011).

During the past, the primary objective of horticulture was to increase the yield and productivity. However, high quality is even more important than total yield for attaining competitiveness in modern horticulture, arising from the beneficial role of vegetables in human diet. New investigations have created a chance to improve fruit quality based on an accurate genotype selection, optimization of environmental conditions and agricultural practices such as water management, fertilization strategies, growth system, harvesting stage and grafting (Michałoić, Buczkowska 2009, Jadczak et al. 2010, Rouphael et al. 2010, Buczkowska, Michałoić 2012, Majkowska-Gadomska, Wróblewska-Wierzbicka 2013). In the past, grafting was used in vegetable crops to limit the effects of soil pathogens (Lee 1994). According to recent studies, grafting is a useful technique to enhance nutrient uptake, increase yields, avoid diseases and improve stress tolerance because of the vigorous root system of the rootstocks (Santa-Cruz et al. 2002, Chen et al. 2003, Bletsos 2006, Ahmedi et al. 2007, Erismann et al. 2008, Martinez-Rodriguez et al. 2008, Johkan et al. 2009).

The aim of this work was to estimate the effect of growth media and grafting on the yield and fruit quality of tomato in greenhouse cultivation.

MATERIAL AND METHODS

In 2010 and 2011, an experiment the tomato cultivar Admiro F_1 on was carried out at a Warsaw University of Life Sciences greenhouse with controlled microclimate. Half of the plants were grafted on the rootstock Maxifort. Tomato seeds were germinated in rockwool plugs. Grafting of one batch of plants was performed when seedlings developed 1 – 2 leaves, using the standard procedure in horticultural practice. After the graft was established, grafted and non-grafted seedlings were transferred to rockwool pots. When the first truss was visible, plants were transplanted on two different types of growing medium slabs. Tomatoes were cultivated on organic medium coconut fiber slabs (manufactured by Ceres Intern.), and rockwool slabs (Grodan BV), commonly used as the standard growing medium for tomato. Slab dimensions in all the cases were 100 x 15 x 7.5 cm (length x width x height). Plants were trained on a single stem up a string according to the high wire system for a long extended growing cycle with a mean density of 2.7 plants m^{-2} in the whole greenhouse. Tomatoes were fertigated by a computer controlled drip-irrigation system and fertilized with similar doses of macro- and micronutrients, according to the levels recommended for tomato. The amount of the nutrient supply ranged from 0.07 to 0.2 dm^{3} per plant and was adjusted to the plant growth phase, light conditions as well as growing medium. Nutrient concentration in the solution, EC (electro-conductivity) and pH were continuously controlled and kept at uniform levels for all experimental objects. The concentration of nutrients (in mg$\cdot$dm^{-3}) was as follows: N-NO$_3$ – 210, P – 60, K – 340, Mg – 50, Ca – 200, Fe – 2, Mn – 0.6, B – 0.3, Cu – 0.15, Zn – 0.3, Mo – 0.05. The experiment was established in a random design, in three replicates, with 8 plants in each.

At harvest, fruits were collected to determine their yield and quality. Total yield and fruit structure in the total yield were investigated. Fruits for quality evaluation were harvested at the full coloured maturity stage on two different harvest dates: the end of June and the end of September. At each harvest time, 40 fruits were collected from each combination. One batch was examined for the content of vitamin C (ascorbic acid) using the Tillmans' method, based on 2.6-dichlorophenol-indophenol reduction; the total soluble solids (TSS) content was determined with a digital refractometer, the titratable acidity (TA) was measured with the potentiometric method according to the Polish Norm PN-90 A-75 101/04 and total sugars were analysed according to the Luff-Schoorl method. Dry matter was determined by drying fruit samples until stable weight in an oven at 105^{o}C. The content of nitrates (NO$_3$) was determined spectrophotometrically, with the Fiastar device (Tecator, Sweden), using the wavelength of 440 nm; the content of P was assayed with the colorimetric test, while the concentrations of of K and Ca were determined with the flame method.

Sensory analysis was carried out using the profile method (QDA). This was achieved by a team of 20 trained panelists in two duplicates. The following attributes were evaluated: tomato odour, strange odour, skin hardness, flesh hardness, flesh juiciness, tomato flavour, taste (sweet, sour, strange) and overall quality. Each panelist marked his evaluation of the investigated sample on a scale – a segment of a straight line with border marks. The marked notes were converted to numerical values in the stipulated units from 0 to 10.

Statistical analysis was performed using three-way analysis of variance (Anova). Detailed comparison of means was done using the Tukey's test at the significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The results reveal that years of cultivation and the applied growing media significantly affected the yield and fruit weight distribution in tomato cultivation. A higher yield was characteristic for plants cultivated in 2011 as compared to 2010. Also, plants grown on rockwool slabs yielded higher than on coconut fiber slabs. On the other hand, no significant differences were observed in the yield of tomato from grafted and non-grafted plants (Table 1). In 2011, a higher yield was obtained in Class A (weight 48-93 g) and Class B (weight 93-144 g), but in 2010 more fruits belonged to Class BB (weight 144-245 g). The yield of the biggest fruit of the Class BBB (246-484 g) was on the same level in both years of cultivation. The investigated substrate also significantly affected the fruit weight. Tomato plants growing on rockwool slabs produced a bigger yield of fruits in Classes BB and BBB, which were characterized by bigger weight, while plants cultivated on coconut fiber slabs produced a bigger yield of fruits in Classes A and B, with the smaller weight of a single fruit. These results may suggest that plants growing on coconut fiber slabs have tendency of producing smaller fruits in contrast to plants cultivated on rockwool slabs. Grafted and non-grafted plants were characterized by a similar fruit weight distribution, except fruits with the smallest weight (Class A), where yield was significantly higher from non-grafted than from grafted plants ones, which shows that grafted plants produced fewer small fruits than non-grafted plants (Table 1). Similar results were obtained by PASSIM et al. (2005) and POGONYL et al. (2005), who showed that grafted tomato and eggplant plants yielded bigger fruits than their non-grafted counterparts.

Table 1

The yielding of tomato in relation to cultivation and growing factors

Factor		Total yield (dt ha^{-1})	Structure of fruit in total yield (dt ha^{-1})			
			A	B	BB	BBB
Year of cultivation	2010	33.08 *b**	2.21 *b*	13.59 *b*	15.79 *a*	1.56 *a*
	2011	37.79 *a*	4.12 *a*	17.42 *a*	12.42 *b*	1.60 *a*
Substrates	rockwool	36.51 *a*	2.62 *b*	15.01 *b*	15.17 *a*	2.14 *a*
	coconut fiber	34.37 *b*	3.71 *a*	15.91 *a*	13.07 *b*	1.03 *b*
Seedlings production	non grafting	35.22 *a*	3.39 *a*	15.59 *a*	13.73 *a*	1.52 *a*
	grafting	35.65 *a*	2.94 *b*	15.34 *a*	14.48 *a*	1.65 *a*

BBB – fruit weight: 246-484 g, BB – fruit weight: 144-245 g, B – fruit weight: 93-144 g, A – fruit weight: 48-93 g

* Means separated at 5% level; same letters assigned to homogenous groups (the Tukey's test), within combination of fruit class and separately: year, growing medium, seedlings production.

The results of the current investigations concerning fruit chemical composition showed the harvest date had a stronger effect on the fruit quality than to the substrate used for cultivation and grafting. Fruits from plants cultivated on coconut fiber were characterized by a significantly higher content of vitamin C, while a higher phosphorus content was observed in fruits from the cultivation on rockwool slabs. The content of the remaining chemical compounds in tomato fruit was at a similar level irrespective of the substrate (Table 2). Chemical composition of fruits obtained from grafted and

Table 2

The content of chemical composition in tomato fruits (FW) – mean for 2010-2011

Factor	Specification	Dry matter (g 100 g^{-1})	Total sugars (g 100 g^{-1})	TSS (^{0}B)	Vitamin C (mg kg^{-1})	TA (g 100g^{-1})	TSS/TA	N-NO_3 (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})
Substrates	rockwool	4.751 *a**	1.990 *a*	4.45 *a*	256.9 *b*	0.33 *a*	6.26 *a*	0.049 *a*	0.159 *a*	1.675 *a*	0.032 *a*
	coconut fiber	4.590 *a*	1.991 *a*	4.26 *a*	306.1 *a*	0.32 *a*	6.27 *a*	0.046 *a*	0.146 *b*	1.675 *a*	0.035 *a*
Seedlings productin	non grafting	4.582 *a*	2.103 *a*	4.32 *a*	286.5 *a*	0.33 *a*	6.59 *a*	0.047 *a*	0.147 *a*	1.640 *a*	0.033 *a*
	grafting	4.751 *a*	1.880 *b*	4.40 *a*	276.4 *a*	0.33 *a*	5.93 *a*	0.048 *a*	0.158 *a*	1.713 *a*	0.034 *a*
Time of harvest	June	4.690 *a*	2.141 *a*	4.23 *b*	253.7 *b*	0.31 *a*	6.93 *a*	0.037 *b*	0.161 *a*	1.733 *a*	0.043 *a*
	September	4.641 *a*	1.852 *b*	4.48 *a*	309.2 *a*	0.35 *a*	5.60 *b*	0.059 *a*	0.144 *b*	1.620 *b*	0.024 *b*

* Means separated at 5 % level; same letters assigned to homogenous groups (the Tukey's test), one factor analysis: year, growing medium, seedlings production.

non grafted plants was on a similar level except the total sugars content; a higher sugars content was observed in fruits from non grafted plants (Table 2). Fruits picked in June were characterized by a higher content of total sugars, phosphorus, potassium and calcium and a higher total sugars to titratable acidity (TS/TA) while fruits picked in September showed a higher content of total soluble solids, nitrates and vitamin C. Seasonal variations in vitamin C content directly correlated with temperature variations were observed in greenhouse-grown tomatoes (Liptay at al. 1986). Also, several reports demonstrated that fruit increase their ascorbic acid levels in response to light (Davey et al. 2000, Dumas et. al. 2003). On the other hand, Raffo et al. (2006) reported no correlation between the antioxidant content and mean solar radiation or average temperature. Furthermore, Hernández et al. (2008) maintained that the sampling period is a more influential factor than a cultivar or cultivation methods in the differentiation chemical characteristics of tomato samples. In our study, the remaining chemical compounds were at a similar level irrespective of the harvest date (Table 2).

The results of the profile assessment of tomato fruits are shown in Figure 1 as ‘a quality map’, in the space created by the first two main components PC 1

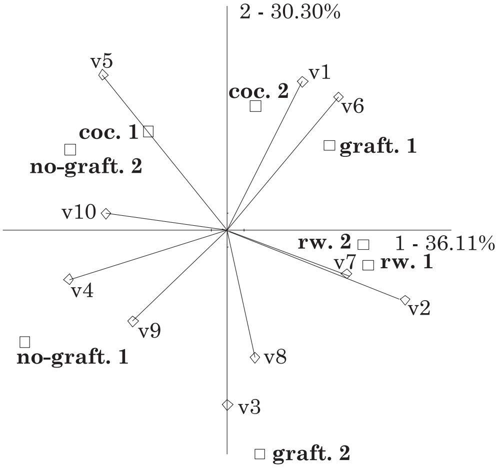

Fig. 1. PCA projection of similarities differences and of sensory quality of fruit tomato (2010-2011). The factors of cultivation (points marks numbers): rw. 1 – rockwool first term of harvest, rw. 2 – rockwool second term of harvest, coc. 1 – coconut fiber first term of harvest, coc. 2 – coconut fiber second term of harvest, no-graft. 1 – non-grafted plants first term of harvest, no-graft. 2 – non-grafted plants second term of harvest, graft. 1 – grafted plants first term of harvest graft. 2 – grafted plants second term of harvest. Attributes evaluated (vectors marks numbers): v1-tomato smell, v2-strange smell, v3-tough skin, v4-flesh texture, v5-juiciness of flesh, v6- tomato taste, v7-sour taste, v8 –sweet taste, v9 – strange taste, v10 – overall quality

and PC 2, which show 66.41% of the variability in the analysed cultivar's sensory quality. The position of analyzed samples of fruits from different combinations on the chart proves their variability in regard to the analysed taste, smell and texture attributes. High notes of tomato flavour and taste were given to fruits from the cultivation on coconut fiber slabs and picked in September and fruits from grafted plants picked in June. Fruits obtained from plants cultivated on rockwool slabs picked at both harvest dates were given high notes for sour taste. High marks for skin firmness and sweet taste were obtained by fruits which were picked in September from grafted plants. Fruits from non-grafted plants picked in June had high notes for flesh firmness and foreign taste, traits which are responsible for a lower quality of tomato fruits. The highest notes for the total quality assessment and flesh juiciness were scored by fruits from plants cultivated on coconut fiber slabs which were picked in June and fruits from non-grafted plants harvested in September. This chart shows that the positive and negative attributes mostly depended on the harvest time and, to a lesser degree, on the growing media and grafting (Matsuzone et al. 1996, Di Gioia et al. 2010).

CONCLUSION

The total yield of tomato fruits was higher from plants cultivated on rockwool slabs than on coconut fiber slabs, which showed that at present rockwool is a a better substrate for tomato cultivated all year round.

The fruit weight distribution was affected by the substrate. Plants cultivated on rockwool slabs were characterized by a higher fruit weight while those cultivated on coconut fiber slabs showed a tendency for growing smaler fruit. There were no significant differences in the fruit weight distributions between grafted and non-grafted plants.

The chemical composition and sensory quality were mainly affected by the fruit harvest date and, to a lesser degree, by the substratum that was used and by grafting.

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PHENOLIC ACIDS IMPROVE THE ANTIOXIDANT ACTIVITY OF CERULOPLASMIN ISOLATED FROM PLASMA OF HEALTHY VOLUNTEERS AND ATHEROSCLEROTIC PATIENTS*

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Abstract

The aim of the present study was to estimate the ability of individual phenolic acids to eliminate Fe(II) ions from the solution. Moreover, the influence of phenolic acids on the ferroxidase activity of ceruloplasmin (Cp) isolated from blood plasma of healthy volunteers (Cp_C) and patients with atherosclerosis obliterans (Cp_{AO}) was established *in vitro*. Phenolic acids demonstrated a ferroxidase-like activity, i.e. the ability to eliminate Fe(II) ions, within the studied concentration range of 2.0-17.0 mol 10^{-5} dm^{-3}, in the following order of decreasing effectiveness: caffeic acid (CA)>ellagic acid (EA)>chlorogenic acid (ChA)>ferulic acid (FA) ≈ *p*-coumaric acid (PcA) = sinapic acid (SA). The study showed that the ability of phenolic acids to eliminate Fe(II)

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ions by oxidation or chelation was related to the structure of the former, to the presence of *orto* -OH groups, especially. Furthermore, the effect of the molar ratio of phenolic acid to Fe(II) ion was observed. EA and ChA, both containing two *orto*-OH groups and the highest number of –OH groups (4 and 5, respectively), demonstrated the greatest ability to eliminate Fe(II) ions, especially at the Fe(II) to phenolic acid molar ratio of 6:1. Phenolic acids added to samples with a constant amount of Cp caused decrease in the concentration of Fe(II) ions. Therefore, it may be assumed that the addition of phenolic acids to Cp_C and Cp_{AO}, even in low concentrations, caused a significant decrease in the concentration of Fe(II) ions.

Key words: caffeic acid, chlorogenic acid, ellagic acid, ferulic acid, sinapic acid, *p*-coumaric acid, atherosclerosis obliterans, ceruloplasmin, ferroxidase activity.

INTRODUCTION

A growing interest in the protective properties of fruits and their components can be ascribed to their potentially beneficial influence on human health. Fruits are known to demonstrate anti-proliferative activities and their consumption is associated with a reduced risk of cardiovascular disease and some types of cancer (LOSSO et al. 2004, ESTAQUIO et al. 2008). Bioactive compounds of fruits, such as phenolic acids, flavonoids, stilbenes and vitamins (HAN et al. 2007, LUGASI et al. 2011, BOBINAITÉ et al. 2012) exhibit various health promoting mechanisms and activities, i.e. antioxidant activity, anti-proliferative activity, free radical scavenging capacity, neuroprotective capacity (AMAROWICZ et al. 2007, BOWEN et al. 2010, FORTALEZAS et al. 2010).

Polyphenols, a class of compounds present in fruits, are known to be good chelators and oxidants of Fe(II) ions, and may inhibit metal-dependent processes generating reactive oxygen species (ROS). Iron ions, Fe(II) particularly, exhibit the ability to generate free radicals ($O_2^{\cdot -}$ or $^{\cdot}OH$), which are capable of oxidizing of biological molecules because of their high redox potential. In the Fenton reaction, Fe(II) is oxidized by hydrogen peroxide to Fe(III) and OH is produced. It has been demonstrated that Cu(I) and other transition metals can also react with hydrogen peroxide and generate ROS. The efficiency of this reaction increases in pathological conditions, often accompanied by an increased concentration of unbound Fe(II) ions. Oxidation of Fe(II) to Fe(III) ions followed by subsequent binding by transferrin and ferritin is catalyzed by ceruloplasmin (Cp), the most important plasma antioxidant.

The ferroxidase activity of Cp seems to be enhanced by polyphenols, yet the interaction between polyphenolic compounds and Cp or any other endogenous antioxidants remains understudied (BUDZYŃ et al. 2009). In our previous study, a ferroxidase-like activity was demonstrated by raspberry seed extract (RSE) (GRYSZCZYŃSKA et al. 2009). An addition of RSE to Cp, isolated from serum of healthy subjects, caused a dose-dependent decrease of Fe(II) concentration. This was due to polyphenolic compounds present in RSE, which eliminated Fe(II) ions by oxidation or chelation. The analysis of the profile of phenolic compounds in extracts from different raspberry cultivars demonstrated that gallic acid (GalA), ChA, EA, 4-hydroxybenzoic acid (4-HbA), CA, PcA, FA, and gentisic acid (GenA) played the most significant antioxidant role among the phenolic acids (Moyer et al. 2002, Zhang et al. 2010).

Therefore, ChA, CA, EA, FA, PcA, and SA, as the most prevalent and effective components of plants and plant products, were selected for this study.

A particularly intensified production of free radicals and ROS occurs as a result of upsetting the balance between antioxidative and prooxidative processes. This imbalance, accompanied by lower activity of antioxidant enzymes and reduced concentration of low-molecular antioxidants, plays a significant role in the pathogenesis of many diseases. Oxidative stress is one of the risk factors of many pathological conditions, including atherosclerosis obliterans (AO) (Majewski et al. 2007). The occlusion of the lower limbs due to atherosclerosis may lead to moderate or critical ischaemia of the lower limbs. Reperfusion injuries and ischaemic changes in tissues entail the formation of various reactive oxygen species (ROS). The latter are resistant to elimination, due to insufficient adaptive mechanisms of the organism, and are produced when the body's antioxidant status is significantly deteriorated (Harris 1992).

The analysis of plasma from patients with and without atherosclerosis, carried out in the present study, is expected to clarify to what extent phenolic compounds support enzymes or low-molecular antioxidants in their fight against free radicals and ROS, in a situation when the disease causes an excessive use or an insufficient production of endogenous antioxidants.

The purpose of the study was to determine individual abilities of these compounds to eliminate free Fe(II) ions, and to assess their effect on the ferroxidase activity of Cp isolated from blood plasma of healthy volunteers (Cp_C) and patients with atherosclerosis obliterans (Cp_{AO}) *in vitro.*

MATERIAL AND METHODS

Chemicals

$(NH_4)_2 Fe(SO_4)_2 \cdot 6H_2O$ (Mohr's salt), $(NH_4)_2 SO_4$, NaCl, KH_2PO_4, K_2HPO_4, CH_3COOH, CH_3COONa, chloroform and ethanol were purchased from POCh Spółka Akcyjna (Gliwice, Poland). 3-(2-Pyridyl)-5,6-diphenyl–1,2,4-triazine-4',4''-disulfonic acid sodium salt (ferrozine), histidine, DEAE-Sephadex A-25 chloride form, CA, EA, ChA, PcA, SA, FA, apotransferrin, acetic acid, sodium acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents and solvents were of analytical grade of purity.

Preparation of phenolic acid solutions

The basic solutions of CA, ChA, FA, PcA were prepared in high purity water. The basic solution of SA was prepared in methanol: H_2O (1:4,v:v) and EA was dissolved in methanol.

Isolation and purification of ceruloplasmin from human plasma samples

For the purpose of the study, Cp was isolated from blood plasma samples of healthy volunteers and patients with AO (the volume of 0.1 dm^{-3} combined from 20 subjects in each studied group). Prior approval for the study had been given by the Ethics Committee of Poznan University of Medical Sciences (decision N° 614/12). Cp_C and Cp_{AO} were isolated and purified according to the procedure detailed previously (Gryszczyńska et al. 2009). The purification procedure yielded an essentially pure preparation of Cp_C and Cp_{AO}, and the absorbance A_{610}/A_{280} ratio reached the value of 0.036 and 0.031, respectively. Cp_C and Cp_{AO} solutions were prepared in PBS buffer (0.05 mol dm^{-3}, pH 7.38). PBS was chosen because the phosphate buffer represents one of the main components of the physiological buffering system.

The ferroxidase activity Cp_C and Cp_{AO}

The ferroxidase activity of Cp was measured spectrophotometrically, using the Fe(II)-histidine complex and ferrozine [disodium salt of 3-(2-pyridyl)--5,6-bis (4-phenylosulfonic acid)-1,2,4-triazine)] as a chromogenic reagent. Histidine, a possible chelator of Fe(II) *in vivo,* was chosen to bind Fe(II) in a stable and low-molecular complex in order to avoid iron autooxidation *in vitro*. In the presence of Cp, Fe(II) ions are oxidized to Fe(III) and the remaining amount of Fe(II) forms a more stable complex with ferrozine, yielding a product measured spectrophotometrically (Juan, aust 1998). The ferroxidase activity of Cp_C and Cp_{AO} in the concentration range of 22-66 mg dm^{-3} was measured according to the procedure described by Gryszczyńska et al. (2009) and expressed as a change in Fe(II) concentration in a sample.

The effect of phenolic acids on the ferroxidase activity of Cp_C and Cp_{AO}

The effect of phenolic acids on the ferroxidase activity of Cp_C and Cp_{AO} was measured in a mixture of Cp_{AO} or Cp_C of a given concentration (22; 33; 44; 66 mg dm^{-3}) and variable concentrations of phenolic acids (2.0-17.0 mol 10^{-5} dm^{-3}). Appropriate volumes of Cp and phenolic acid solution were added to test tubes and handled according to the procedure described by Gryszczyńska et al. (2009).

Determination of the ability of phenolic acids to oxidize Fe(II) ions

The ability of phenolic acids to oxidize Fe(II) ions was measured according to the procedure described by Johanson et al. (1967). This micromethod was used for the determination of the serum concentration of ceruloplasmin and based on the oxidation reaction of Fe (II) to Fe (III) by Cp under defined conditions. The absorbance of orange complex of Fe(III):transferrin was measured spectrophotometrically at λ=460 nm. This method was applied in order to test the capacity of phenolic compounds to oxidize Fe(II) ions. The concentration of Fe(III):transferrin was calculated from the formula:

$$\text{Fe(III):transferrin} = [(A - A_0) - (C - C_{0)}] / \varepsilon\ t\ m \quad (\mu M)$$

where:

A_0 – absorbance value at 0 minute,

A – absorbance value after 1 minute of reaction,

C_0 – absorbance value of blank sample at 0 minute,

C – absorbance value of blank sample after 1 minute of reaction,

ε – micromolar absorption coefficient, ε=0.0025 ($dm^3\ \mu mol^{-1} cm^{-1}$),

t – reaction time in minutes,

m – concentration of phenolic acid ($\mu mol\ dm^{-3}$).

The study of the ability of phenolic compounds to oxidize Fe(II) ions was carried out for two molar proportions of Fe(II) ions to phenolic acids: 6:1 (A) and 1:1 (B). The solutions were prepared by combining 0.00017 dm^{-3} of acetate buffer (1.2 M; pH 6.0), 0.000250 dm^{-3} apotransferrin solution (2.0%), and an appropriate volume of phenolic acid solution, subsequently replenished with water to obtain 0.0007 dm^{-3} of mixture in a cuvette. The cuvette was placed in a spectrophotometer for 3 minutes at 30ºC. Then, 0.0003 dm^{-3} of Fe(II) ion solution (Mohr's salt, 0.22 mmol dm^{-3}) was added to the cuvette and the initial absorbance was measured immediately (A_0). The measurment was repeated after 1 minute (A). At the same time, a blank was prepared by combining 0.00017 dm^{-3} of buffer solution, 0.00025 dm^{-3} of apotransferrin solution and 0.00028 dm^{-3} of water. The initial absorbance (C_0) was measured following the addition of Fe(II) ion solution to the blank and then the measurement was repeated after 1 minute (C).

Statistical analysis

The results were expressed as means ± standard deviation. Statistical differences were estimated by using Student's *t*-test. The significance level was accepted at $p < 0.01$.

RESULTS AND DISCUSSION

The study showed the cooperation of Cp with phenolic acids in Fe(II) ions elimination from solution and the effect of chronic arterial occlusion of the lower limbs due to atherosclerosis on the ferroxidase activity of Cp. The ferroxidase activity of Cp_C (Figure 1*a*), expressed as ΔFe(II), was lower than that of Cp_{AO} in the dose of 66 mg dm^{-3} (Figure 1*b*) – $p < 0.001$.

The abilities of individual phenolic acids to eliminate Fe(II) and their effect on the ferroxidase activity of both Cp_C and Cp_{AO} was analyzed. CA, ChA and EA were shown to be more capable of eliminating Fe(II) ions (at 17.0 mol 10^{-5} dm^{-3}) when compared to SA, PcA and FA. The addition of individual phenolic acids to the solution with a constant amount of Cp_C or Cp_{AO} caused a significant decrease in Fe(II), shown as ΔFe(II) in Figure 1*a* and Figure 1*b*, respectively. The effect of phenolic acids on the ferroxidase activity of Cp_C or Cp_{AO} was dose-dependent for each of Cp concentration. However, the addition of higher concentrations (6.0 mol 10^{-5} dm^{-3} and more) of phenolic acids, especially CA, EA and ChA, to the samples with 66 mg dm^{-3} of Cp_C or Cp_{AO} caused less efficient Fe(II) elimination in both groups.

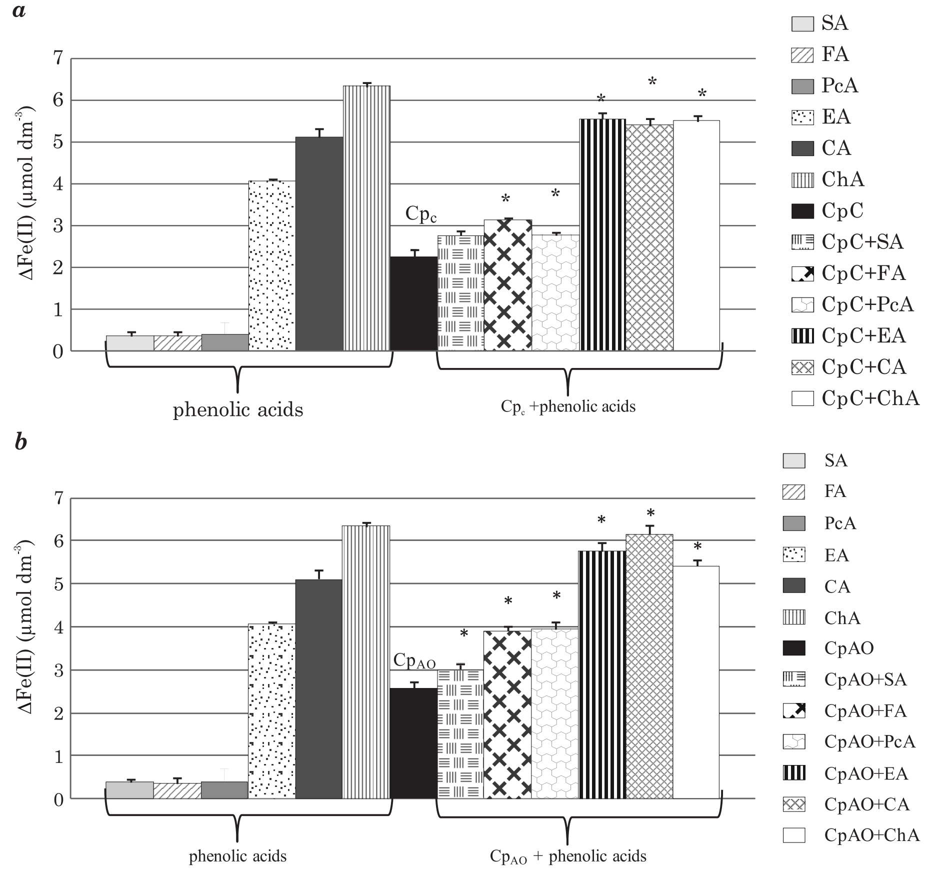

Fig. 1. The effect of phenolic acids (17 mol 10^{-5} dm^{-3}) on the ferroxidase activity of Cp_C (*a*) or Cp_{AO} (*b*) (66 mg dm^{-3}) (* $p < 0.001$ – significant differences *vs* samples with constant amount of Cp_C or Cp_{AO} without phenolic acids); CA – caffeic acid, EA – ellagic acid, ChA – chlorogenic acid, SA – sinapic acid, PcA – *p*-coumaric acid, FA – ferulic acid

The highest concentrations of Fe(II) eliminated by phenolic acids with and without the presence of Cp_C or Cp_{AO} in a model solution were evaluated. For this purpose, the parameters of the regression curves of the reciprocal of concentration of the selected phenolic acids and the reciprocal of ΔFe(II) were calculated (Table 1). The maximum decreases in Fe(II) concentration obtained for CA, EA, ChA, PcA, SA and FA were 64.10; 48.80; 7.44; 0.40; 0.40; 0.40 μmol dm^{-3}, respectively. On the other hand, in the presence of Cp_C or Cp_{AO}, the calculated ΔFe(II) values were lower for CA, EA, ChA and higher for SA, FA, and PcA.

Table 1

Data for $\Delta Fe(II)_{max}$ caused exclusively by a phenolic acid in the presence of Cp_C or Cp_{AO} calculated in the solution of Cp_C/ phenolic acid or Cp_{AO}/phenolic acid

Cp (mg dm^{-3})	Cp_C		Cp_{AO}	
	double reciprocal curve $y = ax + b^*$	$1/b = \Delta Fe(II)_{max}$	double reciprocal curve $y = ax + b^*$	$1/b = \Delta Fe(II)_{max}$
Cp without phenolic acids	$y = 26.619x + 0.0222$	45.05	$y = 10.86x + 0.2258$	4.43
CA	$y = 2.522x + 0.0156$	64.10	$y = 2.522x + 0.0156$	64.10
CA + Cp	$y = 0.2600x + 0.1709$	5.85	$y = 0.0460x + 0.1611$	6.20
EA	$y = 3.5265x + 0.0205$	48.80	$y = 3.5265x + 0.0205$	48.80
EA + Cp	$y = 0.3287x + 0.1628$	6.14	$y = 0.3613x + 0.1447$	6.91
ChA	$y = 0.3886x + 0.1344$	7.44	$y = 0.3886x + 0.1344$	7.44
ChA + Cp	$y = 0.2213x + 0.1826$	5.47	$y = 0.0955x + 0.1896$	5.27
SA	$y = 6.7516x + 2.3519$	0.40	$y = 6.7516x + 2.3519$	0.40
SA + Cp	$y = 0.2129x + 0.3568$	2.80	$y = 0.0792x + 0.3471$	2.88
PcA	$y = 6.2206x + 2.6961$	0.40	$y = 6.2206x + 2.6961$	0.40
PcA + Cp	$y = 0.1833x + 0.3564$	2.80	$y = 0.1021x + 0.2504$	3.99
FA	$y = 9.879x + 2.4191$	0.41	$y = 9.879x + 2.4191$	0.41
FA +Cp	$y = 0.0863x + 0.3163$	3.16	$y = 0.0405x + 0.2698$	3.71

* $y = ax + b \rightarrow 1/\Delta Fe(II) = 1/[\text{phenolic acid}] + 1/\Delta Fe(II)_{max}$
when $x = 0 \rightarrow 1/b = \Delta Fe(II)_{max}$ is the maximum of a possible decrease in Fe(II) concentration

In the next step, the ability of individual phenolic acids to oxidize Fe(II) and the formation of Fe(III)-transferrin complex were estimated. It was shown (Figure 2.) that EA and ChA demonstrated the greatest ability to oxidize Fe(II) ions and thus binding Fe(III) with transferrin. The lower capacity was calculated for CA and the lowest ability to oxidize Fe(II) ions was shown by SA, PcA and FA. Moreover, the ability to eliminate Fe(II) ions was related to the Fe(II)/

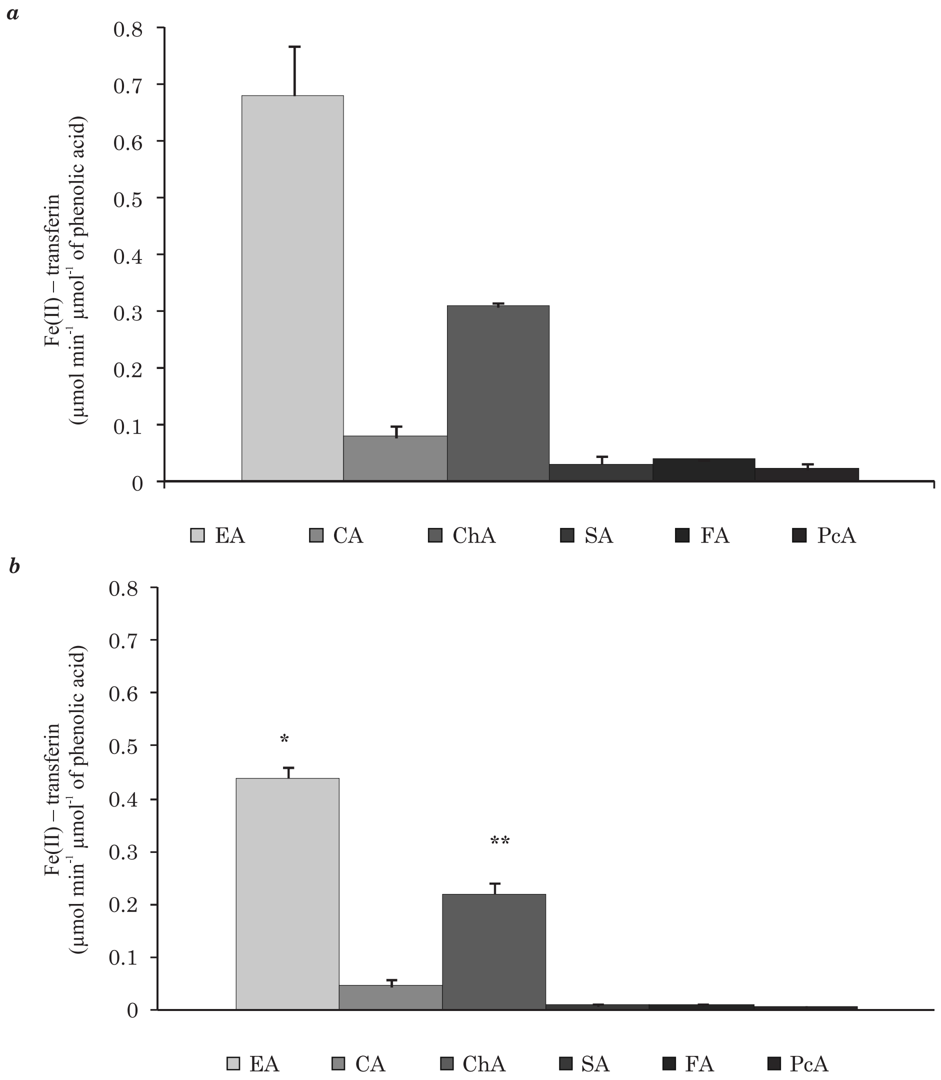

Fig. 2. Formation of a Fe(III):transferrin complex due to the oxidation of Fe(II) ions in the presence of phenolic acids and at the Fe(II)/phenolic acid molar ratio of 6:1(*a*) and 1:1(*b*). (* $p < 0.001$ significant difference *vs*. figure a for ellagic acid; ** $P<0.01$ significant difference *vs*. figure a for chlorogenic acid)

phenolic acid molar ratio. The analysis of data presented in Figure 2 shows that the 6:1 molar ratio is much more favourable than the 1:1 one.

The chosen phenolic acids differ in terms of their chemical structure, i.e. the number of OH groups in a molecule, mutual arrangement thereof, and the presence of aromatic rings. Therefore, some differences in the reactivity of phenolic acids with Fe(II) ions were expected to occur.

The results demonstrated that all phenolic acids were able to eliminate unbounded Fe(II) ions by oxidation or chelation, but in different manner. The addition of CA, EA and ChA, even in low concentrations (2.0; 4.0; 6.0 mol 10^{-5} dm^{-3}), to solution containing Cp caused a significant increase in ΔFe(II). When the concentration of phenolic acids increased, along with the concentration of Cp_C or Cp_{AO}, the competition for the substrate, i.e. free Fe(II) ions, was shown to have intensified. The oxidative activity of Cp_{AO} was higher when compared to Cp_C. The study also revealed that values of $\Delta Fe(II)_{max}$ obtained for samples containing only CA, EA, or ChA were higher than for tsamples in which either Cp_{AO} or Cp_C were present. Although these considerations confirm the competition of CA, EA, ChA and Cp for Fe(II) ions, it is very likely that the addition of the phenolic acids at higher concentrations might even replace Cp as an antioxidant. This effect may be particularly desirable in chronic disease, which probably entails a greater exploitation of antioxidants and necessitates a higher activity of antioxidant enzymes. The next chosen phenolic acids, SA, PcA, and FA, showed significantly weaker ability to eliminate free Fe(II) ions, which confirms that Cp plays a predominant role in decreasing the concentration of Fe(II) ions in the experimental model. The presence of one OH group only, and thus the lack of an *orto*-OH group arrangement, seems to exert an influence on the ability of these acids to chelate or oxidize Fe(II) ions.

The elimination of Fe(II) ions by chelation with phenolic acids was confirmed by using ferrozine. The presence of the phenolic acid perturbs the Fe(II)-ferrozine complex formation and indicates their higher chelating activity (GÜLĘIN 2006). Various studies have demonstrated that compounds containing two or more –OH, -SH, -COOH, -C=O, -S- groups show a metal chelating ability (YUAN et al. 2005, ANDJELKOVIĆ et al. 2006). However, oxidation of Fe(II) is an important mechanism in the elimination of this ion. Phenolic acids increase the rate of Fe(II) oxidation to Fe(III) and form complexes with Fe(II), thus preventing the Fenton reaction, which was suggested by Lopes and co-workers (LOPES et al. 1999). In this study, oxidation of Fe(II) ions by phenolic acids was proven by measuring the concentration of Fe(III):transferrin complex. Higher concentrations of this complex were observed in the presence of EA, ChA and CA than of SA, PcA and FA. The results of other studies indicate that phenolic acids containing gallolyl moieties i.e. 3 adjacent OH groups, favour the autooxidation of Fe(II) ions. This moiety is present both in CA and ChA, thus the highest concentration of Fe(III):transferrin complex in their presence was found. EA, which is a dimer of GalA, demonstrates the highest efficiency to oxidize of Fe(II) ions, as revealed by Chvátalová (CHVÁLATOVÁ et al. 2008). The presence of two *ortho*-OH groups in the structure of EA may favour the oxidation of Fe(II) ions. It seems probable that acids containing one OH group only (3-hydroxybenzoic acid, 4-HbA and 4-hydroxy-3-methoxybenzoic acid) decrease the oxidation of Fe(II) ions with an efficiency similar to that of ascorbic acid. This observation is important for SA, PcA and FA, derivatives of cinnamic acid, containing only one OH and or one methoxyl group. Furthermore, our results indicate that the ability of phenolic acids to oxidize Fe(II) ions depends on their molar ratio, too. The oxidation of Fe (II) is favoured when the Fe(II) to phenolic acid ratio is higher (i.e. equals 6:1), which leads to the more efficient binding of Fe(III) by apotransferrin. Phenolic acids favour the oxidation of Fe(II) when their concentration is much lower than the initial concentration of Fe(II) ions. It was shown that CA and protocatechuic acids are the best oxidants if their concentration constitutes only 0.4% of the initial concentration of Fe(II) (Chválatová et al. 2008).

CONCLUSIONS

The ability of phenolic acids to chelate or oxidize Fe(II) ions, followed by Fe(III) ions binding to apotransferrin and ferritin, may represent a beneficial mechanism that could assist in the elimination of unbound Fe(II) ions by ceruloplasmin in the human body. Phenolic acids added to solution of Cp_{AO} and Cp_{C} at variable concentrations increase the elimination of Fe(II) ions and demonstrate a ferroxidase-like activity in the order of decreasing effectiveness: CA>EA>ChA>FA ≈PcA=SA.

It was shown that the ability of phenolic acids to eliminate Fe(II) ions by oxidation or chelation was associated with their structures, the presence of *orto*-OH groups in particular. Furthermore, the influence of phenolic acid/ Fe(II) ion ratio on the oxidative ability of phenolic acids was observed. EA and ChA, containing two *orto*-OH groups and the highest number of –OH groups (4 and 5, respectively), show the highest ability to eliminate Fe(II) ions, especially at the Fe(II)/phenolic acid molar ratio of 6:1.

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DISTRIBUTION OF CARBON AND NITROGEN FORMS IN HISTOSOLS OF HEADWATER AREAS – A CASE STUDY FROM THE VALLEY OF THE KAMIENNA CREEK (NORTHERN POLAND)

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Abstract

The aim of the study was to recognize the vertical variability in the content of different forms of carbon and nitrogen in Histosols of a forest spring niche located in the upper course of the Kamienna Creek (the Słupia River catchment). Soil samples were taken from three profiles and analyzed with standard methods used in the soil science. Analyses included the degree of peat mass decomposition, content of soil organic matter, pH, content of total organic carbon (TOC), total nitrogen (TN) and forms of carbon and nitrogen after sequential extraction in 0.25 mol KCl dm^{-3}, 0.25 mol H_2SO_4 dm^{-3} and 2.5 mol H_2SO_4 dm^{-3}. The following fractions were isolated: nonhydrolyzable carbon (NHC) and nitrogen (NHN), weakly hydrolyzable carbon (WHC) and nitrogen (WHN), easy hydrolyzable carbon (EHC) and nitrogen (EHN), dissolved organic nitrogen (DON), and its ammonium (NH_4-N) and nitrate (NO_3-N) form.

The Histosols were t up to 0.9 m hick. The degree of peat mass decomposition was 3-9. The content of organic matter ranged from 317.3 to 829.0 g kg^{-1}, and TOC from 162.2 to 459.5 g kg^{-1}. The soils were acid at pH_{H2O} equal 5.6-6.5. The NHC form predominated in TOC. The content of the form was 117.3-399.7 g kg^{-1}, and contribution in TOC 72.3-89.2%. Soils contained 17.1-41.7 g kg^{-1} of WHC (4.5-10.6% in TOC), and 27.3-62.2 g kg^{-1} EHC (6.4-17.2% in TOC). The soils were rich in total nitrogen (TN), whose content was 11.1-33.6 g kg^{-1}. The content of NHN was 5.50-18.89 g kg^{-1} (37.18-69.84% in TN), WHN 4.28-14.17 g kg^{-1} (21.23-43.72 in TN), EHN 1.16-8.02 g kg^{-1} (8.06-24.32% in TN), and DON ranged from 0.029-0.394 g kg^{-1} (0.10-1.20% in TN). The concentration of NH_4-N was 0.043-0.337 g kg^{-1}, and NO_3-N 0.003-0.012 g kg^{-1}. Similar regularities in the vertical distribution of the investigated forms of carbon and nitrogen were observed in every soil profile. In general, an increase in the nonhydrolyzable forms of carbon and nitrogen and a decrease in EHC, EHN, DON and NH_4-N were observed with depth. The maximum concentration of EHC, EHN, DON and NH_4-N found in bog horizons is probably an

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effect of the highest intensity of biochemical processes in the topsoil and the influx of fresh litterfall. A constant underground flow of water and leaching are the factors which caused a low contribution of labile forms of C and N in lower parts of the soil profiles.

Key words: headwater areas, Histosols, carbon forms, nitrogen forms.

INTRODUCTION

Headwater areas, as transition zones between underground and superficial parts of a water cycle in river catchments, play an important role in the functioning of geosystems (Chapman et al 1993, Mazurek 2006, Jekatierynczuk-Rudczyk 2007, Jonczak 2011). Water, soil and plant communities play the key role in the functioning of spring niches, and interactions between these components are very close and anisotropic (Devito et al 1996, Foster et al 2001, Davies et al 2005, Karlsson et al 2005, Szymczyk et al 2010, Daniels et al 2012, Mazurek 2012). The water outflow rate affects the degree of swamping in the vicinity of water seepages, while its chemical composition has a strong impact on species-composition of plant communities. Plants are a source of litterfall, whose accumulation under the lasting surplus of water leads to the formation of Histosols. Physical and chemical properties of soils are conditioned by the character of past and contemporary plant communities and the chemistry of supplying water. On the other hand, the soils influence the direction and range of transformations in water chemistry during the flow through a niche (Jekatierynczuk-Rudczyk 2007, Mazurek 2012). The soils of spring niches are usually rich in organic matter, which affects their physical, chemical, sorptive and buffer properties (Jonczak 2010, Jonczak, Cysewska 2010). Soil organic matter (SOM) is present in the soils in different forms – from fresh litterfall, through humus to soluble (labile) organic compounds. Litterfall is a primary source of SOM, and dissolved organic matter (DOM) is a product of its microbiological decomposition and a substrate in humification process. Thus, the content of different forms of SOM resulted from the rate of microbiological decomposition of litterfall, humification and leaching, which in turn was strongly affected by soil properties and many environmental and anthropogenic factors. Carbon and nitrogen (beside oxygen and hydrogen) are major components of SOM. Bearing in mind permanent flow of water through Histosols of headwater areas, especially in horizons over mineral bed, we should expect in general low, but vertically differentiated content of labile forms of carbon and nitrogen.

The aim of our study was to recognize the vertical variability in the content of different forms of carbon and nitrogen in Histosols of forest spring niche located in the upper course of Kamienna Creek.

MATERIAL AND METHODS

The studies were conducted in northern Poland, in the valley of the Kamienna Creek – a left tributary of the Słupia River. The Kamienna Creek valley is a deep headwater area, carved in the Holocene sands and loams and rich in water seepages and spring niches, which are the subject of our studies. This paper presents results of the studies conducted in an afforested spring niche located in the upper course of the river, near Łysomice (54°19′ N; 17°10′ E). This spring niche is a riparian area overgrown with 40-86 years-old alder trees (*Alnus glutinosa*) and having a very species rich herbaceous plant cover. iIn total, there were 106 species of vascular plants, 17 species of mosses and 8 species of liverworts identified in the niche in 2012. This is a lowland peatbog area with Histosols of the thickness to about 90 cm. In the summer of 2012, three soil profiles were uncovered, in which the soils described and sampled. Soil samples were taken from 10 cm layers, up to the mineral bed. The degree of peat mass decomposition (H) was determined in field on the von Post's scale (Grosse-Brauckmann 1990). Soil samples were dried at 40°C, ground and analyzed. The following properties were analyzed:

- pH potentiometrically (Elmetron CP-401) in H_2O and 1mol dm^{-3} KCl (in the soil:water/KCl ratio of 1:10);
- soil organic matter (SOM) content as loss on ignition at 450°C;
- total organic carbon (TOC) content with the Alten method;
- total nitrogen (TN) content with the Kjeldahl method in a distilling unit VELP UDK-127;
- the content of carbon and nitrogen forms after sequential extraction in 0.25 mol KCl dm^{-3}, 0.25 mol H_2SO_4 dm^{-3} and 2.5 mol H_2SO_4 dm^{-3} (Becher, Kalembasa 2011).

Based on the extractions, the following fractions of carbon and nitrogen were isolated:

- easy hydrolyzable carbon (EHC) – after extraction with 0.25 mol H_2SO_4 dm^{-3};
- weakly hydrolyzable carbon (WHC) – after extraction with 2.5 mol H_2SO_4 dm^{-3};
- nonhydrolyzable carbon (NHC) – was calculated as TOC- EHC-WHC;
- nitrate nitrogen (NO_3-N) - after extraction with 0.25 mol KCl dm^{-3};
- ammonium nitrogen (NH_4-N) - after extraction with 0.25 mol KCl dm^3;
- dissolved organic nitrogen (DON) – the content of Kjeldahl nitrogen after extraction with 0.25 mol KCl dm^{-3} – NH_4N;
- easy hydrolyzable nitrogen (EHN) – after extraction with 0.25 mol H_2SO_4 dm^{-3};
- weakly hydrolyzable nitrogen (WHN) – after extraction with 2.5 mol H_2SO_4 dm^{-3};
- nonhydrolyzable nitrogen (NHN) – was calculated as TN-NH_4-N–DON–EHN-WHN.

The content of carbon in extracts was analyzed with the Tiurin method after evaporation of a sample, NO_3-N was determined colorimetrically with sodium salicylate, NH_4-N was assayed by distillation and organic nitrogen was tested by the Kjeldahl method in a distilling unit VELP UDK-127. Correlation coefficients between the content of carbon and nitrogen forms and some properties of the soils were calculated using Statistica software.

RESULTS AND DISCUSSION

The surplus of water, which is typical for headwater areas, creates conditions for the development of Histosols. The tickness of soils is determined mainly by the basic microrelief in the vicinity of water seepages and the species composition of plant communities. The Histosols subjected to our investigations were thick up to 90 cm and built from alder-sedge peat, whose rate of decomposition on the von Post's scale (Grosse-Brauckmann 1990) was 3-9 (Table 1). The soils contained 317.3-829.0 g kg^{-1} of SOM, 162.2-459.5 g kg^{-1} TOC and 11.08-33.59 g kg^{-1} TN. The TOC:TN ratio ranged from 12.6:1 to 22.0:1. The soils were acid at pH_{H2O} from 5.6 to 6.5 and pH_{KCl} from 5.0 to 6.1.

It is well known that labile forms of SOM, carbon and nitrogen play an important role in the functioning of natural and modified ecosystems, as well as in some soil-forming processes (e.g. Qualls, Haines 1991, Hayes, Moore 1992, Schulten, Schnitzer 1998, Jonczak 2012). Concentrations and pools of the forms in soils are varied among different ecosystems (Hu et al. 1997). Decaying litterfall in forest and other natural ecosystems, and some organic fertilizers in agroecosystems (Gondek 2007, Kalembasa, Beher 2012) are the most important sources of the components in the environment. The content of dissolved organic carbon (DOC), as well as its leaching intensity is a good indicator of the ecological condition of soils and the direction of ongoing processes as a response to the impact of different environmental and anthropogenic factors (Hu et al. 1997, Chertov, Komarov 1997, Dawson et al. 2008, Remeš, Kulhavý 2009, Jonczak, Parzych 2012). The investigated Histosols were rich in SOM, acting as substrate for labile forms of carbon, but the constant water surplus did not favour its mineralization. Finally, the observed concentrations of EHC were rather low, ranging from 27.3 to 62.2 g kg^{-1} (Table 2). EHC accounted 6.4-17.2% of TOC. In general, the highest concentration as well as the contribution of this form in TOC occurred in the 0-30 cm layer, while the lowest ones were in layers lying on the mineral bed. The observed vertical distribution of EHC is an effect of the varied biological activity in soil profiles (which peaked in bog horizons) and the intensive leaching of labile forms from the zones with a constant flow of groundwater. The content of WHC ranged from 17.1 to 41.7 g kg^{-1} (4.5-10.6% of TOC). NHC was the major fraction of carbon in the investigated soils, with concentrations from 117.3 to 399.7 g kg^{-1} and contribution in TOC equal

Table 1

Selected properties of the soils

Depth (cm)	H von Post	SOM (g kg^{-1})	TOC (g kg^{-1})	TN (g kg^{-1})	TOC:TN	pH_{H_2O}	pH_{KCl}
Profile 1							
0-10	9	725.0	421.9	33.0	12.8	6.1	5.5
10-20	9	741.0	423.4	33.6	12.6	6.0	5.4
20-30	9	753.0	443.5	32.4	13.7	5.8	5.3
30-40	5	705.9	438.9	30.1	14.6	5.8	5.3
40-50	5	611.8	384.2	26.3	14.6	5.9	5.4
50-60	6	538.0	325.7	21.8	15.0	5.6	5.3
Profile 2							
0-10	9	810.8	436.6	32.9	13.3	6.5	6.0
10-20	5	803.8	422.2	32.0	13.2	6.4	5.8
20-30	3	813.5	459.5	30.5	15.1	6.5	6.1
30-40	7	795.4	448.1	29.7	15.1	6.5	6.0
40-50	6	721.6	451.2	30.8	14.7	6.4	5.9
50-60	9	633.3	379.1	25.9	14.6	6.5	6.0
60-70	6	678.3	458.7	27.8	16.5	5.9	5.6
70-80	5	620.1	427.8	19.5	22.0	5.6	5.3
80-90	4	634.6	425.3	21.5	19.8	5.6	5.4
Profile 3							
0-10	9	805.2	443.7	29.7	15.1	5.9	5.2
10-20	8	829.0	447.8	29.3	15.2	5.6	5.0
20-30	8	794.6	442.4	33.2	13.2	5.6	5.1
30-40	8	721.8	411.7	28.6	14.4	5.9	5.2
40-50	9	734.7	389.2	29.3	13.3	5.8	5.2
50-60	8	735.6	394.2	31.1	12.7	5.8	5.4
60-70	7	588.6	356.8	19.8	17.6	5.9	5.5
70-80	7	413.7	240.6	13.1	17.6	6.0	5.6
80-90	9	317.3	162.2	11.1	13.5	5.9	5.5

72.3-89.2% (Table 2). The concentrations of EHC, WHC and NHC were closely related to the content of SOM (Table 3).

Almost all of the nitrogen contained in soils is closely associated with SOM. It is present in soils as a component of different organic compounds and in mineral forms - ammonium, nitrates and nitrites. Mineral nitrogen usually constitutes a fraction of percent in total nitrogen, but as a bioavailable form of the element, it plays the key role in the functioning of plant

Table 2

The content of carbon forms in the soils and their contribution in TOC

Depth (cm)	Content (g kg^{-1})			Contribution in TOC (%)		
	EHC	WHC	NHC	EHC	WHC	NHC
Profile 1						
0-10	60.5	35.2	326.2	14.3	8.3	77.4
10-20	62.2	30.3	330.9	14.7	7.2	78.1
20-30	49.7	34.7	359.1	11.2	7.8	81.0
30-40	41.3	34.4	363.2	9.4	7.8	82.8
40-50	33.6	22.4	328.2	8.8	5.8	85.4
50-60	33.5	22.0	270.2	10.3	6.8	82.9
Profile 2						
0-10	50.5	36.2	349.9	11.6	8.3	80.1
10-20	50.6	31.8	339.8	12.0	7.5	80.5
20-30	51.4	33.1	375.0	11.2	7.2	81.6
30-40	43.0	34.7	370.4	9.6	7.7	82.7
40-50	40.9	31.2	379.2	9.1	6.9	84.0
50-60	33.2	29.0	316.9	8.8	7.6	83.6
60-70	31.5	27.5	399.7	6.9	6.0	87.1
70-80	27.3	19.1	381.5	6.4	4.5	89.2
80-90	27.8	20.4	377.1	6.5	4.8	88.7
Profile 3						
0-10	50.5	36.5	356.7	11.4	8.2	80.4
10-20	50.6	38.7	358.5	11.3	8.6	80.1
20-30	51.4	37.9	353.1	11.6	8.6	79.8
30-40	43.0	34.3	334.5	10.4	8.3	81.2
40-50	40.9	35.1	313.2	10.5	9.0	80.5
50-60	33.2	41.7	319.2	8.4	10.6	81.0
60-70	31.5	28.7	296.5	8.8	8.1	83.1
70-80	27.3	22.9	190.4	11.3	9.5	79.1
80-90	27.8	17.1	117.3	17.2	10.6	72.3

communities and soil microorganisms. It is also a limiting factor in this respect. Mineral forms of nitrogen, especially NO_3-N, are highly mobile in the environment, which is a frequent reason of water pollution in agroecosystems. A lower rate of the mineralization of organic nitrogen due to water excess, uptake by plant roots and microorganisms as well as intensive leaching is the most decisive factor causing a very low concentration of NO_3-N in the studied soils, despite the high content of TN. The content of the form ranged from 0.003 to 0.012 g kg^{-1} (0.01-0.04% in TN). Low concentrations

Table 3

Correlation coefficients between the content (g kg^{-1}) of carbon and nitrogen forms and some properties of the soils (in bold correlations statistically significant at $p < 0.05$)

Specification	EHC	WHC	NHC	NO$_3$-N	NH$_4$-N	DON	EHN	WHN	NHN
SOM (%)	**0.727**	**0.813**	**0.810**	0.263	**0.535**	0.258	**0.671**	**0.713**	**0.860**
TOC (%)	**0.529**	**0.586**	**0.979**	0.260	0.388	0.204	**0.547**	**0.554**	**0.875**
TN (%)	**0.794**	**0.810**	**0.706**	0.299	**0.673**	0.344	**0.843**	**0.868**	**0.801**
TOC:TN	**-0.611**	**-0.584**	0.146	-0.033	**-0.570**	-0.219	**-0.671**	**-0.714**	-0.185
pH$_{H2O}$	0.272	0.190	0.088	0.294	0.280	0.288	0.215	0.303	0.169
H	0.292	0.290	0.297	0.276	0.328	0.188	**0.475**	**0.505**	0.225

Table 4

The content of nitrogen forms in the soils and their contribution in TN

Depth (cm)	Content (g kg^{-1})						Contribution in TN (%)					
	NO$_3$-N	NH$_4$-N	DON	EHN	WHN	NHN	NO$_3$-N	NH$_4$-N	DON	EHN	WHN	NHN
Profile 1												
0-10	0.011	0.337	0.394	8.02	11.31	12.91	0.03	1.02	1.20	24.32	34.30	39.13
10-20	0.008	0.246	0.267	7.94	11.28	13.85	0.02	0.73	0.80	23.63	33.59	41.23
20-30	0.007	0.182	0.120	5.88	14.17	12.05	0.02	0.56	0.37	18.14	43.72	37.18
30-40	0.005	0.174	0.029	5.34	8.62	15.95	0.02	0.58	0.10	17.73	28.63	52.96
40-50	0.004	0.099	0.093	3.74	9.15	13.20	0.02	0.38	0.35	14.24	34.81	50.21
50-60	0.004	0.091	0.056	3.72	7.16	10.72	0.02	0.42	0.26	17.09	32.93	49.29
Profile 2												
0-10	0.012	0.305	0.269	5.25	11.81	15.29	0.04	0.93	0.82	15.93	35.87	46.42
10-20	0.008	0.189	0.190	5.06	9.69	16.81	0.03	0.59	0.60	15.82	30.34	52.63
20-30	0.005	0.149	0.127	4.44	9.42	16.37	0.02	0.49	0.42	14.55	30.88	53.65
30-40	0.004	0.104	0.085	3.97	9.23	16.35	0.02	0.35	0.29	13.34	31.04	54.97
40-50	0.006	0.110	0.105	4.22	9.28	17.06	0.02	0.36	0.34	13.72	30.14	55.42
50-60	0.007	0.081	0.099	3.69	8.20	13.87	0.03	0.31	0.38	14.22	31.61	53.46
60-70	0.004	0.085	0.167	2.99	7.42	17.13	0.02	0.30	0.60	10.76	26.68	61.64
70-80	0.008	0.043	0.149	1.63	4.60	13.06	0.04	0.22	0.76	8.35	23.63	67.00
80-90	0.008	0.067	0.112	1.73	4.57	15.02	0.04	0.31	0.52	8.06	21.23	69.84
Profile 3												
0-10	0.007	0.241	0.182	5.19	8.55	15.57	0.02	0.81	0.61	17.46	28.74	52.35
10-20	0.003	0.073	0.100	4.68	9.28	15.18	0.01	0.25	0.34	15.97	31.65	51.78
20-30	0.004	0.201	0.075	4.78	9.24	18.89	0.01	0.61	0.23	14.40	27.83	56.93
30-40	0.008	0.169	0.148	4.39	9.05	14.81	0.03	0.59	0.52	15.37	31.67	51.82
40-50	0.005	0.102	0.105	4.43	9.24	15.38	0.02	0.35	0.36	15.13	31.58	52.56
50-60	0.007	0.120	0.125	3.63	10.56	16.70	0.02	0.38	0.40	11.65	33.91	53.63
60-70	0.006	0.094	0.098	1.90	6.52	11.15	0.03	0.48	0.49	9.62	33.00	56.39
70-80	0.006	0.069	0.141	1.23	4.95	6.66	0.04	0.53	1.08	9.45	37.92	50.98
80-90	0.003	0.056	0.083	1.16	4.28	5.50	0.03	0.51	0.75	10.50	38.58	49.63

of the form of nitrogen were also observed by JONCZAK (2012) in the soils of spring niches in the Jarosławianka Creek valley (0.003-0.018 g kg^{-1}), and is probably a characteristic feature of soils of headwater areas. Much higher

Table 5

The values of EHC:EHN, WHC:WHN and NHC:NHN ratios

Depth (cm)	EHC:EHN	WHC:WHN	NHC:NHN
Profile 1			
0-10	6.9	3.1	25.3
10-20	7.4	2.7	23.9
20-30	8.0	2.4	29.8
30-40	7.4	4.0	22.8
40-50	8.5	2.4	24.9
50-60	8.7	3.1	25.2
Profile 2			
0-10	8.7	3.1	22.9
10-20	9.3	3.3	20.2
20-30	10.9	3.5	22.9
30-40	10.3	3.8	22.6
40-50	9.2	3.4	22.2
50-60	8.6	3.5	22.9
60-70	9.7	3.7	23.3
70-80	14.9	4.1	29.2
80-90	14.5	4.5	25.1
Profile 3			
0-10	9.9	4.3	22.9
10-20	10.1	4.2	23.6
20-30	9.4	4.1	18.7
30-40	9.1	3.8	22.6
40-50	9.1	3.8	20.4
50-60	9.0	4.0	19.1
60-70	10.8	4.4	26.6
70-80	11.7	4.6	28.6
80-90	11.4	4.0	21.3

concentrations were noticed by Kalembasa and Becher (2009) in peat-muck soils in the Liviec River valley (E Poland) – 0.053-0.090 g·kg^{-1}.

The content of NH_4-N in the studied soils was 0.043-0.337 g kg^{-1} (0.22-1.02% in TN) – Table 4. Both the content of NH_4-N and its contribution in TN were the highest in the surface horizons of the soils (0-10 cm) and decreased with depth. The observed concentrations of the form were higher than noticed by Kalembasa and Becher (2009), who noticed 0.137-0.200 g kg^{-1} in M_t horizons and 0.095-0.158 g kg^{-1} in O_{tni} horizons of peat-muck soils. DON is the most labile form, being also most susceptible to mineralization of organic nitrogen. In the analyzed soils, DON contributed 0.10-1.20% in TN, and its concentration ranged from 0.029 to 0.394 g kg^{-1}. The highest content of the form was observed in the surface layer.

The contribution of hydrolyzable forms of nitrogen to TN was 29.29-61.86%. WHN predominated in the pool, with the content 1.4-4.0-fold higher than EHN. The content of hydrolyzable forms of nitrogen decreased with depth. The content of EHN and WHN was significantly positively related to the content of SOM, TOC, TN and degree of the decomposition of peat mass, being negatively correlated to the TOC:TN ratio (Table 3). NHN contributed 37.18-69.84% to TN, with concentrations of 5.50-18.89 g kg^{-1}. The observed dominance of hydrolyzable and nonhydrolyzable forms in the pool of TN is consistent with the data of other authors (HERSEMANN 1987, SHARPLEY, SMITH 1995, SULCE et al 1996 KALEMBASA, BECHER 2009, BECHER, KALEMBASA 2011). A specific feature of the investigated Histosols is a high dominance of nonhydrolyzable forms over hydrolyzable ones, which can be a result of intensive leaching of labile forms of elements by the permanent flow of groundwater.

The significant differences between EHC:EHN, WHC:WHN and NHC: NHN suggest variability in the content of nitrogen in fractions of organic compounds characterized by different susceptibility to hydrolysis. The lowest C:N ratios were observed in weakly hydrolyzable fractions (2.4:1-4.6:1), whereas the highest ones appeared in nonhydrolyzable ones (18.7:1-29.8:1) – Table 5. The lowest ratios of C:N in weakly hydrolyzable fractions can be to some extent a result of the hydrolysis of bodies of microorganisms, which are relatively resistant to hydrolysis and rich in nitrogen, hence being a significant source of soil nitrogen.

CONCLUSIONS

The results of our studies show that specific environmental conditions of headwater areas have a strong impact on associated organic soils, their properties and vertical distribution of the forms of carbon and nitrogen. The investigated Histosols were rich in SOM (317.3-829.0 g kg^{-1}) and TOC (162.2-459.5 g kg^{-1}). NHC dominated in the pool, with the contribution from 72.3 to 89.2% in TOC. EHC accounted for 6.4-17.2% of TOC (27.3-62.2 g kg^{-1}). The content of TN was 11.08-33.59 g kg^{-1}. Mineral nitrogen constituted 0.26-1.05% of TN and NH_4-N dominated in the pool with concentrations of 0.043-0.337 g kg^{-1} and the contribution in mineral nitrogen from 84.55 to 98.27%. In general, the observed concentrations of NO_3-N were lower and those of NH_4-N higher than observed in organic soils by other authors. Hydrolyzable or nonhydrolyzable forms of nitrogen were major components of TN with the contributions 29.29-61.86% and 37.18-69.84%, respectively, which is consistent with the data of other authors. WHN predominated in the pool of hydrolyzable nitrogen, and its content was 1.4-4.0-fold higher than EHN. Quantitative proportions between hydrolyzable and nonhydrolyzable forms of nitrogen varied vertically. An increase in the content of nonhydrolyzable and a decrease in hydrolyzable forms occyrred with depth. The vertical distribution of nitrogen forms (probably typical for the soils of headwater areas) is an effect of the vertically varied intensity of biochemical processes (maximum one in bog horizons) and intensive leaching of the soils over the mineral bed caused by groundwater. The content of EHC, WHC, NHC, NH_4-N, EHN, WHN and NHN in most cases was significantly positively related to the content of SOM, TOC, TN, but negatively to the TOC:TN ratio.

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CONTENT OF MACRO- AND MICROELEMENTS IN GOAT MILK IN RELATION TO THE LACTATION STAGE AND REGION OF PRODUCTION*

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Abstract

The content of macro- and microelements in milk depends on a variety of environmental, genetic and physiological factors. The aim of the study was to determine the effect of the region of production and stage of lactation, associated with the production season, on the content of selected macro- and microelements, including potentially toxic elements, in goat milk. The material consisted of goat milk samples collected from farms in two regions of Poland: 74 samples from central-eastern Poland (the Provinces of Lublin and Świętokrzyskie) and 149 from southern Poland (the Bieszczady mountains). The milk was collected in three seasons: winter (75 samples), summer (111) and autumn-winter (37). In each milk sample, the percentage of casein, protein, fat, lactose and dry matter was determined, as well as the concentrations of K, Ca, Na, Mg, Zn, Fe, Cu and Mn. K, Ca, Na, Mg and Zn using a Varian 240FS AA spectrometer, by the flame atomic absorption technique, while Fe, Cu and Mn were determined in a graphite furnace with the Zeeman background correction, using a Varian 240Z AA spectrometer. The goats' production peak was in the summer, when they were at pasture. From mid-lactation they produced milk with significantly ($p \leq 0.01$) higher concentration of components. Milk obtained in stage I of lactation (winter feeding) was the richest source of Zn, Fe and Cu, while stage II milk (summer feeding) had the highest K content, and stage III milk (autumn-winter feeding) had the highest content of Ca, Na, Mg and Mn. Concentration of Zn, Fe and Cu decreased over the course of lactation. Milk obtained in the Bieszczady mountain region had significantly ($p \leq 0.01$) higher content of dry matter, fat and protein. It also contained significantly ($p \leq 0.01$) more Ca, Na, and Mg, and less K and Zn in comparison with the milk of the goats raised in central-eastern Poland. The highest positive correlation coefficients were noted between the content of Ca and Mg, Zn and Fe, Zn and Cu, Na and Mg, and Fe and Cu, while K content was negatively correlated with that of Na, Ca and Mg ($p \leq 0,001$).

Key words: goat milk, macro- and microelements, lactation stage, production season, region of production.

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INTRODUCTION

Milk is a food of particular interest, both in terms of its nutritional value and its effect on health. Its high calcium content is the main argument put forward in milk promotion campaigns for maintaining the privileged position of milk in the daily diet (Hozyasz, Słowik 2013).

In Poland, most available milk is obtained from cows, but some small quantities originate from goats. Barłowska et al. (2013) demonstrated that goat milk is a more valuable source of calcium, potassium, iron, copper and manganese than cow milk. Studies by other authors confirm this observation, having detected more magnesium and zinc than in cow milk (Belewu, Aiyegbusi 2002, Soliman 2005, Park et al. 2007, Ceballos et al. 2009, Zamberlin et al. 2012). Thus goat milk can be an alternative source of calcium as well as other elements.

Nevertheless, the chemical composition of milk, including the content of macro- and microelements, is not constant. It depends on a variety of environmental, genetic and physiological factors (Danków, Pikul 2011).

The aim of the study was to determine the effect of the region of production and the stage of lactation (associated with the production season) on the content of selected macro- and microelements in goat milk.

MATERIAL AND METHODS

The material for the study consisted of 223 goat milk samples collected from two regions of Poland: 74 samples from central-eastern Poland (the Provinces of Lubelskie and Świętokrzyskie) and 149 from southern Poland (the Bieszczady mountains). The milk was collected during three production seasons, which were closely linked to the stage of lactation: winter – the beginning of lactation (75 samples), summer – the peak of lactation (111) and autumn-winter – the end of lactation (37).

The milk was transported in thermal bags with freezer packs to the laboratory of the *Department of Commodity Science and Processing of Raw Animal Materials*, University of Life Sciences in Lublin (AOAC 2000*b*).

The chemical composition of the milk was evaluated in an Infrared Milk Analyzer. The percentages of fat, protein, lactose and dry matter were determined. The casein content was measured by the Walker method according to AOAC (2000a). The concentration of selected macro- and microelements was determined by atomic absorption spectrometry. 1 ml of milk was poured into each polytetrafluoroethylene flask together with 6 ml suprapure nitric acid (65%). The solutions were mineralized under increased pressure in a CEM MARS 5 Xpress microwave digester. Then, the mineralized samples were quantitatively transferred using deionized water into 25 cm^3 volumetric flasks.

For the determination of Ca, Mg, K and Na, buffer was added to the samples according to Schinkel (10 g L^{-1} CsCl + 100 g L-1 La), so that the final solution contained 1% of the buffer. The concentrations of K, Ca, Na, Mg and Zn were determined on a Varian 240FS AA spectrometer, using the flame atomic absorption technique (air-acetylene flame). Fe, Cu and Mn were determined in a graphite furnace with the Zeeman background correction (in argon atmosphere) using a Varian 240Z AA spectrometer. Parallel to the experimental samples, the certified reference material NCS ZC 73015 Milk Powder and blank samples were analysed. The recovery of certified reference material for the analyzed elements ranged from 95 to 105%. Standard deviation between replicate measurements was no more than 5%.

The results were analysed statistically using Statistica ver. 6 (StatSoft Inc. 2003). Significance of differences between means was determined using the Tukey's test, at p (α) = 0.05 and $p = 0.01$. The Pearson's correlation coefficients between concentrations of macro- and microelements were calculated as well.

RESULTS AND DISCUSSION

In goats, as in sheep, the sexual activity is seasonal. Hence, the stage of lactation is closely linked to the season of production. Lactation usually begins in the first months of the year, that is during the winter feeding period, when goats are fed on roughage. After they are turned out to pasture, daily milk yield increases, and later (in the autumn months) it decreases steadily until the dry period. Table 1 shows that in the early lactation period (early spring – winter feeding) the mean daily yield of goats was 1.44 kg of milk. The peak production (2.04 kg) was noted in the summer, when goats were at pasture, but it fell to 1.16 kg per day near the end of lactation (autumn-winter feeding). From mid-lactation, goats produced milk with significantly ($p \leq 0.01$) higher concentrations of milk components. The lowest levels of these components were noted in the milk from the initial lactation period. A similar relationship was observed by Králíčková et al. (2012), who found that daily milk yield decreased from the beginning to the end of lactation, while the content of dry matter, protein, fat and casein increased from day 190 to the end of lactation.Strzałkowska et al. (2009) compared daily milk yield and the basic chemical composition of milk from Polish White Improved goats during three stages of lactation (day 60, 120 and 200). The highest milk yield was noted on day 120 of lactation (1.38 kg), with the lowest content of fat (3.38%) and dry matter (11.83%). The lowest protein content (2.98%), however, was observed on day 60 after parturition. Mestawet et al. (2012), in contrast, found that the concentration of dry matter, fat and protein in goat milk was significantly higher ($p < 0.001$) in the early and late stages of lactation than in the middle one.

Analysis of the effect of the production region on production parameters among goats revealed significant differences ($p \leq 0.01$) only in the content of dry matter, fat and protein, with higher levels noted in the milk from the Bieszczady mountain region (Table 1).

Table 1

Daily milk yield and basic chemical composition of the goat milk ($\overline{x}$, SD)

Specification	Stage of lactation (feeding period)			Region of production	
	stage I (winter-feeding)	stage II (summer-feeding)	stage III (autumn-winter feeding)	the southern-Poland (the Bieszczady mountains)	the central-eastern Poland (Lubelskie and Świętokrzyskie Provinces)
n	75	111	37	149	74
Daily yield of milk (kg)	1.44^{A} 0.90	2.04^{B} 1.04	1.16^{A} 0.88	1.66 1.10	1.77 0.88
Casein (%)	2.03^{A} 0.61	2.48^{B} 0.27	2.54^{B} 0.34	2.30 0.55	2.42 0.25
Protein (%)	2.85^{A} 0.62	3.12^{B} 0.45	3.14^{B} 0.40	3.17^{Y} 0.46	2.77^{X} 0.53
Fat (%)	3.43^{A} 0.89	3.70^{B} 0.61	3.79^{B} 0.48	3.82^{Y} 0.70	3.22^{X} 0.54
Lactose (%)	4.48^{B} 0.42	4.33^{A} 0.30	4.51^{B} 0.50	4.42 0.38	4.39 0.39
Drymater (%)	11.46^{A} 1.35	11.86^{B} 1.15	12.15^{B} 1.12	12.12^{Y} 1.25	11.08^{X} 0.85

A, *B* – differences between stage of lactation (feeding period) significant at $p \leq 0.01$;
a, *b* – significant at $p \leq 0.05$; *X*, *Y* – differences between production regions significant at $p \leq 0.01$;
x, *y* – significant at $p \leq 0.05$

Concentrations of most of the tested elements were found to vary over the course of lactation, which was linked to the season of production (Table 2). Milk collected during lactation stage II was the richest source of potassium (1938.8 mg L^{-1}), while milk from the final stage contained most calcium, sodium and magnesium. The lowest concentrations of these elements were noted in milk collected during stage II of lactation (the production peak), which was confirmed statistically ($p \leq 0.01$).

Zinc, copper and iron decreased over the course of lactation. The difference between lactation stages I and III was 0.92 mg L^{-1} and 0.065 mg L^{-1} ($p \leq 0.01$) for zinc and copper, respectively, and 0.136 mg L^{-1} for iron, although in the case of iron the difference was statistically insignificant, probably due to its substantial variability. A reverse tendency was noted in the case of manganese, whose concentration was the lowest at the beginning

Table 2

Content of selected mineral elements in goat milk ($\overline{x}$, SD)

Specification	Stage of lactation (feeding period)			Region of production	
	stage I (winter-feeding)	stage II (summer-feeding)	stage III (autumn-winter feeding)	the southern Poland (Bieszczady mountains)	the central eastern Poland (Lubelskie and Świętokrzyskie Provinces)
n	75	111	37	149	74
K (mg L^{-1})	1845.2^{a} 345.8	1938.8^{b} 241.5	1769.1^{a} 225.2	1835.1^{X} 252.3	1968.0^{Y} 325.3
Ca (mg L^{-1})	1190.5^{B} 460.5	1049.8^{A} 199.4	1241.3^{B} 337.7	1182.0^{Y} 367.3	1022.0^{X} 245.1
Na (mg L^{-1})	346.0^{Ab} 187.8	294.1^{Aa} 60.1	464.5^{Bc} 190.8	366.9^{Y} 156.3	285.4^{X} 127.3
Mg (mg L^{-1})	121.0^{Ab} 32.0	112.5^{Aa} 17.3	169.4^{B} 33.9	132.6^{Y} 35.4	109.1^{X} 19.8
Zn (mg L^{-1})	3.32^{B} 1.58	2.73^{A} 1.09	2.40^{A} 1.18	2.56^{X} 0.99	3.50^{Y} 1.67
Fe (mg L^{-1})	0.701 0.352	0.642 0.345	0.565 0.341	0.641 0.335	0.666 0.376
Cu (mg L^{-1})	0.161^{B} 0.095	0.113^{A} 0.061	0.096^{A} 0.044	0.124 0.063	0.131 0.96
Mn (mg L^{-1})	0.056^{A} 0.018	0.086^{B} 0.044	0.125^{C} 0.058	0.085 0.051	0.078 0.035

A, *B* – differences between lactation stage (feeding period) significant at $p \leq 0.01$;
a, *b*, *c* – significant at $p \leq 0.05$; *X*, *Y* – differences between production region significant at $p \leq 0.01$;
x, *y* – significant at $p \leq 0.05$

of lactation (0.056 mg L^{-1}), but more than twice as high at the final stage of lactation – 0.125 mg L^{-1} ($p \leq 0.01$).

The results of our study are confirmed by MESTAWET et al. (2012), who report that the Ca concentration in goat milk was significantly higher ($p < 0.001$) in the first and last stages of lactation than in the middle stage. The Na and Mg content was the highest in the final stage, while Zn decreased over the course of lactation.TRANCOSO et al. (2009) evaluated the content of macro- and microelements in goat milk over the course of lactation from November to May, but did not observe any clear trends. The milk collected in December (second month of lactation) had the highest manganese content, while the milk from January (third month) was the richest in sodium, calcium, magnesium and iron. The milk with the highest concentration of heavy metals, i.e. zinc and copper, was collected in November. Only zinc was found to be decreasing over the course of lactation, which is confirmed by the present study. A study by AL-WABEL et al. (2008) on milk collected from goats raised in the dry climate of Saudi Arabia, determined the concentrations of Ca (751.7 mg kg^{-1}), K (123.85 mg kg^{-1}) and Na (101.3 mg kg^{-1}) in the second month of lactation, hence much lower than the ones noted in the present study, and a higher content of Fe (4.908 mg kg^{-1}), Mn (1.129 mg kg^{-1}) and Cu (0.570 mg kg^{-1}). Barłowska et al. (2013) report that the production season had a significant effect on most of the elements tested. The milk produced by goats contained more calcium, sodium, magnesium and copper in the autumn-winter season, and higher levels of potassium in the spring-summer season. An Italian study by Coni et al. (1996) found that goat milk collected in the summer had a higher content of copper, iron and magnesium, while winter milk had higher levels of manganese and zinc. Kondyli et al. (2007) also observed seasonal changes in the most important macroelements, i.e. Ca, P and K, and microelements, i.e. Cu and Zn.

The data in Table 2 show that the milk of goats raised in the Bieszczadys contained significantly ($p \leq 0.01$) more calcium, sodium, and magnesium, while the milk from central-eastern Poland had a higher concentration of potassium. Higher concentrations of the heavy metals tested, i.e. zinc and copper, were noted in the milk from the central-eastern region of Poland (3.50 mg L^{-1} and 0.131 mg L^{-1}, respectively), in comparison with the Bieszczady mountains (2.56 mg L^{-1} and 0.124 mg L^{-1}). However, the differences were statistically significant ($p \leq 0.01$) only in the case of Zn. Dobrzański et al. (2009) found a substantially higher copper concentration in goat milk than in the present study, both in industrial regions (0.672 mg L^{-1}), and in regions regarded as unpolluted (0.249 mg L^{-1}). Król et al. (2012), in a study on cow milk, also found that the season and region of production had a significant effect on content of Zn, Cu, Mn, Cd and Pb, but not Fe.

The correlation coefficients presented in Table 3 between concentrations of macro- and microelements show a positive correlation between calcium and magnesium (r = 0.65***), calcium and copper (r = 0.22***), zinc and iron (r = 0.42***), zinc and copper (r = 0.46***), sodium and magnesium (r = 0.41***) and iron and copper (r = 0.48***). The potassium content in

Table 3

Correlation coefficients between content of macro- and microelements in the goat milk (mg L^{-1})

Elements	K	Ca	Na	Mg	Zn	Fe	Mn
Ca	-0.36***	-					
Na	-0.52***	0.05	-				
Mg	-0.32***	0.65***	0.41***	-			
Zn	0.13*	0.20**	-0.06	0.11	-		
Fe	-0.10	0.15*	0.03	0.12	0.42***	-	
Mn	0.06	-0.02	0.11	0.20**	-0.04	-0.11	-
Cu	-0.05	0.22***	0.01	0.07	0.46***	0.48***	-0.21***

* values significant at $p \leq 0.05$; ** at $p \leq 0.01$; *** at $p \leq 0.001$

goat milk, on the other hand, was negatively correlated with that of sodium (r = -0.52***), calcium (r = -0.36***) and magnesium (r = -0.32***), and the manganese content - with that of copper (r = -0.21***). Pilarczyk et al. (2013) evaluated milk of Holstein-Friesian and Simmental cows, obtaining high correlation coefficients between the content of magnesium and calcium (r = 0.62** and r = 0.89***) and between zinc and copper (r = 0.46* and r = 0.57**), which is confirmed by the present study. Sikirić et al. (2003) report higher correlation coefficients between Cu and Fe (r = 0.613), Cu and Zn (r = 0.629) and Cu and Ca (r = 0.629) than those observed in the present study.

CONCLUSIONS

1. The highest milk yield was observed during the summer season, when the goats were at pasture. From mid-lactation, the concentration of milk components increased significantly ($p \leq 0.01$). The milk from the Bieszczady region had a significantly ($p \leq 0.01$) higher content of dry matter, fat and protein compared with the Lubelskie and Świętokrzyskie Provinces.
2. Milk obtained from goats in stage I of lactation (winter feeding) was the richest source of Zn, Fe and Cu, while milk from stage II (summer feeding) was the highest in K, and milk from stage III (autumn-winter feeding) was the highest in Ca, Na, Mg and Mn. Concentrations of zinc, iron, and copper decreased over the course of lactation.
3. Milk obtained from goats raised in the Bieszczady mountain region contained significantly ($p \leq 0.01$) more Ca, Na and Mg, and less K and Zn than from the goats kept in central-eastern Poland.
4. The highest positive correlation coefficients were noted between the content of Ca and Mg, Zn and Fe, Zn and Cu, Na and Mg, and Fe and Cu, whereas the K content was negatively correlated with that of Na, Ca and Mg.

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EFFECT OF MANGANESE ON NUTRIENT CONTENT IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) LEAVES

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Abstract

Manganese (Mn) is a microelement, but it is also a heavy metal whose excess may have a toxic effect on plants. The aim of the study was to evaluate the effect of an application of increasing Mn concentrations added to a nutrient solution on the content of macro- and micronutrients in tomato leaves (*Lycopersicon esculentum* Mill., cv. Alboney F_1 and Emotion F_1). Plants were grown in rockwool using a nutrient solution with the following content of manganese (mg dm^{-3}): 0.06; 0.3; 0.6; 1.2 (experiment I, in 2008-2011); 2.4, 4.8; 9.6; 19.2 mg dm^{-3} (experiment II, in 2012) – designated the symbols Mn-0; Mn-0.3; Mn-0.6; Mn-1.2; Mn-2.4; Mn-4.8; Mn-9.6; Mn-19.2. The nutrient solution used for plant fertigation had the following chemical composition (mg dm^{-3}): N-NH_4 2.2, N-NO_3 230, P 50, K 430, Ca 145, Mg 65, Cl 35, S-SO_4 120, Fe 2.48, Zn 0.50, Cu 0.07, pH 5.50, EC 3.00 mS cm^{-1}. Manganese significantly influenced the content of other macro- and microelements in leaves. In variant Mn-0, the content of N, P, K, Ca, Mg, Mn decreased, while that of of Fe, Zn, Cu was higher; in Mn-1.2, the content of N, Mg, Fe, Zn decreased and that of P, K, Ca, Mn increased compared with the variants which ensured optimal yielding. In the range of manganese nutrition from Mn-4.8 to Mn-19.2, N, K, Mg, Fe, Zn, Cu were lower and the content of P and Mn was higher (above optimal). The cultivar significantly modified the nutrient status of plants concerning nitrogen (for Mn-9.6 and Mn-19.2), phosphorus (for Mn-1.2 and Mn-2.4), potassium (for Mn-4.8 and Mn-9.6), calcium (for Mn-0, Mn-1.2, Mn-9.6), magnesium (Mn-0 and Mn-0.6), iron (Mn-0, Mn-0.3, Mn-9.6), manganese (Mn-0.3, Mn-1.2, Mn-2.4, Mn-19.2), iron (Mn-0, Mn-0.3, Mn-9.6), zinc (Mn-0.6, Mn-1.2, Mn-2.4, Mn-9.6) and copper (Mn-2.4, Mn-4.8, Mn-9.6, Mn-19.2). Briefly, both deficit or excess manganese nutrition could induce disorders in the uptake of other nutrients, which may influence plant yielding.

Key words: manganese, index parts, hydroponic, nutrient status.

INTRODUCTION

Manganese (Mn) is a metallic microelement serving several physiological functions in plants (Lidon et al. 2004, Ducic, Polle 2005, Humphries

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et al. 2007). This nutrient may have both a synergistic and antagonistic effect on the uptake of other nutrients, e.g. iron, zinc and copper. Typically, competition is found between ions of a similar diameter, such as manganese (0.075 nm) and iron (0.065 nm) or calcium (0.099 nm) (MARSCHNER 1998). When present in prevailing amounts, manganese may be a strong antagonist towards many nutrients. The same author claimed that the range of optimal manganese concentrations is very narrow, in which it resembles other microelements. According to HORST (1988), the natural defence process in plant organisms against stress evoked by this element involves accumulation of Mn in cell sites which are physiologically rather inactive. SHENKER et al. (2004) found a significant reduction of zinc translocation in plants under the influence of manganese. Very often manganese leads to symptoms of deficits of other nutrients, i.e. calcium, magnesium and iron (LEE 1972, HORST, MARSCHNER 1978*a*, FOY et al. 1981, FLEMING 1989). KASRAEI et al. (1996) indicated that within a certain nutritional range manganese may have a negative effect on the uptake of potassium and sodium as well as phosphorus. GALVEZ et al. (1989) indicated that a higher level of manganese in a nutrient solution depresses the uptake of potassium, calcium, magnesium, zinc, copper and silicon, causing a simultaneous increase in phosphorus. Excess manganese may reduce the magnesium uptake by as much as 50% (KAZDA, ZNACEK 1989). At the same time, SAVVAS et al. (2009) stated the lack of any effect of manganese on the content of potassium, calcium and magnesium, at a simultaneous reduction of the iron and zinc content. In the Chinese cabbage culture exposed to the influence of manganese LEE et al. (2011) observed a reduced content of magnesium and calcium as well as absence of significant changes in the potassium content in outer leaves; in inner leaves there was an increase in the nitrogen and phosphorus content accompanied by a decrease in potassium. CLARK (1982) reported that intensive nutrition of plants with manganese leads to a decrease in the content of potassium, calcium, magnesium, zinc and silicon together with a simultaneous increase in phosphorus. LANDI, FAGIOLI (1983) showed no effect of manganese on copper uptake by maize roots. KOZIK et al. (2008) stated that an increasing content of manganese in the substrate had a significant effect on the zinc nutrient status in lettuce causing its deterioration, but no such an effect on iron and copper. In certain conditions, some ions (e.g. silicon, iron, calcium, magnesium) may reduce toxicity of manganese to plants (HEENAN, CARTER 1975, HORST, MARSCHNER 1978b, LOHNIS 1960, OSAWA, IKEDA 1976).

The aim of this study was to evaluate the effect of increasing manganese concentrations applied in a nutrient solution used for fertigation on the content of macro- and microelements in leaves of tomato.

MATERIAL AND METHODS

In 2008-2012, plant growing experiments were conducted in a greenhouse of the Department of Plant Nutrition, the Poznań University of Life Sciences. The facilities were equipped with a modern, computer-controlled fertigation system and energy-conservation curtains. The detailed methodology was presented earlier (Kleiber 2014). The experiments were conducted on two cultivars of tomato (*Lycopersicon esculentum* Mill.): Alboney F_1 and Emotion F_1, and with 8 levels of manganese nutrition (2 factors: A: manganese nutrition; B: cultivar). Plants were grown in standard rockwool (100 x 15 x 7.5 cm; V 11.25 dm^3; 60 kg m^{-3}) at a stocking density of 2.5 plants m^{-2}. The experiments were set in a completely randomized block design with 4 replications (4 plants were 1 replication). Biological pest control was applied. All cultivation measures were performed in accordance with the current recommendations for tomato growing (Adamicki et al. 2005).

Seeds were sown to cultivation cups in the 1st decade of March in each year of the study. After 2-3 weeks, seedlings were transplanted to rockwool cubes (10×10×10 cm). Transplants were moved to permanent beds in mid -April. The experiment was concluded on 30 September in each year of the study. Following their transplantation to the permanent site, tomato plants were fertigated with a standard nutrient solution of the following chemical composition (in mg dm^{-3}): 2.2 $N\text{-}NH_4$, 230 $N\text{-}NO_3$, 50 P, 430 K, 145 Ca, 65 Mg, 35 Cl, 120 $S\text{-}SO_4$, 2.48 Fe, 0.50 Zn, 0.07 Cu. The pH was 5.50 and EC was 3.00 mS cm^{-1}. The following levels of plant nutrition with manganese were studied: 0.06, 0.3, 0.6, 1.2 mg dm^{-3} (experiment I, in 2008-2011 year), 2.4, 4.8, 9.6, 19.2 mg dm^{-3} (experiment II, in 2012) – denoted respectively as Mn-0, Mn-0.3, Mn-0.6, Mn-1.2, Mn-2.4, Mn-4.8, Mn-9.6 and Mn-19.2. The manganese content in Mn-0 corresponds to the content of this ion in water used to prepare the nutrient solution for plant fertigation. Manganese sulfate ($MnSO_4$ H_2O, 32.3% Mn) was the source of manganese in the other tested combinations. The nutrient solution dose depended on the development phase of plants and climatic conditions. In the period of intensive plant yielding and high temperatures (months June – July), 3.0-3.5 dm^3 nutrient solution per plant were applied daily, in 15–20 single doses at 20–30% outflowing of drainage solution.

In the course of the experiments, samples of index parts (8th-9th fully expanded leaves counting from the apex) were collected at monthly intervals (15.06, 15.07 and 16.08 each of the years of the study). One bulk sample comprised 12 leaves. Collected leaves were dried at 45-50°C and then ground. For assays of total nitrogen, phosphorus, potassium, calcium and magnesium, the plant material was mineralized in concentrated sulfuric acid (IUNG 1972). After mineralization of the plant samples, chemical analyses were performed using the following methods: N-total according to Kjeldahl in a Parnas-Wagner distillation apparatus, P by colorimetry with ammonium molybdate, and K, Ca, Mg by atomic absorption spectrometry (in a Carl Zeiss Jena apparatus). For determinations of total iron, manganese, zinc and copper, the plant material was mineralized in a mixture of dioxonitric and tetraoxochloric acids (3:1 v/v). After mineralization, Fe, Mn, Zn and Cu were determined according to ASA. Results of chemical analyses of leaves to determine their content of macro- and microelements were subjected to analysis of variance, independently for each experiment, using the Duncan test at a significance level of $a = 0.05$.

RESULTS ANS DISCUSSION

In both experiments. a significant influence of manganese nutrition on the nitrogen content in index parts of tomato was found (Table 1). In Experiment I, the lowest content of that macroelement was found in Mn-0 and

Table 1

The influence of manganese nutrition on macroelement content in tomato leaves (express in % in d.m.)

Cultivar	Experiment I				Experiment II			
	Mn-0	Mn-0.3	Mn-0.6	Mn-1.2	Mn-2.4	Mn-4.8	Mn-9.6	Mn-19.2
N								
Alboney F_1	3.52 *ab*	3.85 *b*	3.48 *ab*	3.37 *a*	3.21 *cd*	3.01 *bc*	3.12 *cd*	2.87 *b*
Emotion F_1	3.27 *a*	3.59 *ab*	3.56 *ab*	3.34 *a*	3.27 *d*	3.15 *cd*	2.87 *b*	2.59 *a*
Mean	3.40 *A*	3.72 *B*	3.52 *AB*	3.36 *A*	3.24 *C*	3.08 *B*	3.00 *B*	2.73 *A*
P								
Alboney F_1	0.57 *a*	0.66 *b*	0.73 *bc*	0.93 *d*	1.01 *cd*	0.97 *bc*	0.96 *bc*	1.05 *de*
Emotion F_1	0.49 *a*	0.74 *bc*	0.76 *bc*	0.80 *c*	0.84 *a*	0.91 *ab*	1.04 *c-e*	1.11 *e*
Mean	0.53 *A*	0.70 *B*	0.75 *B*	0.87 *C*	0.93 *A*	0.94 *A*	1.00 *B*	1.08 *C*
K								
Alboney F_1	4.33 *a*	5.12 *b*	5.57 *c*	5.93 *de*	5.48 *e*	5.23 *d*	4.25 *c*	4.14 *a-c*
Emotion F_1	4.56 *a*	4.99 *b*	5.77 *cd*	6.14 *e*	5.42 *de*	4.20 *bc*	3.97 *ab*	3.92 *a*
Mean	4.45 *A*	5.06 *B*	5.67 *C*	6.04 *D*	5.45 *C*	4.72 *B*	4.11 *A*	4.03 *A*
Ca								
Alboney F_1	2.55 *a*	2.94 *bc*	3.32 *ef*	3.44 *f*	3.47 *bc*	3.59 *c*	4.02 *d*	3.45 *a-c*
Emotion F_1	2.83 *b*	3.05 *cd*	3.28 *ef*	3.21 *de*	3.35 *a-c*	3.43 *a-c*	3.25 *ab*	3.19 *a*
Mean	2.69 *A*	3.00 *B*	3.30 *C*	3.33 *C*	3.41 *AB*	3.51 *BC*	3.64 *C*	3.32 *A*
Mg								
Alboney F_1	0.73 *b*	0.79 *b-d*	0.89 *e*	0.77 *b-d*	0.65 *e*	0.61 de	0.55 *cd*	0.46 *ab*
Emotion F_1	0.65 *a*	0.83 *de*	0.80 *cd*	0.73 *bc*	0.64 e	0.62 *de*	0.50 *bc*	0.41 *a*
Mean	0.69 *A*	0.81 *C*	0.85 *C*	0.75 *B*	0.65 C	0.62 *C*	0.53 *B*	0.44 *A*

Key for Tables 1 and 3: within rows, means marked with different capital letters differ significantly (separately for each experiment); within rows and columns, means marked with different small letters differ significantly (separately for each experiment)

Mn-1.2 variants (3.40 and 3.36% N, respectively), while significantly the highest one appeared in Mn-0.3 (3.72 %). Within the studied ranges there were no differences between the cultivars. A decreasing nitrogen tendency was found in Experiment II, including significant differences between the cultivars in variants Mn-9.6 and 19.2. The content of nitrogen in tomato leaves determined by other scientists is varied (Table 2). Nurzyński (2006) and Chohura, Komosa (2003a), who used standard nutrient solution in tomato cultivation on rockwool, found similar concentrations of the aforementioned nutrient; in contrast, Jarosz, Horodko (2004) determined a higher content. In turn, Pawlińska, Komosa (2006) and Jarosz, Dzida (2011) studied the EC effect in tomato cultivation in rockwool and also recorded higher levels of nitrogen in tomato leaves.

Also Kowalska (2004) in her studies on the effect of nutrition levels with sulfate sulfur in tomato detected a higher mean content of nitrogen (for the analysed substrates) at the phase of 4th cluster fruit setting. A wider range of nitrogen content in tomato leaves was given by Kreij et al. (1990), Plank (1999), Campbel (2000) and the Agric. Service (2001). Generally, when confronting results of my study with the cited literature, it may be stated that at the most intensive plant manganese nutrition the nitrogen nutrient status is reduced below optimal levels recommended for tomato.

In contrast to nitrogen, significant upward trends were shown in both experiments for the content of phosphorus in index parts (Table 1). The cultivars significantly influenced the nutrient status only in the case of Mn-1.2 and Mn-2.4. Some authors found a lower content of that nutrient than determined herein ensuring optimal plant yielding (Kleiber 2014) – 0.70-0.75% P. Similar concentrations of phosphorus in tomato leaves were reported by Pawlińska, Komosa (2006) and Jarosz (2006) while a wider range was reported

Table 2

Content of macroelements according to other authors (express in % in d.m. of leaves)

Source	Nutrient content (average or range)				
	N	P	K	Ca	Mg
Agric. Service... (2001)	3.5-5.0	0.3-0.65	3.5-4.5	1.0-3.0	0.35-1.00
Campbel (2000)	3.5-5.0	0.3-0.7	3.0-4.5	1.0-2.0	0.3-0.8
Chohura, Komosa (2003a)	3.31-3.89	0.36-0.47	5.02-5.54	7.08-7.47	0.45-0.69
Jarosz (2006)	2.95-2.96	0.82-0.83	4.41-4.42	5.26-5.41	0.55-0.59
Jarosz, Horodko (2004)	4.20	0.85	5.32	3.31	0.60
Jarosz, Dzida (2011)	3.97-4.27	0.47-0.51	4.30-5.11	2.12-2.98	0.27-0.36
Kowalska (2004)	4.83-4.99	0.51-0.74	3.99-4.08	3.13-3.36	0.65-0.69
Kreij et al. (1990)	2.8-4.2	0.30-0.46	3.5-5.1	1.6-3.2	0.36-0.50
Nurzyński (2006)	3.83	0.41	5.45	2.97	0.28
Pawlińska, Komosa (2006)	4.22-4.27	0.74-0.78	6.15-6.27	-	-
Plank (1999)	3.5-5.0	0.5-1.0	3.5-5.0	0.9-1.8	0.5-1.0

by Plank (1999), Campbel (2000). The cited findings indicate that the tomato nutrition tested in this study (above 1.00% P at Mn-19.2) has not been recorded in the research conducted to date.

Contrary tendencies of the potassium content in tomato leaves were found in this study: within the range of manganese nutrition up to Mn-1.2, the potassium content was significantly increasing, but from Mn-2.4 to Mn-19.2 it was significantly decreasing (Table 1). The cultivar factor significantly modified the nutrient status only in case of Mn-4.8 and 9.6. Generally, the best yielding plants contained between 5.06-5.67% K in index parts (mean values). Similar concentrations of potassium in leaves were reported by Jarosz, Horodko (2004) and Nurzyński (2006) – Table 2. Most of the cited authors showed a lower content of that nutrient (Table 2). More potassium in leaves was found only by Pawlińska, Komosa (2006). Despite the contradictory trends for potassium in leaves, depending on the level of manganese, the plant nutrition level with this nutrient was appropriate within the analysed range.

A significant upward trends was observed in both experiments for the content of calcium in index parts of tomato (Table 1) – except Mn-19.2 in Exp. II. The cultivars significantly varied the calcium content for Mn-0, Mn-1.2 and Mn-9.6. The best plant yielding occurred within 3.00 to 3.30% Ca in index parts (mean from 2 cultivars). Many authors showed a different content of calcium in tomato leaves (Jarosz, Horodko 2004, Nurzyński 2006) (Table 2). Similar calcium concentrations were cited by Jarosz, Dzida (2011). In turn Chohura, Komosa (2003a) and Jarosz (2006) cited higher levels of that nutrient. Kowalska (2004) claimed that intensive tomato nutrition with sulfate sulfur has a significant effect reducing the calcium content in leaves. Markedly lower contents of calcium in leaves of tomato were cited by other authors (Kreij et al. 1990, Plank 1999, Campbel 2000, Agric. Service... 2001).

Like potassium, the magnesium content in tomato leaves demonstrated oposing tendencies (Table 1). Within the manganese nutrition range from Mn-0 to Mn-0.6 it was significantly increasing, while from Mn-2.4 to Mn-19.2, there was a significant decrease in the Mg in tomato leaves. The cultivar factor significantly varied the magnesium content for Mn-0 and Mn-0.6. The best yielding of tomato was recorded for 0.81%-0.85% Mg (mean). Many authors found a lower content of magnesium in tomato leaves (Table 2) while a wider range was determined by Campbel (2000) and Agric. Service ... (2001). The literature data implicate that even in the case of toxic manganese nutrition, the content of magnesium in tomato leaves did not drop below the level observed in studies conducted to date.

In both experiments, a significant decreasing trend of the iron content in index parts was found (Table 3). The cultivar significantly differentiated the iron content in the case of Mn-0, Mn-0.3 and Mn-9.6. The best plant yielding was recorded at the content of iron from 159.6 to 181.0 mg Fe kg^{-1} (mean from 2 cultivars). The determined iron content in leaves up to Mn-1.2 was similar to the results most frequently reported in literature (Table 4). References show a wider (Chohura, Komosa 2003b, Chohura et al. 2006) as well as a narrower range of the iron content (Kreij et al. 1990, Kowalska 2004). In turn, Atherton et al. (1986) recommended the content of this nutrient exceeding 60.0 mg Fe kg^{-1}, which was met in both experiments (except for Mn-19.2). A much wider optimal range for this microelement was reported by Plank (1999), Campbel (2000) and Agric. Service… (2001).

Increasing manganese nutrition caused the accumulation of that micronutrient in leaves (Table 3). The cultivar significantly modified the manganese status for Mn-0.3, Mn-1.2, Mn-2.4 and Mn-19.2. The optimal plant yielding was achieved for Alboney F_1 when manganese ranged from 175.3-260.7 mg kg^{-1}. An optimal content of manganese for Emotion F_1 was 263.8 mg kg^{-1}. In this study, the content of manganese determined in leaves within the range up to Mn-1.2 was similar to the one reported by Campbel (2000), Uchida (2000) and Chohura, Komosa (2003*b*) – Table 4. The manganese content up to Mn-0.3 was consistent with the range of this nutrient shown by Agric. Service ... (2001), while for Mn-0 it resembled that given by Kreij et al. (1990) and Plank (1999). In turn, Breś, Ruprik (2007) detected a lower content of manganese in leaves of small-fruited tomatoes grown on coir. Chohura et al. (2006) determined the manganese content of 338.9 mg kg^{-1}

Table 3

The influence of manganese nutrition on content of chosen microelements in tomato leaves (express in mg kg^{-1} d.m.)

Cultivar	Experiment I				Experiment II			
	Mn-0	Mn-0.3	Mn-0.6	Mn-1.2	Mn-2.4	Mn-4.8	Mn-9.6	Mn-19.2
Fe								
Alboney F_1	202.7 *e*	187.2 *d*	162.7 *b*	130.9 *a*	77.8 *c*	76.9 *c*	77.6 *c*	57.9 *a*
Emotion F_1	227.5 *f*	174.9 *c*	156.4 *b*	125.5 *a*	77.6 *c*	74.5 *c*	64.0 *b*	53.8 *a*
Mean	215.1 *D*	181.0 *C*	159.6 *B*	128.2 *A*	77.7 *C*	75.7 *C*	70.8 *B*	55.9 *A*
Mn								
Alboney F_1	62.9 *a*	175.3 *b*	260.7 *d*	290.8 *e*	424.0 *a*	464.4 *c*	472.0 *c*	471.4 *c*
Emotion F_1	71.1 *a*	229.7 *c*	263.8 *d*	313.3 *f*	446.2 *b*	459.4 *bc*	465.9 *c*	489.5 *d*
Mean	67.0 *A*	202.5 *B*	262.3 *C*	302.1 *D*	435.1 *A*	461.9 *B*	469.0 *B*	480.5 *C*
Zn								
Alboney F_1	43.90 *e*	40.20 *d*	31.50 *c*	30.10 *bc*	26.50 *e*	19.40 *c*	18.90 *c*	12.80 *a*
Emotion F_1	45.10 *e*	37.80 *d*	27.00 *ab*	25.50 *a*	23.30 *d*	17.20 *bc*	15.30 *b*	10.40 *a*
Mean	44.50 *C*	39.00 *B*	29.30 *A*	27.80 *A*	24.90 *C*	18.30 *B*	17.10 *B*	11.60 *A*
Cu								
Alboney F_1	25.42 *e*	19.10 *d*	14.70 *ab*	13.20 *a*	13.65 *d*	13.10 *cd*	13.45 *d*	8.12 *a*
Emotion F_1	24.40 *e*	17.35 *cd*	15.94 *bc*	15.00 *ab*	14.86 *e*	14.78 *e*	12.35 *c*	10.99 *b*
Mean	24.91 *C*	18.23 *B*	15.32 *A*	14.10 *A*	14.26 *C*	13.94 *C*	12.90 *B*	9.56 *A*

in tomato leaves, with no symptoms of its toxicity on plants. Savvas et al. (2009) showed that within the range up to 5.5 mg Mn dm^{-3} nutrient solution, the content of this micronutrient in leaves increases significantly from 36 to 280 mg Mn kg^{-1}, at a simultaneous significant deterioration of the iron and zinc nutrient status of plants.

Similarly to iron, a significant decrease in the zinc content under the tested Mn nutrition was found (Table 3). The cultivar significantly differed the zinc status for Mn-0.6, Mn-1.2, Mn-2.4 and Mn-9.6. The best plant performance was obtained with the zinc content between 29.30-39.00 mg kg^{-1} of leaves. Literature shows a wider range of zinc in leaves (Kreij et al. 1990, Plank 1999, Campbel 2000, Agric. Service… 2001, Chohura, Komosa 2003*b*) (Table 4). A similar content of zinc was shown by Kowalska (2004). The content of this nutrient in leaves found in the present study up to the level of Mn-2.4 fell within the ranges of content cited in literature; however, above this level it fell within a lower range than recommended for tomato.

Table 4

Content of microelements according to other authors (express in mg kg^{-1} d.m. of leaves)

Source	Nutrient content (average or range)			
	Fe	Mn	Zn	Cu
Agric. Service…(2001)	50-300	25-200	18-80	5-35
Atherton et al. (1986)	>60	237.0	38.0	9.68
Breś, Ruprik (2007) mod.	80.0-120.4	70.6-190.9	66.9-102.1	8.70-15.62
Campbel (2000)	45-300	30-300	18-75	5-30
Chohura, Komosa (2003b)	85.5-161.9	252.0-273.3	33.8-75.8	10.23-13.84
Chohura et al. (2006)	97.1-205.3	338.9	28.3-53.7	7.73-12.65
Kowalska (2004) mod.	136.7-141.6	115.6-137.0	40.6-47.5	33.02-36.60
Kreij et al. (1990)	84-112	54-165	54-76	6
Plank (1999)	50-300	50-100	20-100	8-20
Uchida (2000)	60-300	50-250	-	-

Analogously to iron and zinc, a significant decreasing content of copper was found (Table 3) with significant differences between the cultivars in experiment II. The best yielding of plants was accompanied by a copper content within the range of 15.32-18.23 mg Cu kg^{-1}. Chohura et al. (2006) and Chohura, Komosa (2003b) recorded less copper in leaves (Table 4). Markedly wider ranges of concentrations of this nutrient were given by Plank (1999), Campbel (2000) or Kowalska (2004).

There is more than one reason for decreased tomato yielding under Mn -stress caused by the accumulation of manganese. Generally, excessive or toxic manganese concentrations influence negatively the plant nutrition. The highest Mn-concentrations cause the accumulation of this ion, toxic symptoms on the plants and probably symptoms of the deficit of other nutrients, occurring for example on leaves (Kleiber 2014). Marschner (1998) showed that manganese is an antagonistic ion to iron and calcium. This study confirmed a decreasing content of iron, whereas calcium generally presented increasing trends. In turn. Galvez et al. (1989) claimed that higher manganese levels reduced the uptake of potassium, calcium, magnesium, zinc, copper, which is generally verified for zinc and copper. As for magnesium, both increasing (Exp. I – lower concentration of Mn) and decreasing (Exp. II – higher concentration of Mn) trends were found. The findings were similar for potassium, whose content grew up to Mn-1.2 and decreased under higher Mn-levels. Excess manganese may reduce the magnesium (Kazda, Znacek 1989) or magnesium/calcium uptake (Lee et al. 2011). In contrast to my results, Savvas et al. (2009) did not find anu effect of manganese on the content of potassium, calcium and magnesium, although they confirmed a reduction of the iron and zinc content. A lower zinc content under manganese stress was also found by Shenker et al. (2004). Similarly to the current research, a positive effect of manganese on the phosphorus status was found by Clark (1982) and Galvez et al. (1989). Clark (1982) reported also that intensive Mn nutrition reduced the uptake of potassium, calcium, magnesium and zinc. In contrast to my studies, Landi, Fagioli (1983) found no Mn-related effect on the copper uptake by maize roots. Breś et al. (2012) demonstrated an antagonistic effect between manganese and potassium, iron and zinc.

Savvas et al. (2009) and Kleiber (2014) reported that manganese nutrition had a significant influence on tomato yielding, while Chohura et al. (2009) and Kołota et al. (2013) claimed that in research on microelements the form of ions is also important. An optimal content of manganese in a nutrient solution is varied depending on a cultivar (Kleiber 2014). The highest marketable fruit yield of cv. Alboney F_1 is obtained using a nutrient solution with the manganese content in the range 0.3-0.6 mg Mn dm^{-3}, but in the case of cv. Emotion F_1, the yield for Mn-0.3 was significantly lower than for Mn-0.6. Regarding the content of Mn in water used to prepare the nutrient solution (without addition of manganese sulphate), the author found visual deficit symptoms on leaves. Within the range of Mn from 4.8 to 19.2 mg dm^{-3} toxicity symptoms appeared on the plants. The content of manganese in a nutrient solution equal 1.2 or 2.4 mg dm^{-3} is excessive, while that of 4.8 mg dm^{-3} and higher is toxic. In experiment I, the most possible chemical reasons of yield decrease in Mn-0 variant was a significant decrease of N, P, K, Ca, Mg, Mn with a simultaneous significant increase of Fe, Zn, Cu compared with the variants ensuring optimal yielding. In treatment Mn-1.2, N, Mg, Fe, Zn were found to have decreased significantly, while P, K, Ca, Mn were significantly increased in relation to the variants providing optimal yielding. In the case of combinations $\geq$ 4.8 mg Mn dm^{-3} a lower content of N, K, Mg, Fe, Zn, Cu was determined together with a higher (above optimal) content of P and Mn. Visual symptoms on the plants could be the result of disorders in the uptake of the mentioned nutrients.

Manganese is taken up by plants as Mn^{2+} cations. Although it is a heavy metal, it may appear in plant tissues in concentrations higher than necessary for the proper functioning of organisms. Manganese as a nutrient has many physiological functions, including participation in a number of enzymes: Mn-catalase, dehydrogenase, decarboxylases, hydroxylases, acid phosphatases, transferases, SOD superoxide. Furthermore, it is present in xylogens, flavanols, and PS II complex-protein. Of particular importance is the share of micro-fission reactions of water in the light phase of photosynthesis (DUCIC, POLLE 2005, HUMPHRIES et al. 2007, KOZŁOWSKA et al. 2007, BREŚ et al. 2012). Excessive manganese nutrition may interfere with this physiological process and with the nutrient uptake, wich could be a reason for worse yielding.

CONCLUSIONS

1. Manganese nutrition significantly influenced the content of macro- and microelements in leaves.
2. Tomato cultivars significantly modified the content of nitrogen (for Mn-9.6 and Mn-19.2) phosphorus (for Mn-1.2 and Mn-2.4), potassium (for Mn-4.8 and Mn-9.6), calcium (for Mn-0, Mn-1.2, Mn-9.6), magnesium (Mn-0 and Mn-0.6), iron (Mn-0, Mn-0.3, Mn-9.6), manganese (Mn-0.3, Mn-1.2, Mn-2.4, Mn-19.2), iron (Mn-0, Mn-0.3, Mn-9.6), zinc (Mn-0.6, Mn-1.2, Mn-2.4, Mn-9.6) and copper (Mn-2.4, Mn-4.8, Mn-9.6, Mn-19.2) in leaves.

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ATTEMPT AT AN APPLICATION OF NEURAL NETWORKS FOR ASSESSMENT OF THE NITROGEN CONTENT IN MEADOW SWARD ON THE BASIS OF LONG-TERM FERTILIZER EXPERIMENTS

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Abstract

On the basis of long-term fertilizer experiment, conducted since 1968, an attempt was made to verify the nitrogen content with the use of a neural network, in terms of yields from subsequent cuts. The experiment is located at Czarny Potok village near Krynica (20°54′ E; 49°24′ N) on the altitude of about 720 m a.s.l., at the foot of Jaworzyna Krynicka Mt., in the south-eastern Beskid Sądecki mountain range, on a slope with 7° inclination and NNE aspect. The experiment was set up in 1968 on a natural type of mountain meadow of mat-grass (*Nardus stricta* L.) and red fescue (*Festuca rubra* L.) with a large share of dicotyledonous plants. The soil was classified to acid brown soils developed from the Magura sandstone with the texture of light silt loam. Since autumn 1985, the experiment has been conducted in two series, with the same level of fertilization: without liming (0 Ca) and limed (+Ca). Liming was repeated in 1995. The first liming was conducted with a dose calculated on the basis of 0.5 Hh value, the second one was established according to the total hydrolytic acidity. Mineral fertilization was discontinued in 1974 - 1975 and in 1993 - 1994, when the experiment was limited to an assessment of the sward yield and its chemical composition. The experiment comprises 8 treatments with five replications, receiving either nitrogen or phosphorus fertilization (90 kg N or 39.24 kg P ha^{-1}) and (39.24 kg P ha^{-1} and 124.5 kg K ha^{-1}) against the PK background, nitrogen in two forms (ammonium nitrate and urea) and two doses (90 and 180 N ha^{-1}). In 1968-1980, phosphorous and potassium fertilizers were sown in autumn and since 1981 – in spring. However, potassium (1/2 of the dose) was supplemented in summer after I cut. In 1968 - 1973, thermophosphate was applied, but triple superphosphate has been used since 1976. Over the whole period of the experiment, nitrogen fertilizers have been sown at two dates: 2/3 of the annual dose in the spring at the onset of plant growth and 1/3 of the dose several days after the first cut. A single regenerative treatment with copper (10 kg kg^{-1}) and magnesium (8 kg ha^{-1}) was applied once in 1994. Foliar nutrition (2 dm^3 ha^{-1} applied twice) with the microelement fertilizer Mikrovit-1 has been used since 2000. The microelement fertilizer contains (per 1 dm^3): 23.3 g Mg; 2.3 g Fe; 2.5 g Cu; 2.7 g Mn; 1.8 g Zn; 0.15 g B and 0.1 g Mo. The model was compared with a regression analysis. Statistical analysis was applied for two data sets: the whole data set, i.e. 43 years and 8 treatments, 2 cuts and 2 series – data of the 1st set (*n* = 1376), and a narrow data set, comprising

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exclusively fertilization 90 kg N ha^{-1}, irrespective of the form against the background of PK – 43 years and 2 objects, 2 cuts and 2 series – data of the 2nd set (n = 344). A neural network can be applied in the assessment of the nitrogen content on the basis of yield including subsequent years of nitrogen fertilization and cuts. Neural networks including quantitative and qualitative features are useful for modelling the element content.

Key words: neural networks, nitrogen, sword.

INTRODUCTION

Long-term fertilizer experiments are ascribed a number of aims justifying a need for their continuation. One of these is creating data resources for elaborating and testing simulation models allowing for assessing the influence of agrotechnical factors on soil and plants, and providing practical information for farmers about the ways of improving farming systems and observe sustainability (Bakker et al. 2002, Grzebisz et al. 2010). Due to a number of diverse factors simultaneously affecting the results of an experiment, these problems are difficult to asses unanimously and use in practice. Even experiments on monocultures are susceptible to changeability of the weather conditions. Synthetic indicators of e.g. rainfall in natural conditions are not always strictly related to the yield.

Because of botanic diversification and fluctuation of meadow community species, long-term investigations of grasslands have their own specific character. An attempt at the assessment based on regression in these experiments is usually not unanimous. Seeking an explanation of processes occurring in the environment, also important from the practical point of view, may lead to the application of non-linear solutions based on neural networks (Stastny et al. 2011). The results demonstrated that the neural network model is capable of sugar beet leaf nitrogen estimation with reasonable accuracy (Moghaddam et al. 2010). The new integrated approach (continuum-removed absorption features, the REP and a neural network) could explain 60% of the variation in savanna grass nitrogen concentration on an independent test data set using airborne hyperspectral data (Mutanga, Skidmore 2004).

In practice neural networks require an introduction of verified input and output layer of dependent variable. This requires experimental data and the long term experiment allows to create data bases and fulfil statistical assumptions.

On the basis of long-term fertilizer experiment in Czarny Potok, conducted since 1968, an attempt was made to verify the nitrogen content basing on a neural network considering yields of subsequent cuts. The model was compared with regression analysis.

MATERIALS AND METHODS

The experiment (Mazur, Mazur 1972) is located in Czarny Potok near Krynica (Poland, 20°54′53″ E; 49°24′35″ N) at an altitude of about 720 m a.s.l. at the foot Jaworzyna Krynicka Mt. in the south-eastern massif of the Beskid Sądecki Mts. on aslope with 7° inclination and NNE aspect. The experiment was set up in 1968 on a natural mountain meadow of *Nardus stricta* L. and *Festuca rubra* L. type with a considerable share of the dicotyledonous. The soil from the experimental area was classified to acid brown soils developed from the Magura sandstone with granulometric composition of light silt loam (the following % of fractions: 1-0.1 mm – 40; 0.1-0.02 mm – 37; > 0.02 mm – 23) and characteristic three genetic horizons: AhA (0-20 cm – humus horizon), ABr (21-46 cm – browning horizon) and BbrC (47-75 cm parent rock). Detailed experiment data were presented in the previous papers (Kopeć 2000), in Table 1 and Figure 1.

The one factorial experiments experiment, carried on in 5 replications included 8 fertilizer treatments (Table 2).

Since autumn 1985 the experiment, at the same doses of NPK fertilizers, has been conducted in two series, without liming and with lime application. In 1995 and 2005 liming was repeated. In 1985 and 2005 years the dose of

Table 1

Soil properties before the experiment outset

Layer (cm)	pH_{H_2O}	pH_{KCl}	Hh	Hw	P	K	Exchangeable ions (mg kg^{-1} soil)			
			(cmol (+) kg^{-1} soil)		(mg kg^{-1}soil)		Ca	Mg	Na	K
0-10	5.20	4.38	4.42	0.46	4.8	112.0	680	38.0	20.0	69.0
10-20	5.58	4.48	4.04	0.65	2.6	23.3	540	28.0	18.0	61.0

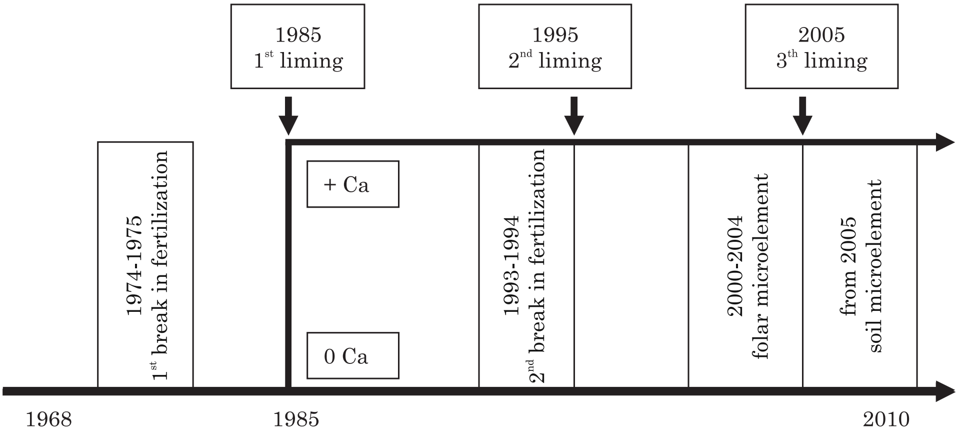

Fig. 1. Scheme of modification of cultivation measures in the experiment

Table 2

Fertilization scheme in the static experiment in Czarny Potok

Fertilizer objects	Annual dose (1985, 1995, 2005) of the element in series 0 Ca and +Ca (kg ha^{-1})			Nitrogenfertilizer	Microelements
	P	K	N		
„0”	-	-	-		B, Cu, Zn, Mn, Co, Mo
N_1	-	-	90	ammonium nitrate	B, Cu, Zn, Mn, Co, Mo
P	39.24	-	-		B, Cu, Zn, Mn, Co, Mo
PK	39.24	124.5	-		B, Cu, Zn, Mn, Co, Mo
PK +N_{1an}	39.24	124.5	90	ammonium nitrate	B, Cu, Zn, Mn, Co, Mo
PK+$N_{1u/an}$	39.24	124.5	90	urea to 2004/ ammonium nitrate from 2005	0 mikroel.*
PK+N_{2an}	39.24	124.5	180	ammonium nitrate	B, Cu, Zn, Mn, Co, Mo
PK+$N_{2u/an}$	39.24	124.5	180	urea to 2004/ ammonium nitrate from 2005	0 mikroel.

* 0 mikroel. – without microelements; P = 90 kg P_2O_5, K = 150 kg K_2O; a.n. – ammonium nitrate; u. – urea; 0 Ca unlimed series; + Ca limed series

limestone was calculated according to 0.5 Hh and in 1995 according to 1 Hh. In the years 1974-1975 and 1993-1994 no mineral fertilization was applied, and the research was limited to determining the sward yield and its chemical composition.

In 1968-1980 phosphorus and potassium fertilizers were applied in autumn. Since 1981 phosphorus fertilizers have been applied in spring, and potassium fertilizers in half dose in spring and half in summer, after the 1st cut. Phosphorus in the years 1968-1973 was in form of calcium thermophosphate (superthomasine) in the years 1975-1992 as triple superphosphate (46%) and since 2005 as enriched superphosphate (40%). In the whole period of the experiment 2/3 of the nitrogen fertilizers annual dose has been in spring at the beginning of vegetation and 1/3 of the dose about two weeks after 1st cut. In 1994, a single dose of 10 kg Cu and 8 kg Mg ha^{-1} in solid fertilizers were applied. In the years 2000-2004 foliar fertilization was conducted (twice 2 dm^3 ha^{-1}) using Mikrovit-1 fertilizer. The fertilizer contained in 1 dm^3: 23.3 g Mg; 2.3 g Fe; 2.5 g Cu; 2.7 g Mn; 1.8 g Zn; 0.15 g B and 0.1 g Mo. In the 2005-2007 periods, 0.5 g B ha^{-1} was supplied to the soil every year, whereas in the spring, 2008, 5 kg Cu, Zn and Mn ha^{-1} and 0.5 kg of Co and Mo ha^{-1} were added.

Vegetation period in the experimental area lasts from April to September (150-190 days). The weather conditions (Table 3) indicate a considerable rainfall variability.

Yields of sward fresh masses were harvested from each plot twice a year (at the turn of June and July and in September), in the initial period from the area of 42 m^2 and when liming was introduced from a 21 m^2 plot. After dry mass determination yield, samples were converted into t d.m. ha^{-1}.

During over a 40-year period of the experiment various aims were set and research hypotheses were put forward. For the sake of presentation, the whole period of research (42 years) was divided into several-year stages (Table 4) according to the applied cultivation measures. The stages cover the year of the measure application as the subsequent years i.e. its direct and residual effect.

Table 3

Statistical characteristics of precipitation and temperatures for the period 1968-2008

Parametr	Precipitation (mm)		Temperature (°C)	
	I–XII	IV–IX	I–XII	IV–IX
Arithmetical mean	876.4	568.5	5.86	12.19
Standard deviation	198.7	138.0	0.87	0.77
Range 25-75% of cases	733.2-990.0	461.5-658.2	5.35-6.30	11.7-12.7

Table 4

Stages of the experiment (Kopeć, Mazur 2011)

Stage No.	Period	Description of the stage
1	1968-1970	3-year period of introducing fertilization
2	1971-1973	3-year period of experiment continuation
3	1974-1976	1st break in fertilization (for two years) and the year of fertilization resumption
4	1977-1980	4-year period after resumption of fertilization
5	1981-1984	4-year period of fertilization continuation
6	1985-1988	4-year period of fertilization continuation after introduction of liming
7	1989-1992	4-year period of fertilization continuation
8	1993-1995	2nd break (2 years), introduction of a single grazing, the year of NPK resumption, Cu and Mg application and repetition of liming
9	1996-1999	4 year of fertilization continuation
10	2000-2004	5-year period of fertilization continuation with annual twofold application of foliar microelement fertilization
11	2005-2007	3-year period of fertilization continuation, replacement of urea by ammonium nitrate, fertilization B applied every year
12	2008-2010	3 year period of fertilization continuation with a single soil treatment with Cu, Zn, Mn, Co and Mo

In the first period, which covered the years 1968-1975, the research focused on the effect of various levels of fertilization on the yield and quality of grassland sward established as a result of natural turfing of abandoned arable land. It was the period of fertilizer industry development in Poland. The agricultural usefulness of ammonium nitrate and urea as a new fertilizer in Poland, has been compared. Nutrient doses were comparatively high; however, plant nutrient requirements for a potential yield were taken into account. At that time agriculture in mountain conditions was rather extensive and intensification process pursued only slowly. Therefore, in the period 1974-1975 fertilization was ceased, assuming a residue effect of applied nutrients. Two years later fertilization was resumed on the same level at the native treatments. The former researches show diversification of botanical composition and soil properties, which indicated a necessity sward liming. After seventeen years, fertilization with sodium carbonate was applied, assuming an improvement in soil pH and supplementing sodium content in the sward. Since 1985, the experiment was conducted in two series: without liming and with limestone application (Figure 1) in 10-year cycles.

Economic transformation, which took place in Poland in the nineties of the 20th century caused a dramatic decline in fertilizer’s consumption in the whole country. It has been decided to mimic these changes on the scale of the experiment. Therefore, another break in fertilization for two years was administered. Another change was to include the single sheep grazing in the experiment. The liming was repeated in 10-year cycles (1995, 2005). While seeking possibilities of restoring yield forming potential in the subsequent years attention was focused on magnesium and microelement deficiencies. Magnesium and copper were supplied once to the soil and then foliar application of the multi microelements fertilizer was used for five years. In 2005, boron was added to the soil and then a set of basic microelements in doses, which should have improved their bioavailability previously limited by a systematic removal of these elements with the yield. The main aim of all agronomic measures was maintaining the grassland productive potential, by their influence on the quality of sward and the soil properties has been analyzed as well. The polygon of the experiment was also used in research on nutrient leaching, changes in humus content, microbial and enthomofauna activity and to determine heavy-metal cycling in agrocenosis.

A module of Statistica 9.0 application (licence for University of Agriculture) was used in statistical analysis (StatSoft, Inc. 2009).

The nitrogen content was considered as the output data for constructing the neural network and regression analysis, whereas dry mass yield was regarded as the quantitative input data and the subsequent years of the experiment, treatments, series and cuts were used as qualitative input data.

RESULTS AND DISCUSSION

In practice there is a need for fast assessment of e.g. protein in animal feed. Protein equivalent is nitrogen and its amount limits the crop yield and its quality (KACORZYK, KASPERCZYK 2006, GRZEBISZ et al. 2008, KULIK 2009, BOJARSZCZUK et al. 2011).

Statistical analysis was applied for two data sets – the whole data set, i.e. 43 years and 8 treatments, 2 cuts and 2 series – data of the1st set (n = 1376) and narrow data set comprising exclusively fertilization 90 kg N, irrespective of the form against the background of PK – 43 years and 2 objects, 2 cuts and 2 series – data of the 2nd set (n = 344).

Despite slight changes in the level of nitrogen content, set of dependencies shown in Figure 2 does not differ significantly for both cuts. With years, nitrogen content was increasing until half of the period of investigations and then was decreasing slightly. In case of lower yields, nitrogen content is higher. Especially in 2nd set biomass increment dilutes nitrogen content.

Linear regression analysis of quantitative features, i.e. nitrogen content in the sward and crop yield describes a small number of cases. Table 5 presents values of the analysis for both data sets.

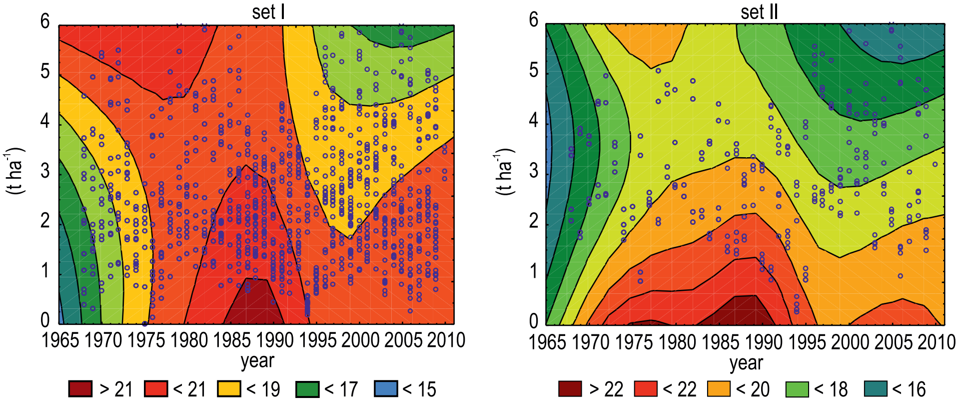

Fig. 2. Nitrogen content in meadow sward (g kg^{-1}) of both series and cuts depending on experiment years and yield for data sets I and II

The ranges of neural networks for both sets were approximate (Tables 5 and 6). Following the analysis, a network called MLP 57-10-1 was selected on the basis of learning coefficients (0.960659) quality, testing quality (0.840155) and validation quality (0.807017).The network was ascribed learning algorithm BFGS 83 with SOS error function and hidden layer initial exponential activation.

Factor sensitivity analysis had the following values: cut – 19.434, years of the experiment – 19.238, treatment – 9.021, series – 2.368, yield – 2.055.

Table 5

Parameters of regression analysis of nitrogen content in sward dependent on crop yield for two data sets

Specification	b^*	St. error. - b^*	b	St. error - b	t	p
Data 1st set	summing up regression of dependent variable: $R = 0.01628$; $R^2 = 0.00026$ $F(1.1343) = 0.35605$ p					
Intercept			19.29367	0.185594	103.9565	0.000000
Yield	-0.016280	0.027284	-0.03765	0.063098	-0.5967	0.550809
Data 2nd set	summing up regression of dependent variable: $R = 0.32071$; $R^2 = 0.10285$ $F(1.324) = 37.146$ p					
Intercept			20.67828	0.407949	50.68839	0.000000
Yield	-0.320710	0.052621	-0.76824	0.126050	-6.09472	0.000000

Table 6

Statistics of data area in neural network

Specification	Test - Learning		Test -Validation		Whole data	
	range	mean/SD	range	mean/SD	range	mean/SD
Set I						
Yield (t ha^{-1}) – quantitative input	0.13-6.09	2.52/1.27	0.38-6.10	2.60/1.44	0.13-6.10	2.53/1.26
Content of N (g kg^{-1}) – quantitative input	10.62-31.40	19.08/3.29	13.00-29.30	19.16/3.04	10.62-31.40	19.10/3.21
Set II – (90 kg N + PK treatments)						
Yield (t ha^{-1}) – quantitative input	2.60-6.09	2.99/1.23	1.10-5.86	3.10/1.12	0.26-6.09	3.02/1.16
Content of N (g kg^{-1}) – quantitative input	10.61-31.40	18.37/2.87	11.12-24.20	18.65/2.69	10.62-31.4	18.37/2.83

The data above show a significant effect of cut and subsequent years of the experiment on nitrogen content. This dependence should be explained by changes in the botanical composition, both regarding the succession registered during the experiment and seasonality of 1st and 2nd data sets. Intensification of fertilization is another factor important for determining nitrogen content. The discussed factor is less conditioned by liming and crop yield.

The network called MLP 49-4-15 was selected for narrow data set on the basis of learning quality coefficients (0.952972), testing quality (0.81568) and validation quality (0.884061). The training algorithm BFGS37 with SOS error function and hidden layer initial linear activation was ascribed to the network.

Factor sensitivity analysis had the following values: years of the experiment – 17.549, cut – 11.603, series – 1.762, yield – 1.339.

The data above evidence a considerable effect of subsequent years of the experiment and cut on nitrogen concentrations. An inversion of sensitive features occurred and diversification of sensitivity level. A difference in botanical composition is less diversified at the same dose than with extreme fertilization considered in 1st set. The sensitivity of liming effect and crop yields look analogously as in case of 1st data set.

Neural networks proved to be particularly good not only for elaboration of short term results (Boniecki 2005), but also in the context of time as an element of data interpretation. Progress in predicting productivity, spatial modelling and spatial-and-time forecasting has been made recently regarding the interpretation of long-term experiment results. Application of neural networks makes possible a deeper analysis, particularly while solving non-linear regressions, forecasting and classification.

CONCLUSIONS

1. Application of a neural network for assessment of nitrogen content on the basis of yield is possible taking into account subsequent years of nitrogen fertilization level and cut.

2. Neural networks are useful for modelling the element content at the application of descriptive quantitative and qualitative features.

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THE TOTAL CONTENT OF NITROGEN IN LEAVES AND WOOD OF TREES GROWING IN THE AREA AFFECTED BY THE GŁOGÓW COPPER SMELTER

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Abstract

Soils affected by industrial emissions of a copper smelter may contain high amounts of heavy metals. Heavy metal infiltration across the soil is a potential source of groundwater contamination. Simultaneously, many ions, especially Cu^{2+} and Pb^{2+}, can be accumulated by plants growing within the emission range. The aim of this study was to determine the influence of high Cu and Pb soil contamination on the total nitrogen content in leaves and wood of trees growing in an area exposed to copper smelter emissions.

Samples of leaves and wood of *Populus robusta* L. and *Betula pendula* L. as well as soil samples were taken from an area affected by industrial emissions, namely from the former sanitary zone of the Głogów Copper Smelter. The samples were collected in 2010. The particle size distribution, pH, organic carbon, total nitrogen and the total content of Cu and Pb in the soil samples were determined. In the plant samples (foliage and trunks), the total nitrogen was assayed. The results were analysed statistically.

The following conclusions were drawn: the litter horizon of soils affected by industrial emissions contains high level of heavy metals (3450-5400 mg Cu kg^{-1}, 1020-1500 mg Pb kg^{-1}), exceeding threshold values for industrial areas. Also the humic horizon is characterised by an increased Cu and Pb content: 174-1530 mg Cu kg^{-1} and 268-702 mg Pb kg^{-1}. The leaves of the tested species contained more nitrogen than the wood, although the birch wood contained more nitrogen then the poplar. There was no difference in the nitrogen content of the annual tree rings of both species. Despite high levels of copper and lead in the tested soils, there was no effect of this factor on the nitrogen content of the leaves, bark and wood of the studied trees.

Key words: nitrogen, copper, lead, copper smelter, industrial pollution.

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INTRODUCTION

The metallurgical industry causes adverse effects on the natural environment. The main factor responsible for environment pollution is the emission of metalliferous dust and gases (Martley et al. 2004). The area around the Głogów Copper Smelter is contaminated with heavy metals, especially with copper and lead (Medyńska et al. 2009, Rosada, Grzesiak 2009, Drzymała, Spychalski 2011). The described situation, persisting for decades, in 1990 forced the Governor of Legnica to establish a sanitary zone around the copper smelter. The sanitary zone originally covered 2,840 ha. The land was bought and the local inhabitants resettled. The soil was limed, and then the whole area was planted with trees: poplar (*Populus robusta* L.) and birch (*Betula pendula* L.). The zone has been under continuous environmental pollution monitoring since then.

The impact of heavy metals on the environment is widely described in literature. The vast majority of reports on this subject relates to an increase in soil pollution with heavy metals (Alloway, Ayres 1997, Kabata-Pendias, Pendias 2001, Mocek et al. 2006, Rosada, Grzesiak 2009). Many studies (Pankovič et al. 2000, Kabata-Pendias, Pendias, 2001, Kabata-Pendias 2004, Xiong et al. 2006, Ke et al. 2007, Shahbaz et al. 2010) show that the uptake of nutrients can be affected by the presence of other elements, including heavy metals (the phenomenon of antagonism and synergism). Some authors (Antosiewicz 2005, Lock et al. 2007, Knapowski et al. 2012) have reported that certain elements such as nitrogen and potassium, calcium and magnesium can reduce copper and lead retrieval by plants or (depending on plant tissues) increase the content of Cu and Pb (Domagała-Świątkiewicz, Gąstoł 2013).

Nitrogen is one of the biogenic elements. It affects the growth and development of plants, and its deficiency interferes with vital processes (photosynthesis, creation and distribution of assimilates, the synthesis of proteins and chlorophyll). The impact of heavy metals on plant metabolism is a well-recognised topic (Garg and Aggarwal 2011, Solanki and Dhankhar 2011). Nieboer and Richardson (1980) described the important mechanism of heavy metal toxicity as a consequence of binding different chemical groups of different compounds, i.e. –SH group. The mechanism of heavy metals forming bonds with enzymes, whose activity is thereby inhibited is a very characteristic development, especially in respect of nitrite and nitrate reductase above a certain threshold (Van Assche, Clijsters 1986, 1990, Arora et al. 2010, Solanki, Dhankhar 2011). Kucharski et al. (2011) noted the significant decrease in the activity of many soil enzymes (dehydrogenase, urease, acid phosphatase, α-glucosidase and arylsulphatase) due to zinc pollution. According to observations made by Alloway and Ayres (1997) and Kabata-Pendias and Pendias (2001), copper may affect the metabolism of nitrogen compounds in plants by raising the level of proteins. Nevertheless, literature references do not exhaust the question of significant correlations between the uptake of respective elements. The influence of heavy metals on the uptake of nitrogen by plants has been discussed in few publications (Hernández et al. 1997, Quariti et al. 1997, Lim et al. 2003, Ke et al. 2007, Shahbaz et al. 2010).

Most scientific papers have focused on microorganisms, herbaceous plants and the behaviour of mosses, whilst there is a shortage research on trees species. The aim of this study was to determine possible changes of the total nitrogen content in leaves and wood of trees growing in an area exposed to industrial emissions, especially on dust high in copper and lead.

MATERIAL AND METHODS

The study took place in the former sanitary zone of the Głogów Copper Smelter (Lower Silesia Province, the western part of Poland). Żukowice I, II and Bogomice are located within the sanitary zone. Żukowice I is about 0.1 km to the SW of the smelter, Żukowice II is about 0.3 km E, and Bogomice is about 1 km NE from the smelter (Figure 1). The control site was located at some distance from the smelter, – in Stypułów, about 15 km NW from the smelter (N: 51°41′44.30″ W: 15°36′13.70″).

Soil samples were taken in June 2010, from all genetic horizons of the soil profile. Each sample was collected as a mixed one, representing material from the whole depth of the horizon. Soil material was air-dried and sieved

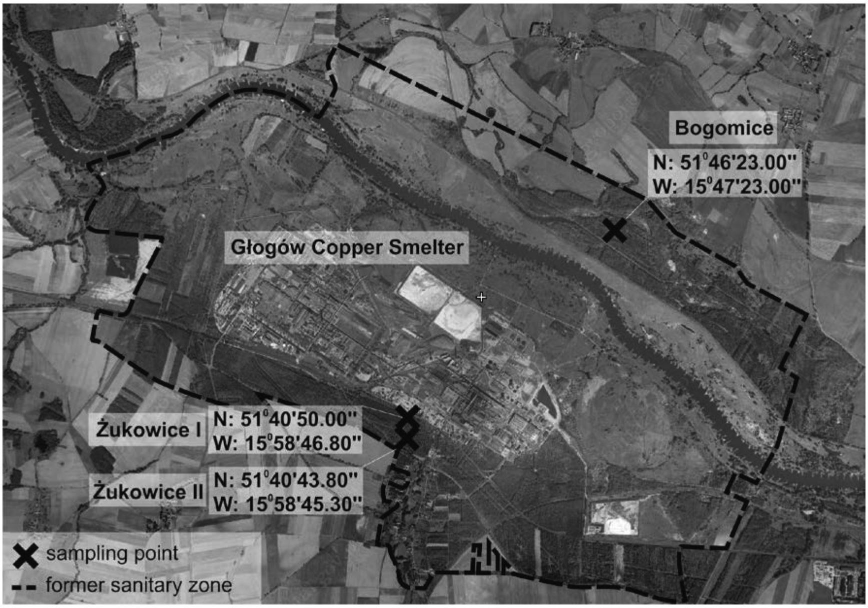

Fig. 1. Location of the site on the Geoportal map (2013)

through a sieve with a mesh diameter of 2.0 mm. Having prepared the material prepared as explained above, the following analyses were made:

- particle size distribution – using the areometric method (Mocek et al. 2006);
- pH – in H_2O and 1M KCl suspension (potentiometrically), soil:liquid 1:2.5 (Mocek et al. 2006);
- organic carbon – using the Tiurin's method (Mocek et al. 2006);
- total nitrogen – using the Kjeldahl method;
- Cu and Pb total content – using atomic absorption spectrometry, after mineralisation in *aqua regia* (PN-ISO 11466:2002).

All the soil analyses were performed in triplicate.

Averaged samples of unwashed leaves of *Populus robusta* L. and *Betula pendula* L. were collected in the autumn of 2010. After drying, the leaves were ground in a mill. After mineralization of the samples in concentrated H_2SO_4, the nitrogen was determined using the Kjeldahl method. The analyses were performed in triplicate. Averaged samples of wood were taken from saw-cut discs at a height of 1.3 m from 3 selected trees at each site. Each disc was divided mechanically into 5-year tree rings. After drying and grinding, the tree samples were mineralized in concentrated H_2SO_4. Nitrogen in the solution was determined with the Kjeldahl method. The analyses were performed in triplicate.

The results were statistically analysed using the Statsoft Statistica 10 procedures.

RESULTS AND DISCUSSION

The results of this study showed a wide variation in soil properties in the area surrounding the Copper Smelter. This is due to fluvial activity (the Odra River Valley), land relief, lithogenesis (mosaic of the sand, loam and organic materials), pedogenesis (Albic Luvisols, Haplic Gleysols, Mollic Fluvisols, Fluvic Cambisols, Fluvic Endogleyic Cambisols, Sapric Histosols, Gleyic Phaeozems, Brunic Arenosols) and also anthropogenic pressure (agriculture, industry, reclamation). The physical and chemical properties of the soils are shown in Table 1. The particle size distribution of the soils covering the sanitary zone is mostly that of sandy loam and loamy sand. The pH of the tested soils varies from acid (Żukowice I, Bogomice), neutral (Stypułów) to alkaline reactions (Żukowice II). This variation is a typical effect of liming as a reclamation technique (Żukowice, Bogomice) and of agricultural activities (Stypułów). In some literature references, information dust emmision increasing thesoil pH can be found (Medyńska-Juraszek, Kabała 2012). Next to the total concentration of heavy metals, the soil reaction is one of the most frequently cites factors implicating bioavailability of heavy metals..

Table 1

Selected properties of soil

Site	Depth	Sand	Silt	Clay	pH		C_{org}	$N_{tot.}$	C:N	$Cu_{tot.}$	$Pb_{tot.}$
		2.0-0.05 (mm)	0.05-0.002 (mm)	< 0.002 (mm)							
	(cm)	(%)			H_2O	1M KCl	(%)			(Mg kg^{-1})	
Żukowice I	3-0	-	-	-	5.9	5.5	-	1.58	-	5400	1320
	0-23	69	31	0	6.2	6	1.33	0.07	19	1530	416
	23-44	63	34	3	5.3	4.9	0.57	0.05	11.4	52	304
	44-59	58	33	9	5.5	5.4	0.54	0.04	13.5	45	79
	59-79	83	11	6	5.9	4.4	0.3	0.03	10	45	76
	>79	82	18	0	6.4	5.9	0.13	0.01	13	38	109
Żukowice II	2-0	85	15	0	6.9	6.6	3.14	0.34	9.2	3450	1020
	0-27	79	21	0	7.8	7	2.13	0.16	13.3	958	268
	27-40	78	22	0	7.8	7.1	1.61	0.14	11.5	41	60
	>40	87	13	0	7.9	7.2	1.26	0.12	10.5	100	75
Bogomice	3-0	-	-	-	6.3	5.9	-	2.1	-	4110	1510
	0-20	65	34	1	5.1	3.8	1.48	0.13	11.4	174	702
	20-37	61	37	2	5.2	3.8	1.35	0.12	11.3	99	114
	37-67	65	34	1	5.7	4.3	1.23	0.1	12.3	85	108
	>67	80	19	1	5.6	4.3	1.26	0.1	12.6	60	106
Stypułów	0-30	90	10	0	7.7	7.2	0.92	0.07	13.1	7	10
	30-70	97	3	0	7.4	6.5	0.14	0.02	7	6	9
	70-90	98	2	0	7.4	6.6	0.17	0.03	5.7	6	9
	>90	79	12	9	6.7	5.2	0.3	0.03	10	7	9

The content of both organic carbon and total nitrogen in the surface and subsurface horizons was significantly higher than in samples from deeper layers. This regularity recurred at all sites. The highest average content of organic carbon was found in the soil profiles from Żukowice II and Bogomice, and the lowest – from Stypułów. The highest average content of total nitrogen was found in Bogomice and Żukowice I, and the lowest – again in Stypułów. Differences in the content of these elements are an effect of genetic variation. One possible reason is the dominance of different plant species, e.g.the dominant species in the former sanitary zone is the poplar (*Populus robusta* L.), and the admixed species is the birch (*Betula pendula* L.). The control site was dominated by the oak. The difference in the carbon and nitrogen content can be habitat-dependent, as was shown by ENOKI, KAWAGUCHI (2000). Statistical analysis showed a highly significant ($P < 0.01$) effect of the sampling depth and the total nitrogen content.

The C: N ratio of the tested soils varied. In most of the samples from the former sanitary zone, it amounted to 12 : 1. A slightly higher C:N ratio was observed in samples taken from the control site, but even in this profile a low C:N index was found. The average ratio in the profiles ranged from 9.2 to 19.0 in the areas affected by industrial emissions and from 5.7 to 13.1 in the control area. The differences are connected with the pedogenesis and land use (including covering with trees) rather than with the industrial influence. The impact of heavy metals on the organic matter mineralisation cannot be confirmed unambiguously. The mineralisation processes may be limited by the diversity, activity and abundance of soil microorganisms (NEILL, GIGNOUX 2006).

The highest concentration of heavy metals was found in the humus and subsurface horizons of the tested soil profiles. This is particularly marked in the site located in the former sanitary zone, where the metal content exceeds the limits for industrial areas in Poland – limits for Cu and Pb: 600 mg kg^{-1} (*Regulation* ... 2002). The horizons located lower in the soil profiles contain copper and lead at levels typical for uncontaminated areas. What is very characteristic for the described area of the sanitary zone is the presence of extremely big differences in the heavy metal content in different places, even the ones located in close proximity. A clear manifestation was the outcome of a comparison with other data from this area – 855-13143 mg Cu kg^{-1} and 585-9181 mg Pb kg^{-1} in the 0 horizon and 200-2875 mg Cu kg^{-1} and 92-723 mg Pb kg^{-1} in the Ap horizon (MEDYŃSKA-JURASZEK, KABAŁA 2012). A much lower heavy metal content was found in the control samples.

The high content of heavy metals in soils covering areas exposed to industrial impact is a well-known fact (KABATA-PENDIAS, PENDIAS 2001, MEDYŃSKA-JURASZEK, KABAŁA 2012). In addition to the main source of pollution in the analyzed former sanitary zone (metal containing dusts), secondary pollution occurs as a result of litter decomposition and dust washed off the leaves into the soil. It is worth mentioning that, according to literature reports, most of the described pollution factors have only exerted a superficial impact (MEDYŃSKA-JURASZEK, KABAŁA 2012). Thus, soil sorption comes to the foreground as a process realised both by clay minerals and organic soil matter. It prevents the heavy metals from penetrating to the deeper soil horizons *via* ground and surface waters. The high content of organic matter in the litter and humus horizons effectively influences the Pb and Cu sorption capability. Statistical analysis showed a significant positive correlation between the content of copper and lead and organic matter in the soil ($r_{\text{Pb-C}} = 0.75$, $r_{\text{Cu-C}} = 0.76$).

Many studies show differences in the nitrogen content in plant tissues. In our analyses performed on the chosen tree species, a significantly higher nitrogen content was found in the bark than in the wood (Figure 2). More nitrogen was found in the poplar bark than in the birch bark. Annual tree-rings showed small differences in the content of this element, although a higher content of nitrogen was found in the birch wood than in the poplar wood. The total nitrogen content in the leaves varied within a wide range (Figure 3). The birch leaves had a higher content of nitrogen than the poplar

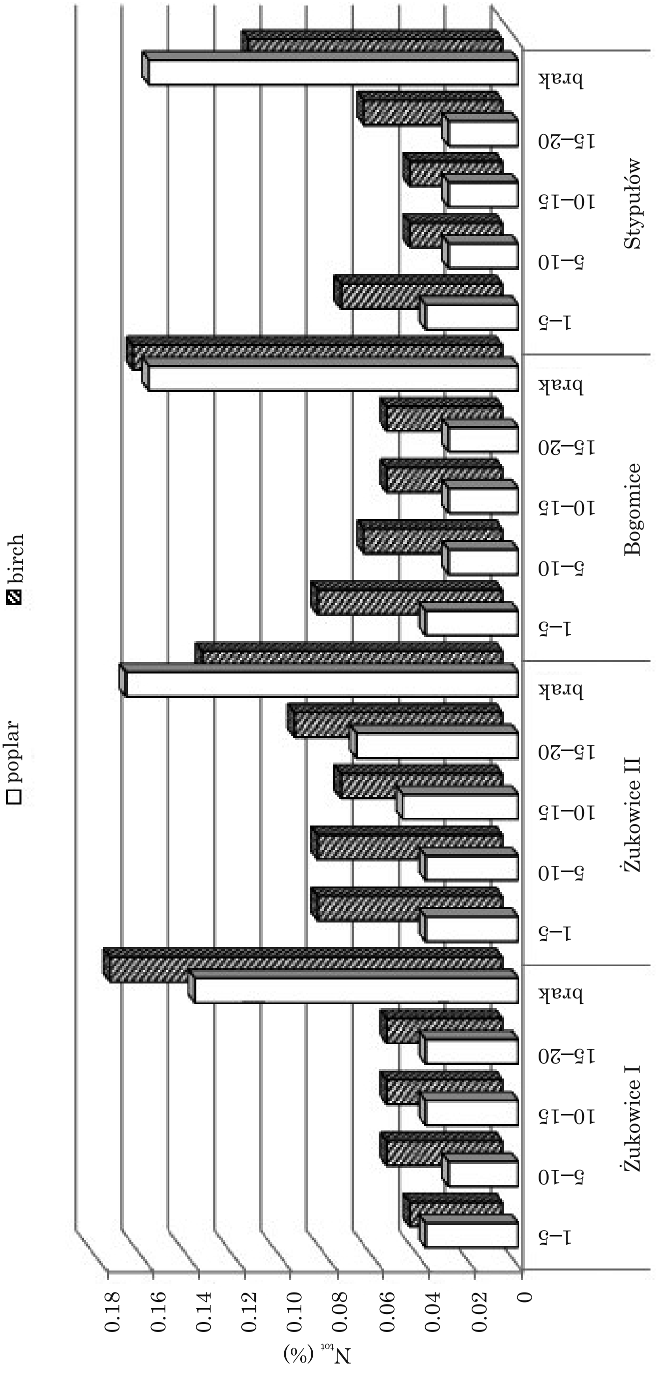

Fig. 2. The nitrogen content in the annual tree-rings of poplar and birch

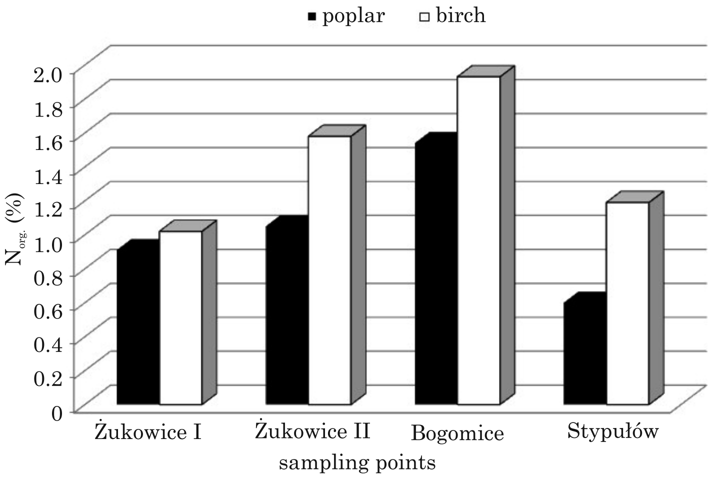

Fig. 3. The nitrogen content of the poplar and birch leaves

ones – differences from 2 to 98% were observed. A higher content was found in young tissues (leaves and bark) than in the wood of the birch and poplar.

The results of the nitrogen content were lower than those found in the literature or both the birch (Saramäki, Hytönen 2004) and the poplar (Fortier et al. 2010). Several authors have indicated that the nitrogen content in plant tissues may be affected by many factors, such as the plant age, season of the year, exposure and nutrient content of the soil (Coll et al. 2010, Fortier et al. 2010).

The statistical analysis showed no relationship between the content of heavy metals and nitrogen in plant samples taken from the areas exposed to the direct impact of the industrial plant (Table 2). A positive correlation was found between the content of nitrogen in the poplar stem and the lead content of the soil (r = 0.99) and also between the content of nitrogen in the birch stem and the copper content of the soil (r = 0.96). In the control area, a correlation between the C content of the soil and the N content of the poplar stem (r = 0.98) and another one between the N content of the soil and the N content of the the poplar stem (r = 0.98) were found. Analysing the behaviour of trees growing in the sanitary zone, a positive relation between the C:N ratio of the soil and the nitrogen content of the stem of birch (r = 0.98) was noted.

The decomposition of organic matter is possible only with the participation of micro-organisms. Their biodiversity and abundance turns into the efficiency and quality of the matter decomposed. Many authors emphasize the reduced activity of the soil micro flora in areas highly contaminated with heavy metals (Alloway, Ayres 1997, Kabata-Pendias, Pendias 2001, Wang et al. 2007). Fertilisation of soils contaminated with heavy metals with nutrients available for microorganisms can be beneficial to the growth and colony size (Peltola et al. 2006, Odlare, Pell 2009). Lime applied in the 1990s

Table 2

Correlation matrices for chosen soil properties and nitrogen content in plant organs

Variable	Correlation coefficient, $p < 0.05$					
	Populus robusta L.			*Betula pendula* L.		
	N_{leafs} (%)	N_{stem} (%)	N_{bark} (%)	N_{leafs} (%)	N_{stem} (%)	N_{bark} (%)
Samples from the former sanitary zone of Głogów Copper Smelter						
pH H_2O	-0.37	0.69*	0.11	0.00	0.67*	-0.79*
pH 1M KCl	-0.55	0.71*	-0.05	-0.23	0.59	-0.65
$C_{org.}$ (%)	0.11	0.19	-0.14	-0.05	-0.07	0.32
$N_{tot.}$ (%)	0.21	0.21	-0.01	0.08	0.09	0.23
C:N	0.09	0.16	-0.19	-0.08	-0.10	0.34
$Cu_{tot.}$ (mg kg^{-1})	-0.16	0.16	-0.07	-0.20	0.04	0.05
Pb_{tot} (mg kg^{-1})	0.13	0.03	0.00	0.04	0.14	0.11
Samples from control site						
pH H_2O	0.00	0.63	0.00	-0.49	0.00	0.00
pH 1M KCl	0.05	0.65	-0.05	-0.44	0.03	0.05
$C_{org.}$ (%)	0.03	0.98*	-0.03	-0.05	0.88	0.03
$N_{tot.}$ (%)	0.18	0.98*	-0.18	0.04	0.83	0.18
C:N	-0.16	0.84	0.16	-0.03	0.98*	-0.16
$Cu_{tot.}$ (mg kg^{-1})	0.00	0.58	0.00	0.30	0.96*	0.00
Pb_{tot} (mg $\cdot kg^{-1}$)	0.00	0.99*	0.00	-0.17	0.78	0.00

* statistically significant

(especially in the first years) might have accelerated the rate of the mineralisation of organic matter and increased the abundance of nitrogen. The soil nitrogen unavailable for microorganisms could have been translocated deeper into the soil profile (in the form of nitrates), released into the atmosphere (in the form of ammonia) or lost in the process of denitrification.

The study on the afforested areas in the vicinity of the Głogów Copper Smelter shows satisfactory condition of the trees. Despite the high heavy metal content in the top horizons of soil, the nitrogen content in the leaves, wood and bark of the tested plants is comparable to the one in the samples taken from the control site. Such an effect, dependent on the biological barriers to the heavy metal uptake by plants has been stated by many authors (MEDYŃSKA et al. 2009). Comparing to the results of other studies, a lower content of nitrogen in tree tissue has been noted (FORTIER et al. 2010, PEARSON et al. 2010, PALVIAINEN, FINÉR 2011).

CONCLUSIONS

1. The topsoil contained more nitrogen, copper and lead than the deeper layers – as the sampling depth increased, the content of the analysed elements decreased.

2. The total nitrogen content in leaves and bark was much higher than in the wood of the tested tree species at all research sites. The birch wood contained more nitrogen than the poplar wood. Annual tree rings did not show differences in the nitrogen content during the growth of the poplars and birches.

3. The influence of the high content of heavy metals in soils affected by industrial emissions on the nitrogen content in the plant tissues was unobserved.

4. Samples taken from the control site with well-balanced soil chemical composition, showed a significant correlation between the content of copper in the soil, and the nitrogen in the leaves of the birch and poplar and the bark of birch. No significant correlations were found between the lead content of the soil of the control area and the nitrogen content of the plant tissues.

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DO WATER FILTERS IMPROVE THE QUALITY OF POTABLE WATER?

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Abstract

It is common knowledge that household water filtration systems cause water demineralisation. However, the available literature lacks data concerning to what extent filters decrease concentrations of chosen mineral components in water. The aim of the study was to estimate the effect of filters mounted on domestic water intakes on the changes in selected water properties. Water samples filtered in a reverse osmosis system and not enriched by further mineralization were taken for the study. Total hardness, concentrations of magnesium, calcium, chloride and nitrate ions and water pH and conductivity were determined after filtration and compared with the same parameters in water from the waterworks. Randomly chosen water samples used for the study were taken from sites in north-eastern Poland. The analyses were carried out according to current standards. All the studied samples of unfiltered water met the drinking-water quality standards laid down by the Regulation of the Minister of Health. In the study, it was demonstrated that water filters significantly decreased electrolytic conductivity as well as the concentrations of magnesium and calcium ions (decisive for water hardness) and chlorides. The analysed filters also increased water acidity. Drinking filtered water decreases the share of water in the recommended dietary allowance (RDA) for calcium and magnesium and may result in many diseases, which is discussed in the paper. No positive effect of water filters on nitrate concentrations was found. The authors proved that installing household water treatment devices seems unnecessary, especially when water is purchased from municipal waterworks and tested as safe for human consumption.

Key words: unfiltered water, filtered water, total hardness, magnesium, calcium, chlorides, nitrates, electrolytic conductivity, water pH.

INTRODUCTION

Assessment of potable water quality is standardised in Poland by the Regulation of the Minister of Health of 29 March 2007 on the requirements pertaining to the quality of potable water (*Regulation*... 2007 with subsequent amendments *Regulation*... 2010). The document takes into account

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the recommendations given in the Council Directive 98/83 EC on the quality of water intended for human consumption. It contains i.a. strictly defined chemical requirements which potable water should conform to. Total hardness, concentrations of magnesium, chlorides and nitrates and electrolytic conductivity and pH are among standardised chemical indices.

Total hardness is determined by the content of divalent cations, mainly by calcium and magnesium. Both are considered macroelements. Calcium and magnesium are indispensable components of human diet – they both participate in many physiological processes in the human organism at subcellular, cellular and tissue levels. Their deficit or excess leads to hypocalcemia and hypomagnesemia, respectively (Kołłątaj, Szewczyk 2006, Pasternak et al. 2010, Grzebisz 2011, Błaszczyk, Duda-Chodak 2013).

Magnesium activates over 300 enzymatic reactions. It participates in the transformation of proteins, lipids, nucleic acids; magnesium is also an activator of enzymes associated with the transfer of phosphate groups, it participates in reactions with ATP, in nucleic DNA transcription, in translation of mRNA in ribosomes and finally it stabilises cell membranes. The Recommended Dietary Allowance (RDA) for magnesium is 300 - 350 mg for adults, being even higher for children and adolescents, breastfeeding and pregnant women and persons exposed to stress or intensively exercising (Knypl 2005, Pasternak et al. 2010, Grzebisz 2011, Błaszczyk, Duda-Chodak 2013).

Calcium is the basic component of bone structure. It plays an important role in the contraction of smooth, skeletal and heart muscles, participates in blood coagulation, affects the synthesis and release of antibodies, the synthesis of structural and regulatory proteins and release of some hormones (e.g. insulin) (Kołłątaj, Szewczyk 2006). The Recommended Dietary Allowance (RDA) of calcium for adult humans is 1000 mg (Januszko et al. 2012).

Daily supply of calcium and magnesium with drinking water should constitute at least 15% of the RDA. The ratio of Ca^{2+} to Mg^{2+} ions is also important. The molar Ca to Mg ratio equal 2 ensures optimum assimilation of both elements by the human body (Wojtaszek 2006).

The presence of chlorides, mainly as NaCl, in food maintains proper osmotic pressure in cells. Chlorides are the main component of digestive acids in the stomach. They maintain acid-base equilibrium and activate some enzymes,like amylase. Chloride ions are ingested in the form of salts, mainly as NaCl. The recommended daily NaCl consumption is 3-6 g (Suliburska 2010).

Nitrates are undesirable in excessive amounts in natural water. In the human alimentary tract, they are easily reduced to nitrites, which cause methemoglobinemy (the so-called "blue baby syndrome") and hypertension, increased infant mortality, diabetes, central nervous system birth defects, spontaneous abortions, respiratory tract infections and changes in the immune system (Fewtrell 2004). The presence of nitrates in human diet may result in the formation of N-nitrozo compounds, many of which are cancerogenic (Nowak, Libudzisz 2008). The Joint FAO/WHO Expert Committee on

Food Additives (JECFA 2003) and Commission Regulation (*Commission* ... 2011) established the Acceptable Daily Intake (ADI) of nitrates at 0-3.7 mg kg^{-1} body weight.

The Regulation of the Minister of Health on the quality of water intended for human consumption (*Regulation* ... 2007) imposes an obligation on the State Sanitary Inspection to issue regional assessments of water quality and to estimate health risk of consumers. The content of nitrates in drinking water should not exceed 50 mg dm^{-3}, concentrations of chlorides should be less than 250 mg dm^{-3}, total hardness – 60-500 mg $CaCO_3$ dm^{-3}, concentration of magnesium 30-125 mg dm^{-3}, electrolytic conductivity should not exceed 2500 mS cm^{-1} and pH 6.5-9.5. The calcium concentration in drinking water is not limited but its content determines water hardness and concentrations of calcium and magnesium in waters are significantly and positively correlated (CZEKAŁA et al. 2011).

Water filters have recently been often installed in many households to improve the quality of water intended for consumption. Filter producers asssure their clients that they will have water of excellent quality (DERKOWSKA-SITARZ, ADAMCZYK-LORENC 2008). Some studies (DROBNIK 2002, DROBNIK, LATOUR 2005, DERKOWSKA-SITARZ, ADAMCZYK 2008) underlined that the consumption of demineralized water may disturb the electrolytic equilibrium in an organism and exert negative effects on consumers' health. There is no information, however, to what extent filters decrease concentrations of selected ions in water.

Analyses undertaken within this study were aimed at estimating the effect of water filters on chemical properties of drinking water, with a specific focus on the indices like total hardness, concentrations of magnesium, calcium, chlorides, nitrates and water pH and electrolytic conductivity.

MATERIAL AND METHODS

Water samples were taken at random from 13 localities. In 10 sampling sites (1 – Biała Podlaska, 2 – Ełk, 3 – Hajnówka, 4 – Harachwosty, 5 – Korczew, 6 – Ozorów, 7 – Radzyń Podlaski, 8 – Siedlce, 9 – Warszawa Bemowo, 10 – Warszawa Centrum) water originated from municipal waterworks. In three other sites (11 – Biała Podlaska, 12 – Ruda Wolińska, 13 – Żeliszew Duży) water was sampled from farm wells. Water samples in sites 11 and 13 were taken from dug wells at depths of 15 and 5 m, respectively; in site 12 the sample was taken from a drilled well at a depth of 20 m. In each sampling site, water was collected before and after the instalation of a filter, and at two sites this meant a time gap of ca. 1 month. The analyses were carried out in 2012 and samples from the farm wells were collected in July – August of that year. In total, 26 water samples were collected and each sample was analysed twice. Water samples filtered in a reversed osmosis system and not enriched with additional mineralization were taken for the study. Total hardness, concentrations of calcium and magnesium (titration with sodium versenate) chlorides (argentometric method) nitrates (spectrophotometric method), electrolytic conductivity (with conductivity meter) and pH (potentiometrically) were determined in water samples according to recommendations given in the Regulation of the Minister of Environment (*Regulation* ... 2011).

The distribution of the data was tested with the Shapiro-Wilk test. The non-parametric Mann-Whitney U test was used to compare chemical parameters determined before and after the filters. Correlations between the analysed parameters were calculated with the Spearman rank correlation. All calculations were performed with the Statistica 10 software.

RESULTS

The results of determinations of particular components are illustrated in Figures 1 and 2. Table 1 shows values of the analysed parameters and statistical data. The analysed waters were characterised by a great variability of the components. In most samples, water hardness was between 200 and 300 mg $CaCO_3$ dm^{-3}. The water from site 7 was the hardest, while that from site 12 was the least hard. Water hardness was mainly determined by the concentration of calcium ions ($r_s = 0.868$, $p < 0.05$). In most unfiltered water

Table 1

Statistical parameters of analyses of water samples before (BF) and after filter (AF)

Parameter	Before (BF)/ after (AF) filter	Median	Min	Max	Z	p
$CaCO_3$ (mg dm^{-3})	BF	264.2	146.1	311.3	4.205	<0.0001
	AF	34.00	14.12	198.7		
Mg^{2+} (mg dm^{-3})	BF	9.648	4.538	21.10	4.205	<0.0001
	AF	3.400	1.148	4.860		
Ca^{2+} (mg dm^{-3})	BF	82.24	50.92	113,8	4.103	<0.0001
	AF	9.620	2.510	72,58		
Cl^- (mg dm^{-3})	BF	18.30	3.500	107.0	2.308	0.021
	AF	5.400	1.400	25.50		
NO_3^- (mg dm^{-3})	BF	2.892	0.654	30.55	1.385	0.166
	AF	1.408	0.473	8.414		
Electrolytic conductivity (μS cm^{-1})	BF	555.0	332.0	758.0	4.154	<0.0001
	AF	67.00	17.70	452.0		
pH	BF	7.390	6.810	7.840	4.206	<0.0001
	AF	6.520	4.410	6.930		

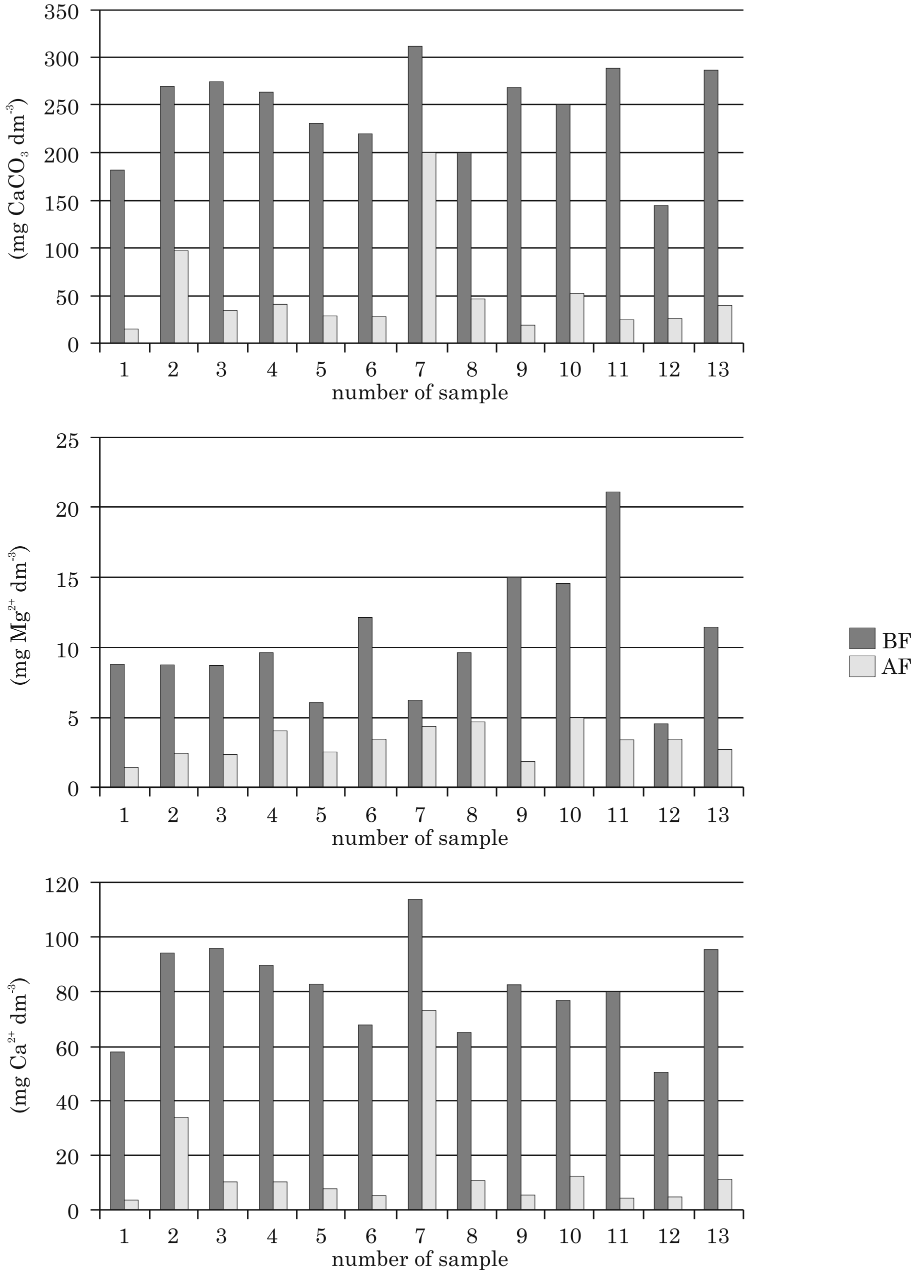

Fig.1.The results of analyses of total hardness and concentrations of magnesium and calcium ions in water before (BF) and after (AF) filter

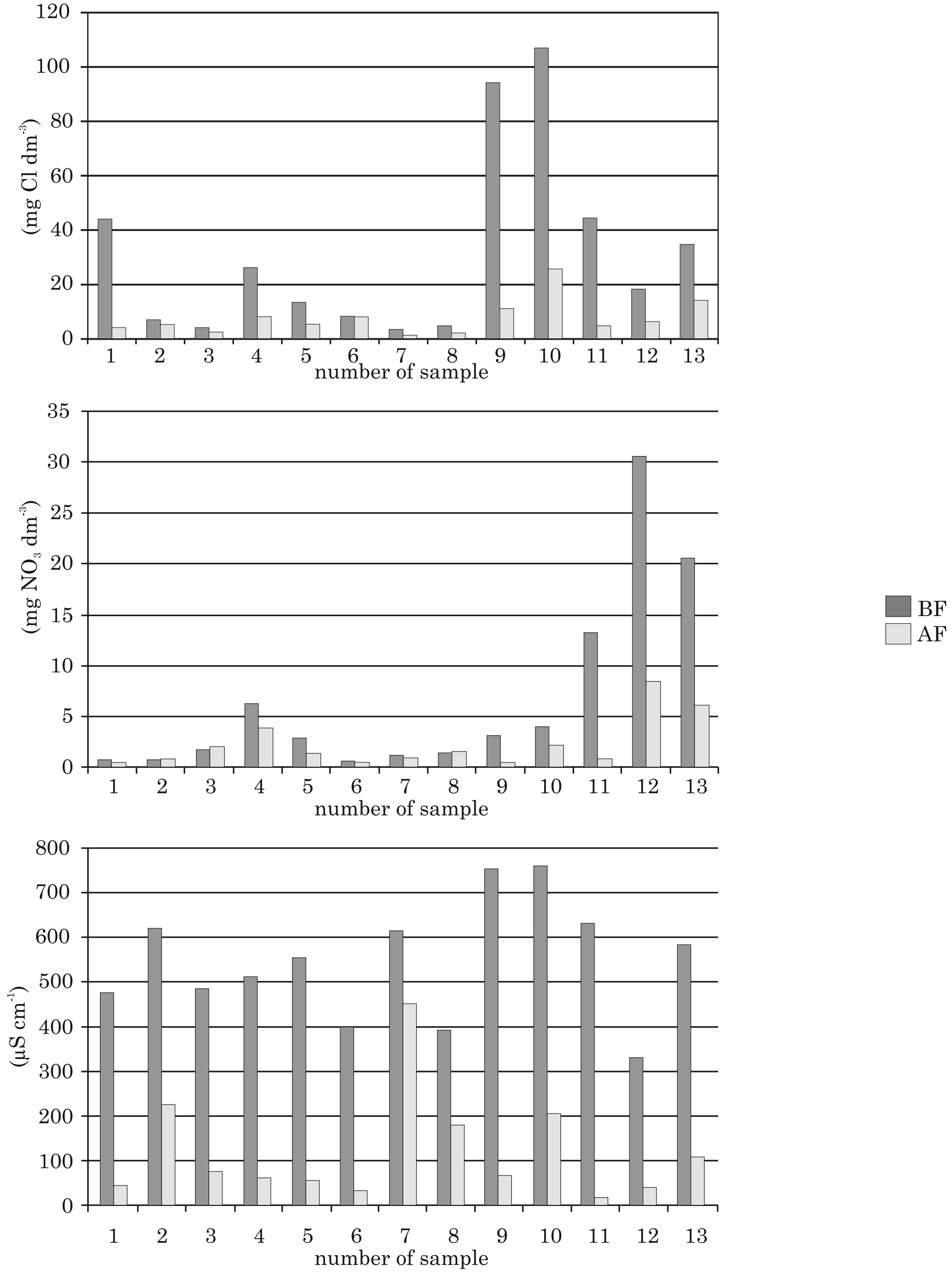

Fig. 2. The results of the analyses concentrations of chloride and nitrate ions and electrolytic conductivity in water before (BF) and after (AF) filter

samples, the calcium concentration exceeded 80 mg dm^{-3}, while in filtered waters this concentration was below 15 mg dm^{-3}. In five samples of unfiltered water, the concentration of magnesium was above 10 mg dm^{-3} and in none of the filtered samples, the magnesium concentration exceeded 5 mg dm^{-3}. The water filters decreased water hardness by 87% on average, including the decrease of Ca ions by 88% and Mg ions by 65% (Figure 1). The Ca : Mg molar ratio was 4.050 and 1.592 in unfiltered and filtered waters, respectively. Using water filters also resulted in the decrease of anion concentrations. This was particularly visible at high concentrations of chlorides and nitrates (Figure 2). The highest concentrations of chlorides (above 90 mg dm^{-3}) were noted in samples from the Warsaw waterworks (sites 9 and 10). Concentrations of nitrates in nine analysed samples of tap water did not exceed 5 mg dm^{-3}. The highest nitrate concentrations (above 10 mg dm^{-3}) were recorded in samples from farm wells (sites 11-13). The process of filtering affected the concentrations of chlorides (mean decrease by 70%) more than nitrates (mean decrease by 50%).

The highest electrolytic conductivity (above 700 μS cm^{-1}) was found in samples from the Warsaw waterworks. In most analysed water samples, the conductivity fell in the range from 400 to 650 μS cm^{-1}. Electrolytic conductivity in filtered waters decreased by *ca* 88% (Figure 2) compared with unfiltered waters. An increase of water acidity was also noted in filtered waters – the mean pH decreased from 7.39 to 6.52. Statistically significant differences of the mean values of water indices between unfiltered and filtered waters were found for all analysed parameters except nitrates (Table 1). Data presented in Figure 2 demonstrate higher concentrations of nitrates in water from farm wells (number of samples 11-13) than in waters from municipal waterworks (number of samples 1-10).

DISCUSSION

The results indicate that water filters installed in household water intakes substantially alter the chemical composition of drinking water. Changes manifest themselves in the decrease of electrolytic conductivity and total hardness due to decreased concentrations of calcium and magnesium. The analyses of total hardness of unfiltered water revealed that the inhabitants of northeast Poland drink water of medium hardness. Filtered water was classified, after Biszof (2010), as very soft ($CaCO_3$ concentration below 100 mg dm^{-3}). Total hardness of unfiltered water in the range from 60 to 500 mg $CaCO_3$ meets the norms set by the Minister of Health (*Regulation* ... 2007). After filtration, however, the concentration of $CaCO_3$ in waters from 11 intakes did not fulfil this requirement. The analytical results indicate that groundwater in the north-east of Poland, irrespective of a sampling site, had very low concentrations of magnesium (no more than 15 mg dm^{-3}).

The magnesium concentration in unfiltered waters was below the obligatory standards – 30-125 mg dm^{-3} (*Regulation* ... 2007). Filtration additionally decreases concentrations of both calcium and magnesium ions.

Long-term consumption of water poor in calcium and magnesium may result in disturbances in the electrolyte equilibrium. Deficit of magnesium may cause diseases of the cardiovascular system, disturbances of the cardiac cycle, weaknesses, vertigos and muscle contraction (GRZEBISZ 2011, BŁASZCZYK, DUDA-CHODAK 2013, CIEŚLEWICZ et al. 2013). Pathological conditions associated with systemic magnesium deficiencies may be associated with disturbances of numerous neurophysiological processes, manifesting themselves in migraine (RYBICKA et al. 2012). The symptoms of calcium deficiency include excessive neural and muscular excitability (contractions of eyelids, larynx, coronary arteries) and disturbances the cardiovascular system disorders (e.g. ventricular dysrhytmia). Chronic hypocalcemia leads to osteoporosis (MORR et al. 2006), increases the risk of brain stroke and raises blood pressure (JORDE, BONAA 2000). Supplementing the diet with both elements is very important. As underlined by KNYPL (2005), the ions of magnesium, calcium and potassium given simultaneously to geriatric patients interact synergistically and positively affect the functions of the cardiovascular system.

Magnesium and calcium are much better assimilated from water than from food (JANUSZKO et al. 2012), and drinking soft water devoid of these elements may pose a much greater risk than consumption of hard water (DERKOWSKA-SITARZ, ADAMCZYK-LORENC 2008). Consumption of highly mineralised waters, as underlined by SALOMON and REGULSKA-ILOW (2013), supplies consumers with appropriate amounts of calcium and magnesium ions and in some cases may even cover the daily demand for these elements.

Research shows that the consumption of 2 litres of unfiltered water covers 10-20% of the daily demand for calcium and 7-14% – for magnesium. Drinking filtered water decreases the contribution of water in RDAs of both elements by several times. Because the magnesium concentrations in unfiltered water were below the level recommended by the Ministry of Health (*Regulation* ... 2007), it is advisable to supplement the diet with magnesium in northeastern Poland. Supplementing the diet with both calcium and magnesium is recommended in the case of drinking filtered water.

Optimum assimilation of calcium and magnesium by the human organism takes place at the 2:1 molar ratio of Ca:Mg in water (WOJTASZEK 2006). Water filters favourably alter the Ca:Mg ratio, but this is unimportant when the concentrations of both elements are low (WOJTASZEK 2006).

The use of water filters significantly decreased water pH. This effect is directly associated with the concentration of bicarbonate ions co-existing with calcium and magnesium ions in drinking water. Waters rich in bicarbonates alkalise stomach content (exerting positive effect in hyperacidity) and in the initial stages of diabetes they decrease the concentration of glucose in blood and urine (WOJTASZEK 2006).

Noteworthy, water components analysed in randomly collected samples of unfiltered water conformed to the requirements for the quality of water intended for human consumption also with respect to chlorides and nitrates (*Regulation* ... 2007).

The concentrations of chlorides in most samples were within the range typical for the hydrogeochemical background, which is 2-60 mg dm^{-3} for Polish groundwaters (*Regulation* ... 2008). Of particular interest was a high (above 90 mg dm^{-3}) concentration of chlorides in samples from the Warsaw waterworks (samples 9 and 10), apparently caused by drinking water chlorination. Samples of water from farm wells showed elevated concentrations of chlorides and nitrates compared with those from most waterworks intakes. Concentrations of nitrates above the groundwater hydrogeochemical background (0-5 mg dm^{-3}) indicate their anthropogenic origin. Due to high concentrations of nitrates, water from farm wells, irrespective of their depth, is of worse quality than water from municipal waterworks intakes.

The results reported here indicate that the installed water filters did not significantly decrease nitrate concentrations in water. In some cases (samples 2, 3 and 8), a slight increase of nitrate concentrations in filtered water was noted. Much attention is paid in the literature to the presence of nitrates in drinking water due to their negative effect on human health (FAO/WHO 2003, Fewtrell 2004, Szczerbiński et al. 2006). Ou results showed that installed water filters did not significantly decrease nitrate concentrations in water samples but favourably decreased the Ca:Mg ratio. However, as pointed out by Wojtaszek (2006), the ratio is of no importance when concentrations of both elements are low.

Noteworthy, water from municipal waterworks is controlled by the State Sanitary Inspectorate and its consumption should not pose a health risk. This was confirmed by results of studies made by Wojtyła-Bucior and Marcinkowski (2010) in Wielkopolska. Water from farm wells is not monitored by sanitary services. In some cases, its regular consumption may pose a health risk to consumers, mainly because of nitrate concentrations exceeding the standards (Raczuk 2010, Raczuk et al. 2013). The claims of manufacturers of water filters that their product may improve water quality mislead consumers. Water filters installed in household water intakes mainly demineralise drinking water (Derkowska-Sitarz, Adamczyk-Lorenc 2008), which was also confirmed in this study. Irrespective of an intake, filtration of water in households without its supplementation with calcium and magnesium markedly decreases the concentration of ions important for proper functioning of the human organism.

CONCLUSIONS

Water filters without additional mineralization installed in households:

- significantly decrease total hardness, concentrations of magnesium, calcium and chloride ions and electrolytic conductivity. They also decrease water pH but do not statistical significantly change nitrate concentrations in drinking water,
- worsen the drinking water quality due to unfavorably decreased concentrations of calcium and magnesium and water pH.

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THERMOPHYSICAL AND CHEMICAL PROPERTIES OF BIOMASS OBTAINED FROM WILLOW COPPICE CULTIVATED IN ONE- AND THREE-YEAR ROTATION CYCLES*

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Abstract

Most of the energy today is obtained from fossil fuels, which are becoming more expensive and less available. Energy from biomass produced on agricultural land is an alternative option. Energy crops should guarantee high yield and good quality parameters, associated with their use in energy production. This study analysed the thermophysical and chemical properties of biomass obtained from 15 new clones of willow selected in the Department of Plant Breeding and Seed Production of the University of Warmia and Mazury in Olsztyn. The plants were cultivated in one- and three-year rotation cycles, run in 2009-2011 at two research stations: in Bałdy and in Łężany. The energy content as well as elemental and physical properties of biomass were analysed. The higher heating value was better in biomass from one-year shoots (on average 19.66 MJ kg^{-1} d.m.). The highest value of this parameter was recorded for the clone of *Salix acutifolia* UWM 093 (20.04 MJ kg^{-1} d.m.). The higher heating value in biomass of three-year old clones was on average lower by 0.06 MJ kg^{-1} d.m. The lower heating value in biomass increased in longer willow coppice harvest cycles. The highest lower heating value was recorded for the clone UWM 035 of *Salix pentandra* (9.27 MJ kg^{-1}) harvested in a three-year cycle, whereas the lowest one was achieved by the clone of *Salix dasyclados* UWM 155 (7.55 MJ kg^{-1}) harvested in a one--year cycle. The average moisture content in three-year shoots was 50.01% d.m., being higher by 2.31% in one-year shoots. The ash content was lower in biomass harvested in three-year rotation. In conclusion, willow biomass obtained in a three-year harvest cycle contains less of undesirable elements and proves to be better quality fuel than biomass obtained in a one-year harvest cycle.

Key words: higher and lower heating value, thermophysical properties, chemical properties, short rotation coppices, willow biomass, harvest cycles.

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INTRODUCTION

Apart from food and environmental safety, energy safety is one of the key factors to the existence and development of our civilisation. Most of the energy today is obtained from fossil fuels, which are becoming more expensive and less available. Biomass is an alternative option. Its annual potential for use as an energy source is estimated to be 2900 EJ year^{-1} (Krzyżaniak et al. 2013). The EU Directive 2009/28/EC and the Energy Policy of Poland define it as a target to raise the share of renewable energy sources in the total energy consumption to 15% by 2020 and to 20% by 2030. Importantly, it is to be expected that the consumption of forest biomass for energy purposes is likely to decrease as woodlands are to be protected against excessive exploitation. Another goal is to promote sustainable biomass production on agricultural land for energy purposes (including biofuels) in order to avoid competition between renewable energy production and agriculture. Moreover, efforts will be made to increase diversification of energy sources and to create the optimum conditions for the development of distributed energy generation, based on locally available matter. Therefore, great hopes are placed in energy crop production.

Energy crops should ensure high yield and good quality parameters, associated with their use in energy production. Other features, very important in agriculture, are a high yield of dry matter per hectare, low energy consumption and production cost, low content of contaminants and low fertilisation needs (Mckendry 2002).

The total area of land under perennial energy crops in Poland is small (10,202 ha). It is worth underlining that of all domestic sources of "green" energy, solid biomass will continue to be the main fuel in the generation of renewable energy. The trees and shrubs used as sources of energy that are most frequently mentioned in literature include: willow (*Salix* L.), poplar (*Populus* L.) and black locust (*Robinia pseudoacacia* L.) (Grünewald et al. 2009, Stolarski et al. 2011, Serapiglia et al. 2012, Buchholz, Volk 2013).

Plantations of tree species, established on agricultural land, are referred to as short rotation coppice. Wood in such systems is harvested every one to six years. The biomass is usually of better quality than forest biomass or industrial and communal waste. The type of biomass fuel, including its physical and chemical characteristics, affects the entire process of its utilisation (transport, technology of combustion, gas emission and generating solid waste). Therefore, the characteristics of biomass as fuel should include a low moisture content, high energy content, adequate proportion of fixed carbon to volatile matter, low ash content and an appropriate content of alkaline metals. Importantly, values of the above parameters differ depending on the biomass type – straw, perennial semi-wood plants, wood (Mckendry 2002, Stolarski et al. 2014). On the other hand, the thermophysical and chemical properties of wood biomass will differ depending on the species, cultivar and clone, plant organs, harvest cycle duration (e.g. 1-3 years), fertilisation technology and the habitat conditions (ADEGBIDI et al. 2001, THARAKAN et al. 2003, CHIN et al. 2013, KRZYŻANIAK et al. 2014).

The aim of this study was to determine the thermophysical and chemical properties of biomass of 15 new clones of willow cultivated in one- and three -year rotation cycles.

MATERIAL AND METHODS

Field experiments

Experiments on willow coppice were carried out in 2009-2011, in two research stations of the University of Warmia and Mazury in Olsztyn (UWM), located in Bałdy and in Łężany Two controlled field experiments involved 15 willow clones from the collection of the Department of Plant Breeding and Seed Production of UWM:

1. *Salix acutifolia* UWM 093.
2. *Salix alba* UWM 095.
3. *Salix dasyclados* UWM 155.
4. *Salix fragilis* UWM 195.
5. *Salix pentandra* UWM 035.
6. *Salix triandra* UWM 198.
7. Seven clones of *Salix viminalis:* UWM 006, UWM 063, UWM 067, UWM 144, UWM 145, UWM 146, UWM 196.
8. *Salix viminalis* x *Salix amygdalina* UWM 054.
9. *Salix viminalis* x *Salix purpurea* UWM 033.

In each experiment, clones were planted in a completely randomized design in three replicates. At the research station in Bałdy (53°35′ N, 21°08′ E), the field experiment was started in 2008. The plantation was set up on mud -muck soil developed on calcareous gyttia lying on the loamy subsoil. The soil was classified as soil IVb in the Polish soil classification system. The groundwater table was determined at below 80 cm. Cuttings (25 cm long) were planted in the second decade of April, at the density of 48 thousand pieces per ha. The plants were harvested in annual cycles (2009, 2010, 2011). At the research station in Łężany (53°58′ N, 20°36′ E), the field experiment was commenced in 2008. The soil under the experiment was classified as incomplete proper brown soil developed from medium loam on light silty clay. In agronomic terms, it was classified as IVa class, defective wheat complex. The groundwater table was below 150 cm. Cuttings (25 cm long) were planted in the third decade of April, at 25 thousand pieces per ha. The crops were harvested in a three-year cycle (2011).

Laboratory analyses

Representative samples of biomass were taken from the plots during harvest. To this end, entire willow shoots were cut into pieces and placed in plastic bags. The samples were then transported to the laboratory of the Department of Plant Breeding and Seed Production of the UWM. Immediately afterwards (no later than one day after collection and storage in a fridge, to preserve the initial moisture content), thermophysical and chemical properties of the biomass were determined. All determinations were conducted in three replications.

The moisture content was determined by the drying-weighing method, in accordance with Polish Standard PN-80/G-04511 (drying at 105±2°C until constant weight). The higher heating value (HHV) of the biomass of the willow clones was determined in accordance with PN-81/G-04513 in an IKA C2000 calorimeter by the dynamic method. The HHV and moisture content of biomass were used to calculate the lower heating value (LHV). The content of ash, volatile matter and fixed carbon was determined with a thermogravimetric analyser ELTRA TGA THERMOSTEP. This device operates in accordance with the following standards: ASTM D-5142, D-3173, D-3174, D-3175 and PN-G-04560:1998, PN- ISO 562. The carbon, hydrogen and sulphur content in willow biomass was determined with an Eltra CHS 500 automatic analyser, intended for concurrent determination of those elements. The device works in line with the following standards: ISO-10694, ASTM E 1915-97, D-1552, D-4239, D-1619, DIN EN 13137 as well as PN-G-04584 and PN-G-04517. The chlorine content in biomass was determined with the Eschka mixture. The nitrogen content in biomass was determined by the Kjeldahl method (modified by Zinneke) with a K-435 mineraliser and a B-324 BUCHI distiller. The content values of the elements were used to calculate the oxygen content.

The results of determinations of the moisture content, HHV and LHV are presented in tables for all the clones used in the experiment. The content of ash, solid and volatile parts, as well as the concentrations of C, H, S, N, Cl and O are shown in figures as mean, minimum and maximum values for plants cultivated in one- and three-year cycles, which was due to editorial considerations.

Statistical analysis

The experiment results were analysed using Statistica 9.1 PL software (StatSoft Inc.). The arithmetic mean and standard deviation were calculated for all examined features. The analysis of variance (Anova) was applied and the Tukey test (HSD) was used to determine homogenous groups (the level of significance of $\alpha = 0.05$) and correlation coefficients between the examined attributes.

RESULTS AND DISCUSSION

The average HHV of willow shoots in both harvest cycles was 19.63 MJ kg^{-1} d.m. (Table 1). The significantly highest HHV was recorded for the clone UWM 093 of *Salix acutifolia* (19.90 MJ kg^{-1} d.m.). A homogenous group with the significantly lowest value of the feature included UWM 198 *Salix triandra* (19.32 MJ kg^{-1} d.m.). Higher average values of the HHV were recorded for the clones harvested in the one-year cycle (19.66 MJ kg^{-1} d.m.), being lower by 0.06 MJ kg^{-1} d.m. for the three-year cycle. The significantly highest HHV was recorded for UWM 093 *Salix acutifolia* in the one-year harvest cycle (20.04 MJ kg^{-1} d.m.). Another homogenous group included the UWM 035 *Salix pentandra* clone in the same harvest cycle (19.88 MJ kg^{-1} d.m.). A homogenous group with the significantly lowest HHV included UWM 198 *Salix triandra* in the three-year harvest cycle. The HHV was significantly positively correlated with the content of carbon, nitrogen, sulphur and with the content of fixed carbon (Table 2).

Table 1

Higher Heating Value (MJ kg^{-1} d.m.) of willow biomass cultivated in one-year and three-year rotation cycles

Species	Clone	Annual harvest cycle	Triennial harvest cycle	Average
S. acutifolia	UWM 093	20.04±0.09 *a*	19.76±0.02 *c*	19.90±0.15 *a*
S. alba	UWM 095	19.58±0.07 *e*	19.64±0.00 *d*	19.61±0.03 *d*
S. dasyclados	UWM 155	19.53±0.14 *f*	19.45± 0.02 *g*	19.49±0.05 *f*
S. fragilis	UWM 195	19.56±0.09 *f*	19.42±0.04 *g*	19.49±0.08 *f*
S. *pentandra*	UWM 035	19.88±0.05 *b*	19.84±0.05 *b*	19.86±0.04 *b*
S. triandra	UWM 198	19.46±0.12 *g*	19.18±0.01 *h*	19.32±0.15 *g*
S. viminalis	UWM 006	19.68±0.17 *c*	19.58±0.03 *e*	19.63±0.06 *d*
	UWM 063	19.53±0.11 *f*	19.56±0.02 *f*	19.54±0.02 *e*
	UWM 067	19.56±0.10 *f*	19.50±0.04 *f*	19.53±0.04 *e*
	UWM 144	19.60±0.08 *e*	19.80±0.03 *b*	19.70±0.11 *c*
	UWM 145	19.73±0.04 *c*	19.75±0.03 *c*	19.74±0.03 *c*
	UWM 146	19.69±0.04 *c*	19.60±0.01 *e*	19.65±0.05 *d*
	UWM 196	19.66±0.10 *d*	19.59±0.01 *e*	19.62±0.04 *d*
S. viminalis x *S. amygdalina*	UWM 054	19.66±0.10 *d*	19.46±0.00 *g*	19.56±0.11 *e*
S. viminalis x *S. purpurea*	UWM 033	19.72±0.15 *c*	19.82±0.01 *b*	19.77±0.06 *c*
Mean		19.66±0,17 *a*	19.60±0.18 *b*	19.63±0.16

± standard error of the mean; *a*, *b*, *c*… homogenous groups

Table 2

Pearson correlations coefficients between analysed biomass features

Specification	Moisture content	HHV	LHV	Fixed carbon	Volatile matter	Ash	C	H	N	S	Cl
Moisture content*	1.00	0.04	**0.98**	**0.52**	**-0.53**	**0.52**	-0.15	**-0.51**	**0.55**	**0.54**	**0.43**
HHV	0.04	1.00	0.14	**0.23**	**-0.26**	0.07	**0.22**	0.06	**0.28**	**0.34**	0.11
LHV	**-0.98**	0.14	1.00	**-0.47**	**0.48**	**-0.50**	**0.19**	**0.51**	**-0.49**	**-0.47**	**-0.41**
Fixed carbon	**0.52**	**0.23**	**-0.47**	1.00	**-0.97**	**0.65**	0.14	**-0.39**	**0.87**	**0.77**	**0.61**
Volatile matter	**-0.53**	**-0.26**	**0.48**	**-0.97**	1.00	**-0.77**	-0.14	**0.30**	**-0.88**	**-0.76**	**-0.58**
Ash	**0.52**	0.07	**-0.50**	**0.65**	**-0.77**	1.00	-0.02	**-0.26**	**0.69**	**0.56**	**0.44**
C	-0.15	**0.22**	**0.19**	0.14	-0.14	-0.02	1.00	**0.28**	**0.23**	-0.11	-0.11
H	**-0.51**	0.06	**0.51**	**-0.39**	**0.30**	**-0.26**	**0.28**	1.00	**-0.34**	**-0.39**	**-0.38**
N	**0.55**	**0.28**	**-0.49**	**0.87**	**-0.88**	**0.69**	**0.23**	**-0.34**	1.00	**0.76**	**0.53**
S	**0.54**	**0.34**	**-0.47**	**0.77**	**-0.76**	**0.56**	-0.11	**-0.39**	**0.76**	1.00	**0.47**
Cl	**0.43**	0.11	**-0.41**	**0.61**	**-0.58**	**0.44**	-0.11	**-0.38**	**0.53**	**0.47**	1.00

* Significat correlations (p value ≤0,05) are indicated in bold

The average moisture content of willow shoots was 51.17% in both harvest cycles (Table 3). The significantly highest moisture content was found in the biomass of the UWM 155 *Salix dasyclados* clone (54.18%). The value of this characteristic was the lowest in UWM 035 *Salix pentandra* (48.50%). The other clones made up 5 homogenous groups with the moisture content of biomass ranging from 53.17 to 49.12%. A significantly higher moisture content in biomass (52.32%) was recorded in the one-year harvest cycle for all the clones. This property was lower in value by 2.31% in the three-year harvest cycle The significantly highest moisture content was found in biomass of UWM 155 *Salix dasyclados* in the one-year harvest cycle (54.56%), while being significantly the lowest in biomass of UWM 196 *Salix viminalis* in the three-year harvest cycle (47.06%). The same homogenous group included UWM 195 *Salix fragilis*, UWM 198 *Salix triandra*, UWM 035 *Salix pentandra* and UWM 063 *Salix viminalis*, all cultivated in the three-year harvest cycle.

The average LHV of willow biomass was 8.34 MJ kg^{-1} (Table 4). The significantly highest value of this property was recorded for biomass of UWM 035 *Salix pentandra* (9.04 MJ kg^{-1}). Another homogenous group included UWM 063 *Salix viminalis*, UWM 093 *Salix acutifolia* and UWM 196 *Salix viminalis*. The LHV for their biomass was 8.64, 8.73 and 8.78 MJ kg^{-1}, respectively. The other clones made up four homogenous groups with the LHV ranging from 8.43 MJ kg^{-1} (UWM 195 *Salix fragilis*) to 7.61 MJ kg^{-1} (UWM 155 *Salix dasyclados*). Regarding the harvest cycles, a higher LHV

Table 3

Moisture content (%) of willow biomass cultivated in one-year and three-year rotation cycles

Species	Clone	Annual harvest cycle	Triennial harvest cycle	Average
S. acutifolia	UWM 093	51.01±1.90 f	49.02±0.03 *g*	50.02±1.07 *e*
S. alba	UWM 095	52.93±1.25 *c*	51.35±0.18 *f*	52.14±0.86 *c*
S. dasyclados	UWM 155	54.56±2.83 *a*	53.79±0.07 *b*	54.18±0.52 *a*
S. fragilis	UWM 195	52.62±1.29 *d*	48.18±0.20 *h*	50.40±2.39 *e*
S. *pentandra*	UWM 035	49.56±1.17 *g*	47.44±0.06 *h*	48.50±1.14 g
S. triandra	UWM 198	51.98±1.61 *e*	48.25±0.05 *h*	50.11±2.01 *e*
S. viminalis	UWM 006	52.31±1.22 *d*	51.05±0.21 *f*	51.68±0.73 *c*
	UWM 063	51.44±0.80 *e*	47.82±0.20 *h*	49.63±1.95 *f*
	UWM 067	52.84±1.92 *c*	51.22±0.00 *f*	52.03±0.98 *c*
	UWM 144	53.79±1.66 *b*	52.55±0.41 *d*	53.17±0.83 *b*
	UWM 145	53.52±2.10 *b*	52.41±0.37 *d*	52.97±0.81 *b*
	UWM 146	52.91±1.96 *c*	49.47±0.16 *g*	51.19±1.85 *d*
	UWM 196	51.17±0.98 *f*	47.06±0.17 *h*	49.12±2.21 *f*
S. viminalis x *S. amygdalina*	UWM 054	50.89±0.70 *f*	50.60±0.21 *g*	50.74±0.21 *d*
S. viminalis x *S. purpurea*	UWM 033	53.29±1.22 *b*	49.99±0.14 *g*	51.64±1.77 *c*
Mean		52.32±2.00 *a*	50.01±2.01 *b*	51.17±2.06

± standard error of the mean; *a*, *b*, *c*… homogenous groups

was recorded for biomass harvested in the three-year cycle (8.58 MJ kg^{-1} on average). The significantly highest value of the feature (9.27 MJ kg^{-1} for UWM 035 *Salix pentandra*) was determined in the same harvest cycle. On the other hand, the significantly lowest LHV was found for UWM 155 *Salix dasyclados* (7.55 MJ kg^{-1}) obtained in a one-year harvest cycle. The LHV was significantly negatively correlated with the biomass moisture content, fixed carbon, the content of ash, nitrogen, sulphur and chlorine (Table 2).

The average ash content in biomass from willow shoots was 12.8 g kg^{-1} d.m. for both harvest cycles. The significantly highest value of the feature was recorded for UWM 195 *Salix fragilis* (15.9 g kg^{-1} d.m.), whereas the lowest one was determined for UWM 198 *Salix triandra* (11.0 g kg^{-1} d.m.). The content of ash in the biomass of willow coppice harvested in a one-year cycle was higher by 3.0 g kg^{-1} d.m. than in that harvested in a three-year cycle (Figure 1). The significantly highest content was found in biomass of UWM 195 *Salix fragilis* harvested in the one-year cycle (19.7 g kg^{-1} d.m.). The significantly lowest ash content was determined in biomass of UWM 033 *Salix viminalis* x *S. purpurea*, harvested in the three-year cycle (9.2 g kg^{-1} d.m.).

The content of fixed carbon in the biomass was 208.3 g kg^{-1} d.m. in both harvest cycles. The significantly highest value of the parameter was deter-

Table 4

Lower heating value (MJ kg^{-1}) of willow biomass cultivated in one-year and three-year rotation cycles

Species	Clone	Annual harvest cycle	Triennial harvest cycle	Average
S. acutifolia	UWM 093	8.57±0.46 *d*	8.88±0.00 *b*	8.73±0.16 *b*
S. alba	UWM 095	7.93±0.29 *h*	8.30±0.04 *e*	8.11±0.20 *d*
S. dasyclados	UWM 155	7.55±0.65 *i*	7.68±0.01 *i*	7.61±0.10 *f*
S. fragilis	UWM 195	7.98±0.29 *h*	8.89±0.02 *b*	8.43±0.49 *c*
S. *pentandra*	UWM 035	8.82±0.25 *b*	9.27±0.01 *a*	9.04±0.24 *a*
S. triandra	UWM 198	8.08±0.33 *g*	8.75±0.01 *d*	8.41±0.36 *c*
S. viminalis	UWM 006	8.11±0.23 *g*	8.34±0.06 *e*	8.22±0.14 *c*
	UWM 063	8.23±0.21 *f*	9.04±0.04 *a*	8.64±0.43 *b*
	UWM 067	7.94±0.40 *h*	8.26±0.02 *f*	8.10±0.20 *d*
	UWM 144	7.75±0.38 *i*	8.11±0.10 *g*	7.93±0.23 *e*
	UWM 145	7.86±0.47 *h*	8.12±0.07 *g*	7.99±0.18 *e*
	UWM 146	7.98±0.44 *h*	8.70±0.04 *d*	8.34±0.39 *c*
	UWM 196	8.35±0.18 *e*	9.22±0.03 *a*	8.78±0.47 *b*
S. viminalis x *S. amygdalina*	UWM 054	8.41±0.12 *e*	8.38±0.04 *e*	8.40±0.04 *c*
S. viminalis x *S. purpurea*	UWM 033	7.91±0.27 *h*	8.69±0.04 *d*	8.30±0.42 *c*
Mean		8.10±0.47 *b*	8.58±0.44 *a*	8.34±0.46

± standard error of the mean; *a*, *b*, *c*… homogenous groups

mined in biomass of UWM 196 *Salix viminalis* (217.0 g kg^{-1} d.m.). The other clones made up seven homogenous groups with the content of this element ranging from 212.1 g kg^{-1} d.m. (UWM 145 *Salix viminalis*) to 202.8 g kg^{-1} d.m. (UWM 195 *Salix fragilis*). With respect to the harvest cycles, the significantly highest fixed carbon content was found in biomass of willow cultivated in the one-year cycle (218.9 g kg^{-1} d.m.) – Figure 1. The value of this characteristic was 21.3 g kg^{-1} d.m. lower in biomass harvested from plants cultivated in the three-year cycle. The significantly highest value was determined for UWM 054 *Salix viminalis* x *Salix amygdalina*, cultivated in the one-year cycle. Another homogenous group was composed of the clones UWM 196 and UWM 145 *Salix viminalis* (both 244.8 g kg^{-1} d.m.) and UWM 033 *Salix viminalis* x *S. purpurea* (223.3 g kg^{-1} d.m.) in the same harvest cycle. The significantly lowest content of fixed carbon was determined in biomass of UWM 195 *Salix fragilis* cultivated in the three-year cycle.

The content of volatile matter in biomass of willow cultivated in both harvest cycles averaged 778.7 g kg^{-1} d.m. The significantly highest value of this feature was found for UWM 198 *Salix triandra* (786.7 g kg^{-1}d.m.). The

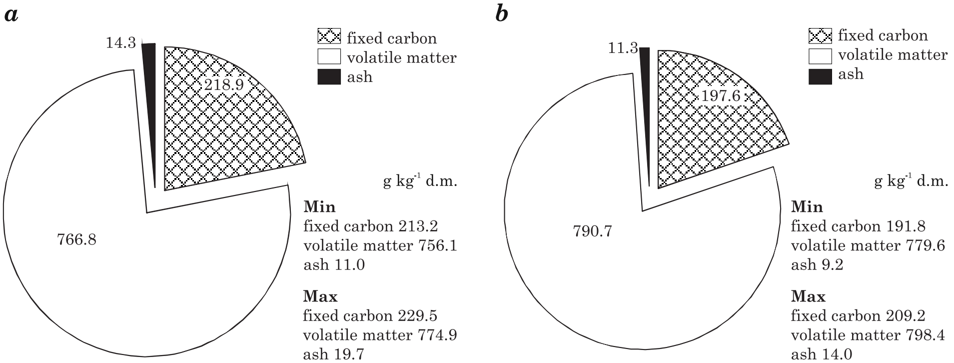

Fig. 1. Fixed carbon, volatile matter and ash content (g kg^{-1}) in biomass of willow cultivated in one-year (*a*) and three-year (*b*) rotation cycles

other clones made up seven homogenous groups with values ranging from 783.3 g kg^{-1} d.m. (UWM 035 *Salix pentandra*) to 770.7 g kg^{-1} d.m. (UWM 196 *Salix viminalis*). The biomass of willow cultivated in the three-year harvest cycle contained more volatile matter (by an average of 23.9 g kg^{-1} d.m.) than the biomass of plants cultivated in the one-year cycle (Figure 1). The content of this component was the highest in biomass of UWM 198 *Salix triandra* cultivated in a three-year cycle. The same homogenous group also included UWM 054 *Salix viminalis* x *S. amygdalina* cultivated in the three-year rotation. The other clones made up 10 homogenous groups, with the volatile matter content ranging from 796.5 g kg^{-1} d.m. to the significantly lowest value of 756.1 g kg^{-1} d.m. (UWM 054 *Salix viminalis x S. amygdalina*, in the one-year harvest cycle).

The average content of carbon element in biomass of plants cultivated in both harvest cycles was 509.1 g kg^{-1} d.m., with values ranging from 522.3 to 501.2 g kg^{-1} d.m. Biomass harvested in the one-year cycle contained 1.4 g kg^{-1} d.m. more of this component than in the three-year cycle (Figure 2). The highest carbon content was found in biomass of UWM 093 *Salix acutifolia* (527.5 g kg^{-1} d.m.) cultivated in the one-year cycle, and the lowest one was in UWM 144 *Salix viminalis* from the three-year harvest cycle.

The hydrogen content in biomass of both harvest cycles averaged 63.0 g kg^{-1} d.m. The highest content of this element was found in biomass of UWM 093 *Salix acutifolia.* The other clones made up five homogenous groups with the hydrogen content ranging from 63.9 to 61.8 g kg^{-1} d.m. The biomass of willow harvested in the three-year cycle contained more hydrogen (63.5 g kg^{-1} d.m.) than in the one-year cycle (62.5 g kg^{-1} d.m.) – Figure 2. The significantly highest content of this element was found in biomass of UWM 093 *Salix acutifolia* cultivated in the three-year cycle (67.6 g kg^{-1} d.m.). The highest content of this element in plants cultivated in the one-year cycle was not qualified until the third homogenous group, being lower by 3.9 g kg^{-1} d.m. The significantly lowest hydrogen content througho-

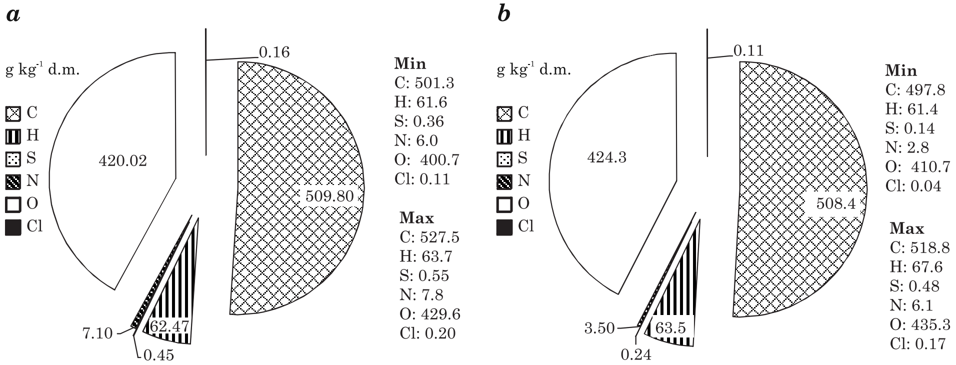

Fig. 2. Elemental analysis of willow biomass (g kg^{-1}) cultivated in one-year (*a*) and three-year (*b*) rotation cycles

ut the whole experiment was found in biomass of UWM 067 *Salix viminalis* cultivated in the three-year cycle.

The nitrogen content in willow biomass averaged 5.3 g kg^{-1} d.m. The significantly highest content of the element was found in biomass of UWM 095 *Salix alba* (6.9 g kg^{-1} d.m.). On the other hand, UWM 144 *Salix viminalis* contained the significantly smallest amount of this element (4.4 g kg^{-1} d.m.). The biomass of willow cultivated in the one-year harvest cycle contained, on average, twice as much nitrogen as the biomass of clones grown in the three -year harvest cycle (Figure 2). The significantly highest content of nitrogen was found in the biomass of UWM 145 and UWM 146 *Salix viminalis* (7.8 g kg^{-1} d.m.) from the one-year harvest cycle. The same homogenous group contained four other clones cultivated in the same harvest cycle. The significantly lowest nitrogen content was found in the clones cultivated in the three-year harvest cycle: UWM 196 and UWM 144 (2.8 g kg^{-1} d.m.), both of the *Salix viminalis* species.

The content of sulphur in the biomass of the clones used in the experiment, in both harvest cycles, averaged 0.34 g kg^{-1} d.m. The highest content of this element was found in the biomass of UWM 144 *Salix viminalis* (0.45 g kg^{-1} d.m.), being significantly the lowest in the biomass of UWM *Salix triandra* (0.13 g kg^{-1} d.m.). As for the harvest cycles, the sulphur content in biomass of willow cultivated in the one-year cycle (Figure 2) was nearly double than that from the three-year cycle. The significantly highest content of this parameter was found in the biomass of UWM 095 *Salix alba* cultivated in the one-year cycle (0.55 g kg^{-1} d.m.). The same homogenous group included UWM 035 *Salix pentandra*. The other clones made up six homogenous groups with the content of sulphur ranging from 0.49 g kg^{-1} d.m. in UWM 146 *Salix viminalis* and UWM 033 *Salix viminalis* x *S. purpurea* (cultivated in the one-year harvest cycle) to 0.14 g kg^{-1} d.m. in UWM 155 *Salix dasyclados* (in the three-year harvest cycle).

The average chlorine content in the biomass of clones cultivated in the one-year and three-year harvest cycles was 0.14 g kg^{-1} d.m. The significantly highest content of this element was determined in the biomass of UWM 195 *Salix fragilis* and UWM 196 *Salix viminalis* (0.17 g kg^{-1} d.m.). The same homogenous group also included UWM 095 *Salix alba* and UWM 155 *Salix dasyclados* with the chlorine content lower by 0.01 g kg^{-1} d.m. The other clones made up three homogenous groups. The significantly lowest chlorine content was determined in the biomass of UWM 054 *Salix viminalis* x *S. amygdalina.* The biomass of the clones cultivated in the one-year cycle contained more chlorine (0.16 g kg^{-1} d.m.) than in the three-year cycle (0.11 g kg^{-1} d.m.) – Figure 2. The significantly highest Cl content was found in biomass of UWM 196 *Salix viminalis* cultivated in the one-year harvest cycle (0.20 g kg^{-1} d.m.). A homogenous group with the lowest chlorine content was made up of UWM 067 *Salix viminalis* and UWM 054 *Salix viminalis* x *S. amygdalina* cultivated in the three-year cycle (0.05 and 0.04 g kg^{-1} d.m., respectively).

The amount of energy (higher and lower heating value) as well as the thermophysical and chemical composition of biomass (carbon, sulphur, hydrogen, nitrogen as well as ash and moisture content) are very important characteristics of biomass used for energy purpose and production of biomaterials. For example, ash and chlorine formed in the process of biomass combustion (responsible for particulate emission and corrosion), affect the operation of boilers, installation safety and its later use or utilisation. The composition of feedstock also influences the quality of bio-products obtained by thermochemical conversions (WILSON et al. 2013).

The higher heating value determined in this study was slightly higher when biomass was grown in the one-year harvest cycle. The higher HHV of one-year willow shoots resulted from those shoots having more bark and, consequently, more lignin than older plants (KLASNJA et al. 2002, KOMOROWICZ et al. 2009). FIJAŁKOWSKA and STYSZKO (2011) showed that the HHV varied in biomass of 9 willow clones, ranging from 18.45 to 18.77 MJ kg^{-1} d.m. STOLARSKI (2009) examined 5 willow cultivars: Duotur and Corda (*Salix alba*), Tur, Turbo and UWM 046 (*Salix viminalis*) and found the lower and higher heating values to be diverse as well. The HHV averaged 19.2 MJ kg^{-1}. The highest HHV was determined in biomass of cv. Tur. The HHV of willow biomass is high compared to other types of biomass. For example, the HHV of stems of Virginia mallow ranged from 17.17 to 18.50 MJ kg^{-1} d.m. (SZYSZLAK-BARGLOWICZ et al. 2012). On the other hand, the HHV of giant miscanthus, most frequently cultivated as an energy crop, ranged from 16.58 to 17.96 MJ kg^{-1} d.m. (KOMOROWICZ et al. 2009).

The moisture content of willow biomass in this study decreased as the harvest cycle was prolonged. This relationship was confirmed by STOLARSKI (2009) for willow grown in one-, two-, and three-year harvest cycles. The moisture content in willow wood was high compared to the biomass of perennial semi-wood plants and grasses. The moisture content in Virginia mallow harvested in December was 25.7%, thus being much lower than in willow shoots – 52.9% on average (BORKOWSKA 2005). In addition, the moisture content in grasses is lower than in willow (as determined in this study), varying from 16.0 to 26.2% depending on a species (Stolarski 2008).

The lower heating value (the amount of energy which can be utilised) was equal to 8.58 MJ kg^{-1} in this study, when willow was harvested in the three-year cycle and 8.10 MJ kg^{-1} in the one-year harvest cycle. Therefore, it increased as the harvest cycle duration was extended from one to three years. This characteristc was strongly and negatively correlated to the moisture content in biomass. The LHV of willow wood immediately after harvest is lower compared to that of perennial plants or grasses. However, willow shoots can be stored (seasoned) to reduce their moisture content by as much as 70-80% (Gigler et al. 2000).

The ash content in biomass harvested every three years was lower by 3.0 g kg^{-1} d.m. compared to the one-year harvest cycle. The ash content decreasing with an increasing harvest cycle duration has been confirmed in other studies (Klasnja et al. 2002, Komorowicz et al. 2009, Stolarski 2009, Krzyżaniak et al. 2014). The relationship is associated with less bark vs wood in older (thicker) shoots. The value of ash content determined in wood of *Salix alba* ranged from 5.2 to 8.9 g kg^{-1} d.m. On the other hand, its value in the bark of shoots ranged from 47.7 to 59.4 g kg^{-1} d.m. The authors mentioned that the share of bark in willow biomass was from 26.7% in a one-year harvest cycle down to just 16.7% in a two-year cycle (Klasnja et al. 2002). Tharakan et al. (2003) determined an average ash content in biomass of 30 clones of willow cultivated in a three-year harvest cycle to be 20 g kg^{-1} d.m. The value ranged from 13 in *Salix purpurea* 94003 to 27 g kg^{-1} d.m. in the hybrid *S. erio* 39 x *S. petiolaris* 47 S599. Interestingly, willow wood contains less ash than perennial plants or grasses, where the values can be up to 30-50 g kg^{-1} d.m. (Tharakan et al. 2003, Kalembasa 2006, Stolarski 2008, Brosse et al. 2012).

The content of undesirable elements, such as nitrogen, sulphur and chlorine, in willow biomass decreased with the duration of a harvest cycle changed from one to three years. However, willow biomass harvested in shorter cycles still contains less sulphur and chlorine than grass and semi-wood species (Kalembasa et al. 2005, Stolarski et al. 2014). This is important for the environment and technologies, because chlorine causes corrosion whereas sulphur and nitrogen are responsible for the emission of hazardous gases (sulphur and nitrogen oxides) to the atmosphere in the process of biomass combustion. For energy producers, it is essential to secure a supply of good quality energy feedstock from a documented source e.g. forest or agricultural biomass, which – for example in Poland – is a necessary condition for obtaining a ‘green certificate’. That is why power generating companies are interested in receiving so-called ‘fuel cards’, which contain information about the termophysical and chemical properties of biomass (Szymanowicz 2011). Another equally important issue in the search for good quality and composition of solid biomass fuels is the low cost of generating electricity from combustion or co-combustion of this feedstock, as compared to other renewable energy sources (Lüschen, Madlener 2013). This makes biomass an energy source. Additionally lignocellulose biomass can be used for production of second generation ethanol or synthesis gas, which can also be used for the production of biofuels, heat or electricity (TIJMENSEN et al. 2002, NANDA et al. 2012, STOLARSKI et al. 2013).

CONCLUSIONS

The demand for biomass in Poland and in the European Union will continue to rise because of the binding regulations pertaining to the use of renewable sources of energy. A large share of biomass for energy generation should be produced in a sustainable manner, saving nature valuable areas. Some of the biomass will be recovered from waste, by-products and residues of the wood processing industry and agriculture, but most will be derived from dedicated energy crops, e.g. short rotation willow coppice. It is presumed that such plantations will be established on farmland of worse quality, less suitable for growing food or fodder crops. Thus, biomass will be grown in compliance with the sustainability requirements but it will be of better quality than biomass derived from waste or residues.

The study presented in this paper has been performed to determine the quality of biomass from 15 new clones of willow grown in a one- and three-year harvest cycle. The analyses have demonstrated that the biomass of plants harvested in the one-year cycle was characterized by higher HHV. However, a higher moisture content in woody matter means a lower LHV (that is usable energy) compared to biomass from plants harvested in the three-year cycle. Also, the content of undesirable components in fuel (ash, sulphur, nitrogen and chlorine) was lower in the biomass of plants grown in the three-year rotation cycle. Thus, the willow biomass obtained in the three -year harvest cycle contained less of undesirable elements and proved to be better quality fuel than biomass obtained in the one-year harvest cycle. The research on the biomass quality from willow short rotation coppice, presented above, can be useful for energy industry and for selection of proper feedstock, suitable for requirements of a given facility or the conversion method it employs. These results are part of the research on Polish varieties and clones of willow developed at the Department of Plant Breeding and Seed Production of the University of Warmia and Mazury in Olsztyn. The whole research framework comprises studies on productivity of plants, economic efficiency, energy generation efficiency, and the effect of cultivation of energy crops on the environment. These issues will be discussed in the following articles.

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INFLUENCE OF THE SELECTED ANTIBIOTIC ON ABSORPTION PROCESS OF Mg^{2+} IONS FROM SOLID DISPERSIONS CONTAINING GLUTAMATE MAGNESIUM SALTS

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Abstract

Numerous papers concerning the research on the role of magnesium in an organism and the effects of its deficiency indicate many causes and mechanisms leading to a negative balance of this macroelement. One of the causes of magnesium deficiency in systems may be its malabsorption from the digestive track resulting from taking compounds complexing magnesium ions or using drugs destructive to saprophytic flora.

The results of our experiments testing *in vitro* the influence of β-lactam of penicillin G on the process of Mg^{2+} ions absorption through the intestinal membrane from solid dispersions containing phosphatidylcholine (PC 45) and the salts of magnesium glutamate and its derivatives from glycine and arginine ligands are presented in this paper.

In order to make solid dispersions containing glutamate magnesium salts, auxiliary substances, polyvinylpyrrolidone (PVP) and phosphatidylcholine (PC45), were used. For the solid dispersions obtained by the Hansch method, partition coefficient in the system *n* - octanol/phosphate buffer was determined. Log P was calculated for the examined dispersions.

Research on the degree of absorption of Mg^{2+} ions solid dispersions, which was the subject of the experiment, was done by the *in vitro* method, making use of a model of the intestine (ileum) of a rat. The evaluation of penicillin G influence on the degree of absorption of Mg^{2+} ions from solid dispersions containing magnesium salts was carried out by the above method.

The results of experiments indicate that an additional ligand (glycine, arginine) in the structure of magnesium glutamate particle significantly influences the change of the value of of the kinetic absorption parameter of Mg^{2+} ions from solid dispersions containing magnesium salts. Introducing an additional ligand induces an accelerated rate of diffusion of the examined salts through the intestine.

It has been found that penicillin G hinders the degree of Mg^{2+} ions absorption of the examined solid dispersions in the small intestine.

Key words: magnesium glutamate, amino acids: glycine, arginine, penicillin G, solid dispersion, log P, absorption.

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INTRODUCTION

An antibiotic therapy may weaken the immune system and induce magnesium deficiency. In such a situation, antibiotics are sometimes administered with magnesium supplements. Inorganic magnesium ions reduce the bioavailability of antibiotics (Satterwhite et al. 1992). The latest research results on interactions of antibiotics with minerals show that calcium, zinc, magnesium and iron compounds can interact with tetracycline, preventing absorption of an antibiotic. Arayne et al. (2005) described that magnesium forms insoluble complexes with tetracyclines. Guz and Bugla-Płoskońska (2007) explain that the inhibitory activity of tetracyclines is the effect of chelation of Ca^{2+} and Mg^{2+}. Medications from the antacida group diminish the absorption of antibiotics and limitate their efficacy. Simultaneous administration of both vitamin and mineral medications plus tetracycline causes undesirable reactions in and organism. Aminoglycoside antibiotics worsen Mg excretion in urine, causing or exacerbating its shortage (Sandstrom 2001). Lactam antibiotics significantly affect the process of methylation of the carboxyl groups in the protein, lowering the ascorbic acid (vitamin C) content (Rodriguez 1991, Guz, Bugla-Płoskońska 2007). Suliburska (2010) showed that there is interaction between the drug and the diet, which influences the process of absorption and metabolism. Inhibited and delayed absorption of penicillin and amoxicillin has been noticed in the presence of ingested food. This negative effect on drug absorption is explained by creation of their insoluble forms.

Solid dispersions are systems which enable one to control release of a therapeutic substance. A choice of the suitable method for making solid dispersions, including the type and amount of the carrier, are decisive for the availability of a givem pharmaceutical (Dhiman et al. 2012, Ahuja et al. 2012). The up-to-date research on absorption shows that phosphatidylcholine (PC 45) and polyvinylpyrrolidone (PVP) are good carriers of Mg^{2+} ions from solid dispersions containing magnesium salts (Marcoin 2006, Marcoin, Szulc-Musioł 2009, 2011). Phosphatidylcholine (PC45) was a natural carrier which can be biodegradable and metabolized since it is a part of biological membranes. Polyvinylpyrrolidone (PVP) the hydrophilic synthetic polimer was the carrier which enhanced the solubility of drugs. Dua et al. 2010, prepared solid dispersions of aceclofenac with various hydrophilic carriers such as: PVP, ureamannitol, and PVP/VA-64. with an aim to improve their dissolution. Other researches (Rao et al. 2010) also the solid dispersion technique are methods proved to be improving the dissolution and bioavailability of simvastatin. According to patent (Curatolo et al. 2012) solid dispersions containing sparingly soluble drug and PVP, HPMC (hydroxypropyl methylcellulose acetate succinate), HPC (hydroxypropyl cellulose) provide water solubility and availability in the used medium. Teberekidis et al. (2006), Dhiman et al. (2012), showed that the improvement of the solubility of felodipineis a result of the formation of hydrogen bonding between PVP and the drug.

The subject of this paper is to evaluate the absorption of Mg^{2+} by an *in vitro* method from solid dispersions containing magnesium salts such as: $MgGlu)_{2,}$ Mg(Glu-Gly) and Mg(Glu-Arg) and carriers PVP or PC45. Evaluation of penicillin G influence on degree absorption of Mg^{2+} from solid dispersions containing magnesium glutamate salts and phosphatidylcholine (PC 45) was made.

MATERIALS AND METHODS

The following were the subject of the experiment:

- magnesium glutamate-$Mg(Glu)_2$, $(C_5H_8\ O_4N)_2Mg$; mol.wt. – 316,33
- magnesium glycine-glutamate-Mg(Glu-Gly), $C_7H_{12}O_6N_2Mg$; mol.wt. – 244,32
- magnesium arginine-glutamate-Mg(Glu-Arg), $C_{11}H_{21}O_6N_5Mg$; mol.wt. – 343,36
- penicillin G benzatine salt - Sigma Chemical Co. (Lot 50H0450)

The auxiliary substances such as polyvinylpyrrolidone (PVP by Serva) and phosphatidylcholine 45% (PC 45 by Lucas Meyer, Ltd) were used in order to produce solid dispersions containing the above magnesium salts. All the chemicals were of the analytical reagent grade.

The synthesis of magnesium glutamate was carried out according to the procedure previously described by Marcoin and Ryszka 1991. The magnesium glutamate-glycine Mg(Glu-Gly) and magnesium glutamate-arginine Mg(Glu-Arg) were obtained through modification structure of the magnesium glutamate with the ligand of glycine or arginine (Figure 1). The synthesis of

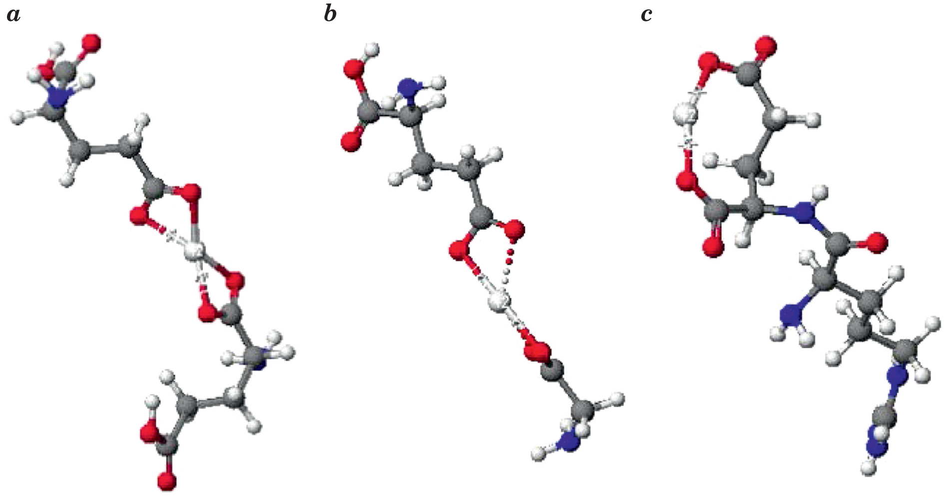

Fig. 1 $Mg(Glu)_2$ – *a*, Mg(Glu-Gly) – *b*, Mg(Glu-Arg) – *c* these compounds was carried out similarly to the compounds described in previous research work (Marcoin, Szulc-Musioł 2011). The content of magnesium in the salts was measured by atomic absorption spectrophotometry (Carl Zeiss Jena model AAF 3) at the wavelength of 258.2 nm.

Solid dispersions containing magnesium salts were prepared by FP VIII method. After previous micronization each magnesium salt was mixed with the selected carrier (PVP or PC 45) in a molar ratio (1:10) and dissolved in ethanol. The solid dsipersions were isolated from the solution after evaporation of ethanol and drying under vacuum and which unified by a sieve (1,0 mm).

Partition coefficient o/w was determined for the solid dispersions, according to the Hansch theory and also the log P as alipophilicity parameter.

The absorption of Mg^{2+} from magnesium salts was carried out on an *in vitro* model (the method described previously (Marcoin, Szulc-Musioł 2002) in which the absorption area wasthe small intestine (ileum) of arat. Similar procedure of the study of absorption of Mg^{2+} ions from solid dispersion scontaining magnesium salts with addition of PC45 carrier in the presence of penicillin G (50 mg) was carried out. The study was approved by the Bioethics Committee of the Medical University of Silesia. Constant (k) rate of absorption and of absorption half time ($t_{50\%}$) were also calculated. These results are considered significant statistically verified using the Anova followed by Kruskal-Wallis test or else Post Hoc multiple comparisons were made $p \leq 0.05$.

RESULTS AND DISCUSSION

Lipophilic properties of magnesium salts were determined by means of the partition coefficient of log P for the *n*-octanol/phosphate buffer system. The calculated partition coefficient (log P) values for the solid dispersions containing the examined magnesium salts with PVP or PC 45 are presented in Table 1. Based on these results, the modification of the structure of the parent ligand of glycine or arginine affects the growth of the log P values. In the case of Mg(Glu-Gly), an increase in the log P value for the parent compound is 0.175, whereas for Mg(Glu-Arg), the difference is greater, up to 0.965. Analysis the results showed that introduction of an additional ligand, such as glycine or arginine, into a molecule of $Mg(Glu)_2$ influences lipophilic properties of the examined salts. The chain lengthened with a $(-CH_{2}-)$ group

Table 1

Values of log P of the partition coefficient for solid dispersions containing magnesium salts

Log P	$Mg(Glu)_2$	Mg(Glu-Gly)	Mg(Glu-Arg)	$Mg(Glu)_2$		Mg(Glu-Gly)		Mg(Glu-Arg)	
	-1.061	-0.886	-0.096	PVP	PC45	PVP	PC 45	PVP	PC45
				-0.768	0.148	-0.078	0.176	-0.026	0.250

as well as the presence of the nucleophilic group (-NH_2) are the factors conditioning intercellular and intermolecular interactions.

The use of PVP or PC 45 as carriers in solid dispersions significantly contributed to the increase in the value of log P, thus raising the hydrophobicity of these systems.

It needs to be noted that the log P values for the solid dispersions with the addition of PC 45 were higher in each case. The impact of the interaction between the carriers and magnesium salts was noted.The calculated log P value for the solid dispersions containing Mg(Glu-Arg) with the addition of the PC 45 carrier (log P = 0.250) is higher than the log P value for solid dispersions containing Mg(Glu-Arg) and PVP (log P = -0.026). The calculated log P value for the solid dispersion, containing the Mg(Glu-Gly) PC45 carrier increased by 0.290 units compared with the log P values for the dispersion of Mg(Glu-Gly) without PC45. Use of PC 45 as a carrier in forming of solid dispersion containing Mg(Glu-Arg) causes an increase of the value of log P.

Phosphatidylcholine (PC 45) as a carrier significantly improves lipophilicity (increased log P values).To study the absorption of Mg^{2+} ions from solid dispersion in the presence of the selected antibiotic, the PC 45 carrier was used. Other authors who carried out studies of solid dispersions of various drugs using other carriers also obtained positive results. The addition of phosphatidyl choline to solid dispersion containing active ingredients increases dissolution. Other authors (Law 1992, Marsac 2008) explained the presence of intermolecular hydrogen bonding as a factor creating an amorphous form of a crystal structure drug. Bikiaris et al. (2005) as well as Jagadeesan et al. (2013) used phospatidylcholine for solid dispersions of poorly water-soluble drugs and determined parameters of physicochemical properties. They found that addition of phosphatidylcholine increased the dissolution rate and consequently improved bioavailability of the examined drugs. Singh et al. (2011) were those who also reported that addition of the carrier may contribute to increasing dissolution rates and consequently better bioavailability of the drug.

Table 2 shows the mean value of pharmacokinetic parameters in the process of Mg^{2+} ions absorption from solid dispersions containing magnesium glutamate salts with PC 45.

Table 2

Kinetic parameters of Mg^{2+} absorption in the small intestine from solid dispersions containing magnesium salts

Solid dispersions	k 10^{-3} (min)	$t_{50\%}$ (h)	Total amount (%) of absorberd Mg^{2+} within 2 h of the experiment	(±) SD	V (%)
Mg $(Glu)_2$	1.569	7.361	12.70	0.67	5.27
Mg (Glu-Gly)	1.843	6.266	15.28	0.73	4.77
Mg(Glu-Arg)	1.742	5.842	16.28	0.66	4.05

k – absorption rate constant, $t_{50\%}$ – absorption half-time, V – variance, SD – standard deviation at $p \leq 0.05$

In the course of our experiments, it was established that Mg^{2+} absorption can take place according to two independent transport mechanisms. The first one employs a passive diffusion process, while the other one occurs by facilitated diffusion from the intestinal lumen into epithelial cells, followed by the flow from cells into blood by means of a mechanism depending on the energy supply. The absorption of Mg^{2+} ions by the small intestine of a rat is in agreement with first order kinetics. When modified with glycine Mg(Glu-Gly), an increase of the constant rate of absorption was observed (k = $1.843 \cdot 10^{-3}$ min) in comparison with the matrix compound $Mg(Glu)_2$ (k = $1.569 \cdot 10^{-3}$), whereas the absorption half-time ($t_{50\%}$) is shorter for Mg(Glu-Gly) ($t_{50\%}$ = 6.266 h) than for solid dispersions containing $Mg(Glu)_2$ ($t_{50\%}$ = 7.361 h). The calculated values of absorption rate (k) and absorption time 50% for solid dispersions containing Mg(Glu-Arg) (k = $1.742.10^{-3}$, $t_{50\%}$ = 5.842 h) are lower than the analogous values for solid dispersions containing Mg(Glu-Gly).

Modification with an arginine ligand also influences beneficially the profile of absorption parameters: the amount of absorbed Mg^{2+} ions during 2 h Mg(Glu-Arg) was 16.28% versus 12.70% for the $Mg(Glu)_2$ matrix compound in the case of Mg(Glu-Gly), this value was 15.28%. Structural properties significantly influenced the effect of lipid phase (cellular membranes). Moreover, parameters of the surface, volume and molecular mass affect the ability of an active substance to penetrate through the lipid barrier.

The analysis of the research results on absorption of Mg^{2+} ions from solid dispersions containing the examined salts and in presence of penicillin G showed that pharmacokinetic profile changes significantly depended on the type of salt (cf. Table 3). The results of our analysis showed that the absorption rate constant (k) for solid dispersions containing $Mg(Glu)_2$, Mg(Glu-Gly) and Mg(Glu-Arg) salts in the presence of penicillin G causes a decrease of k by 0.888, 0.287 and 0.028, respectively, compared with solid dispersions containing these salts without penicillin G. The calculated values of absorption half-time ($t_{50\%}$) for the examined solid dispersions with $Mg(Glu)_2$, Mg(Glu-Gly) and Mg(Glu-Arg) increased by about 9.602 h, 1.145 h, 0.895 h, respectively, compared with the reference system. The amount of absorbed

Table 3

Kinetic parameters of Mg^{2+} absorption in the small intestine from solid dispersions containing magnesium salts in the presence penicillin G

Solid dispersions	k 10^{-3} (min)	$t_{50\%}$ (h)	Total amount (%) of absorbed Mg^{2+} within 2 h experiment	(±) SD	V (%)
Mg $(Glu)_2$ + penicillin G	0.681	16.963	9.80	0.942	9.612
Mg (Glu-Gly) + penicillin G	1.556	7.411	12.31	0.54	4.38
Mg(Glu-Arg) + penicillin G	1.714	6.737	14.21	4.48	3.15

Explanations see Table 2

Mg^{2+} after 2 hours of the experiment was significantly ($p \leq 0.05$) increased for solid dispersions in the presence of penicillin G with $Mg(Glu)_2$ by 2.90%, with Mg(Glu-Gly) by 2.97% and with Mg(Glu-Arg) by about 2.07 % compared with the reference system. On the basis of the completed experiment, an interaction was found in the phase of the pharmacokinetic process of Mg^{2+} ion absorption from the examined dispersions.

Values of kinetic parameters of Mg^{2+} absorption from solid dispersions containing the examined glutamate magnesium salts were lower than the values of kinetic absorption parameters of Mg^{2+} absorption from solid dispersions without penicillin G.

In brief, penicillin G hinders the process of Mg^{2+} ion absorption from the examined dispersions.

CONCLUSION

Modifying the structure of Mg(Glu)2 with an additional ligand glycine or arginine significantly improves the kinetic profile of the absorption of Mg^{2+} ions from solid dispersions containing magnesium salts of the carrier PC 45.

The presence of β-lactam antibiotic inhibits the Mg^{2+} absorption process from solid dispersions, which was the subject of our research.

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INFLUENCE OF FOLIAR FERTILISATION WITH CALCIUM FERTILISERS ON THE FIRMNESS AND CHEMICAL COMPOSITION OF TWO HIGHBUSH BLUEBERRY CULTIVARS*

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Abstract

Maintaining the high quality of fruits after harvest, which amongst other things is determined by firmness, is the aim producers strive after. Calcium, which fruits can be enriched with also in an extraradicular manner, is mostly responsible for the mechanical resistance of fruits. In the research conducted at the Laboratory of Orcharding, West Pomeranian University of Technology in Szczecin, the influence of foliar calcium fertilisers on the quality of fruits from two cultivars of highbush blueberry was studied. The firmness, size and chemical composition of fruits were examined (the content of mineral and organic compounds, especially polyphenols). At the fruit growth stage, bushes were sprayed 4 times with calcium solutions every 10 days, starting from the first decade of June. All fertilisers caused an increase in calcium concentrations in fruits (0.12-0.15 g 100 g^{-1}), as compared with the control (0.09 g 100 g^{-1}). The weight of 100 fruits collected from bushes sprayed with the Lebosol, Folanx and Calcinit fertilisers was the highest and it ranged from 327 to 365 g, and the Sunrise cultivar fruits were larger. The firmest (391 and 415 G mm) and the most resistant to mechanical damage (132 and 114 G mm) were the fruits sprayed with the calcium chloride and Folanx fertilisers, while fruits sprayed with Folanx and Calcinit were characterised by the highest polyphenol content (300 and 313 mg 100 g^{-1}), and anthocyanins, among which delphinidin-3-galactoside predominated, constituted the largest group of compounds (77-80%).

The Calcinit and Lebosol Calcium Forte fertilisers had the greatest influence on the change of chemical composition of the fruits, while the Folanx fertiliser had the greatest influence on the increase in fruit firmness. All calcium-based fertilisers decreased the acidity of fruits and, except for calcium chloride, they increased the nitrite level.

Key words: anthocyanins, foliar fertilization, flavonols, macro- and microelements, *Vaccinium corymbosum*.

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INTRODUCTION

Highbush blueberry (*Vaccinum corymbosum* L.) is mostly grown for the fresh fruit market in Poland. Its bushes have specific habitat and soil requirements, for example acidic peat soils are the best, light sandy soils are possible if the demand for water and nutrients is satisfied. Highbush cultivars are derived from the genus *Vaccinium*, which grows wildly in soils characterised by a low nutrient content. As a result, fertilising requirements of highbush blueberry are relatively modest, as compared with other fruit crops. However, intensive cultivation influencing high yields and the use of organic litter determine the necessity of fertilisation to keep the content of micro- and macronutrients in the soil and the plants at a constant level (Pormale et al. 2009).

Maintaining the high quality of fruits after harvesting, after the storage period as well as during marketing activities, is a goal that producers want to achieve. Firmness is one of the indicators of fruit freshness and attractiveness. Mechanical resistance of the skin and the flesh largely depends on the condition of cellular membranes and, in particular, the chemical composition of pectins binding plant cells together to form tissues. Calcium is mostly responsible as it contributes to the stabilisation of cellular membranes as a component of phospholipids, thus influencing their permeability (White, Broadley 2003). Calcium deficits or calcium transport disorders cause a large number of physiological diseases, which lower the storage and commercial value of such fruits (Shear 1975). Plant roots absorb calcium from the soil solution in the form of the Ca^{2+} ion (White 2001, Clarkson 2003); however, the number of free calcium ions is limited by the high humus content, in which organic acids form chelated bindings with Ca^{2+}. The low pH of the substrate is also a problem, as it stimulates high Al^{3+}, Fe^{2+}, Mn^{2+} concentrations, which make it difficult to absorb calcium ions (Haynes 1986). Thus, supply of calcium fertilisers by spraying the plants could be an alternative (Wójcik 2004). Calcium delivered to leaves by means of spraying increased the concentration of this nutrient in fruits by 17% (Saure 2005, Rosen et al. 2006). Therefore, the best results are observed when solution of a calcium preparation is applied directly onto the fruit surface. Young fruits are characterised by the highest calcium absorption (Schlegel, Schönherr 2002), as the permeability of the cuticle is the highest at that time (Petit-Jimenez et al. 2009), and properly functioning stomata are an easy route for Ca^{2+} absorption. The time of treatment is also very important. The movement of calcium salts largely depends on air humidity. An increase in humidity from 50% to 90% resulted in twice as high a rate of $Ca(NO_3)_2$ absorption through the cuticle layer (Schönherr 2001).

The influence of foliar application of calcium fertilisers with diverse chemical composition on the quality of highbush blueberry fruits, their size, firmness and chemical composition was studied.

MATERIAL AND METHODS

The studies were carried out in 2010-2012, in the Laboratory of Orcharding at the Department of Horticulture of the West Pomeranian University of Technology in Szczecin. The experiment was conducted at a 60 ha production plantation located in the area of Szczecin. Blueberry bushes were planted in the spacing of 1.2 x 2.0, in the podzolic soil of the VI valuation class. The experiment followed a randomised sub–block design (3 blocks, 15 plants in each block). The content analysis of the soil minerals showed a very high level of magnesium, medium levels of phosphorus and calcium, and a low level of potassium. In spring, the nitrogen fertilization was applied at a dose of 45 kg N ha^{-1}.

Within the growing period, the bushes were sprayed 4 times with calcium solutions (every 10 days from the first decade in June), starting from the fruit setting until the full wetting of the foliage. Liquid calcium fertilizers, designed for foliar fertilization of plants and fruit, were used, together with the Silwet®Gold preparation enhancing the adhesion properties.

- Folanx® Ca29 – solution 1% (LANXESS Distribution GmbH);
- Calcium chloride – solution 0.5% (Inowrocławskie Zakłady Chemiczne Soda Mątwy S.A.) $CaCl_2$ – 78-80%, NaCl – 3-3.5%;
- Lebosol Calcium Forte – solution 1% (Lebosol Dünger GmbH);
- Ca – 13.5%, Mn – 1,5%, Zn – 0.5%;
- Calcinit – solution 0.5% (Yara Poland) – Ca – 19.0%, N 15.5%, (NO_3 – 14.5%, NH_4 – 1.0%);
- control – spraying water.

Physical features of fruits (fruit size, firmness, puncture resistance of the skin) and soluble solids, titratable acidity, pH, total polyphenol, L-ascorbic acid and NO_3 were measured on fresh berries immediately after the harvest. Phenolics and mineral composition samples that were kept frozen (-32°C) in polyethylene bags (2 x 500 g) until analyzed.

Fruit diameter, firmness and puncture resistance of the skin were measured with a FirmTech2 apparatus (BioWorks, USA) on 100 randomly selected berries from each replicate, and the latter was expressed as a gram-force causing the fruit surface to sag by 1 mm. A puncture were made using a stamp with a diameter of 3 mm.The fruit weight (three replicates of 100 fruit) was measured on a RADWAG WPX 4500 electronic scales (0.01 g accuracy).

To obtain juice, berries (two replicates of 250 g) were macerated at 50°C for 60 minutes, with the addition of the PT 400 Pektopol enzyme at a dose of 400 mg kg^{-1} of fruits. After the completion of the enzymatic processing, the pulp was pressed using a hydraulic press at a pressure of 3 MPa (Oszmianski, Wojdyło 2005). Titratable acidity was determined by titration of the water extract of juice with 0.1 N NaOH to the end point of pH 8.1, measured with a pH-meter Elmetron 501 (PN). The content of soluble solids was determined with an PAL1 KonicaMinolta refractometer. L-ascorbic acid and NO_3 concentrations were measured with a RQflex 10 reflectometer Merck (Ochmian et al. 2012).

In unwashed fruits, after mineralization in H_2SO_4 and H_2O_2, the total N content was determined with the Kjeldahl method. The content of K and Ca was measured with atomic emission spectrometry, whereas the Mg content was determined with flame atomic absorption spectroscopy using an SAA Solaar. The phosphorus content was determined with the Barton method at the wavelength of 470 nm, whereas sulphur was assayed with the turbidimetric method at 490 nm employing a spectrophotometer Marcel s 330 PRO. The content of microelements (Cu, Zn, Mn, Fe), after mineralization in $HClO_4$ and HNO_3, was measured with flame atomic absorption spectroscopy on an SAA Solaar (IUNG 1972).

The HPLC analyses of polyphenols were carried out with an HPLC apparatus consisting of a Merck-Hitachi L-7455 diode array detector (DAD) and quaternary pump L-119 7100 equipped with the D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan). The runs were monitored for phenolic acids at 320 nm, flavonols and luteolin glucoside at 360 nm, and anthocyanin glycosides at 520 nm. Retention times and spectra were compared to that of pure standards and total polyphenols content was expressed as mg per 100 g fruit tissue. Standards of anthocyanidin glycosides were obtained from Polyphenols Laboratories (Norway), while those for phenolic acids and flavonols were purchased from Extrasynthese (France).

In order to determine the significance of differences, a two-factor analysis of variance was carried out, followed by the assessment of the significance of differences using the Tukey's test. To determine the relation between the applied fertlizers and fruit firmness, chemical compositions and phenolic content, the results were subjected to an agglomerative cluster analysis and classified into groups in a hierarchical order by means of the Ward's method. The statistical analyses were performed using the Statistica software.

RESULTS AND DISCUSSION

The firmness of fruits, and especially their size, is a very important factor determining the yield quality and its durability while on the market. The results showed high variability of this parameter after using calcium-based foliar fertilisers (Table 1). The foliar fertilisers, except for calcium chloride, allowed for obtaining larger fruits, as compared to the control. The largest fruits were collected from bushes sprayed with Lebosol (the average weight of 100 fruits was 365 g) and with Calcinit (333 g), the height of the fruits was 16.7 mm and 15.7 mm, respectively. Bushes treated with calcium chloride were characterised by the smallest fruits (the average height 14.0 mm,

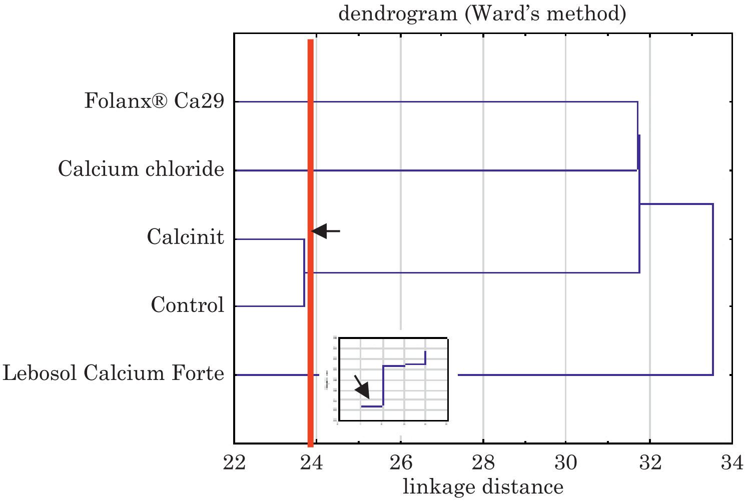
Table 1

The firmness and size of highbush blueberry in relation to the fertilisation and cultivars (average values for the years 2010-2012)

Fertilizer (AxB)	Cultivar (A)	Mass of 100 fruits (g)	Measured on height axis		Measured on diameter axis		
			height (mm)	firmness (G mm)	diameter (mm)	firmness (G mm)	puncture (G mm)
Folanx® Ca29	Brigitta	302	14.4	435	20.9	236	134
Calcium chloride		255	14.1	407	18.5	217	115
Lebosol Calcium Forte		369	15.3	341	21.1	174	101
Calcinit		321	14.8	384	20.3	208	122
Control		299	14.3	389	18.8	193	109
Mean		**309**	**14.6**	**391**	**19.9**	**206**	**116**
Folanx® Ca29	Sunrise	352	14.4	394	22.0	196	129
Calcium chloride		292	13.8	375	18.9	177	113
Lebosol Calcium Forte		361	18.0	322	22.0	151	82
Calcinit		345	16.6	319	21.8	164	89
Control		311	15.7	359	20.4	168	94
Mean		**332**	**15.7**	**354**	**21.0**	**171**	**101**
Folanx® Ca29	The mean for fertilizer (B)	**327**	**14.4**	**415**	**21.5**	**216**	**132**
Calcium chloride		**274**	**14.0**	**391**	**18.7**	**197**	**114**
Lebosol Calcium Forte		**365**	**16.7**	**332**	**21.6**	**163**	**92**
Calcinit		**333**	**15.7**	**352**	**21.1**	**186**	**106**
Control		**305**	**15.0**	**374**	**19.6**	**181**	**102**
LSD$\alpha_{0,05}$		**A-22; B-27; AxB-33**	**A-0.8; B-1.1; AxB-1.3**	**A-28; B-32; AxB-37**	**A-0.7; B-0.9; AxB-1.2**	**A-25; B-29; AxB-36**	**A-16; B-19; AxB-22**

and the diameter 18.7 mm). The firmness of fruits determines, amongst other things, the resistance to mechanical damage. It is assumed that this phenomenon occurs to a lesser extent in fruits with a good calcium content (Carl, Sams 1993, Fallahi et al. 1997). Reports concerning the influence of calcium on the firmness of blueberry fruits do not render unambiguous results. Fruits soaked in a calcium solution after harvesting were less susceptible to crushing (Hanson et al. 1993). Also, fruits from the 'O'Neal' cultivar treated with calcium were firmer than fruits from the control bushes. However, this relationship was not confirmed by 'Bluecrop' fruits (Angeletti et al. 2010). The results obtained are also ambiguous, which is confirmed by an analysis of concentrations, the activity of the Calcinit fertiliser did not affect fruit firmness (Figure 1). Fruits sprayed with the Lebosol fertiliser had the highest calcium content (Table 2); however, they were the least firm. Undoubtedly, their size had an influence on this, large fruits are less firm. However, blueberries treated with the Folanx fertiliser contained a lot of calcium and were characterised by high firmness, both in the diameter and in the height and fruit resistance to mechanical damage (129-134 G mm). Regardless of the fertilisers used, fruits of the tested cultivars were characterised by a lower firmness as compared to fruits from the 'Sierra' (440) and 'Patriot' cultivars (512 G mm) (Ochmian et al. 2007, 2009*b*).

The chemical composition of fruits is an equally important parameter determining fruit quality. It affects both the taste and the health promoting properties of fruits. The analyses showed that cv. Brigitta fruits were characterised by a higher content of organic acids and a lower extract content (Table 3). Moreover, it was shown that all foliar fertilisers used, regardless of the cultivar, had an influence on the reduction of fruit acidity (from 0.35



The vertical line indicate the cut-off used to form the grups

Fig. 1. Dendrogram of cluster analysis for fertilizers based on average for firmness

Table 2

The influence of calcium-based fertilisers on the macronutrient content in highbush blueberry fruits(g 100 g^{-1}f.w.)

Fertilizer (AxB)	Cultivar (A)	Content in fruits (g 100 g^{-1} f.w.)				
		Ca	**N**	**P**	**K**	**Mg**
Folanx® Ca29	Brigitta	0.16	0.53	0.11	0.45	0.06
Calcium chloride		0.15	0.36	0.11	0.49	0.03
Lebosol Calcium Forte		0.18	0.39	0.09	0.67	0.04
Calcinit		0.15	0.74	0.07	0.65	0.04
Control		0.11	0.35	0.08	0.54	0.03
Mean		**0.15**	**0.47**	**0.09**	**0.56**	**0.04**
Folanx® Ca29	Sunrise	0.11	0.85	0.14	0.62	0.07
Calcium chloride		0.09	0.52	0.14	0.50	0.04
Lebosol Calcium Forte		0.12	0.60	0.11	0.71	0.06
Calcinit		0.09	1.19	0.09	0.62	0.06
Control		0.06	0.51	0.09	0.50	0.04
Mean		**0.09**	**0.73**	**0.11**	**0.59**	**0.05**
Folanx® Ca29	The mean for fertilizer (B)	**0.14**	**0.69**	**0.12**	**0.54**	**0.07**
Calcium chloride		**0.12**	**0.44**	**0.12**	**0.49**	**0.04**
Lebosol Calcium Forte		**0.15**	**0.49**	**0.10**	**0.69**	**0.05**
Calcinit		**0.12**	**0.96**	**0.08**	**0.64**	**0.05**
Control		**0.09**	**0.43**	**0.09**	**0.52**	**0.04**
LSD$\alpha_{0,05}$		**A-0.03. B-0.04 AxB-0.06**	**A-0.19. B-0.23 AxB-0.28**	**A-0.04. B-0.05 AxB-0.08**	**A-0.07. B-0.10 AxB-0.12**	**A-0.01. B-0.02 AxB-0.04**

Table 3

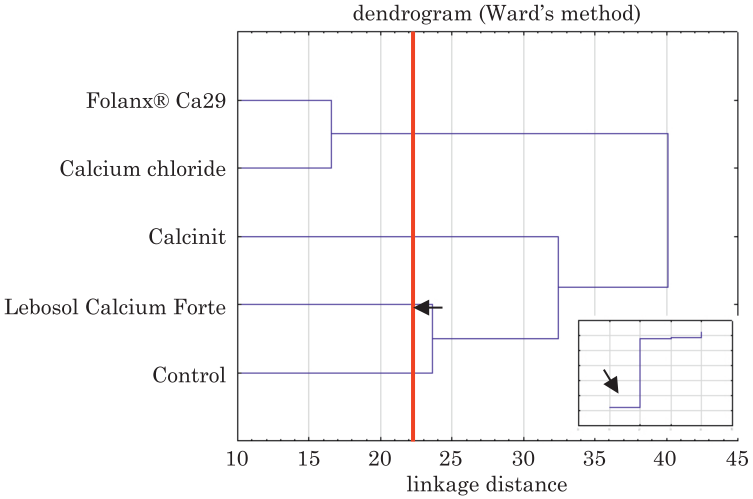
Some characteristics of the fruits of highbush blueberry in dependence on fertilizing and cultivar (mean for 2010–2012)

Fertilizer (AxB)	Cultivar (A)	Titratable acidity (g 100 mL^{-1})	Soluble solids (%)	NO$_3$ (mg 1000 mL^{-1})	L-ascorbic acid (mg 1000 mL^{-1})	Juice pH
Folanx® Ca29	Brigitta	0.78	14.7	39.6	95	3.86
Calcium chloride		0.87	13.9	29.2	102	3.75
Lebosol Calcium Forte		0.85	13.6	42.4	120	3.84
Calcinit		0.74	14.9	47.8	84	3.81
Control		0.96	13.8	31.5	126	3.67
Mean		**0.84**	**14.2**	**38.1**	**105**	**3.79**
Folanx® Ca29	Sunrise	0.35	15.7	32.7	71	3.85
Calcium chloride		0.42	15.2	18.3	107	3.89
Lebosol Calcium Forte		0.44	15.1	31.0	92	3.94
Calcinit		0.38	15.2	34.3	68	3.97
Control		0.55	15.5	17.3	84	3.82
Mean		**0.43**	**15.3**	**26.7**	**84**	**3.89**
Folanx® Ca29	The mean for fertilizer (B)	**0.57**	**15.2**	**36.2**	**83**	**3.86**
Calcium chloride		**0.65**	**14.6**	**23.8**	**105**	**3.82**
Lebosol Calcium Forte		**0.65**	**14.4**	**36.7**	**106**	**3.89**
Calcinit		**0.56**	**15.1**	**41.1**	**76**	**3.89**
Control		**0.76**	**14.7**	**24.4**	**105**	**3.75**
LSD$\alpha_{0,05}$		**A-0.08 . B-0.09 AxB-0.11**	**A-0.4 . B-0.5 AxB-0.7**	**A-4.3 . B-5.6 AxB-7.9**	**A-13 . B-19 AxB-24**	**A-0.11 . B-0.13 AxB-0.19**

to 0.87 g 100 mL^{-1}), as compared with the control (cv. Sunrise 0.55 and Brigitta 0.96 g 100 mL^{-1}, respectively) and a slight increase in the pH of the juice. These parameters are similar to those obtained by Duan et al. (2011). The influence of the fertilisers used on the extract content was diverse. The extract content in the cultivar Sunrise fruit was at a similar level (15.1-15.7%), while in cv. Brigitta fruits, the extract content was the highest in bushes sprayed with the Calcinit and Folanx fertilisers (14.9 and 14.7%). In studies by other authors, the extract content in fruits from the cultivars under analysis ranged from 10% to 14.6% (Prior et al. 1998, Skupień 2006, Duan et al. 2011).

The use of calcium fertilisers did not increased the L-ascorbic acid content, and in fruits collected from bushes sprayed with the Calcinit and Folanx fertilisers, it was found to be at a level (76 and 83 mg 1000 mL^{-1}) lower than the control (105 mg 1000 mL^{-1}). The use of Calcinit, Lebosol and Folanx fertilisers, on the other hand, had an influence on a higher nitrate content (on average 36.2241.1 mg 1000 mL^{-1}) in fruits from both cultivars, as compared to control fruits (24.4 mg 1000 mL^{-1}). Nevertheless, these are levels which are allowed for consumption even by children, according to the Polish standards.Similar levels were identified by other authors: from 15.5 to 34.7 mg NO_3 in 1000 g fruits (Ochmian et al. 2009*a*, *b*, 2010). To show similarities in the action of fertilisers, an analysis of concentrations was performed between the fertilisers used and the content of SS, TA, NO_3 and L-ascorbic acid (Figure 2). All of the fertilisers affected the analysed features, and the action of Folanx and Calcinit was similar.

Owing to a high content of phenolic compounds as well as other biologically active substances, dark fruits are valued as products with pro-health

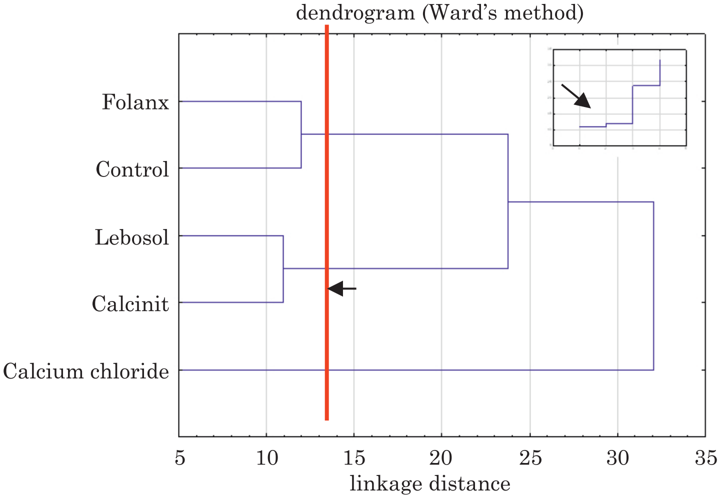


The vertical line indicate the cut-off used to form the grups

Fig. 2. Dendrogram of cluster analysis for fertilizers based on average for chemical compositions

properties. Polyphenols are products of the secondary synthesis which protect from ultraviolet radiation and pathogens (De Gara et al. 2003, Hodges et al. 2004). Their content largely depends on the cultivar (Mikkonen et al. 2001) and fruit ripeness (Wang, Jiao 2001). In the authors' research, fruits from cv. Sunrise were richer in polyphenolic compounds (on average 314 mg 100 mL^{-1}) than from cv. Brigitta (241 mg 100 mL^{-1}) – Table 4. Those levels were similar to those reported by Moyer et al. (2002): 274 mg, and Prior et al. (1998): 305 mg, but lower than in fruits tested by Ochmian et al. (2009*b*, *c*): 375 mg, and Bunea et al. (2011): 424 mg 100 g^{-1}.

It was found that the fertilisers did not have the same influence on this feature. The lowest polyphenolic content was found in cv. Brigitta fruits, control and sprayed with Folanx fertiliser, and in berries from the cultivar Sunrice collected from objects sprayed with calcium chloride. Fruits of both cultivars sprayed with Lebosol and Calcinit were characterised by the highest content of these compounds. A similar influence of these fertilisers was confirmed by an analysis of concentrations (Figure 3). In highbush blueberry fruits, anthocyanins made up approx. 80% of all determined phenolic compounds. Among anthocyanins, the amount of delphinidin-3-galactoside was the highest, especially in fruits sprayed with Lebosol and Calcinit fertilisers (Table 5). In total, delphinidins made up approx. 50% of all determined anthocyanins. In research by Lohachoompol et al. (2008), this group of compounds consitituted 34-36% of all anthocyanins. In fruits collected from these bushes, a large amount of chlorogenic acid, which is a strong natural antioxidant, was also determined. The largest amounts of flavonoids, on the other hand, were found in cv. Brigitta fruits sprayed with calcium chloride and in cv. Sunrise' fruits sprayed with Lebosol.



The vertical line indicate the cut-off used to form the grups

Fig. 3. Dendrogram of cluster analysis for fertilizers based on average for phenolic compositions

Table 4

The influence of calcium-based fertilisers on the polyphenol content in highbush blueberry fruits(mg 100 g^{-1} f.w.)

Fertilizer (AxB)	Cultivar (A)	Que-3-gal	Que-3-glu	Que-3-ram	Kem-3-rut	**Total flawonols**	**Neochlorogenic acid**	**Total anthocyanins (Table 5)**	**SUME**
Folanx® Ca29	Brigitta	9.0	3.32	0.87	0.45	**13.7**	**26.8**	**171**	**212**
Calcium chloride		16.4	1.47	1.62	0.89	**20.4**	**38.1**	**183**	**242**
Lebosol Calcium Forte		10.2	3.54	1.94	1.32	**17.0**	**33.8**	**211**	**262**
Calcinit		9.3	1.87	0.99	1.11	**13.2**	**43.5**	**206**	**263**
Control		10.8	1.73	1.35	0.57	**14.5**	**29.5**	**184**	**228**
Mean		**11.1**	**2.39**	**1.35**	**0.87**	**15.7**	**34.3**	**191**	**241**
Folanx® Ca29	Sunrise	10.7	3.85	1.51	2.05	**18.1**	**40.4**	**238**	**297**
Calcium chloride		12.0	1.92	2.35	1.53	**17.8**	**32.8**	**205**	**256**
Lebosol Calcium Forte		19.2	5.00	2.11	2.44	**28.7**	**54.6**	**255**	**338**
Calcinit		12.9	2.60	3.48	1.69	**20.6**	**60.1**	**282**	**363**
Control		17.7	3.14	1.86	0.97	**23.7**	**46.7**	**247**	**317**
Mean		**14.5**	**3.30**	**2.26**	**1.74**	**21.8**	**46.9**	**245**	**314**
Folanx® Ca29	The mean for ferti- lizer (B)	9.9	3.59	1.19	1.25	**15.9**	**33.6**	**204**	**254**
Calcium chloride		14.2	1.70	1.99	1.21	**19.1**	**35.4**	**194**	**249**
Lebosol Calcium Forte		14.7	4.27	2.03	1.88	**22.9**	**44.2**	**233**	**300**
Calcinit		11.1	2.24	2.24	1.40	**16.9**	**51.8**	**244**	**313**
Control		14.2	2.44	1.61	0.77	**19.1**	**38.1**	**215**	**273**

LSD$\alpha_{0,05}$ for: total flawonols A-2.6; B-3.1; AxB-3.6; neochlorogenic acid A-3.7; B-4.2; AxB-4.9; total anthocyanins A-16; B-19; AxB-26; SUME A-17; B-21; AxB-25

The results of the analyses confirmed the influence of the fertilisers used on the content of some macro- and micronutrients in fruits (Tables 2 and 6). Generally, fruits from the Brigitta cultivar contained more calcium (0.15 g 100 g^{-1}) than fruits from the Sunrise cultivar (0.09 g 100 g^{-1}). All the tested foliar fertilisers increased its concentration in fruits from both cultivars (Table 2). The fruits of cv. Brigitta sprayed with Lebosol had the highest calcium content (0.18 g 100 g^{-1}). In other experiments, the use of foliar fertilisation with calcium also increased the level of this component in fruits (STÜCKRATH et al. 2008, ANGELETTI et al. 2010). The content of this component obtained in this study was at a comparable level to that found by EICHHOLZ et al. (2011), while being much higher than reported by HANSON (1995), who determined it as 0.04 g 100 g^{-1}.

A considerable increase in nitrogen levels was observed in fruits from both tested cultivars collected from bushes sprayed with Calcinit fertiliser. The composition of the fertiliser had undoubtedly some influence, as it contains nitrogen. It is also reflected by elevated nitrate levels (Table 3). However, no influence of the fertilisers on the phosphorus content in fruits was observed. Phosphorus levels ranged from 0.07 to 0.14 g 100 g^{-1}. In contrast, the use of Lebosol and Calcinit fertilisers resulted in increased levels of potassium in fruits (0.69 and 0.64 g 100 g^{-1}). The content of this ingredient in fruits sprayed with calcium chloride or Folanx fertiliser was at a similar level as in the control (from 0.49 to 0.54 g 100 g^{-1}). An increased level of magnesium was observed only in fruits which were sprayed with Folanx fertiliser (0.07 g 100 g^{-1}), and it was at a similar level in the other fruits (0.04-0.05 g 100 g^{-1}). The macronutrient content, except for nitrogen, in the analyzed fruits was at a similar level as in fruits from the cultivars Sierra and Patriot examined by OCHMIAN et al. (2009*a*,*b*, 2010).

Fruits from the Sunrise cultivar contained more copper, zinc and manganese than fruits from the Brigitta cultivar (Table 6). It was found that the influence of the fertilisers used on the micronutrient content varied. The largest amount of copper was found in fruits collected from bushes sprayed with calcium chloride, while the zinc content was the highest when Lebosol was used. Calcinit and Folanx, on the other hand, increased the manganese levels; additionally, fruits treated with Calcinit were characterised by the highest iron content. SKUPIEŃ (2004) found a considerably lower content of copper and zinc in highbush blueberry fruits (1.08-1.30 and 0.17-0.30 mg 100 g^{-1}).

CONCLUSIONS

The application of foliar calcium fertilisers affected the quality of highbush blueberry fruits; however, the results depended on the fertiliser used. The main purpose of calcium supplementation is to fortifiy the mechanical resistance of fruits. Not all fertilisers contributed to the achievement of this effect, despite

Table 5

The influence of calcium-based fertilisers on the anthocyanin content in highbush blueberry fruits(mg 100 g^{-1}f.w.)

Fertilizer (AxB)	Cultivar (A)	Del-3-ara	Del-3-gal	Del-3-glu	Cy-3-ara	Cy-3-gal	Cy-3-glu	Pet-3-ara	Pet-3-gal	Pet-3-glu	Peo-3-ara	Peo-3-gal	Peo-3-glu	Mal-3-ara	Mal-3-gal	Mal-3-glu
Folanx® Ca29	Brigitta	20.9	50.9	4.27	2.89	9.32	4.99	7.60	10.59	3.47	0.97	26.6	17.8	6.74	2.01	1.54
Calcium chloride		31.8	56.6	2.46	3.78	5.06	8.33	5.27	11.66	5.70	1.40	20.1	16.7	10.92	0.89	2.06
Lebosol Calcium		24.4	62.8	1.61	2.86	11.21	11.57	4.22	16.61	6.80	0.53	35.9	19.0	12.41	1.01	0.31
Calcinit		44.0	60.9	4.55	5.69	14.74	7.26	11.60	8.05	5.26	0.86	15.6	12.3	12.14	2.86	0.58
Control		23.2	56.0	4.48	3.18	10.16	5.48	7.29	9.53	3.89	1.07	29.2	19.6	7.42	2.21	1.69
Mean		**28.9**	**57.5**	**3.47**	**3.68**	**10.10**	**7.53**	**7.20**	**11.29**	**5.03**	**0.97**	**25.5**	**17.1**	**9.93**	**1.79**	**1.24**
Folanx® Ca29	Sunrise	55.4	64.9	3.73	4.20	7.62	9.26	5.85	12.96	6.33	1.56	26.3	18.6	12.14	8.59	0.32
Calcium chloride		25.8	62.2	4.98	3.53	11.29	6.09	8.10	10.59	4.32	1.19	32.5	21.8	8.24	2.45	1.88
Lebosol Calcium		46.8	81.0	3.81	4.79	16.16	7.72	13.89	12.74	10.44	0.36	19.4	15.5	15.33	6.20	0.73
Calcinit		52.0	89.1	5.50	5.27	17.61	8.49	17.09	11.47	11.69	0.40	20.3	17.1	16.86	7.82	0.80
Control		62.9	67.2	1.65	3.82	15.58	8.42	6.10	14.40	5.66	1.42	23.9	16.9	11.03	7.81	0.29
Mean		**48.6**	**72.9**	**3.93**	**4.32**	**13.65**	**8.00**	**10.21**	**12.43**	**7.69**	**0.98**	**24.5**	**18.0**	**12.72**	**6.57**	**0.80**
Folanx® Ca29	The mean for fertilizer (B)	38.2	57.9	4.00	3.54	8.47	7.12	6.72	11.78	4.90	1.26	26.5	18.2	9.44	5.30	0.93
Calcium chloride		28.8	59.4	3.72	3.65	8.18	7.21	6.69	11.13	5.01	1.29	26.3	19.3	9.58	1.67	1.97
Lebosol Calcium		35.6	71.9	2.71	3.83	13.68	9.65	9.06	14.68	8.62	0.45	27.6	17.3	13.87	3.60	0.52
Calcinit		48.0	75.0	5.03	5.48	16.18	7.88	14.35	9.76	8.48	0.63	17.9	14.7	14.50	5.34	0.69
Control		43.1	61.6	3.06	3.50	12.87	6.95	6.69	11.97	4.77	1.24	26.6	18.3	9.22	5.01	0.99

Table 6

The influence of calcium-based fertilisers on the micronutrient content in highbush blueberry fruits (mg 100 g^{-1})

Fertilizer (AxB)	Cultivar (A)	**Content in fruits (mg 100 g^{-1} f.w.)**			
		Cu	**Zn**	**Mn**	**Fe**
Folanx® Ca29	Brigitta	1.54	4.55	18.79	40.33
Calcium chloride		2.35	5.33	9.43	31.48
Lebosol Calcium Forte		1.36	8.47	23.62	21.94
Calcinit		1.52	5.02	11.55	53.07
Control		1.82	3.74	7.37	25.61
Mean		**1.72**	**5.42**	**14.15**	**34.48**
Folanx® Ca29	Sunrise	2.10	7.56	41.52	23.27
Calcium chloride		3.21	8.86	20.79	27.83
Lebosol Calcium Forte		1.86	14.08	25.50	43.86
Calcinit		2.08	8.34	52.18	56.45
Control		2.26	5.65	14.83	24.70
Mean		**2.30**	**8.90**	**30.97**	**35.22**
Folanx® Ca29	The mean for fertilizer (B)	**1.82**	**6.06**	**30.15**	**31.81**
Calcium chloride		**2.78**	**7.10**	**15.13**	**29.65**
Lebosol Calcium Forte		**1.61**	**11.28**	**24.57**	**32.90**
Calcinit		**1.80**	**6.68**	**31.86**	**54.73**
Control		**2.04**	**4.70**	**11.09**	**25.15**
LSD$\alpha_{0,05}$		**A-0.19; B-0.22; AxB-0.31**	**A-2.17; B-2.34; AxB-3.46**	**A-13.2; B-16.5; AxB-19.0**	**A-9.4; B-13.1; AxB-17.7**

increasing the calcium content in fruits, especially ones sprayed with Lebosol fertiliser. Bushes sprayed with Lebosol and Calcinit fertilisers were characterised by the largest fruits; however, these fertilisers did not improve fruit firmness as compared to the control. They were, however, characterised by the highest content of valuable polyphenols. Fruits collected from bushes sprayed with Folanx fertilisers and calcium chloride were the firmest and the most resistant to mechanical damage. The latter fertiliser, however, had the least advantageous effect on the fruit size. All calcium–based fertilisers reduced the acidity of fruits and nearly all, except for calcium chloride, contributed to the accumulation of larger amounts of harmful nitrates.

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THE MINERAL PROFILE OF WINTER OILSEED RAPE IN CRITICAL GROWTH STAGES – POTASSIUM

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Abstract

Sustainable management of nitrogen in the oilseed rape canopy depends, in the first place, on the supply of potassium during critical yield formation stages. The K status assessment in plants was made in the 2008, 2009, and 2010 seasons. The one-factorial experiment to verify the formulated hypothesis, consisting of six treatments, was as follows: control (C), NP, NPK, NPK+MgS – $^{1}/_{3}$ rate of the total planned dose applied in spring (NPKMgS1), NPK+1.0 MgS rate in autumn (NPKMgMgS2), NPK+MgS – $^{2}/_{3}$ in autumn + $^{1}/_{3}$ in spring) (NPKMgS3). Plant samples were taken at three stages: full rosette (BBCH 30), the onset of flowering (BBCH 61), maturity (BBCH 89). At each measurement, the harvested sample was partitioned, in accordance with the stage of development, into subsamples of leaves (BBCH 30), stems and leaves (BBCH 61), stems + pericarp (trashed silique) and seeds (BBCH 89). The biomass yield as well as the potassium concentration and content were determined in each part of the plant. The yield forming effect of potassium absorbed by plants during the critical growth stages was decisive for the yield of seeds, as resulted from path and stepwise analyses. The study showed that the N management in oilseed rape canopy was significantly governed by the K management just before and after flowering. An elevated K supply in the first period, leading to the N saturated status of plant parts, resulted in seed density reduction, in turn decreasing the size of the K seed-sink. The seed yield of oilseed rape significantly depended on the net K content increase in vegetative parts of the crop during the seed-filling period. It can be therefore concluded that an adequate supply of K up to the physiological maturity of oilseed rape is a prerequisite of high yield.

Key words: potassium, plant parts, K partitioning, yield structural components, yield.

INTRODUCTION

In agronomic practice, the key concept of sustainable nitrogen management focuses most frequently on nitrogen levels. This simplified approach is acceptable provided an ample supply of other nutrients. Potassium deficien-

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cy is the key cause of an inadequate utilization of the applied nitrogen by plants, resulting in lower and unstable yields, as documented recently for central European countries (Grzebisz et al. 2012*a*). A sound management of nitrogen based on a balanced supply of nutrients, such as phosphorus, potassium and magnesium, is expected to result in and increased uptake and improved utilization of elements (Grzebisz et al. 2010).

The demand of crop canopy for nitrogen is significantly governed by its stage of growth, and the rate of biomass accumulation. The following three cardinal periods of the crop plant growth are distinguished, based on the rate and trends of dry matter accumulation, i.e. i) the foundation period (FP), ending at the rosette stage, ii) the construction period (CP), ending at the onset of flowering, iii) the yield forming period ending at the plant physiological maturity. Each period exerts a specific impact on the crop plant performance (Sylvester-Bradley et al. 2002). The third stage is of crucial importance for the final yield because of its impact on the number of pods and seeds per pod, termed as seed density (Diepenbrock 2000).

It has been well recognized that an insufficient supply of potassium to a crop leads to the decreased rate of the crop's growth, as documented by Grzebisz et al. (2012*b*) for sugar beet. The yield forming effect of nitrogen, as documented by Szczepaniak et al. (2014) for maize and Szczepaniak and Potarzycki (2014) for wheat, considerably depends on potassium fertilizing patterns. Despite the well-known physiological background and the recognized dynamics of the accumulation of both nutrients, we still lack information about the potassium impact on the formation of yield components of oilseed rape. The course of potassium accumulation by oilseed rape and its impact on yield are poorly understood. The pot experiment by Rose et al. (2008), based on an optimum supply of nutrients, showed that the potassium accumulation in canola peaked around flowering. The field experiment by Barłóg et al. (2005) showed that potassium accumulates progressively up to the stage of green pods.

The objective of the current study was to define the potassium accumulation pattern and its redistribution among key parts of oilseed rape in cardinal stages of growth. The key aim was to evaluate the effect of the potassium content in the crop parts during the season on i) the development of key structural components of yield, ii) yield of seeds produced by the main raceme and by secondary branches.

MATERIAL AND METHODS

The general characteristics of the experimental field have been described by Szczepaniak (2014). Plants for determinations of dry mater and potassium concentration were sampled from an area of one m^2 in three consecutive stages of oilseed growth, according to the BBCH scale: 30, 61 and 89. At each measurement date, the harvested plant sample was partitioned, in accordance with the stage of development, into subsamples of leaves (BBCH 30), stems and leaves (BBCH 61), stems + pericarps (trashed siliques) and seeds (BBCH 89), and then dried (65°C). Plant material for mineral element determination was mineralized at 640°C and the ash was next dissolved in 33% HNO_3. The potassium concentration was determined by flame photometry. The results are expressed on a dry matter (DM) basis. The content of the nutrient in each plant part was calculated by multiplying its concentration and the respective biomass of a crop part.

The experimental data were subjected to conventional analysis of variance using the computer program Statistica 7. The differences between treatments were evaluated with the Tukey's test. In tables and figures, results of the *F* test (***, **, *) indicate significance at the $P < 0.1\%$, 1%, and 5%, respectively. Path analysis was conducted based on correlation coefficients and taking the yield of seed and/or its structural components as effects and the nitrogen accumulation in plant parts as independent variables. A path diagram showing direct and indirect path coefficients was elaborated based on the highest value of the correlation coefficient for a particular set of variables. In the second step of the diagnostic procedure, stepwise regression was applied to define an optimal set of variables for a given crop characteristic. In the computing procedure, a consecutive variable was added to the multiple linear regressions stepwise. The best regression model was chosen based on the highest *F*-value for the entire model and significance of all independent variables (Konys, Wiśniewski 1984).

Acronyms applied in the paper:

MBY – yield of the main branch (inflorescence),
SBY – yield of secondary branches,
TSY – total yield of seeds,
SMB – number of siliques per main branch,
SSB – number of siliques per secondary branch,
SSMB – number of seeds per silique of the main branch,
SSSB – number of seeds per silique of the secondary branch,
K_{RE} – potassium content in oilseed rape at the rosette stage,
K_{LE} – potassium content in leaves of oilseed rape at the onset of flowering,
K_{SH} – potassium content in shoots of oilseed rape at the onset of flowering,
K_{ST} – potassium content in the stem of oilseed rape at maturity,
K_{SE} – potassium content in seeds of oilseed rape at maturity,
K_n – K net uptake by oilseed rape in the period BBCH 61 - BBCH 89,
B_n – oilseed rape biomass net increase in the period BBCH 61 - BBCH 89.

RESULTS AND DISCUSSION

The potassium concentration in oilseed rape throughout the growing season was affected significantly by both factors, years and fertilizing treatments (Table 1). At the rosette stage, the K concentration was much lower in the control plants. In all the years, it showed a significant response to the applied nitrogen and phosphorus (NP). The resultant increase was year-specific. In 2008, the highest increase appeared in plants grown in the treatment with split dose of magnesium sulfate (NPKMgS3), while in 2009, the maximum K concentration was revealed in plants fertilized with NPK. In 2010, the highest K concentration was noted for the NP treatment. Addition of other nutrients such as K and MgS did not affected K concentration increase. At the beginning of flowering, the K concentration in leaves was year -to-year different. In 2009, the K concentration fluctuated around 2.4%. In 2009, it was much higher, rising up in response to fertilizer K, more strongly owing to the split application of magnesium sulfate. The same trend, but on a much higher level, was noted in shoots. However, in 2008, the maximum K concentration was characteristic of the treatment with magnesium applied in autumn, and in 2009 the highest K concentration was induced by the NPK treatment. The main cause of the year-to-year variability of K concentrations

Table 1

Statistical evaluation of potassium concentration in oilseed rape organs during critical growth stages (g kg^{-1} DM)

Factor	Factor level	BBCH 30	BBCH 61		BBCH 89	
		LE	LE	SH	ST	SE
Fertilization treatments (FT)	AC	34.6^{a}	26.8^{a}	30.3^{a}	16.9^{a}	8.2^{a}
	NP	45.0^{b}	32.5^{bc}	37.8^{b}	22.1^{b}	8.6^{ab}
	NPK	47.2^{b}	34.3^{bcd}	42.4^{c}	23.7^{bc}	9.2^{b}
	NPKMgS1	48.1^{b}	36.6^{cd}	42.8^{c}	25.8^{c}	8.8^{ab}
	NPKMgS2	47.7^{b}	31.5^{b}	38.9^{bc}	22.7^{bc}	9.0^{ab}
	NPKMgS3	49.4^{b}	37.1^{d}	41.2^{bc}	25.3^{bc}	9.2^{b}
Years (Y)	2008	4.16^{a}	24.0^{a}	32.6^{a}	26.2^{c}	9.0^{b}
	2009	46.3^{b}	43.2^{c}	44.9^{c}	24.0^{b}	8.1^{a}
	2010	48.1^{b}	32.1^{b}	39.3^{b}	18.0^{c}	9.5^{c}
Fertilization treatments (FT)		***	***	***	***	**
Years (Y)		***	***	***	***	***
FT x Y		*	***	**	ns	ns

LE, SH, ST, SE – leaves, shoots, straw, seeds, respectively;
F – probability values: *,**, *** of 0.05, 0.01, 0.001, ns – not significantly different;
^{a} the same letters mean the lack of significant differences.

was the course of weather. The amount of precipitation in the three spring months (April, May and June) was about 100 mm in 2008, 190 mm in 2009 and 138 mm in 2010. The long-term average is 136 mm (SZCZEPANIAK 2014). The analyzed relationship between the K concentration in leaves and N accumulation in leaves showed a curvilinear course:

$$N_{LE} = -0.692K_{LE}^{2} + 8.594K_{LE} - 8.412 \quad \text{for } n = 18,\ R^2 = 0.68 \text{ and } p < 0.000.$$

Quite a different regression model was achieved for the relationship between the K concentration in shoots and the nitrogen content in oilseed rape vegetative parts at the onset of flowering:

$$N_{LE} = 5.349K_{SH} - 8.952 \qquad \text{for } n = 18,\ R^2 = 0.84 \text{ and } p < 0.000,$$
$$N_{SH} = 4.034K_{SH} - 5.81 \qquad \text{for } n = 18,\ R^2 = 0.52 \text{ and } p < 0.000.$$

The above regression models inform us that the K concentration in leaves obtained a saturation-like status, raising up the N content in leaves to the K optimum of 6.2%. The linear model indicates the unsaturated status of nitrogen. This discrepancy probably results from the limited size of leaves for nitrogen accumulation and, at the same time, the continuous growth of branches during the flowering phase, resulting in an increasing nitrogen accumulation capacity.

The amount of potassium accumulated in parts of oilseed rape during the cardinal growth stages was significantly affected by years and fertilizing treatments (Table 2). Interaction, however, was noted only at the beginning

Table 2

Statistical evaluation of potassium accumulation and distribution in oilseed rape plant organs during critical growth stages (g m^{-2})

Factor	Factor level	BBCH 30	BBCH 61		BBCH 89	
		LE	LE	SH	ST	SE
Fertilization treatments (FT)	AC	7.71^{a}	3.26^{a}	12.00^{a}	17.36^{a}	2.35^{a}
	NP	14.05^{b}	6.52^{b}	20.23^{b}	28.42^{b}	3.25^{b}
	NPK	14.38^{b}	7.64^{b}	22.94^{b}	36.37^{bc}	3.93^{c}
	NPKMgS1	14.61^{b}	7.93^{b}	20.78^{b}	33.45^{bc}	4.12^{c}
	NPKMgS2	14.16^{b}	6.41^{b}	20.95^{b}	36.16^{bc}	3.61^{bc}
	NPKMgS3	13.02^{b}	8.05^{b}	22.91^{b}	36.87^{bc}	3.98^{c}
Years (Y)	2008	11.39^{b}	3.49^{a}	14.21^{a}	42.52^{c}	4.28^{c}
	2009	13.72^{b}	11.89^{b}	30.52^{b}	29.73^{b}	3.44^{b}
	2010	13.84^{b}	4.53^{a}	17.19^{a}	22.07^{a}	2.91^{a}
Fertilization treatments (FT)		***	***	***	***	***
Years (Y)		*	***	****	***	***
FT x Y		ns	**	***	**	ns

key under Table 1

of flowering and for the stem at the physiological maturity. In the period of stem elongation, the content of potassium underwent a significant change versus the beginning of flowering (Figure 1). The key reason of the observed variation was the course of weather, only slightly modified by the fertilizing treatments. In 2009, the content of potassium in leaves was around 3 times higher than in the other years. Its content in the control plant was on the same level as noted for the best treatments in the other years. The application of fertilizers resulted in its increase: 2-forld for NP, 2.-7-fold for NPK and 3-fold for NPKMgS1. The N content in leaves and in shoots showed a curvilinear response to the K content in leaves:

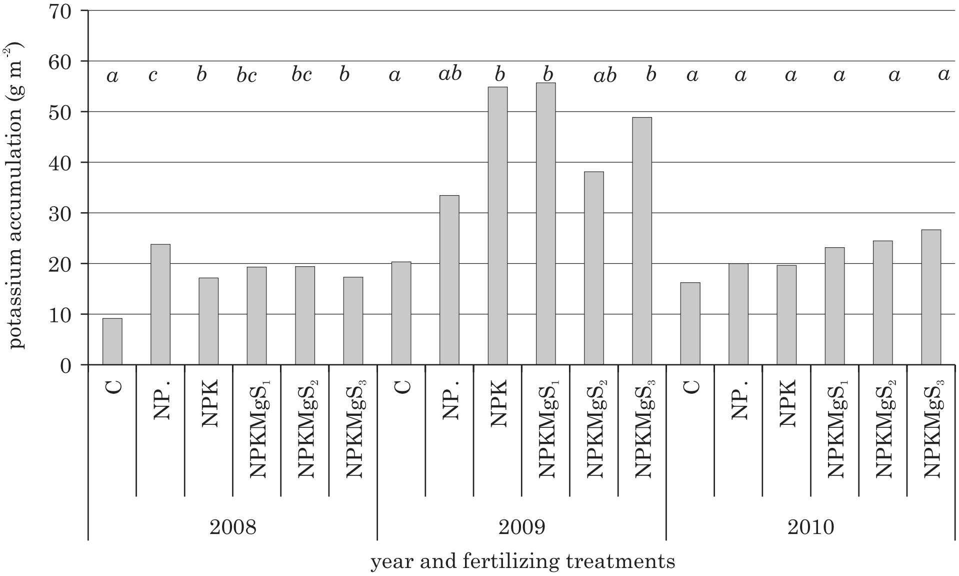

Fig. 1. Effect of fertilizing treatments on potassium accumulation in oilseed rape canopy at the beginning of flowering; ^{a} the same letters mean the lack of significant differences

$$N_{LE} = -0.095K_{LE}^2 + 2.495K_{LE} + 1.307 \quad \text{for } n = 18,\ R^2 = 0.85 \text{ and } p < 0.000,$$
$$N_{SH} = -0.071K_{LE}^2 + 2.194K_{LE} + 2.237 \quad \text{for } n = 18,\ R^2 = 0.88 \text{ and } p < 0.000.$$

The same pattern of K accumulation was observed in shoots, with the maximum values recorded in the NPK treatment. The analysis of the impact of the K_{SH} content on the nitrogen accumulation course in leaves and in stems showed an identical trend as for K_{LE}:

$$N_{LE} = -0.017K_{SH}^2 + 1.256K_{SH} - 5.49 \quad \text{for } n = 18,\ R^2 = 0.93 \text{ and } p < 0.000,$$
$$N_{SH} = -0.01K_{SH}^2 + 0.933K_{SK} -6.445 \quad \text{for } n = 18,\ R^2 = 0.84 \text{ and } p < 0.000.$$

The regression models show a saturation-like status of nitrogen in response to the increased K supply to oilseed plants at the onset of flowering.

This implicates that excess of available potassium leads to an elevated accumulation of nitrogen, in turn boosting the growth of the vegetative biomass of oilseed rape at the expense of seed setting (Szczepaniak 2014).

The amount of potassium accumulated in the stem at physiological maturity was significantly affected by the weather and slightly modified by the fertilizing treatments (Figure 2). In 2008, the content of K in this plant organ showed a considerable response to the increasing input of nutrients, reaching the maximum in the NPKMgS1 treatment. In turn, in 2009, the total K amount in straw was 30% lower on average than 2008, reaching the maximum in the NPK treatment. In the third year, 2010, the same trend was observed as in 2008, but the amount of K in straw was 50% lower. The effect of the fertilizing treatments on the K content in seeds increased stepwise in accordance to the number of applied nutrients.

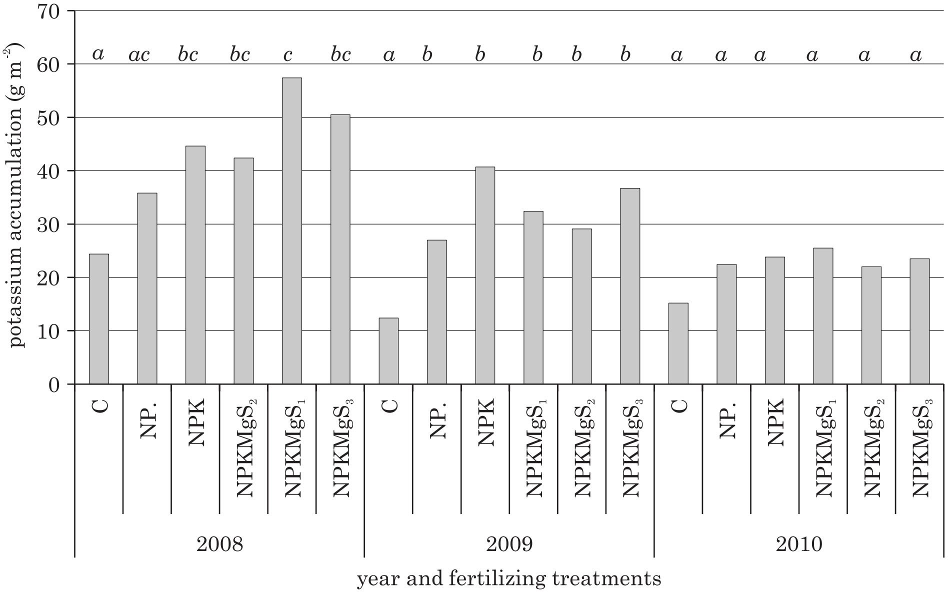

Fig. 2. Effect of fertilizng treatments on potassium accumulation in oilseed rape postharvest residues; [a] the same letters mean the lack of significant differences

The K content in stems at the onset of flowering exerted the highest direct effect on the yield of the main inflorescence. However, it was significantly depressed by the indirect impact of the other variables. On the other hand, a moderate direct effect of the potassium content in seeds, through the impact of the other variables, became the key factor defining the yield produced by the principal branch (Table 3). Its effect was modified the most by the K content in straw (Figure 3*a*). The optimum sets of variables, as results from the stepwise analysis, except K_{SE}, were slightly different:

$$\mathrm{MBY} = 0.234\mathrm{K}_{\mathrm{SE}} + 0.45 \qquad \text{for } n = 18,\ R^2 = 0.39 \text{ and } p < 0.0053,$$

$$\mathrm{MBY} = 0.037\mathrm{K}_{\mathrm{SH}} + 0.211\mathrm{K}_{\mathrm{SE}} + 0.28 \qquad \text{for } n = 18,\ R^2 = 0.65 \text{ and } p < 0.0035,$$

$$\mathrm{MBY} = -0.048\mathrm{K}_{\mathrm{RE}} + 0.055\mathrm{K}_{\mathrm{SH}} + 0.247\mathrm{K}_{\mathrm{SE}} + 0.665 \qquad \text{for } n = 18,\ R^2 = 0.79 \text{ and } p < 0.00006.$$

This set of equations clearly indicates that the straw K content is an important factor influencing seed yields. The yield of seeds produced by secondary branches was most strongly affected by the K content in straw, due to the indirect effect of K in seeds (Figure 3*b*, Table 3). Thus, it can be concluded that the degree of K-seed sink saturation depended on the K supply from vegetative plant parts during the seed filling:

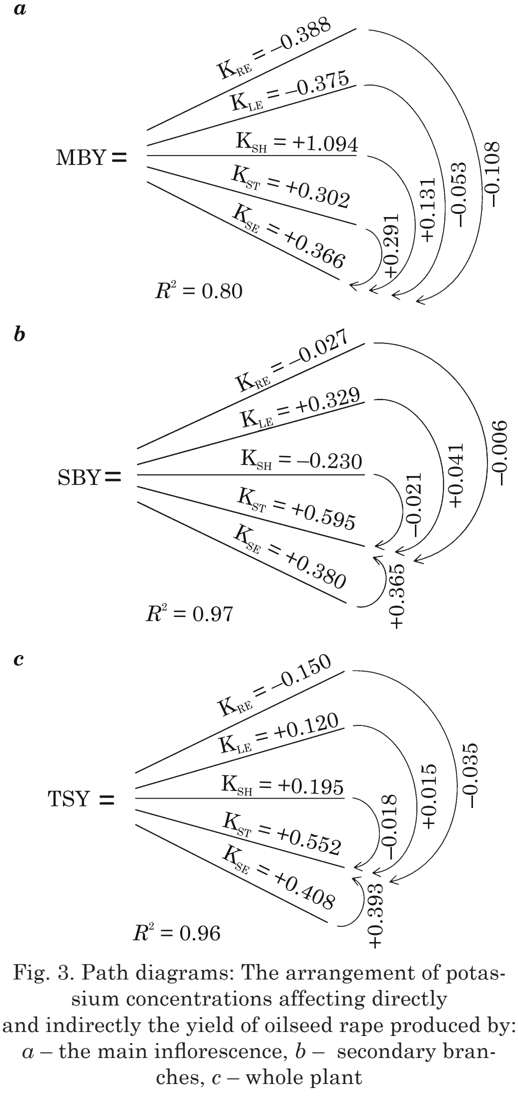

Fig. 3. Path diagrams: The arrangement of potassium concentrations affecting directly and indirectly the yield of oilseed rape produced by: *a* – the main inflorescence, *b* – secondary branches, *c* – whole plant

Table 3

Components of the path analysis of the impact of K concentrations on oilseed rape yield, $n = 18$

Yield type/ K variable	MBY		SBY		TSY	
	DI*	IDs**	DI	IDs	DI	IDs
K_{RE}	-0.388	0.546	-0.027	0.331	-0.150	0.433
K_{LE}	-0.375	0.896	0.329	-0.112	0.120	0.218
K_{SH}	1.094	-0.511	-0.230	0.404	0.195	0.131
K_{ST}	0.302	0.313	0.595	0.379	0.552	0.390
K_{SE}	0.366	0.260	0.380	0.584	0.408	0.530

* direct effect, ** sum of indirect effects

$$SBY = 0.059K_{ST} + 0.885 \quad \text{for } n = 18, R^2 = 0.95 \text{ and } p < 0.00000.$$

The above regression model implicitly indicates the importance of the K post-flowering management in the oilseed rape canopy on the yield produced by secondary branches. The dominant effect of this plant part was corroborated by the analysis of the varied K impact on the total yield of seeds (Figure 3c). It depended on the K content in straw, as revealed by the stepwise analysis, which highlighted the decisive effect of K in straw on yield:

$$TYS = 0.076K_{ST} + 1.638 \quad \text{for } n = 18, R^2 = 0.89 \text{ and } p < 0.00000,$$
$$TYS = 0.053K_{SH} + 0.074K_{ST} + 1.134 \text{ for } n = 18, R^2 = 0.97 \text{ and } p < 0.00000.$$

In order to explain the dominant effect of K_{SH} on oilseed rape yield, the potassium content in plant parts throughout the season was related to the size of its structural components. The number of siliques on the main stem was affected directly by the K content in straw (Figure 4a). However, it was significantly modified by indirect effects of the other variables, most markedly by K accumulated in seeds (Table 4). The negative signs of K in seeds can be explained by the insufficient K-sink seed capacity, which in fact depended on the limited K supply from stems. The stepwise analysis revealed a significant but negative effect of the K content in oilseed rape at the rosette stage on the number of siliques on the main inflorescence. To attain high accuracy of the developed model, an additional set of variables is required, including K in both the rosette and the stems:

$$SMB = 0.463K_{ST} + 31.81 \quad \text{for } n = 18, R^2 = 0.55 \text{ and } p < 0.00044,$$
$$SMB = -0.865K_{RE} + 0.515K_{ST} +41.42 \text{ for } n = 18, R^2 = 0.67 \text{ and } p < 0.00027,$$
$$SMB = -1.386K_{RE} + 0.621K_{SH} + 0.525K_{ST} + 43.72$$
$$\text{for } n = 18, R^2 = 0.76 \text{ and } p < 0.00013.$$

The seed density was taken as the principal variable, affecting the plant's K requirement during the seed-filling period. The highest effect on the number of seeds per silique on the main branch, as indicated by the correlation coefficient, was exerted by the K content in shoots. Its impact was negative, although it was modified the most by the K content in leaves (Figure 4*b*, Table 4). The final seed density on the main branch resulted from the balance between the K content in leaves and shoots at the onset of flowering. At the same time, the stepwise analysis revealed a significant effect of the K content in leaves at the rosette stage:

$$SSMB = 0.675K_{RE} - 0.55K_{SH} + 9.286 \text{ for } n = 0.18, R^2 = 0.39 \text{ and } p < 0.0234.$$

The number of siliques per secondary branch was most distinctly affected by the K content in leaves at the onset of flowering (Figure 3*c*). The trait was significantly modified by the K content in shoots, which affected it indirectly albeit negatively, as indicated by path coefficients (Table 4). The stepwise analysis corroborated the dominating but relatively weak impact of the K content in leaves on the number of siliques:

$$SSB = 2.349K_{LE} + 109.4 \qquad \text{for } n = 18, \ R^2 = 0.42 \text{ and } p < 0.0034.$$

Secondary branches showed the same seed density pattern in response to the K content as the main inflorescence. The key factor, only slightly affected by the other variables, was the K content in shoots at the beginning of flowering and in straw (Figure 4*d*, Table 4). This model was fully corroborated by the stepwise analysis:

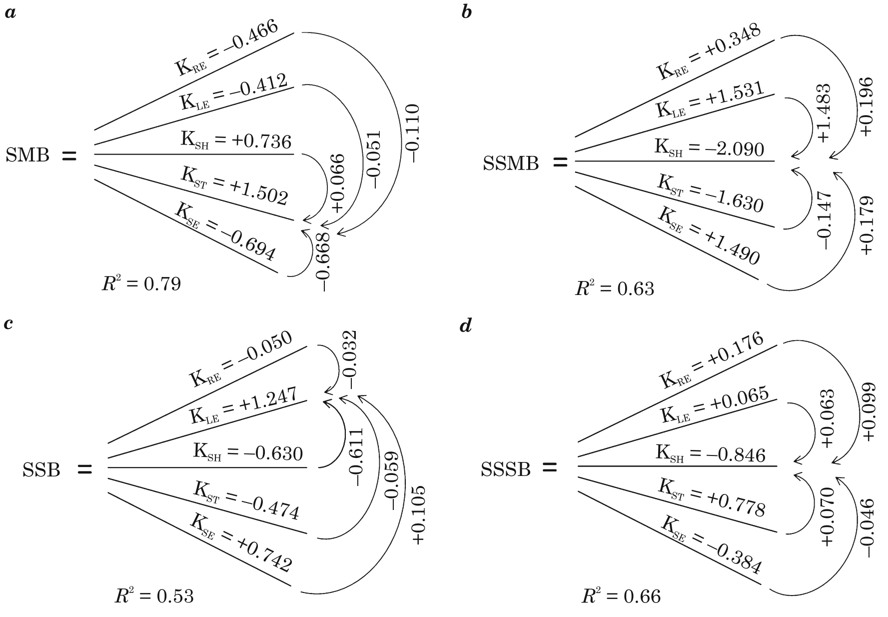

Fig. 4. Path diagrams: The arrangement of potassium concentrations affecting yield structural components:
a – number of siliques per main inflorescence, *b* – number of seeds per silique in the main inflorescence, *c* – number of siliques on the secondary branch, *d* – number of seeds per silique on the secondary branch

Table 4

Components of path analysis of the impact of K concentrations on oilseed rape yield structural components, $n = 18$

Yield component /K variable	SMB		SSMB		SSB		SSSB	
	DI*	IDs**	DI	IDs	DI	IDs	DI	IDs
K_{RE}	-0.466	0.306	-0.050	0.553	0.348	-0.148	0.176	-0.359
K_{LE}	-0.412	0.497	1.247	-0.598	1.531	-1.787	0.065	-0.664
K_{SH}	0.736	-0.609	-0.630	1.226	-2.090	1.709	-0.846	0.186
K_{ST}	1.502	-0.762	-0.474	0.799	-1.630	1.516	0.778	-0.397
K_{SE}	-0.694	1.345	0.742	-0.368	1.490	-1.504	-0.384	0.705

* direct effect, ** sum of indirect effects

$$SSSB = -0.6K_{SH} + 14.38 \quad \text{for } n = 18,\ R^2 = 0.44 \text{ and } p < 0.0029,$$

$$SSSB = -0.636K_{SH} + 0.15K_{ST} + 9.9 \quad \text{for } n = 18,\ R^2 = 0.63 \text{ and } p < 0.00057.$$

It can be concluded, based on the above equations, that the seed density as a prerequisite of final yields depends on the K management pattern in oilseed rape canopy before and just after flowering. It was observed that an increase in the net K accumulation affected significantly the net biomass production, most distinctly in 2008 (Figure 5). This finding implicates the K oilseed rape economy as the key cause of positive correlation between the dry

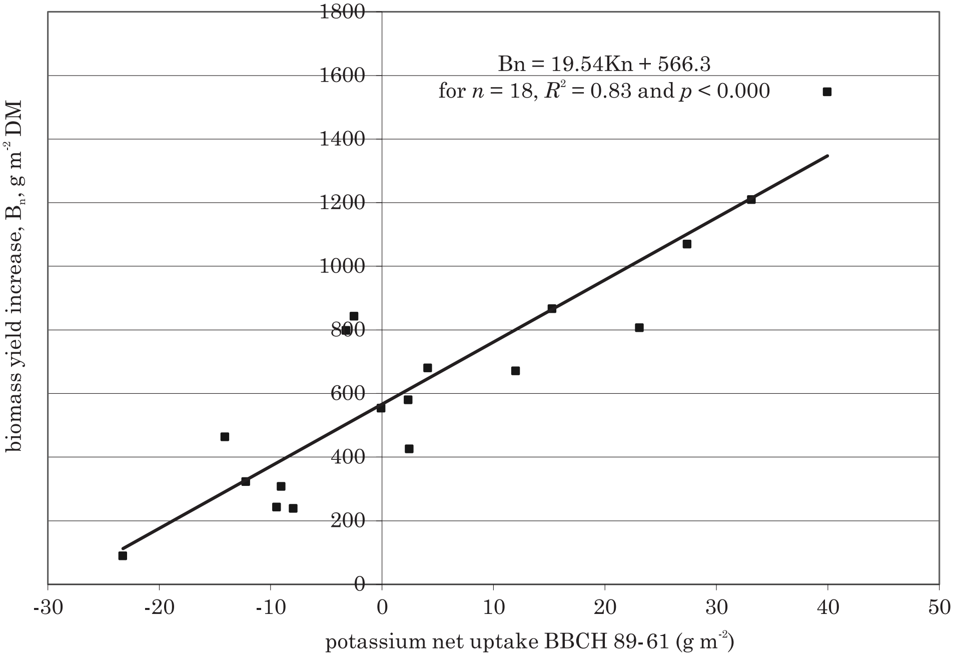

Fig. 5. A net vegetative biomass increase during the seed-filling period as a function of the net potassium accumulation in straw

matter increase during oilseed rape flowering and seed density. In 2009 and, in few instances, in 2010. there was a negative K_n balance. Consequently, the number of seeds per silique on the secondary branch considerably depended on this trait of oilseed rape plants:

$$\text{SSSB} = 0.184\text{K}_n + 9.61 \qquad \text{for } n = 18 \text{ and } R^2 = 0.62 \text{ for } p < 0.00000.$$

This study showed that the yield forming effect of potassium is intrinsic to the seed density on secondary branches. Therefore, the suggestion expressed by Rose et al. (2008), that potassium has a yield effect until the flowering stage was confirmed only under an ample water supply, which occurred in 2009. In general, oilseed production is conducted under rainfed conditions. Oilseed rape, as shown in Figures 1 and 2, requires a huge amount of potassium. The most important factor which determined the K uptake by plants is the soil resources. As reported by Škarpa and Hlušek (2012), the actual content of soluble and exchangeable potassium depends on the weather course during the growing season. However, the negative impact of the weather on this K form can be ameliorated by K fertilizer application. In addition, non-exchangeable K resources should also be considered in making fertilization plans (Grzebisz, Oertli 1993).

CONCLUSION

The study proved that potassium is a major nutritient, which affects the N economy in oilseed rape, formation of yield components and, consequently, oilseed rape yield. Despite fluctuations of the K content during the growing season, the key predictor of yield is the potassium content in stems. This relationship can be explained by the requirements of growing seeds during plant maturation. Therefore, sucessful control of the seed density, which defines the size of K physiological sink, is the challenging task for farmers. The study showed that the pattern of potassium uptake by the crop since the onset of flowering significantly affected the development of structural yield components. Any excess of potassium taken up by oilseed rape before flowering, resulting in the saturation-like model of nitrogen uptake, leads to a significant reduction in the seed density on the main branch and, especially, on secondary branches. The oilseed rape yield, in the light of the above study, depends on the net K content increase in vegetative parts of the crop during the seed-filling period.

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EFFECT OF NUTRIENT SUPPLY FROM DIFFERENT SOURCES ON SOME QUALITY PARAMETERS OF POTATO TUBERS

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Abstract

Application of mineral fertilisers, especially nitrogen ones, increases potato yields. On the other hand, it can depreciate the quality of potato tubers and raise storage loss. With bio-fertilisers like UGmax, farmers can limit the need for mineral fertilisers by activating minerals which occur in soil. Microorganisms contained in UGmax, enriched with the start-nutrition medium, process (compost) residue of the last harvested crop, straw, manure break crops (organic fertilisers), etc. Together with soil minerals, they make humus, a natural environment for soil life as well as a 'store' of nutrients. The aim of the following, 3-year research was to determine changes in the content of vitamin C, nitrogen, phosphorus, potassium and crude protein (nitrogen x 6.25) in cv. Satina potato tubers stored for 6 months indoors at the temperature of 4°C, depending on previous application of soil fertiliser (with/ without UGmax), varied organic matter (without fertiliser, stubble intercrop: pea, straw and farmyard manure (FYM) as well as a half dose of mineral fertilisation as compared with the control. The highest vitamin C content was recorded in potato tubers from the treatments with FYM, full NPK and soil fertiliser. With half the dose of mineral fertilisation, UGmax most effectively increased the vitamin C content in potato tubers in the treatments with FYM and pea. The biggest decrease in the content of that nutrient after 6-month storage appeared in tubers from the plots with FYM, 50% NPK and without UGmax (38.5%). The content of nitrogen, phosphorus, potassium and total protein in tubers significantly depended on the dose of mineral fertilisation, where full NPK increased the content of the analysed nutrients. Moreover, the content of potassium was significantly higher in tubers from the plots with farmyard manure (FYM). The use of the soil fertiliser significantly increased nitrogen and total protein in tubers. After 6-month storage in chambers at + 4°C, the nitrogen, phosphorus and potassium content decreased slightly, on average by 4.9%, 12.4%, 13.1%, respectively.

Key words: vitamin C, nitrogen, phosphorus and potassium, total protein, bio-fertiliser, storage.

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INTRODUCTION

Organic food has been enjoying a growing consumer interest, which *inter alia* necessitates performance of an objective evaluation of potato tuber quality in terms of their chemical composition. For the last ten years, the number of organic farms in Poland has risen by several-fold (Truba at al. 2012). The European Union countries promote organic farming, hence an answer is searched for to the question whether organic potato tubers or those derived from reduced mineral fertilisation plantations where farmyard manure (FYM), green manure as well as plant growth enhancers are applied, achieve a better nutritive value than those grown in other farming systems. The use of microbiological bio-agents (also referred to as 'fertilisers') enhances tuber yielding and chemical composition (Trawczyński, Bogdanowicz 2007). The soil fertiliser UGmax is a microbiological preparation composed of yeasts, lactic acid bacteria, photosynthetic bacteria, *Azotobacter*, *Pseudomonas* and *Actinobacteria*, as well as potassium (3500 mg dm^{-3}), nitrogen (1200 mg dm^{-3}), sulphur (1000 mg dm^{-3}), phosphorus (500 mg dm^{-3}), sodium (200 mg dm^{-3}), magnesium (100 mg dm^{-3}), zinc (20 mg dm^{-3}) and manganese (0.3 mg dm^{-3}) (Trawczyński 2007). The use of UGmax aims at improving physiochemical soil properties. It accelerates the decomposition of post-harvest residue and organic fertilisers, activates nutrients from minerals or insoluble compounds and improves water relations (Długosz et al. 2010). Thus, crops can use elements from mineral fertilisers more efficiently, which in turn allows farmers to lower mineral fertilisation doses, consequently limiting the emission of harmful substances to surface waters. The positive effect on potato tuber yield volumes is also well known (Zarzecka et al. 2011).

The nutritive value of potato tubers is determined by such nutrients which, having been digested and absorbed into blood, are used by the body as a source of energy, building material or a factor controlling life processes (*Law* ... 2009). Examples are vitamin C and proteins. In organic farming, farmyard manure (FYM) and green manure are the source of nitrogen, phosphorus, potassium and trace elements. Plants take up nitrogen up to the share of 50% over a period of 45 days after emergence. Thus, delayed mineralization of FYM and green manure to available nitrogen forms during the plant growth can be a problem, causing adverse effects on the quality and storage life of tubers (Wheatley et al. 2000). The aim of the present research was to verify whether a farming system with limited mineral fertilisation and the use of organic fertilisers as well as soil fertiliser can affect the content of vitamin C, nitrogen, phosphorus, potassium and crude protein content in cv. Satina potato tubers.

MATERIAL AND METHODS

Tubers of the table cultivar Satina N (mid-early potato cultivar) were obtained from experimental plots set up on soil class IV b in a three-factor split-plot design at Mochełek Experimental Station of the University of Technology and Life Sciences in Bydgoszcz. After harvest, they were stored for 6 months in chambers at the temperature of + 4°C and relative air humidity of 95%. For the experiment factors, see Table 1.

Table 1

Research factors

First factor (A): type of organic matter	
Control	without organic fertilisers
Intercrop - pea	at a dose of 40 kg ha^{-1} on experimental plots in autumn after post-harvest treatments and before pre-winter plough
Straw	at a dose of 4 t ha^{-1} on experimental plots in autumn after post-harvest treatments and before pre-winter plough
Farmyard manure - FYM	at a dose of 25 t ha^{-1} in autumn
Second factor (B): mineral fertilisation	
100% dose of NPK fertilisation	applied in spring prior to potato planting, at doses adjusted to the soil richness (low phosphors very low potassium) and nutritive requirements of the plant: 100 kg N ha^{-1}, 100 kg P$_2$O$_5$ ha^{-1}, 150 kg K$_2$O ha^{-1}
50% of NPK fertilisation	
Third factor (C): the application of the bio- fertiliser UGmax	
With bio-fertiliser	The bio-fertiliser was applied in the experiments at three doses: in autumn prior to pre-winter plough on organic matter at the dose of 0.6 l ha^{-1}, in spring prior to tuber planting during land tillage at the amount of 0.3 l ha^{-1}, as foliar fertilisation at the potato plant height of 15-20 cm at the dose of 0.3 l ha^{-1}.
Without bio-fertiliser	

The vitamin C content was assayed with the Tillmans method, by titration of an adequately prepared sample with the titrant of 2.6-dichlorophenoloindophenol, in line with standard PN-90/A-75101/11; the content of total protein was calculated from the content of nitrogen multiplied by 6.25, assayed with the Kjeldahl method on a BüchiLabortechnik B-324 apparatus, after mineralization in concentrated sulphuric acid (VI). The potassium content was determined in the dry weight in potato tubers after mineralization and using atomic absorption spectrophotometry. Phosphorus was assayed by the photometric method. The significance of differences between the results was tested at $a = 0.05$; the data were submitted to analysis of variance with the help of Statistica 8.0.

The course of the weather during the growing season was described with the precipitation sum and coefficient K (Table 2), calculated from the formula $K = 10$ (P/T), which includes the sum of precipitations in (mm) during the analysed time (P) and the sum of mean daily temperatures in (°C) in the same time period (T). Optimal values fall within the range from 1.0 to 2.0. Values of $K > 2.0$ stand for a wet plant growing period with water excess. The meteorological data were collated from records of the Weather Station at

Table 2

Weather conditions during the plant growing season at Mochełek Experiment Station

Years	Rainfall						Sum
	month						
	Apr	May	June	July	Aug	Sept	
2009	0.4	12.4	53.4	118.0	17.6	34.4	236.1
2010	33.8	92.6	18.1	107.4	150.7	74.7	477.3
2011	13.5	35.4	100.8	132.5	67.7	37.0	386.9
Average	15.9	46.8	57.4	119.3	78.7	48.7	366.8
	Temperature						
2009	9.8	12.3	14.5	18.6	18.2	13.7	87.1
2010	7.8	11.5	16.7	21.6	18.4	12.2	88.2
2011	10.5	13.5	17.7	17.5	17.7	14.3	91.2
Average	9.4	12.4	16.3	19.2	18.1	13.4	88.8
	Hydrotermal coefficient *K*						average
2009	0.01	0.32	1.23	2.05	0.61	0.84	0.84
2010	1.44	2.60	0.36	1.60	2.64	2.04	1.78
2011	0.43	0.85	1.90	2.44	1.23	0.86	1.29
Average	0.63	1.25	1.16	2.03	1.50	1.25	1.30

K: 0-0.5 drought; 0.6-1.0 mild drought; 1.1-2.0 moist, optimal conditions; 2.1- wet

Mochełek. The 2009 season showed drought periods during the early plant growth, much moisture afterwards and a spell of semi-dry weather at the end of the growing season. During the 2010 and 2011 growing seasons, the weather was quite wet, thus being optimal for the potato development and growth.

The chemical composition of soil before the onset of the field experiment was similar in each year; the soil was very poor in available forms of potassium and magnesium and poor in available phosphorus (Table 3). Besides, the soil pH implicated slight acidification with trace elements of organic carbon and total nitrogen.

Table 3

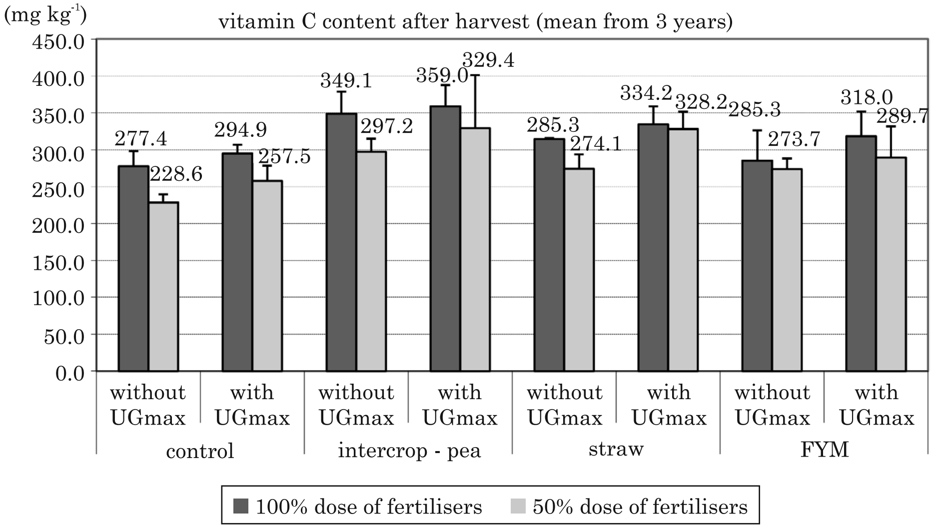
Chemical properties of the soil (mean from years 2009-2011)

Specification	Richness
pH H_2O 6.5 pH KCl 6.0	slightly acid soil
Organic matter 6.95 g kg^{-1}	
Total nitrogen 0.7 g kg^{-1}	
Content of available nutrients (mg kg^{-1}) P - 26.0 K - 45.0 Mg - 16.0	 poor very poor very poor

RESULTS AND DISCUSSION

The human body cannot synthesize vitamin C, which must be supplied with a diet. By eating about 300 g potato tubers per day, the body can be supplied with up to 50% of daily vitamin C requirement (Wichrowska, Pobereżny 2008). Vitamin C is an essential health-enhancing exogenous nutrient for humans, and potatoes are one of the staple foodstuffs supplying this nutrient. Vitamin C plays an essential role in cells maintaining an adequate redox potential, by scavenging reactive forms of oxygen and neutralizing nitrogen produced by the cellular metabolism (Sies et al. 1992). Vitamin C also affects the production of collagen, which accelerates wound healing, increases resistance to infections and, as an antioxidant, offers protection from carcinogenic changes. Its content in potato tubers ranges from 100 to 300 mg kg^{-1} and is mostly cultivar-specific, although considerably depending on the climate conditions during vegetation (Rogozińska 2000, Wichrowska, Pobereżny 2008). With our research results confirmed by the statistical analysis, it was proven that all the experimental factors significantly differentiated the content of vitamin C in potato tubers after harvest (Figure 1). The highest content of that nutrient was reported in potato tubers from the treatments with FYM, a full dose of mineral fertilisation and the bio-fertiliser (359.0 mg kg^{-1}). The vitamin C accumulation was enhanced by an application of UGmax into soil (an average increase by 11% compared with tubers collected from the treatments with no bio-fertiliser) and full NPK. With half the mineral fertilisation dose, UGmax increased the content of vitamin C in potato tubers most effectively in the treatments with the application of FYM and pea (Figure 1).

Similar relationships between fertilisation with FYM and catch crop versus an increasing content of vitamin C in tubers (compared with treatments without organic fertilisation) were reported by Boligłowa and Gleń (2003). Inoculation of soil with microorganisms enhances its fertility, the yielding of plants and the chemical composition of tubers (Sulewska et al. 2005, Trawczyński, Bogdanowicz 2007). After storage, the content of vitamin C in potato tubers derived from the experiment decreased by 33.3%, on average for all the treatments, compared with the content immediately after harvest (Figure 2). The biggest decrease in the nutrient analysed after 6-month storage was recorded in tubers from the plots with the application of FYM, 50% NPK and without UGmax (38.5%). All the research factors applied in the experiment significantly differentiated the level of vitamin C in potato tubers. There were also significant interactions between the application of organic matter as well as mineral fertilisation. Similarly to the findings immediately after harvest, most vitamin C was contained in potato tubers from the treatment with FYM, full mineral fertilisation and soil fertiliser, where the content of the nutrient equalled 255.2 mg kg^{-1} of fresh weight of tubers. The storage period duration as well as temperature and moisture in



LSD $p \geq 0.05$ (Tukey test): A (organic matter) = 60.7

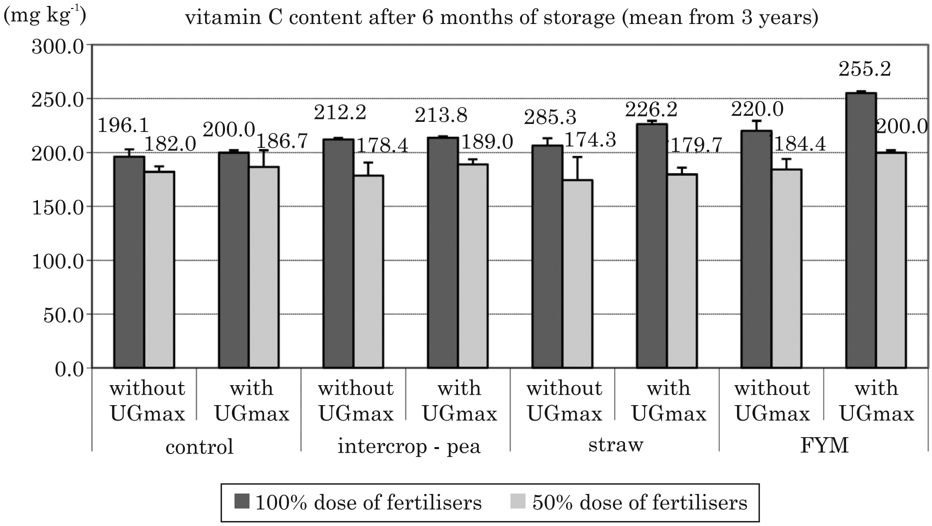
B (mineral fertilisers)= 2.2

C (used UGmax)= 14.9

B/A = n.s. A/B = n.s. C/A = n.s. A/C = n.s. C/B = n.s. B/C = n.s.

n.s. – non significant

Fig. 1. Vitamin C content in potato tubers after harvest depending on research factors (mg kg^{-1} fresh weight) the 2009-2011 average



LSD (Tukey test) $p \geq 0.05$ A (organic matter) = 10.3

B (mineral fertilisers) = 5.8 C (used UGmax) = 7.9

B/A = 11.6 A/B = 12.7 C/A = n.s.

A/C = n.s. C/B = n.s. B/C = n.s.

n.s. – non significant

Fig. 2. Vitamin C content in potato tubers after 6 months of storage depending on research factors (mg kg^{-1} fresh weight) average from the 2009/2010-2011/2012 storage seasons

the storage room can change the content of nutrients, including vitamin C, nitrogen, protein (Gąsiorowska 2000, Wichrowska et al. 2009).

During storage, both forms of vitamin C (ascorbic acid and dehydroascorbic acid) undergo irreversible oxidation to biologically inactive products (2,3- dioxol-L-gluconic acid). The process intensifies during tuber sprouting, hence optimal temperature to minimise vitamin C losses ranges from +4 to +6°C. The content of vitamin C after seven-month storage could decrease by as much as 60-80%.

The results have shown that the content of the macroelements under study was modified by the experimental factors. Nitrogen is an important macroelement in an assessment of the chemical composition of potato tubers. Its amount is mostly determined by organic and mineral fertilisers (Wierzbicka, Trawczyński 2011). In the tuber samples they investigated, the nitrogen content varied dependng on effective microorganisms, cultivars and weather conditions. The highest content of nitrogen in tubers was recorded for very early cultivar Miłek (13.2 g kg^{-1}), and the lowest one for late cultivar Ursus (11.0 g kg^{-1}). The application of effective microorganisms increased the content of nitrogen in tubers by an average of 1.2 g kg^{-1}. Trawczyński and Bogdanowicz (2007) revealed the effect of soil microorganisms contained in soil fertiliser on the content of nitrogen, phosphorus and potassium. As reported by many authors (Gianquinto, Bona 2000, Płaza 2004, Różyło, Pałys 2006, Piikki et al. 2007), each form of fertilisation significantly increased the nitrogen content in tubers. In tubers produced by potatoes fertilised with FYM and NPK, the nitrogen content was 16.5 g kg^{-1}; control tubers (only mineral NPK) had 15.4 gN kg^{-1} (Płaza 2004).

In this study, the nitrogen concentration ranged from 15.86 to 18.30g kg^{-1} dry weight after harvest (Table 4), falling to 15.1 to 17.1 g kg^{-1} of dry weight after 6 months of storage (Table 5). A full dose of mineral fertilisation and applied bio-fertiliser significantly increased the nitrogen content in potato tubers after harvest. The interaction between organic matter and mineral fertilisation was also significant, with a positive effect in tubers from the treatment with pea as intercrop; full mineral fertilisation dose and bio-fertiliser. The effect of organic matter and bio-fertiliser on the nitrogen content in potato tubers during storage was not significant. After 6-month storage in chambers at the temperature of +4°C, the nitrogen content decreased slightly, by an average of 4.9% for treatments. The storage capacity and the system of storage used are very much associated with the carbohydrate metabolism and the substances affecting it as well as the nitrogen metabolism (Mohamed E.O. Elsayed, 2008).

The content of phosphorus in tubers determines the technological value of potato. According to Wierzbicka and Trawczyński (2011), its amount depends on the cultivar and weather conditions. The content of phosphorus in the experiments in which standard fertilisation was applied (Płaza 2004, Zarzecka, Gugała 2010) ranged from 2.5 to 2.9 g kg^{-1} and it was two-fold higher than in those investigated by Wierzbicka and Trawczyński (2011) in organic tubers. Zarzecka and Gugała (2010) also found that more phosphorus

Table 4

Nitrogen, phosphorus and potassium content in potato tubers after harvest depending on research factors (g kg^{-1} dry weight), 2009-2011 average

Factors			Mineral fertilisation (B)						Average		
			100% dose			50% dose					
Organic matter (A)	bio- fertiliser (C)		N	P	K	N	P	K	N	P	K
Control	without soil fertilisers	average from years	17.30	3.26	26.86	16.61	2.26	21.55	16.94	2.76	24.21
		SD	1.14	0.04	0,25	1.14	0.06	0.37	1.14	0.05	0.31
	with soil fertilisers	average from years	17.74	3.36	27.84	16.70	2.31	22.49	17.23	2.83	25.16
		SD	0.50	0.01	1.07	0.14	0.01	0.52	0.32	0.01	0.80
Average			17.52	3.31	27.35	16.66	2.28	22.02	17.09	2.81	25.16
Intercrop-pea	without soil fertilisers	average from years	17.81	3.32	27.59	15.86	2.31	22.39	16.83	2.82	24.99
		SD	0.29	0.02	0.16	0.78	0.01	0.49	0.53	0.02	0.3
	with soil fertilisers	average from years	18.30	3.40	28.29	16.45	2.38	22.97	17.38	2.89	24.99
		SD	0.29	0.02	0.34	0.50	0.01	0.13	0.38	0.01	0.23
average			18.05	3.36	28.41	16.14	2.35	23.02	17.10	2.85	25.71
Straw	without soil fertilisers	average from years	18.14	3.33	27.59	16.54	2.31	23.50	17.34	2.82	25.59
		SD	1.06	0.01	0.25	0.78	0.01	0.49	0.91	0.01	0.37
	with soil fertilisers	average from years	18.00	3.39	28.39	17.34	2.35	24.79	17.68	2.87	26.59
		SD	0.85	0.04	0.34	0.78	0.04	0.23	0.82	0.04	0.29
average			18.08	3.36	28.51	16.94	2.33	24.87	17.52	2.85	26.69
FYM	without soil fertilisers	average from years	16.75	3.48	28.77	16.05	2.44	25.39	16.40	2.96	27.08
		SD	0.35	0.01	0.20	0.21	0.01	0.23	0.29	0.01	0.21
	with soil fertilisers	average from years	16.66	3.59	28.96	16.66	2.50	26.78	16.66	3.04	27.87
		SD	0.21	0.02	0.10	0.26	0.04	0.15	0.24	0.03	0.12
average			16.70	3.53	28.86	16.36	2.47	26.83	16.53	3.00	27.91
Average	without soil fertilisers	average from years	17.50	3.35	27.70	16.26	2.33	23.21	16.88	2.84	25.45
	with soil fertilisers	average from years	17.68	3.44	28.41	16.78	2.40	24.29	17.23	2.92	26.35
Average			17.58	3.51	28.05	16.52	2.46	23.75	17.05	2.98	25.90
LSD (Tukey test) $p \geq 0.05$ n.s. – not significant SD – standard deviation								A	n.s.	n.s.	1.183
								B	0.256	0.994	0.994
								C	0.304	n.s.	n.s.
								B/A	0.496	n.s.	n.s.
								A/B C/A	2.832	n.s.	n.s.
								A/C	n.s.	n.s.	n.s.
								C/B	n.s.	n.s.	n.s.
								B/C	n.s.	n.s.	n.s.

Table 5

Nitrogen, phosphorus and potassium content in potato tubers after 6 months of storage depending on research factors (g kg^{-1} dry weight), average from 2009/2010-2011/2012 storage seasons

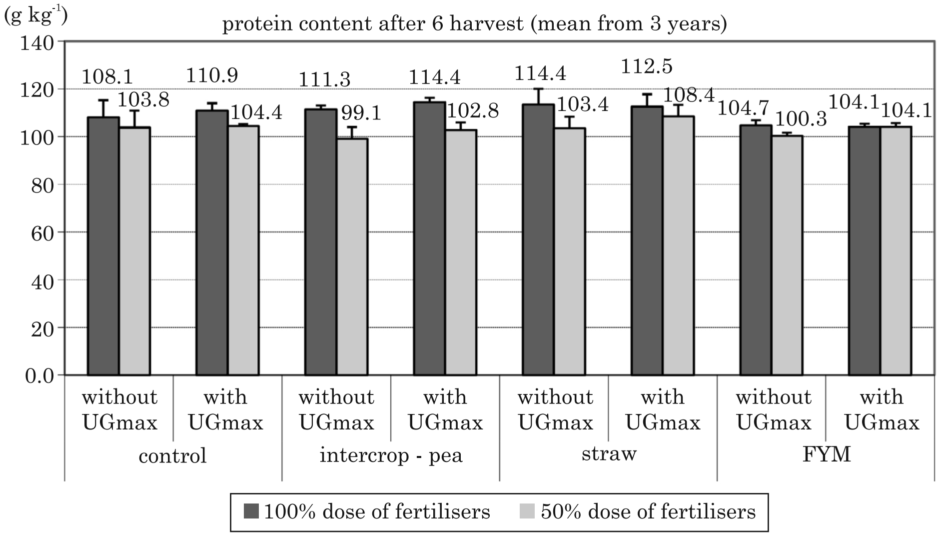
Research factors			Mineral fertilisation (B)						Average		
			100% dose			50% dose					
Organic matter (A)	bio- fertiliser (C)		N	P	K	N	P	K	N	P	K
Control	without soil fertilisers	average from years	16.26	2.97	20.65	15.10	1.93	20.23	15.68	2.45	20.44
		SD	0.35	0.02	0.21	1.14	0.01	0.44	0.74	0.02	0.33
	with soil fertilisers	average from years	16.94	3.00	21.25	15.81	1.98	20.63	16.37	2.49	20.94
		SD	0.21	0.02	0.33	0.29	0.01	0.43	0.24	0.02	0.38
average			16.61	2.99	20.95	15.46	1.96	20.43	16.03	2.47	20.69
Intercrop-pea	without soil fertilisers	average from years	15.55	3.08	21.98	15.86	2.02	21.66	15.70	2.55	21.82
		SD	0.64	0.01	0.16	1.63	0.01	0.23	1.14	0.01	0.19
	with soil fertilisers	average from years	17.06	3.09	22.73	16.26	2.04	22.16	16.66	2.56	22.44
		SD	0.21	0.01	0.32	0.21	0.01	0.38	0.21	0.01	0.35
Average			16.30	3.09	22.35	16.05	2.03	21.91	16.18	2.56	22.13
Straw	without soil fertilisers	average from years	15.90	3.10	22.74	15.30	2.07	22.58	15.60	2.58	22.66
		SD	1.28	0.01	0.23	0.29	0.01	0.41	0,78	0.01	0.32
	with soil fertilisers	average from years	17.10	3.13	23.44	15.90	2.07	23.38	16.50	2.60	23.41
		SD	0.43	0.01	0.21	0.14	0.01	0.41	0,29	0.01	0.31
Average			16.50	3.12	23.09	15.60	2.07	22.98	16.05	2.59	23.03
FYM	without soil fertilisers	average from years	17.34	3.28	24.46	15.86	2.26	23.97	16.61	2.77	24.22
		SD	0.21	0.01	0.44	0.21	0.01	0.11	0.21	0.01	0.28
	with soil fertilizers	average from years	17.06	3.37	24.65	16.00	2.34	23.49	16.53	2.86	24.07
		SD	0.06	0.01	0.33	0.70	0.02	0.35	0.38	0.02	0.34
average			17.20	3.32	24.56	15.92	2.30	23.73	16.56	2.81	24.14
Average	without soil fertilizers	average from years	16.26	3.11	22.46	15.52	2.07	22.11	15.89	2.59	22.28
	with soil fertilisers	average from years	17.04	3.15	23.01	15.98	2.11	22.41	16.51	2.63	22.71
Average			16.66	3.13	22.74	15.76	2.09	22.26	16.21	2.61	22.50
LSD (Tukey test) $p \geq 0.05$ n.s. – not significant								A	n.s.	n.s.	n.s.
								B	0.896	0.994	n.s.
								C	n.s.	n.s.	n.s.
								B/A A/B	n.s.	n.s.	n.s.
								C/A	n.s.	n.s.	n.s.
								A/C	n.s.	n.s.	n.s.
								C/B	n.s.	n.s.	n.s.
								B/C	n.s.	n.s.	n.s.

was contained in potato tubers in simplified cultivation as compared with the traditional one, and the herbicides used for cultivation decreased the content of phosphorus. Other authors [Różyło and Pałys 2006] observed neither the effect of fertilisation nor the type of soil on the content of phosphorus, although in all the objects the phosphorus content was high: from 4.4 to 4.6 g kg^{-1}. In the tubers analysed in our experiment, the mean content of phosphorus for the objects was 3.51 g kg^{-1} of dry weight (Table 4).

The tubers from plants fertilised with full NPK contained significantly more phosphorus (by 30%) than those harvested from the plots with half a dose of mineral fertilisation. A similar tendency was observed, i.e. a higher concentration of phosphorus, after 6 months of storage in tubers from the plots of full mineral fertilisation (Table 5). Manure (FYM) introduced into soil significantly increased the content of potassium in tubers after harvest (Table 3). The concentrations of phosphorus decreased during storage (Table 5) depending on the content after harvest.

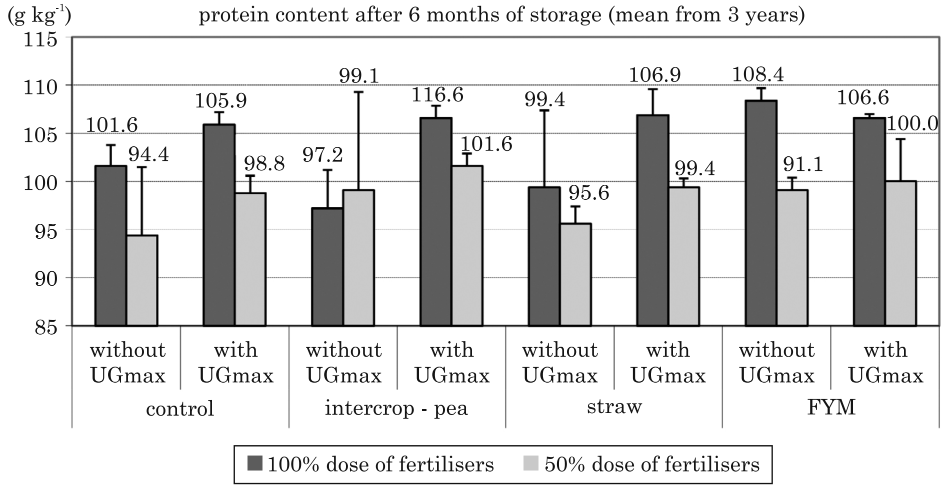
Of all the macroelements, potato tubers contained most potassium. The content of potassium in the dry weight of tubers was 25.9 g kg^{-1} on average and varied as a result of the type of organic and mineral fertilisation applied. The content of potassium in organic tubers investigated by Wierzbicka and Trawczyński (2011) was similar to the level of the potassium content in the fertilisation experiments, being on average 16.3 g kg^{-1} (Zarzecka, Gąsiorowska 2000, Płaza 2004, Prośba-Białczyk, Tajner-Czopek 2006). A higher level of potassium (17.5 g kg^{-1}) was recorded in tubers when mineral NPK fertilisation was applied than without it (15.5 g kg^{-1}) (Prośba-Białczyk, Tajner-Czopek 2006). In the studies which involved organic and mineral fertilisation (Płaza 2004), the mean content of potassium in tubers collected from an object with farmyard manure was 15.6 g kg^{-1} and in tubers from a variant with mineral fertilisation (without FYM) it declined to 14.1 g kg^{-1}. In other research (Różyło, Pałys 2006), the level of potassium was much higher than in the organic tubers investigated by Wierzbicka and Trawczyński (2011). The application of varied mineral-and-organic fertilisation by those authors resulted in a significant increase in the content of potassium (22.2 g kg^{-1}) compared to organic fertilisation (21.6 g kg^{-1}) or to the variant without fertilisation (21.1 g kg^{-1}). In the present research, the significantly highest amount of potassium was recorded in tubers from the plots fertilised with FYM as well as a complete dose of mineral fertilisation (27.91 g kg^{-1}).

Nitrogen compounds occur in tubers at the amount of about 2% converted into protein (N x 6.25), i.e. as the so-called total protein, with the share of pure protein between 35 and 65%. The daily protein requirements for an adult is 1 gram per kilogram of the body weight, e.g. with the body weight of 70 kg one should consume 70 g of protein daily (including 35 g of animal and 35 g of plant protein). Potato protein is a valuable nutrient, although its content in potato is small. The protein quality is determined by essential amino acids it contains; the body cannot synthesize them independently, which is why they must be supplied with food. Out of the eight amino acids people must ingest, seven are found in potato protein: leucine, lysine, isoleucine, phenylalanine, threonine, methionine and valine (WODA-LEŚNIEWSKA 1993, ZGÓRSKA 2002, AHMED et al. 2010). The content of protein considerably depends on the climate conditions during the vegetation period. Dry years enhance the increase in the total protein content in tubers (ROZTROPOWICZ 1989). The protein accumulation in tubers is also affected by genetic traits (WODA-LEŚNIEWSKA 1993) and agrotechnical conditions (SZYNAL, SYKUT 1992, BOLIGŁOWA, DZIENIA 1999, RIVERO 2003, TEKALIGN, HAMMES 2005, BRZOZOWSKA 2008, WICHROWSKA et al. 2009). Based on the results given in Figure 3, the content of total protein in tubers ranged from 99.1 to 114.4 g kg^{-1} of dry weight and significantly depended on mineral fertilisation, where full NPK increased the content of the compound. The application of bio-fertiliser increased the content of total protein in tubers. According to RÓŻYŁO and PAŁYS (2009), potato tubers from organic + mineral objects contained significantly more total proteins than tubers from the remaining objects. After 6-month storage in chambers at the temperature +4°C, the content of total protein decreased slightly, on average for treatments by 2.3% (Figure 4). According to WICHROWSKA et al. (2009) and WICHROWSKA (2013), total protein losses in the tubers stored at +4°C are lower than in chambers at the temperature of +8°C: by 1% after 3 months and by 2.9% after 6 months.



LSD (Tukey test) $p \geq 0.05$ A (organic matter) = n.s. B (mineral fertilisers) =1.6 C (UGmax) =1.9 B/A = 3.1 A/B = 17.7 C/A = n.s. A/C = n.s. C/B = n.s. B/C = n.s.

Fig. 3. Protein content in potato tubers after harvest depending on research factors (mg kg^{-1}dry weight) 2009-2011 average



LSD (Tukey test) $p \geq 0.05$ A (organic matter) = n.s. B (mineral fertilisers) = 5.6 C (UGmax) = n.s. B/A = n.s. A/B = n.s. C/A = n.s. A/C = n.s. C/B = n.s. B/C = n.s.

Fig. 4. Protein content in potato tubers after 6 months of storage depending on research factors (mg kg^{-1} dry weight), average from 2009/2010-2011/2012 storage seasons

CONCLUSIONS

1. A significant effect of the organic matter applied on the content of vitamin C and potassium in potato tubers was demonstrated.

2. Potato tubers from the plots fertilised with a complete dose of mineral fertilisers contained significantly more macroelements than tubers collected from the plots with half the dose of mineral fertilisers.

3. The microorganisms introduced into soil together with the bio-fertiliser caused a significant increase in the content of vitamin C, nitrogen as well as protein in tubers; they also increased the content of phosphorus and potassium in tubers. Beraing this in mind, it is justified to apply bio-agents in potato growing because they can transform the unavailable forms of nutrients into available ones owing to the microorganisms which they contain.

4. The content of nitrogen and protein in tubers significantly depended on the interaction of organic matter as well as a dose of mineral fertilisation introduced to soil. The highest levels of nutrients were found in tubers from the plots fertilised with pea grown as an intercrop, and from the plots with full NPK fertilisation.

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RESPONSE OF PLANKTONIC CYANOBACTERIA AND PERIPHYTON ASSEMBLAGES TO PHYSICOCHEMICAL PROPERTIES OF STORMWATER IN A SHALLOW URBAN LAKE

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Abstract

The aim of this study was to determine the response of planktonic cyanobacteria and periphyton assemblages (periphytic algae in the separator pipes, epilithon and epiphyton) to lamella separator-treated stormwaters in urban Lake Jeziorak Mały in 1997-2003 and 2005. Relationships between periphyton and phytoplankton assemblage and water chemistry variables were analyzed by calculating the Spearman's rank correlation coefficient, and then with canonical correspondence analysis (CCA).

The basic factors favouring the cyanobacterial growth were water temperature and iron, while high chloride concentration limited their development. Cyanobacterial abundance recorded at the separators was half that of the pelagic zone because of a lower water temperature and higher Cl concentration, indicating high algal sensitivity to the considerable velocity and disturbances caused by stormwater effluents. Higher silicon and calcium concentrations at the separators and orthophosphates at sites with stones and gravel showed connection with the growth of diatoms, especially *Fragilaria leptostauron* var. *martyi*, *Diatoma vulgaris* and *Navicula gregaria*. The richness of the dominant cyanobacteria, *Aphanizomenon gracile* and *Limnothrix redekei*, was related with the water temperature, conductivity, pH, and TN. Similarities in the periphyton dynamics in separator pipes and epilithon between the years covered by the study suggest significant influence of separator-treated stormwater on these assemblages, in contrast to epiphyton and phytoplankton in the pelagial zone, where these waters had limited influence.

Key words: rainwater, pollution, algae, nutrients, CCA.

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INTRODUCTION

The growing industrial development of urban areas and the twnetieth-century agriculture contributed to water eutrophication, which accelerates in urban agglomerations when domestic sewage and nutrient-rich stormwater enter shallow lakes (Guzkowska, Gasse 1990, Wichelen et al. 2007). Excess primary nutrient production causes massive growth of some algal species, particularly cyanobacteria, resulting in algal blooms (Reynolds 1984), which deteriorates the ecological status of some lakes (Napiórkowska-Krzebietke et al. 2012). These processes are effectively limited by reducing the nutrient inflow, using lake-basin restoration methods, including hypolimnetic withdrawal (Olszewski 1961, Wichelen et al. 2007) combined with artificial aeration and mixing of bottom waters (Steinberg 1983, Moore et al. 2012), plus the removal of bottom sediments and chemical deactivation of phosphorus (Lossow et al. 2004). Effective lake restoration must take into the lake's morphological and hydrological conditions, pollution sources, catchment use and technical methods appropriate for local conditions (Bernhardt 1987). Polluted stormwaters are often discharged directly into lakes in urbanized catchments where sewerage systems and pipes to collect overland flow and waste water are absent (Guzkowska, Gasse 1990). Separators can be used for stormwater organic substance pretreatment to protect urban lake water.

Owing to their role in aquatic ecosystem, phytoplankton and plant periphyton are good indicators of eutrophication changes in lakes, caused by nutrient inflow. Ecologically, phytoplankton and periphyton contribute to the nutrient cycling and biological productivity of aquatic systems by linking the "bottom-up" and "top-down" processes (Reynolds 1984).

Jeziorak Mały is a eutrophic lake dominated by planktonic cyanobacteria. In 1997, protective restoration measures were implemented, including the installation of lamella separators for stormwater pretreatment. The aim of this paper was to determine the response of cyanobacteria (plankton) and periphyton assemblages to the influx of stormwaters in the urban lake Jeziorak Mały, in 1997-2003 and in 2005. The general problem was whether stormwaters flowing through the separators affected the growth of cyanobacteria (plankton) and periphyton assemblages in this lake. Answers to the following questions were sought to verify it:

(1) Did storm water inflows significantly change the water chemical composition in Jeziorak Mały Lake?

(2) Do differences occur in succession of cyanobacteria at the separator sites and in the pelagic zone?

(3) Are there relationships between periphyton assemblages in the separator pipes and in the epilithon, epiphyton and phytoplankton?

(4) Have the environmental requirements of the dominant periphyton and phytoplankton assemblages changed after the installation of the separators?

MATERIAL AND METHODS

Jeziorak Mały is a typical, shallow (mean depth 3.4 m), eutrophic, urban lake lying in the Mazurian Lakeland, in northeastern Poland (Figure 1). The 26-ha lake is situated in a temperate climate zone. It is connected to Jeziorak Duży Lake (3219 ha area) by a narrow canal (4 m width and 4 m depth). For decades, Jeziorak Mały received untreated municipal sewage from the town of Iława, but since 1991, the effluent has been treated in a local wastewater treatment plant. The ongoing lake water quality improvement began in 1997 and included the installation of separators for pretreatment of stormwater influent and a fountain-based water aeration system. The construction of on the Unicon System lamella separators in the lake's littoral zone started in 1996 and was completed in spring 1997, now preventing untreated stormwater from entering the lake. These separators contain 16 blinder sections, 1200 mm diameter inlet and outlet pipes, and a 10,000 l sedimentation tank to remove petroleum compounds, silt and sand in a separate rainwater sewer system. The efficiency of the separation petroleum products was 97% at the nominal discharge of 160 l s^{-1}. This stormwater pretreatment covered the 70-ha catchment area of Jeziorak Mały Lake (Puh Ekol 1995). The amount of stormwater flowing through the separators depends on monthly precipitation in Warmia and Mazury (Grzesiak, Domańska 1998-2004, 2006). The highest mean total monthly rainfall recorded during the study (1997-2003 and 2005) was 79 mm in summer and 116 mm following torrential rains in July (Figure 2).

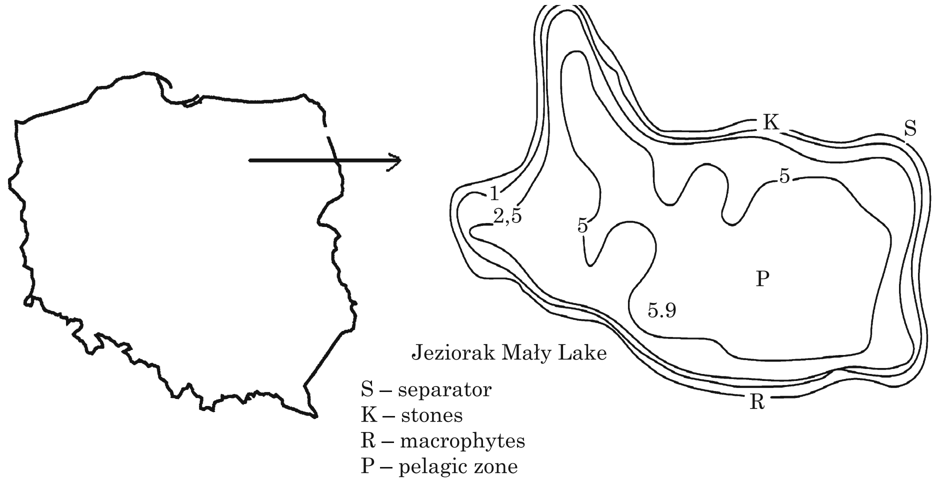

Fig. 1. Morphometric map of Jeziorak Mały Lake

Periphyton samples were collected monthly from April to October in 1997-2003 and 2005, from 3 substrates in the littoral zone, and phytoplankton from the pelagic area of Jeziorak Mały Lake:

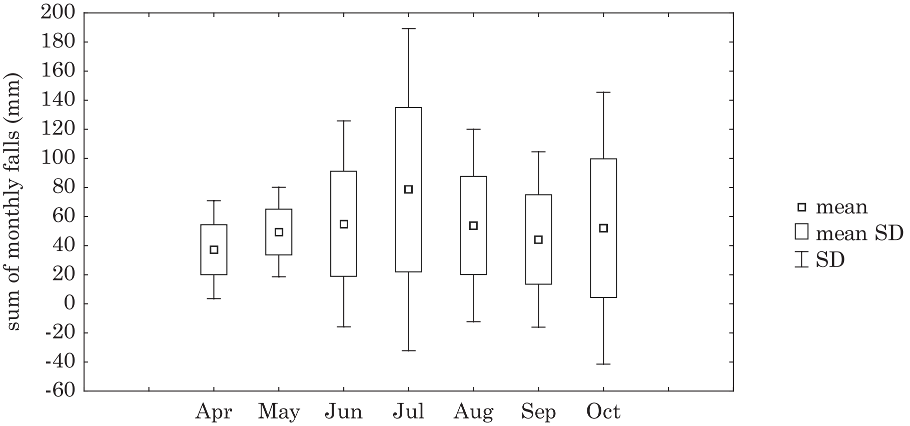

Fig. 2. Sum of monthly rainfalls in Warmia and Mazury (mean and standard deviation – SD) in 1997-2003 and 2005 (Grzesiak, Domańska 1998-2004, 2006)

1) periphyton from the separator pipes draining stormwater (S);
2) epilithon accumulated on stone surfaces in 1997 (K);
3) epiphyton from *Acorus calamus* L. vascular plant leaves (R);
4) phytoplankton from the 1 m euphotic area of the pelagic zone, where the average water transparency in 1997-2003 and 2005 was 0.80 m (P).

Periphyton was scraped from the pipes, from stones (1 cm^2 surface area), and from macrophyte leaves (5 cm lengths). The pipes and stones were often found to be overgrown with *Cladophora glomerata* (L.). Kűtzing filamentous green algae formed a natural substratum for periphytic algae. The periphyton was shaken carefully in distilled water to separate algae from chlorophytic thalli, and the remains were scraped from macrophyte leaves with a knife. Samples were rinsed and preserved in ethanol and formaldehyde solution. Phytoplankton samples were collected from a one-meter surface layer of the pelagic zone using a 5 l Toń plankton sampler, filtered through a 25 μm mesh plankton net, and preserved in Lugol and 4% formaldehyde solution. In total, 124 periphyton and phytoplankton samples were collected. The basic physical and chemical water parameters were measured directly at the sampling sites. The water temperature was exactly 0.1°C, the oxygen content was measured at 0.01 mg dm^{-3} using an HI 9143 oxygen meter, and the pH and conductivity were 1 and 1500 μS cm^{-1} as checked with a CONMET 1 conductometer *in situ.* In the laboratory, the Spectroquant Merck test and a NOVA 400 spectrophotometer were used to determine the following nutrient contents: orthophosphates (0.05-5.00 mg dm^{-3}), silicon (0.005-5.0 mg dm^{-3}), calcium (10-250 mg Ca dm^{-3}), total nitrogen (0.5-15 mg dm^{-3}), iron (0.05-4.0 mg dm^{-3}) and chlorides (2.5-250 mg Cl dm^{-3}).

Phytoplankton and plant periphyton were inspected to determine their qualitative and quantitative characteristics under an Alphaphot YS2 optical microscope at magnifications of lens 10x, 20x, 40x and 100x. These phytoplankton and periphyton samples were composed of prokaryotic (cyanobacteria) and eukaryotic organisms, including diatoms, chlorophytes, dinoflagellates, chrysophytes, and cryptophytes. Diatoms were prepared following the methods of Battarbee (1979). Algal biomass was calculated for bio-volume by comparing the algae with their geometric shapes (Rott 1981). The mean biomass was calculated for 10 individuals of each planktonic and periphytic algal species. Counts in 1 ml samples of periphyton and phytoplankton were determined in 5000 fields of vision with 200× magnification in each planktonic chamber to account for differences in organism densities and their abundance and biomass expressed in identical, basic 1 cm^3 volumes.

The cyanobacterium abundance and species dominance in phytoplankton and periphyton assemblages were correlated with the physical and chemical water parameters using non-parametric methods because these data are not normally distributed. Relationships were confirmed by calculating the Spearman's rank correlation coefficient in a Statistica version 8.0, and then with canonical correspondence analysis (CCA) to relate water chemistry variables to periphyton and phytoplankton dominant species in assemblages. Finally, these relationships were presented on a biplots graph using Canoco for Windows 4.5 software.

RESULTS

In this study, the mean proportion of cyanobacteria in total phytoplankton in the pelagic zone was 72%, ranging from 60% in 1998 to 88% in 2003. The share decreased to 66.02% at the separators in the littoral zone, ranging from 34.15% in 2000 to 87.48% in 1997 (Figure 3*a*). A higher mean abundance of cyanobacteria occurred in the pelagic zone at 48,522 ind. cm^{-3} in 2002, compared to 30,843 ind. cm^{-3} at the separators in 2003 (Figure 3*b*). Statistically positive correlation was shown between the richness of cyanobacteria and both water temperature ($r = 0.46$, N = 119, $p < 0.05$) and iron concentration ($r = 0.57$, N = 36, $p < 0.05$), while negative correlation was detected with chlorides ($r = -0.38$, N = 36, $p < 0.05$). A rapid increase in cyanobacteria was observed in the pelagic zone in June, with 19°C water temperature, maximum total nitrogen of 3.5 mg dm^{-3} and maximum iron concentration of 5.13 mg dm^{-3}. The maximum abundance of cyanobacteria was noted in July at 49,895 ind. cm^{-3}, when the highest water temperature was 25.1°C and the lowest orthophosphate concentration was 0.14 mg dm^{-3}. Cyanobacterial abundance at the separators in the littoral zone was 20,534 ind. cm^{-3} in July, less than half the abundance in the pelagic zone. The littoral zone had a lower water temperature of 20.3°C but a higher maximum chloride concentration of 63 mg dm^{-3} (Figure 4).

Periphytic algal assemblages had the highest mean abundance for periphyton in the pipes (70,535 ind. cm^{-3}) and the lowest for epiphyton

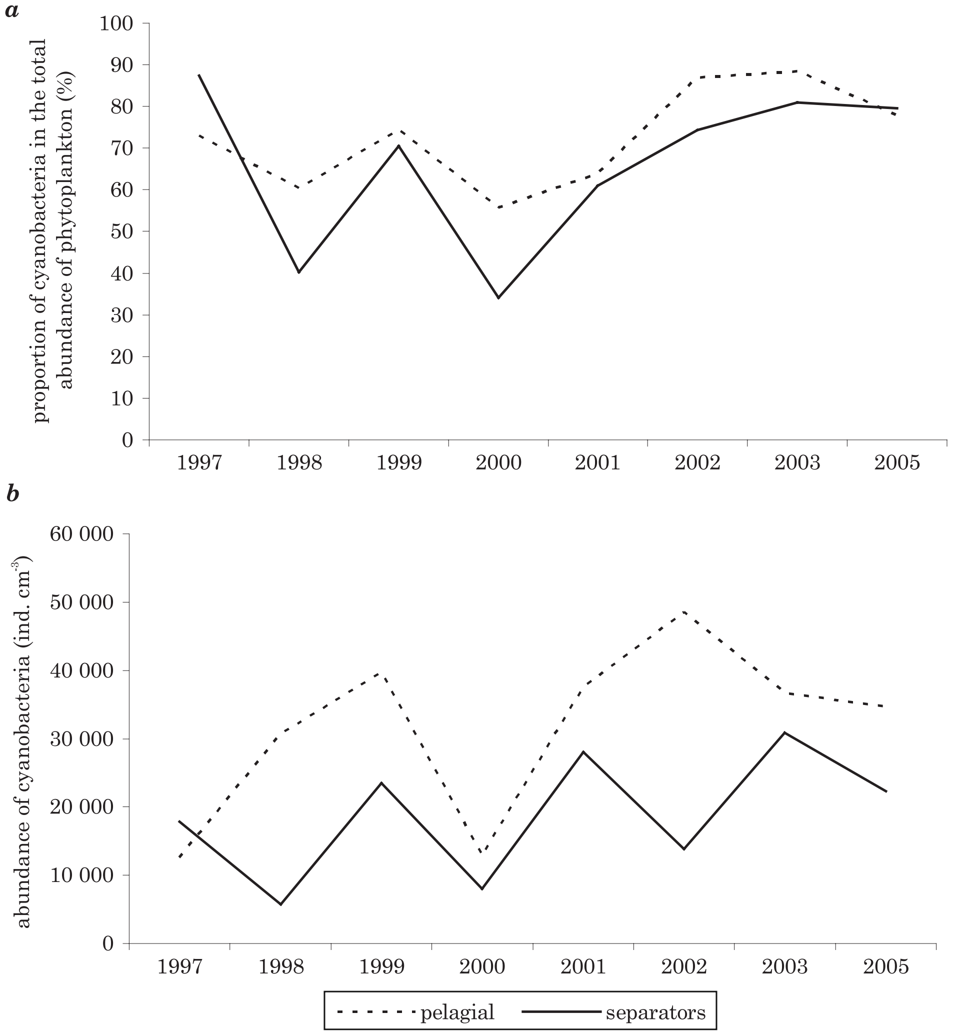

Fig. 3. Proportion of cyanobacteria in total abundance of phytoplankton (*a*) and abundance of cyanobactera (*b*) in 1997-2003 and 2005, in the pelagic zone and at sites with separators in Jeziorak Mały Lake

(39,821 ind. cm^{-3}), and the highest mean biomass for epilithon (0.226 mg cm^{-3}) and the lowest for epiphyton (0.067 mg cm^{-3}). The mean abundance of phytoplankton in the pelagic zone was 31,272 ind. cm^{-3} and its biomass was 0.065 mg cm^{-3} (Table 1). Diatoms dominated periphyton (max. 86% - S), and cyanobacteria outnumbered phytoplankton in both abundance and biomass (73%). Chlorophytes had a significant share in periphyton, with the maximum percetange of 42% in the epilithon, versus 32% of diatoms and 14% of dinoflagellates found in the total phytoplankton.

The following values were recorded (Table 1):

Table 1

Characteristics of periphyton and phytoplankton assemblages and physicochemical parameters of water in Jeziorak Mały Lake (means for the 1997-2003 and 2005 period)

Parameters	Pipes of separators (S)	Stones (K)	Macrophytes (R)	Pelagic zone (P)
Abundance of periphyton/ phytoplankton (ind. cm^{-3})	70,353	65,173	39,821	31,272
Biomass of periphyton/ phytoplankton (mg cm^{-3})	0.175	0.226	0.067	0.065
Water temp (°C)	16.8	19.3	19.2	19.4
Oxygen concentration (mg dm^{-3})	8.17	8.17	8.06	10.33
pH	8.85	8.87	8.98	9.30
Conductivity (mS cm^{-1})	577	469	443	417
PO_4 (mg dm^{-3})	0.41	0.56	0.24	0.32
Si (mg dm^{-3})	1.56	0.77	0.71	1.30
Ca (mg dm^{-3})	136	103	97	82
TN (mg dm^{-3})	3.5	2.8	2.3	1.9
Fe (mg dm^{-3})	2.99	4.36	4.01	3.33
Cl (mg dm^{-3})	51	38	31	23

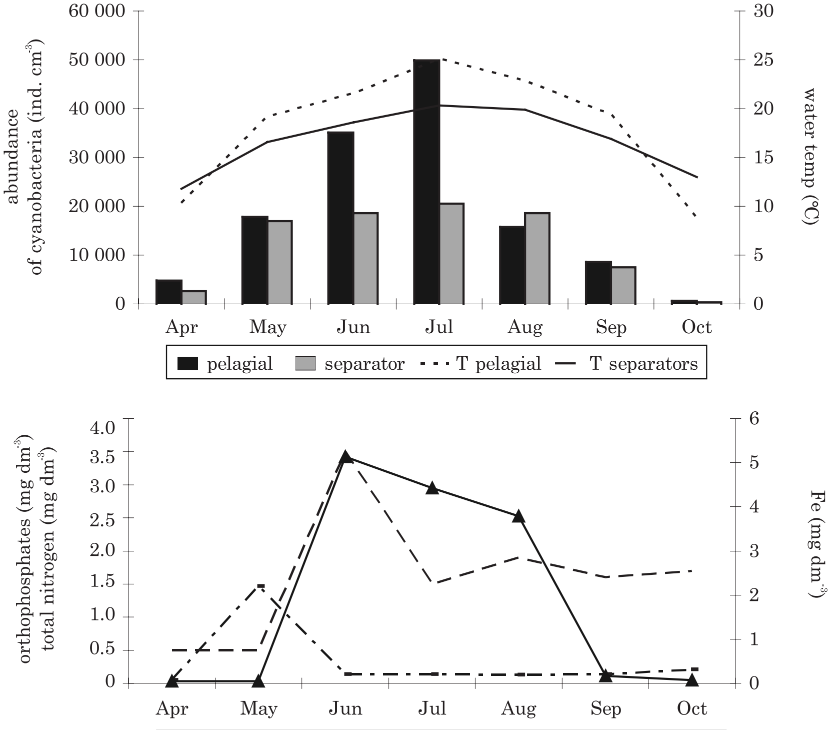

Fig. 4. Changes in abundance of cyanobacteria and water physicochemical parameters from April to October in the pelagic zone and at sites with separators in Jeziorak Mały Lake (means from 1997-2003 and 2005)

(1) at the separators, the lowest mean water temperature was 16.8°C, and the highest water electrolytic conductivity was 577 μS cm^{-1}; additional concentration values were determined for silicon (1.56 mg dm^{-3}), calcium (136 mg dm^{-3}), total nitrogen (3.5 mg dm^{-3}) and chlorides (51 mg dm^{-3});

(2) at sites with stones, the highest mean PO_4 and Fe concentrations were 0.56 mg dm^{-3} and 4.36 mg dm^{-3}, respectively;

(3) in the surface layer of the pelagic zone, the maximum pH values were recorded at 9.3, with 10.33 mg O_2 dm^{-3} of oxygen concentration.

Changes in periphytic assemblages as well as in the phytoplankton abundance and biomass occurred at different orthophosphate concentrations during 1997-2003 and in 2005 (Figure 5a-c). Similar algal dynamics was observed in periphyton sampled from the separator pipes and epilithon. Although the maximum abundance of epilithon (134,644 ind. cm^{-3}) and maximum biomass of both periphytic assemblages (0.648 mg cm^{-3} and 0.521 mg cm^{-3}, respectively) were recorded in 1997, a rapid decrease occurred thereafter. The maximum abundance of periphyton in the pipes was 135,177 ind. cm^{-3} in 2000, at the highest orthophosphate concentration of 0.68 mg dm^{-3}. Changes in PO_4 levels during the study were similar to the changes recorded for periphyton abundance in the separator pipes. Similar dynamics of algal growth were noted between epiphyton and phytoplankton, with the maximum abundance in 2001.

The following diatom species dominated periphytic assemblages: *Navicula gregaria* Donkin, *Nitzschia frustulum* (Kützing) Grunow and *Gomphonema olivaceum* (Hornemann) Brébisson. In turn, the cyanobacterium *Planktolyngbya brevicellularis* Cronberg & Komárek dominated phytoplankton. *Diatoma vulgaris* Bory dominated periphyton biomass in the separator pipes and epilithon; and the epilithon contained the filamentous chlorophytes *Ulothrix tenuissima* Kützing and *Stigeoclonium* sp.

Some correlations between counts of dominant species and water physicochemical parameters were statistically significant at $p < 0.05$):

(1) *G. olivaceum*: a positive correlation with water temperature ($r = 0.42$) in separator pipes, and with PO_4 and Si ($r = 0.52$ and $r = 0.57$) in epilithon;

(2) *F. leptostauron* var. *martyi*: a positive correlation with Si and PO_4 ($r = 0.59$ and $r = 0.40$) in separator pipes;

(3) *D. vulgaris*: a positive correlation with TN in epilithon ($r = 0.59$) and a negative correlation with PO_4 ($r = -0.61$) in separator pipes;

(4) *N. gregaria*: a positive correlation with oxygen ($r = 0.40$) in epiphyton;

(5) *N. frustulum*: a negative correlation with PO_4 ($r = -0.69$) in epiphyton;

(6) *A. gracile*: a positive correlation with pH and TN ($r = 0.71$ and $r = 0.58$), *L. redekei*: with conductivity ($r = 0.64$), and *Peridinium inconspicuum* and *Cryptomonas erosa*: with water temperature

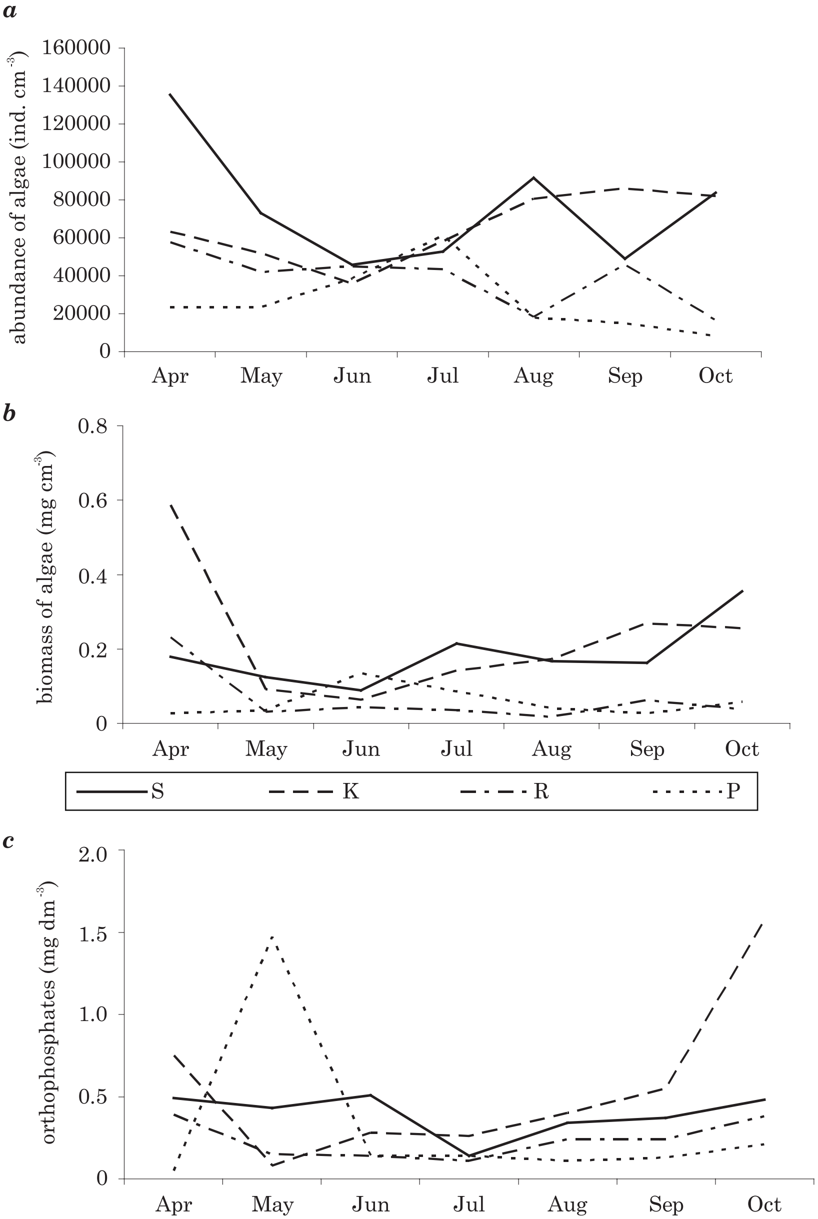

Fig. 5. Relationships between the abundance (*a*) and biomass (*b*) of periphyton assemblages (S – periphyton in separator pipes, K – epilithon, R – epiphyton) and phytoplankton (P) and orthophosphates (*c*), at these sites in Jeziorak Mały Lake in 1997-2003 and 2005

($r = 0.62$ and $r = 0.59$) and negatively correlated with PO_4 ($r = -0.62$ and $r = -0.64$) in phytoplankton.

The periphyton and phytoplankton CCA also showed significant relationships between dominant species and the physicochemical parameters of water (Figure 6).

The statistical analysis used a dataset of 124 samples, 14 dominant species and 9 environmental variables, with the first axis accounting for 49% of the total periphyton species variation in separator pipes, 50% for epilithon, 55% for epiphyton, and 53% for phytoplankton. In the separator pipes, *F. leptostauron* var. *martyi* correlated with PO$_4$ and *G. olivaceum* – with water temperature. In addition, conductivity promoted the occurrence of *D. vulgaris*. Correlations were found between *D. vulgaris* and TN , and *G. olivaceum* and Si and PO$_4$ in the epilithon, while *N. gregaria* correlated only with oxygen in epiphyton. In phytoplankton, *A. gracile* correlated with pH, *L. redekei*

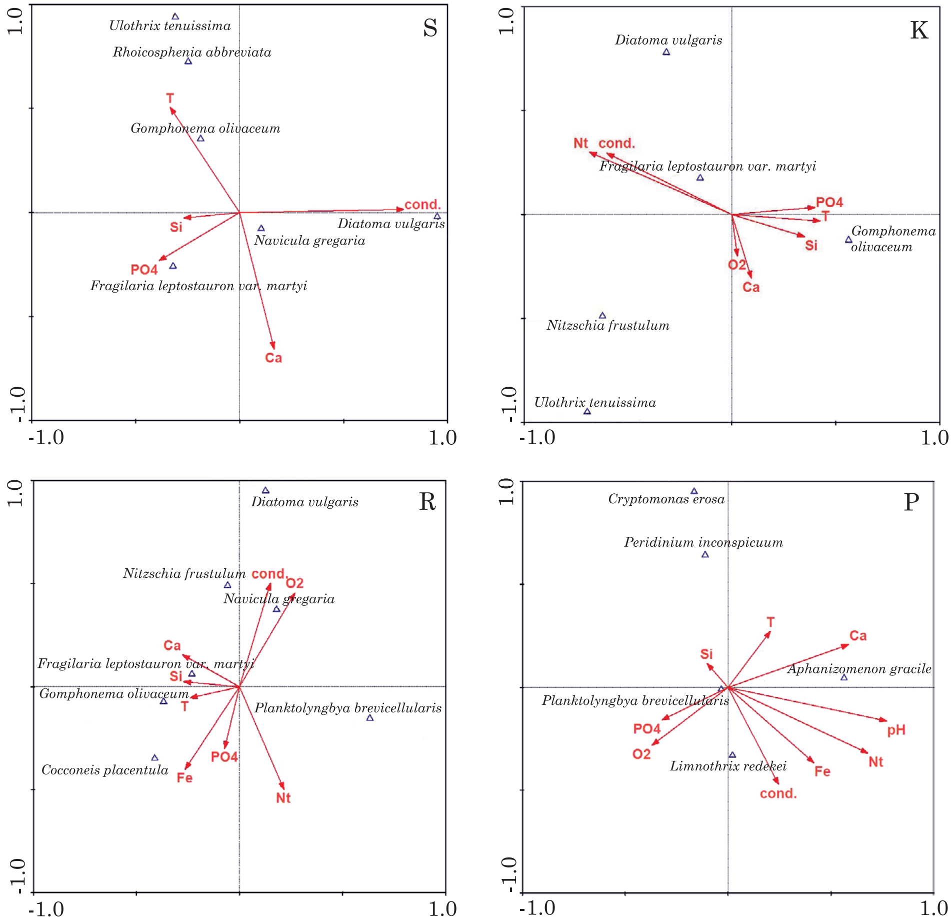

Fig. 6. Biplots of the canonical correspondence analysis (CCA) showing the relationships between dominant species in the periphyton assemblages (S – periphyton in separator pipes, K – epilithon, R – epiphyton) and phytoplankton (P) and the physical and chemical water parameters. (cond. – electrolytic conductivity)

with conductivity, and *Peridinium inconspicuum* and *Cryptomonas erosa* with water temperature.

DISCUSSION

The phytoplankton studies in 1978 showed that Jeziorak Mały was a polytrophic lake, where cyanobacteria dominated, making up over 90% of the total phytoplankton biomass in summer (Spodniewska 1986). Before the implementation of protective and restoration measures in the pelagic zone in 1996, the average percentage of cyanobacteria was above 90% (Zębek 2009), thereafter decreasing to 72% in 1997-2003 and 2005, when stormwater was pretreated by the separators. The share and abundance of cyanobacteria were lower at the separator sites than in the pelagic zone over this period. According to Guzkowska, Gasse (1990), stormwaters environmentally shock the algal growth, especially diatoms, suggesting that cyanobacteria in Jeziorak Mały Lake response to separator-treated stormwater. Analogously to other examinations on urban lakes (Bobin et al. 1989), the impact of stormwaters could be related to precipitation levels, especially in summer, when the highest sum of monthly rainfalls was recorded around this lake.

The mass cyanobacterial growth depends not only on water temperature, nutrient resources, the degree of exposure to wind and water mixing, phosphorus resuspension from sediments and iron accessibility (Reynolds 1984, Berman-Frank et al. 2007), but also on the specific iron, nitrogen, and phosphorus ratio (Burchardt et al. 2007). In this study, high water temperature and iron concentration affected the cyanobacterial growth in the pelagic zone, as supported by the positive correlation between the abundance of these microorganisms and the said water parameters. Additionally, the water temperature of 19°C was identified as the treshold temperature for cyanobacterial growth in the lake, i.e. the temperature at which the abundance of these prokaryotic organisms begin to grow most rapidly (Zębek 2005). The chemical composition of water changed abruptly with a rapid decrease in PO_4, TN and Fe after cyanobacteria attained the maximum number at water temperatures above 19°C, suggesting that the cyanobacterial development is affected by interrelated Fe, TN, and PO_4 concentrations in addition to being dependent on the water temperature.

As observed in other urban lakes, the influence of stormwater flowing through Jeziorak Mały Lake from the separators is so strong that it environmentally shocks aquatic organisms, including cyanobacteria. The changes in water quality in urban lakes are often rapid and extreme because of inflows, which vary in quantity, chemistry and seasonality. This situation is exacerbated by unpredictable occurrences such as building construction and road salting in surrounding catchments (Guzkowska, Gasse 1990). Disturbances in the cyanobacterial biomass growth are also caused by environmental stress, including decreased water temperature and strong mixing (Bobin et al. 1989, Burchardt, Pawlik-Skowrońska 2005, Vincent et al. 2014), as well as the influx of chlorides after winter. In this study, the cyanobacterial abundance recorded at the separators was half that of the pelagic zone because of the lower temperatures from cooler catchment waters and severe water disturbances at the separators. Additionally, the high Cl concentration induced stress in cyanobacterial growth. The stormwater flow from the catchment affected the growth of both cyanobacteria and other algae (Bobin et al. 1989) e.g. periphyton assemblages. In this study, the separators supported the most varied environmental conditions compared to the other sites (Guzkowska, Gasse 1990, Vincent et al. 2014), including the lowest mean water temperature, the highest water electrolytic conductivity and the highest concentrations of Si, Ca, TN, and chlorides. Thus, the flow of polluted stormwater from the catchment area significantly affected the lake water chemistry. Beside increased nutrient concentrations, rainwater had also high levels of Pb, Cu, Zn, Cd, heavy metals and other chemical elements (Szpakowska et al. 2014, Sapek 2014, Zębek 2014). Different changes occurred in the abundance and biomass of periphytic assemblages and phytoplankton related to the PO_4 concentration in 1997-2003 and 2005. Periphyton in the separator pipes and epilithon had similar maximum biomasses, unlike the previously rapid decrease in its abundance and biomass after the separators had been installed. Hence, stormwaters flowing through the separators meant creating environmental conditions similar to those in the pelagic zone and sites with macrophytes, with differences in the algal dynamics resulting from the limited influence of separator-treated stormwater in deeper waters.

After the separators in Jeziorak Mały Lake began operating in 1997, the dominant cyanobacterium species in phytoplankton changed from *Limnothrix redekei* to *Plaktolyngbya brevicellularis*, similarly to other eutrophic lakes (Cronberg, Komarek 1994, Zębek 2005). The periphytic assemblages were dominated by species from genera typical of natural and artificial substrata in eutrophic lakes such as diatoms *Diatoma* spp., *Fragilaria* spp., and *Gomphonema* spp., *Cocconeis* spp., and also filamentous chlorophytes *Stigeoclonium* sp. and *Ulothrix* sp. (Bohr, Miotk 1979, Danilov, Ekuland 2001, Jobgen et al. 2004, Raeder et al. 2010). The periphyton assemblages in the separator pipes, epilithon, and epiphyton were dominated by *N. gregaria*, *N. frustulum*, and *G. olivaceum* diatoms. In addition, *N. gregaria* grew well in the separator pipes because of its mobile form classified as an α-meso-saprobe, which tolerates large amounts of organic matter (Zębek et al. 2012). The CCA showed that changing environmental conditions caused differences in environmental preferences of the dominant and accompanying taxa in the periphytic and phytoplankton assemblages. The periphytic species *F. leptostauron* var. *martyi, G. olivaceum*, and *D. vulgaris* preferred nutrient-rich waters with high concentrations of Si, and PO_4, and TN in the separator pipes and epilithon. The low water temperature at the separator pipes is another factor stimulating the growth of *G. olivaceum*. However, the planktonic species preferred high water temperature, pH and conductivity. Moreover, the negative correlation of *D. vulgaris* in the separator pipes, and *P. inconspicuum* and *C. erosa* in the pelagial with orthophosphates may indicate intensive uptake of this nutrient by these species.

In conclusion, stormwater significantly influenced the development of phytoplankton and periphyton by changing the environmental conditions in Jeziorak Mały Lake, and this led to the reconstruction of the structure and function of the analyzed algal assemblages, with the stronger influence in the littoral than in the pelagic zone. These results highlight the response of planktonic and periphytic algal trophic indicators to stormwater, domestic runoff and sewage, as well as the possibilities to limit toxic cyanobacterial blooms in shallow lakes. These findings are especially important because a massive growth of cynaobacteria is threatening to the water quality of urban water bodies worldwide. As demonstrated by this study, stormwaters also influence the growth of these prokaryotic organisms by changing environmental conditions, such as decreasing water temperature and increasing Cl concentration. These results suggest that the above phenomenon should be incorporated in future management strategies for urban lakes.

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INVESTIGATION ON THE DISTRIBUTION OF MERCURY IN TISSUES AND ORGANS OF WILD BIRDS OBTAINED FROM THE AREA COVERED BY GREATER WARSAW

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Abstract

The material for investigations was composed of samples of tissues and internal organs of 20 birds. The aim of the work was to analyze the mercury distribution in tissues and organs of wild birds. The following body organs were sampled: chest muscles, femoral muscles, heart muscle, bones, intestines, liver, kidney, lungs, gizzard, the mucosa of the stomach and brain. The content of mercury in the samples was determined by the method of atomic absorption spectrometry (AAS). Based on the results and supported by the relevant literature, certain regularities emerged in the distribution of mercury in tissues and organs of wild birds. The organs with the highest accumulation of mercury were the kidney, liver, cardiac muscle and skeletal muscles. The smallest concentration of mercury appeared in the lungs, bones and the brain. The distribution of mercury depended on the tissue, indicating that the alimentary tract is the main gate through which mercury penetrated into birds' organisms, in a way corresponding to their eating prefences. The highest concentrations of this element were ascertained in the little grebe feeding on aquatic organisms and small fish. The lowest concentrations of mercury appeared in town-dwelling pigeons.

Key words: mercury, wild birds, contamination, tissues, organs.

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INTRODUCTION

Rapid development of civilisation which ignores the principles of sustainable development has contributed to the creation of technologies devoid of the so-called clean and zero-waste production. The consequence is environmental pollution with chemical substances on both the local and global scale.

Mercury is an extremely toxic element, although cases of mercury poisoning are only sporadically reported. In the past, it was used in the past as an active ingredient in ointments, parasiticidals, antiseptics, disinfectants, diuretics and fungicides. Mercury can be a source of environmental contamination when present in seed dressing fungicides, anti-slime fungicides in the pulp and paper industries, by-products of burning coal, mine tailings and waste from chlorine and alkali industries

Mercury compounds which are able to migrate to all elements of the natural environment, including terrestrial and aquatic food chains, are the most dangerous. The use of mercury fungicides in agriculture has contributed to the accumulation of mercury in soils. Seed dressing enriched soils with *ca* 10 g Hg ha^{-1}. Plant contamination with mercury in croplands was small. Seed-eating birds are occasionally affected by toxic mercury after feeding on mercury fungicide treated seed. Contamination of granivorous food chain was first noticed in the 1950s in Sweden, when various bird species had disappeared: pigeons, pheasants, partridges and others (BORG 1958, LINDBERG, ODSJÖ 1983).

The second link in this terrestrial food chain was composed of diurnal and nocturnal birds of prey like e.g.: the kestrel, white-tailed eagle, peregrine falcon, goshawk and the eagle owl. Birds of these and other species died or lost reproductive abilities due to the thinning of egg shells and breaking eggs by nesting birds. In many regions of Sweden, kestrels were almost extinct and the number of peregrine falcons and goshawks markedly declined (BORG et al. 1970). During the period when alkyl mercury was used in agriculture in Sweden (1940-1966), elevated mercury levels were reported among many of the common prey species of the peregrine. Samples of pigeons, i.e. wood pigeons, feral pigeons and stock dove (*Columba oenas*), from 1956-63 averaged 10.6 ppm w.w. in mixed kidney-liver tissues, corresponding to a level of about 3.5 ppm w.w. in muscles assuming the ratio between muscles and liver kidney of 0.3 (BORG et al. 1970).

Mercury levels in food webs in southern Sweden were at least 25 times higher during that period compared with residues in 1976-1977. Corresponding high levels were also found in peregrines and the concentrations were probably so high that they lowered both reproduction and survival (LINDBERG, ODSJÖ 1983). The sex of a bird can, in principle, affect exposure to and accumulation of mercury.

One conventional explanation for the sex differences in mercury burden suggests that female birds should have lower concentrations than conspecific males, because breeding females can depurate methylmercury to their eggs. It is true that eggs of several birds from aqueous reservoirs should not be eaten by sensitive humans due to high levels of mercury (EICHLER 1982, BURGER et al. 1997).

Mercury, which is eliminated from the female via egg laying, has been reported to cause abnormal egg laying behaviour, impaired reproduction, slowed duckling growth and altered duckling behaviour in mallard ducks (KHERA 1979, WOLFE et al. 1998, HEINZ, HOFFMAN 2003, BURGESS, MEYER 2008) Ingested mercury is rapidly absorbed by the intestinal tract and stored in the kidney and liver. A similar situation was also observed in other areas (LINDBERG, ODSJÖ 1983).

Mercury also enters aquatic food chains with industrial waste waters and surface runoff from fields. It accumulates in subsequent links of food chains leading to a situation when its concentration in tissues of piscivorous birds goes up to a level that would be lethal to granivorous field birds (SCHEUHAMMER et al. 2009).

Most of the mercury in birds is in the form of methylmercury and comes from the diet. Although the consumption of fish is the main pathway for methylmercury in humans, for there are few people who eat piscivorous birds, such as fish-eating seabirds and ducks (e.g., mergansers), this is a potentially significant source of mercury exposure. Granivorous game birds such as doves, quail and pheasants tend to have low mercury levels and pose a small threat to a human consumer (SCHEUHAMMER 1987, WOLFE et al. 1998, BRASSO, CRISTOL 2008, SCHEUHAMMER et al. 2009)

Even doves that have fed on hazardous waste sites, such as the contaminated lakebed of Par Pond, a Superfund site in South Carolina, have accumulated little mercury (BURGER et al. 1997).

This study was aimed at determining the distribution of mercury in tissues and internal organs of wild birds. It was part of a study on mercury transformation in birds and on critical accumulation of mercury in these animals. The study will facilitae an assessment of the environmental loading by this heavy metal because of the direct dependence of wild birds on local food resources.

MATERIAL AND METHODS

The material consisted of samples of tissues and internal organs of 20 birds. The birds died when crashed against the walls of the Palace of Culture (16 individuals) or were found dead in other places of Warsaw (4 individuals). Collected individuals represented the following species: the song thrush *Turdus philomelos* Brehm 1831(6 birds), the woodcock *Scolopax rusticola* Linnaeus, 1758 (3 birds), the kestrel *Falco tinnunculus* Linnaeus, 1758

(3 birds including one chick), the blackbird *Turdus merula* Linnaeus, 1758 (2 birds). The great spotted woodpecker *Dendrocopus major* Linnaeus, 1758, the feral pigeon *Columbia livia* forma *urbana*, Gmelin, 1789, the icterine warbler *Hippolais icterina* Vieillot, 1817, the crow *Corvus corone* Linnaeus, 1758*,* the skylark *Alauda arvensis* Linnaeus, 1758, and the little grebe *Tachybeptus ruficollis* Pallas, 1764, were represented by one bird each. Pectoral muscles, femoral muscles, heart, bones, intestines, liver, kidney, lungs, gizzard, stomach mucus and brain were taken for analyses. Until analyses, the material was stored in a refrigerator at -21°C.

The content of mercury in studied samples was analysed with atomic absorption spectrophotometry (AAS) using an automatic mercury analyser AMA-254 made by the Czech firm Altec. The apparatus works on the basis of atomic absorption spectrophotometry and is controlled by the AMA computer software. The method consists in measurements of the absorption spectrum of a lamp with a mercury cathode. The sensitivity is 0.01 ng Hg and the measurement range is from 0.05 to 600 ng. The maximum weight of a sample should not exceed 300 mg.

Concentrations of mercury in analysed samples are given in mg per 1 kg of tissue mass. Each analysis was triplicated and the result is a mean of three measurements. The apparatus was calibrated with the solution of polarographically pure mercury in 2% HNO_3. Arithmetic mean, standard deviation, minimum and maximum values were calculated and obtained results were statistically processed using Statgraphic 4 + computer package.

RESULTS AND DISCUSSION

Concentrations of mercury in samples of tissues and organs varied substantially both across tissues and bird species (Table 1) and were related to the feeding habits of birds. High standard deviation from the mean was mainly the result of extremely high mercury concentrations noted in practically all tissues and organs of the little grebe.

Irrespective of the bird species, concentrations of mercury in analysed tissues decreased in the following order: kidneys, liver, heart muscle, pectoral muscles, intestines, femoral muscles, gizzard, lungs, brain, bones and stomach mucus (Table 1).

Absolute amounts of mercury depend on a bird's feeding habit, but within the distinguished groups there were interspecific differences. The lowest mercury concentrations were found in omnivorous birds, which, due to specific urban conditions, included also the feral pigeon, a granivorous bird when feeding in the wild. Relatively low mercury concentrations were noted in the birds of prey represented here by three kestrels (including one chick). Low tissue contamination by mercury in these birds is understood since they all were relatively young (adult kestrels were no more than one year old).

Table 1

The content of Hg in tissues and internal organs of wild birds (mg kg^{-1} fresh weight)

No.	Bird species	Pectoral muscles	Femoral muscles	Heart	Bone	Intestines	Liver	Kidney	Lungs	Gizzard	Stomach mucosus	Brain
1	***Turdus philomelos*** *Turdus philomelos melos*	0.004	0.003	0.006	0.005	0.010	0.018	0.019	0.004	0.003	0.002	0.004
2	***Turdus philomelos***	0.003	0.005	0.004	0.001	0.010	0.012	0.014	0.004	0.003	0.004	0.002
3	***Turdus philomelos***	0.005	0.009	0.016	0.003	0.014	0.015	0.029	0.002	0.003	0.013	0.008
4	***Turdus philomelos***	0.001	0.002	0.002	0.002	0.019	0.011	0.045	0.011	0.007	0.014	0.004
5	***Turdus philomelos***	0.002	0.004	0.013	0.004	0.006	0.050	0.053	0.009	0.007	0.009	0.014
6	***Turdus philomelos***	0.001	0.001	0.002	0.002	0.018	0.032	0.023	0.006	0.003	0.006	0.006
7	***Tachybeptus ruficolis***	0.704	0.454	1.331	0.088	0.783	1.094	1.498	0.619	0.992		
8	***Falco tinnunculus***	0.016	0.011	0.010	0.004	0.033	0.052	0.022	0.019	0.012		0.015
9	***Falco tinnunculus***	0.041	0.030	0.095	0.004	0.094	0.094		0.056	0.001		0.025
10	***Falco tinnunculus***	0.001	0.000	0.001	0.000	0.000	0.001	0.002	0.001	0.001		0.000
11	***Columbia livia***	0.000	0.000	0.000	0.002	0.000	0.000		0.001			
12	***Hippolais icterina***	0.082	0.079	0.101	0.002	0.041	0.118	0.165	0.080	0.058	0.041	0.060
13	***Turdus merula***	0.004	0.003	0.007	0.003	0.006	0.021	0.053	0.003	0.010	0.006	0.004
14	***Turdus merula***	0.013	0.012	0.014	0.006	0.026	0.064	0.052	0.025	0.018	0.029	
15	***Corvus corone***	0.001	0.002	0.002	0.001	0.001	0.005	0.004	0.001	0.002	0.001	
16	***Dendrocopus major***	0.007	0.010	0.008	0.002	0.005	0.010	0.009		0.006		
17	***Alauda arvensis***	0.002	0.004	0.003	0.002	0.006	0.009	0.006	0.004	0.004		
18	***Scolopax rusticola***	0.030	0.015	0.059		0.125	1.377	0.141	0.055	0.043		
19	***Scolopax rusticola***	0.427	0.483	0.421	0.022			0.958				0.027
20	***Scolopax rusticola***	0.129	0.099	0.185	0.016	0.208	0.020	0.354	0.255			0.148
	Mean	0.074	0.061	0.114	0.009	0.078	0.158	0.192	0.064	0.069	0.013	0.024
	Standard deviation	0.177	0.142	0.303	0.020	0.184	0.384	0.398	0.151	0.238	0.013	0.041
	Maximum	0.704	0.483	1.331	0.088	0.783	1.377	1.498	0.619	0.992	0.041	0.148
	Minimum	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.001	0.001	0.001	0.000

Age has a limited effect on mercury concentrations in the birds' tissues. Mercury accumulated in internal tissues is excreted into the feathers and eliminated in the moult period. It is a way to remove mercury from a bird's body. However, most studies quoted by Stewart et al. (1997) found that mercury concentrations in adults were higher than in chicks or subadults.

Distinct differences in mercury contamination were found among birds feeding on invertebrates. The lowest concentrations of Hg were found in the song thrush (*Turdus philomelos*) and the highest – in woodcocks (*Scolapax rusticola*). There were several reasons for such differences. First, the diet of the song thrush is composed mainly of insects and berry fruits while woodcocks feed mainly on earthworms and other soil invertebrates. The origin and age of a bird are also important. The woodcock most severely contaminated with mercury was an adult bird banded in France, where loads of chemicals in agriculture and industries (and hence the environmental contamination) are incomparably higher than in Poland.

In agreement with the current knowledge of mercury cycling and transformation in aquatic ecosystems, the highest concentrations of mercury were found in the little grebe (*Tachybeptus ruficollis*), a bird feeding on aquatic organisms including small fish. Mercury concentration in muscles, liver and kidneys of this bird were by two orders of magnitude higher than in birds from terrestrial ecosystems. Similarly high were mercury concentrations in other tissues and organs. It has been thus confirmed that mercury accumulates in a trophic chain and that mercury in food is the most important source of contamination by this metal in natural conditions (Khera 1979, Lindberg, Odsjö 1983, Scheuhammer 1987, Burgess, Meyer 2008). Fish selectively accumulating mercury in their muscles are the source of contamination for the little grebe.

A very strong correlation was affirmed between the concentrations of mercury in individual tissues and organs, except the accumulation of mercury in the osseous tissue, which was independent from the rigors of this element in soft tissues (Table 2)

Table 2

The correlation coefficients among mercury concentrations in tissues and organs

Tissues, organs	Femoral muscles	Heart	Bones	Liver	Gizzard	Stomach mucosa	Intestine	Kidney	Lungs	Brain
Pectoral muscles	0.99**	0.99**	- 0.24	0.93**	0.99**	0.94**	0.90**	0.95**	0.99**	0.98**
Femoral muscles	-	0.99**	- 0.25	0.93**	0.98**	0.95**	0.90**	0.94**	0.98**	0.98**
Heart		-	- 0.18	0.94**	0.98**	0.95**	0.87**	0.95**	0.97**	0.99**
Bones			-	-0.13	- 0.24	- 0.36	- 0.50	- 0.19	- 0.28	-0.19
Liver				-	0.93**	0.87**	0.79*	0.93**	0.94**	0.97**
Gizzard					-	0,85**	0.87**	0.98**	0.99**	0.98**
Stomach mucosa						-	0,95**	0.95**	0.96**	0.95**
Intestine							-	0.82*	0.92**	0.86**
Kidney								-	0.96**	0.96**
Lungs									-	0.98**

Explanations: * correlation coefficient for two variables r_{xy} significant at $p \le 0.05$, ** correlation coefficient r_{xy} significant at $p \le 0.01$

Earlier studies on mercury contamination of the skin and feathers of wild birds led to a hypothesis that periodical shedding of feathers enables birds to remove mercury excess from the organism. Data relating Mg feather levels to hatchability are more scanty, but in some species reduced hatching was observed in the 5-10 ppm range, while in others the levels of 40-70 ppm in feathers were associated with lowered reproduction (Adley, Brown 1972, Eisler 1987). Using 5 ppm in feathers as a criterion level, Burger and Gochfeld (1997) reported that the common loons were at considerable risk with an average feather mercury level of 10 ppm

CONCLUSIONS

Based on the results and literature data, some regularities may be seen in the distribution of mercury in tissues and organs of wild, free-living birds.

1. Kidneys, liver, heart and skeleton muscles are the organs of largest mercury accumulation.
2. The lowest mercury concentrations are characteristic of lungs, bones and brain.
3. Distribution of mercury in birds' tissues in relation to feeding behaviour indicates that the alimentary tract is the main port of entry for mercury into birds' organisms.
4. The piscivorous little grebe was the most severely contaminated with mercury, while the granivorous feral pigeon was the least affected.

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