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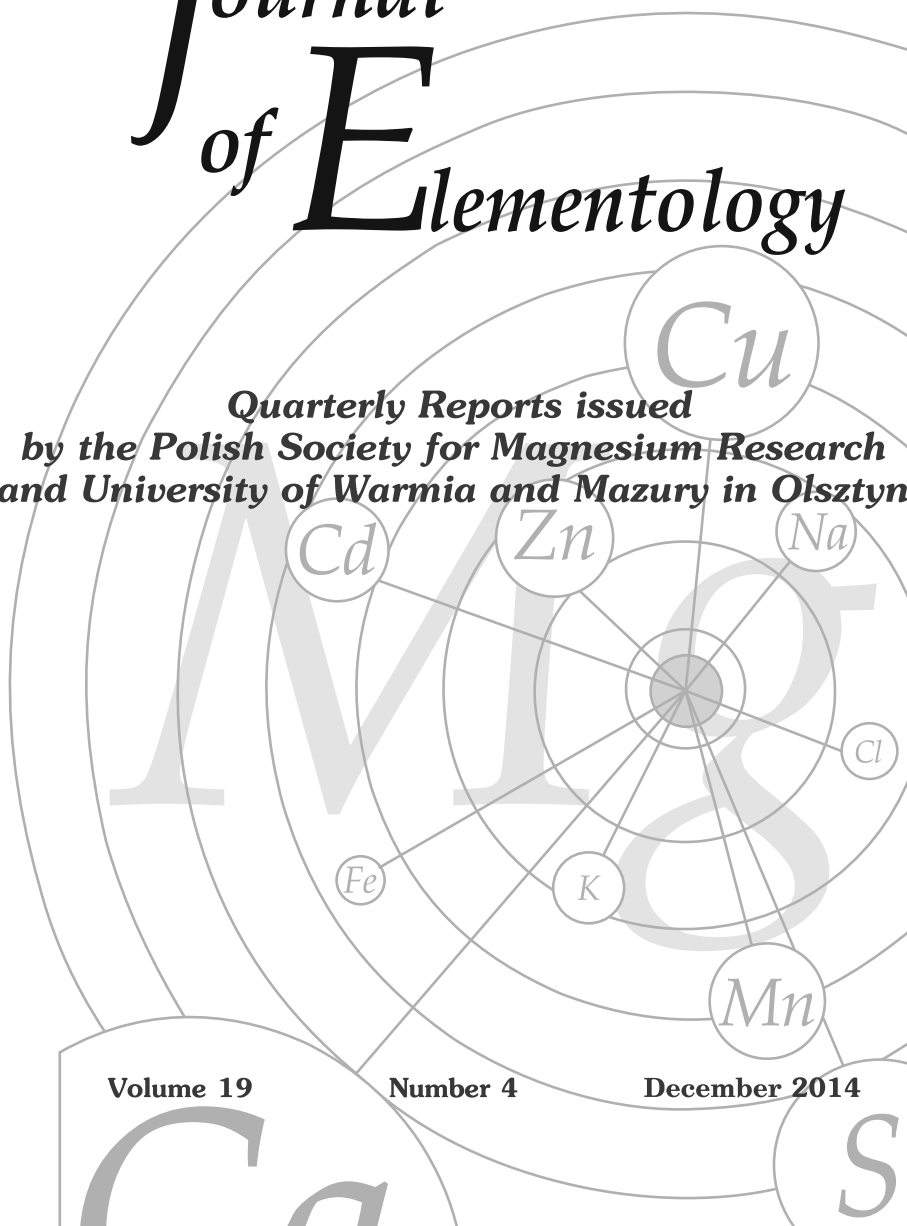
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ORIGINAL PAPERS

**RESISTANCE OF DEHYDROGENASES,
CATALASE, UREASE AND PLANTS TO
SOIL CONTAMINATION WITH ZINC***

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Mirosław Kucharski, Jan Kucharski**

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Abstract

Pot trials on growing plants were conducted in order to determine the resistance of dehydrogenases, catalase and urease as well as the plants themselves to soil contamination with zinc. The experimental variables were: the type of soil (loamy sand and sandy loam), degree of soil pollution with zinc from 0 to 600 mg Zn²⁺ kg⁻¹ d.m., and plant species (oat, spring rape and yellow lupine). Samples of soil were tested to determine the activity of dehydrogenases, catalase and urease as well as its physicochemical properties. Based on the enzymatic activity of soil and the dry matter of harvested plants, the resistance of enzymes and each of the crops was determined to excessive amounts of zinc in soil with different grain-size distribution. It was concluded that zinc contamination significantly inhibited the activity of dehydrogenases, catalase and urease. With respect to their sensitivity to zinc, the enzymes were arranged in the following order: dehydrogenases > urease > catalase. The plant species and grain-size distribution of soil determined the resistance of the enzymes to zinc pollution. Dehydrogenases were most resistant to zinc in soil cropped with oat, urease – in soil under spring rape and catalase – in soil sown with yellow lupine. Dehydrogenases and urease were more resistant to the adverse influence of zinc in sandy loam than in loamy sand, contrary to catalase, which was less vulnerable in loamy sand than in sandy loam. Tolerance of plants to zinc pollution proved to be a species-specific characteristic. Yellow lupine was most sensitive to excess zinc in soil, while oat was most resistant to the said contamination out of the three examined plant species.

Key words: zinc, activity of enzymes, resistance index, oat, yellow lupine, spring rape, soil contamination with zinc.

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OPORNOŚĆ DEHYDROGENAZ, KATALAZY, UREAZY I ROŚLIN NA ZANIECZYSZCZENIE GLEBY CYNKIEM

Abstrakt

W badaniach wegetacyjnych wazonowych, których celem było określenie oporności dehydrogenaz, katalazy i ureazy oraz roślin na zanieczyszczenie gleby cynkiem, czynnikami zmiennymi w doświadczeniu były: rodzaj utworu glebowego (piasek gliniasty i glina piaszczysta), stopień zanieczyszczenia gleby cynkiem (od 0 do 600 mg Zn²⁺ kg⁻¹ s.m. gleby) oraz gatunek uprawianej rośliny (owies, rzepak jary i łubin żółty). W próbkach gleby określono aktywność dehydrogenaz, katalazy i ureazy oraz właściwości fizykochemiczne. Na podstawie aktywności enzymów oraz plonu suchej masy roślin określono oporność enzymów oraz poszczególnych gatunków roślin na nadmierne ilości cynku w glebach o zróżnicowanym składzie granulometrycznym. Stwierdzono, że zanieczyszczenie cynkiem hamuje istotnie aktywność dehydrogenaz, ureazy i katalazy. Enzymy pod względem wrażliwości na cynk uszeregowano następująco: dehydrogenazy > ureaza > katalaza. Gatunek roślin oraz skład granulometryczny gleby determinował oporność enzymów na zanieczyszczenie cynkiem. Dehydrogenazy najbardziej odporne na negatywne działanie cynku były w glebie pod uprawą owsa, ureaza – rzepaku jarego, a katalaza – łubinu żółtego. Dehydrogenazy i ureaza są bardziej odporne na działanie cynku w glinie piaszczystej niż w piasku gliniastym, a katalaza odwrotnie – bardziej odporna w piasku gliniastym niż w glinie piaszczystej. Wrażliwość roślin na zanieczyszczenie cynkiem okazała się być cechą gatunkową. Spośród badanych roślin najbardziej wrażliwy na nadmiar cynku w glebie był łubin żółty, a najmniej – owies.

Słowa kluczowe: cynk, aktywność enzymów, indeks oporności, owies, łubin żółty, rzepak jary, zanieczyszczenie gleby cynkiem.

INTRODUCTION

Zinc is an essential element for all organisms, in which it performs a number of metabolic functions. It occurs in world soils within the range of concentrations from 35 mg to 12,400 mg kg⁻¹ of soil (KABATA-PENDIAS, PENDIAS 2001). This element can occur in excessive amounts in soils in industrial regions and as a point pollutant in soils lying in agricultural regions (BOUSSEN et al. 2013, BRINGMARK et al. 2013, FAMERA et al. 2013). However, when present in excess of the threshold limit, zinc becomes a destructive agent, producing toxic effects on humans and animals (CORDOVA, ALVAREZ-MONA 1995, TAKEDA 2000) and plants (SHUMAKER, BEGONIA 2005, BOROS et al. 2011, GUALA et al. 2013) as well as on soil microorganisms (LANDI et al. 2000, MIKANOVA et al. 2001, RENELLA et al. 2005, BOROWIK et al. 2011) and enzymes (LANDI et al. 2000, BOROS et al. 2011, KUCHARSKI et al. 2011, TREVISAN et al. 2012, WYSZKOWSKA et al. 2013). Many enzymes present inside cells could not function properly without this element. In fact, zinc occurs in over 300 enzymes, which belong to six different classes (McCALL et al. 2000, SEKLER et al. 2007). Its role as a component of metal enzymes should be analyzed in the context of catalytic, structural and regulatory functions. This means that zinc can be essential for the activity of some enzymes, e.g. carbon anhydrase, thermolysine, alkaline phosphatase, dehydrogenases: 3-phosphoglyceric aldehyde, alcohol and glutamine dehydrogenases, and fructobisphosphate aldolase, su-

peroxide dismutase, DNA and RNA polymerase, tRNA transferase. Zinc can stabilize their protein structure and act either as their activator or inhibitor (CORDOVA, ALVAREZ-MONA 1995, SEKLER et al. 2007). These natural functions of zinc could be disrupted when the element occurs in excessive quantities in nature.

Too much zinc in the environment interferes with the metabolism of soil and poses a threat to the proper development of all organisms (DE BROUWERE et al. 2007, MERTENS et al. 2007, WYSZKOWSKA et al. 2008, MORENO 2009). Excessive amounts of zinc depress soil fertility and reduce the activity of enzymes (RENELLA et al. 2005, MIKANOVA 2006, DJUKIC, MANDIC 2006, GULSER, ERDROGAN 2008, VOGELER et al. 2008, LEE et al. 2009, JIANG et al. 2010, COPPOLECCHIA et al. 2011), which are an important biomarker of the quality of soil (HINOJOSA et al. 2008, BOROWIK et al. 2013, WYSZKOWSKA et al. 2013). Thus, being able to recognize all underlying conditions of the effects produced by zinc on the natural environment is important from both the cognitive and utilitarian point of view. For this reason, the present experiment has been conducted in order to determine the impact of zinc present in excessive amounts in soil on the activity of dehydrogenases, catalase and urease. Another objective has been to determine the resistance of these enzymes to soil contamination with zinc.

MATERIAL AND METHODS

Design of the experiment

The trials were set up with five replications and conducted in a greenhouse, in polyethylene pots each with the capacity of 3.5 dm³. The following variables were tested:

- 1) soil type: loamy sand and sandy loam;
- 2) degree of soil contamination with zinc, in mg Zn²⁺ kg⁻¹ d.m. of soil: 0, 150, 450, 600;
- 3) species of the grown plant: oat (*Avena sativa* L.), spring rape (*Brassica napus* L.) and yellow lupine (*Lupinus luteus* L.).

Soil (3 kg per pot) was placed in a polyethylene container, prior to putting into single pots, and polluted with zinc in the form of zinc chloride. NPKMg fertilizers were added. Soil was carefully mixed with the added ingredients and packed into the pots. The same level of fertilization with microelements was used in all the treatments, consisting of N – 100 (yellow lupine was not fertilized with nitrogen), P – 35, K⁺ – 100, Mg²⁺ – 20 mg kg⁻¹ d.m. of soil. Nitrogen was added in the form of CO(NH₂)₂, phosphorus – as KH₂PO₄, potassium – as KH₂PO₄ and KCl, and magnesium – as MgSO₄ · 7H₂O. Once in the pots, soil was added water up to the moisture content equal 60% of capillary water capacity. Finally, the following plants were sown: oat cv. *Kasztan*, spring rape cv. *Huzar* and yellow lupine cv. *Mister*.

After emergence, the plants were thinned, leaving the following per pot: 12 oat, 8 spring rape and 5 yellow lupine plants. The plants were left to grow for 50 days. After harvest, the dry matter yield produced by the plants was determined.

Soil

Two types of soil, both belonging to Eutric Cambisols and collected from the arable humic horizon, were used in the experiment. With respect to their grain-size distribution, the soils were loamy sand and sandy loam. The physicochemical and chemical properties of the soils are specified in Table 1.

Table 1

The physico-chemical and chemical properties of the soil

Property	Loamy sand	Sandy loam
pH _{KCl}	6.7	6.8
Soil texture (μm)	(g kg ⁻¹)	
2000 - 50	755.6	479.2
50 - 2	229.2	487.1
2 < 0	15.2	33.7
	(mmol(+) kg ⁻¹)	
HAC	7.8	5.2
TEB	98.7	131.4
CEC	106.5	136.6
	%	
BS	92.7	96.2
	(g kg ⁻¹)	
C _{organic}	11.0	9.0
N _{total}	0.97	1.14
	(mg kg ⁻¹)	
K _{exchangeable}	180	168
Na _{exchangeable}	28	57
Ca _{exchangeable}	1429	2214
Mg _{exchangeable}	80	50
Zn _{total}	26.8	37.2

Explanations: HAC – hydrolytic acidity, TEB – sum of exchangeable bases Ca⁺⁺, Mg⁺⁺, K⁺, and Na⁺, CEC – cation exchange capacity, BS – base saturation

Determination of the physicochemical and chemical properties of soils

Before the proper experiment, the following properties of the soils were determined:

- 1) grain-size composition of the soil with laser method using a Mastersizer 2000 m;
- 2) pH – by potentiometry in aqueous KCl solution of the concentration of 1 mol KCl dm⁻³ (ISO 10390, 2005);
- 3) hydrolytic acidity (HAC) and total exchangeable base cations (BS) according to KAPPEN, (KLUTE 1996);
- 4) content of organic carbon (C_{org}) – with Tiurin's method (KAWADA 1957);
- 5) content of total nitrogen according to the method by KJELDAHL (ISO 11261:1995),
- 6) content of zinc by flame atomic absorption spectrometry (PN-ISO 11047:2001).
- 7) content of potassium, sodium and calcium exchangeable cations by flame photometry, and the content of magnesium cations by atomic absorption spectrophotometry (BS EN ISO 11260:2011).

Additionally, after harvesting the crops, the analyses specified in points 1 to 5 were repeated in vegetative trials. The results of these determinations were used to calculate correlation coefficients between the activity of soil enzymes and the physicochemical and chemical properties of soil.

Determination of the activity of soil enzymes

Twice during the whole experiment (on day 25 and 50), soil samples were taken from each replicate for determination of the activity of dehydrogenases (EC 1.1), urease (EC 3.5.1.5) and catalase (EC 1.11.1.6). Dehydrogenases were determined according to the method elaborated by ÖHLINGER (1996). TTC (2,3,5-Triphenyl tetrazolium chloride) prepared as 3% aqueous solution served as the substrate for dehydrogenases. Soil was incubated for 24 hours at 37°C. Extinction of produced TFF (triphenyl fomazan) was measured on a Perkin-Elmer Lambda (485 nm) spectrophotometer. The results were converted into µmol of produced TFF kg⁻¹ d.m. of soil h⁻¹.

The activity of catalase was determined according to the protocol described by ALEF and NANNIPIERI (1998). The substrate was composed of 0.3% aqueous solution of H₂O₂. Soil incubation was carried out for 20 minutes at 20°C. Soil filtrate was titrated with 0.02 M aqueous solution of KMnO₄, and the results were converted into mol O₂ kg⁻¹ d.m. of soil h⁻¹.

The activity of urease was measured according to the procedure described by ALEF and NANNIPIERI (1998). The substrate for urease was 10% aqueous solution of urea. Soil was incubated for 3 hours at 37°C. The results were presented in mmol of produced N-NH₄ kg⁻¹ d.m. of soil h⁻¹.

Activities of all the enzymes were presented as means from the dates of determinations.

Calculation methods

The resistance of dehydrogenases, catalase and urease and the tolerance of plants to zinc were derived from the data describing the activity of soil

enzymes and volumes of harvested yields. The following formula, proposed by ORWIN and WARDLE (2004) was applied:

$$RS = 1 - \frac{2 |D_0|}{C_0 + |D_0|},$$

RS – resistance,

C_0 – value of analyzed parameter in control soil,

P_0 – value of analyzed parameter in polluted soil,

$D_0 = C_0 - P_0$.

The RS index ranges from -1 to +1. The RS equal 1 means complete resistance.

Additionally, the extent of the toxic influence of zinc on dehydrogenases, catalase and urease was computed through the determination of a rate of the metal causing a 20% (ED_{20}) decrease in the activity of these enzymes.

Statistical analysis

The results were submitted to statistical processing, which involved the determination of homogenous groups with Tukey's test at the level of significance $p=0.01$. Pearson's simple correlation coefficients were calculated between the degree of soil contamination and the activity of enzymes, as well as the activity of enzymes versus the harvest of plants or the physico-chemical properties of soil. For the assessment of the influence of zinc on the activity of soil enzymes, the principal component analysis (PCA) was employed. All statistical calculations were aided by the software Statistica 10.0 (StatSoft, Inc... 2012).

RESULTS AND DISCUSSION

Activity and resistance of enzymes

The activity of the tested enzymes was determined, to a different degree, by the soil contamination with zinc (Table 2). Zinc was a stronger inhibitor of dehydrogenases and urease than of catalase. Inhibition of the activity of dehydrogenases and urease by excess zinc in soil was also reported by KANITO et al. (2001) or BOROS et al. (2011). In turn, RENELLA et al. (2005) showed that zinc had a less destructive influence on soil enzymes than other metals. Similarly to the findings of the present study, the effect of zinc on different enzymes was not identical. In the authors' own research, zinc had the weakest negative influence on catalase. According to EPELDE et al. (2008), the inhibitory effect of zinc can be counteracted by a proper selection of plants. In the experiment discussed herein, oat was a plant that acted

Table 2

Enzyme activity in soil contaminated with zinc

Dose of Zn ²⁺ (mg kg ⁻¹ soil)	Kind of soil					
	loamy sand			sandy loam		
	kind of plant					
	oat	spring rape	yellow lupine	oat	spring rape	yellow lupine
Dehydrogenases (μmol TFF kg d.m. of soil h ⁻¹)						
0	16.923 ^a	12.954 ^{bcd}	12.945 ^{bcd}	16.528 ^a	11.839 ^d	12.368 ^{cd}
150	13.476 ^{bc}	8.644 ^{ef}	7.501 ^{gh}	13.732 ^b	9.392 ^e	8.163 ^{fg}
450	7.951 ^{fg}	4.403 ^{ij}	3.502 ^{jk}	9.766 ^e	7.602 ^{fgh}	7.659 ^{fg}
600	6.471 ^h	3.331 ^{jk}	3.072 ^k	7.552 ^{fgh}	7.215 ^{gh}	5.260 ⁱ
Average	11.205	7.333	6.755	11.894	9.012	8.363
Catalase (mol O ₂ kg ⁻¹ d.m. of soil h ⁻¹)						
0	0.239 ^{ij}	0.238 ^{ij}	0.223 ^{jk}	0.474 ^a	0.475 ^a	0.494 ^a
150	0.249 ⁱ	0.238 ^{ij}	0.208 ^{kl}	0.421 ^{cd}	0.419 ^{cd}	0.423 ^{bc}
450	0.219 ^k	0.212 ^k	0.179 ^{mn}	0.438 ^b	0.423 ^{bc}	0.406 ^{de}
600	0.207 ^{kl}	0.192 ^{lm}	0.167 ⁿ	0.368 ^h	0.399 ^{ef}	0.387 ^{fg}
Average	0.228	0.220	0.194	0.425	0.429	0.428
Urease (mmol N-NH ₄ kg ⁻¹ d.m. of soil h ⁻¹)						
0	1.351 ⁱ	0.758 ^l	1.251 ^j	3.129 ^a	2.761 ^d	3.063 ^b
150	0.888 ^k	0.686 ^m	0.798 ^l	3.092 ^{ab}	2.975 ^c	2.926 ^c
450	0.612 ⁿ	0.480 ^o	0.445 ^o	2.086 ^g	2.306 ^f	2.520 ^e
600	0.473 ^o	0.361 ^p	0.320 ^p	1.416 ^h	1.470 ^h	1.302 ⁱ
Average	0.831	0.571	0.703	2.431	2.378	2.453

Same letters within a given enzyme, both in columns and in rows, are assigned to homogenous groups.

protectively towards dehydrogenases and urease. However, it was capable of protecting the enzymes only at the lowest degree of soil contamination (150 mg Zn²⁺ kg⁻¹). These relationships can be more easily traced by looking at Figures 1 and 2. The alleviating effect produced by plants with respect to the toxic influence of heavy metals on soil metabolism has been mentioned by other scholars (RENELLA et al. 2006, EPELDE et al. 2008, JIANG et al. 2010, WYSZKOWSKA et al. 2010). Figure 1 indicates that in loamy sand the first two components represent 83.38% of the variance of original variables, with the first component corresponding to 64.44% and the second one – to 64.44% of the said variance. Vectors reflecting the activity of dehydrogenases under all the plants, activity of urease in soil cropped with oat and canola and catalase in soil sown with oat almost reached the edges of the unit circle (a circle of the radius equal 1), which means that they are very well represented by the

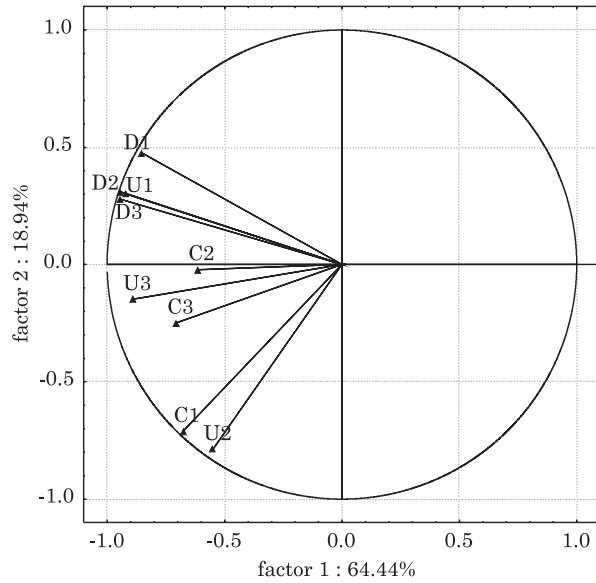


Fig. 1. Enzyme activity in loamy sand contaminated with zinc – PCA method.
Vectors represent the analyzed variables: D – dehydrogenases, C – catalase, U – urease,
1 – oat, 2 – spring rape, 3 – yellow lupine

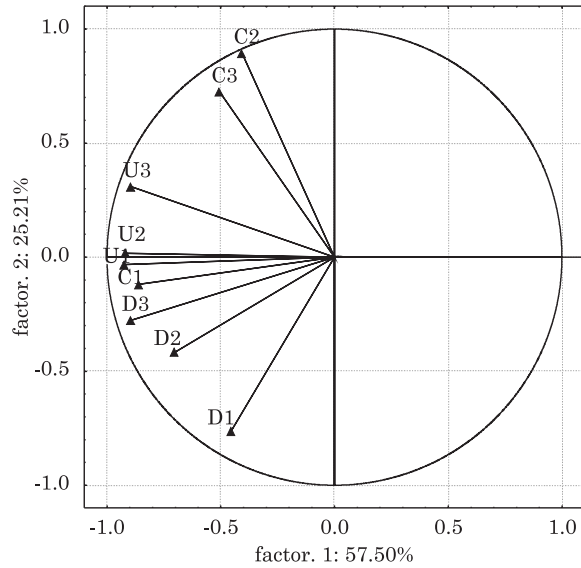


Fig. 2. Enzyme activity in sandy loam contaminated with zinc – PCA method.
Vectors represent the analyzed variables: D – dehydrogenases, C – catalase, U – urease,
1 – oat, 2 – spring rape, 3 – yellow lupine

first two components, which create a set of coordinates. Vectors showing the activity of dehydrogenases, catalase and urease in soil under yellow lupine are located the closest to the first principal component, which proves that the response of these enzymes to soil contamination with zinc was similar and negative, with the original variables being most closely reflected by dehydrogenases and most weakly by catalase.

Also, we were able to detect the negative influence of zinc in soil under spring rape, especially directed against dehydrogenases and urease, with the correlation between dehydrogenases and urease being weaker than in soil under yellow lupine. Vectors representing original variables for dehydrogenases and urease in soil under oat are approximately the same, but the one corresponding to catalase is much more distant. The first principal variable most strongly determined the activity of dehydrogenases in soil under spring rape and most weakly affected the activity of catalase in soil under the same crop. All the values of the vectors representing the activity of the enzyme shaped by the first principal component were negative and ranged from -0.551 for U2 to -0.945 for D1. The second principal component most strongly shaped the U2 and C1 vectors, which assumed negative values.

The contribution of the first and second principal component in reflecting the original variable in sandy loam is slightly different (Figure 2). In this type of soil, both principal components represent 82.71% of variance, with the first one responsible for 57.50% of variance and the second one corresponding to 25.21%. Vectors U1, U2, U3, C1, D2 and D3 gathered along the axis representing the first principal component. They are characterized by high negative adjustment. Vectors C2, C3 and D1 lie along the axis corresponding to the second principal component. Vectors C2 and C3 are characterized by high positive adjustment, while vector D1 is highly negatively adjusted.

Values of the RS index for dehydrogenases (Table 3) show that the resistance of these enzymes to zinc decreased in response to an increasing degree of soil contamination with this element. The mean values suggest that dehydrogenases in sandy loam were more resistant to zinc pollution than the same enzymes in loamy sand. Dehydrogenases can be classified as very sensitive to excess zinc in soil because the RS values even in soils with the lowest dose of zinc ranged from 0.434 to 0.641 in loamy sand and from 0.494 to 0.705 in sandy loam. Dehydrogenases in soil under oat were the most resistant, while those in soil under yellow lupine were the most sensitive to zinc. Dehydrogenases determined in soil under spring rape were characterized by intermediate resistance.

The resistance of catalase (Table 3) to the influence of excessive quantities of zinc in soil was much higher than that of dehydrogenases. In loamy sand with 150 mg Zn²⁺ kg⁻¹, the RS for this enzyme varied from 0.880 to 0.955; in sandy loam, it ranged from 0.789 to 0.804. Following the application of the smallest dose of zinc to soil, no significant effect of the plant species on the RS of catalase was appeared. In loamy sand cropped with oat and

Table 3

Index of soil enzymes resilience (RS) depending on zinc pollution

Dose of Zn ²⁺ (mg kg ⁻¹ soil)	Kind of soil					
	loamy sand			sandy loam		
	kind of plant					
	oat	spring rape	yellow lupine	oat	spring rape	yellow lupine
Dehydrogenases (μmol TFF kg d.m. of soil h ⁻¹)						
150	0.641 ^b	0.497 ^c	0.434 ^d	0.705 ^a	0.693 ^a	0.494 ^c
450	0.330 ^e	0.211 ^h	0.177 ⁱ	0.437 ^d	0.505 ^c	0.452 ^d
600	0.254 ^g	0.151 ^j	0.156 ^{ij}	0.292 ^f	0.496 ^c	0.270 ^{fg}
Average	0.408	0.286	0.256	0.478	0.565	0.405
Catalase (mol O ₂ kg ⁻¹ d.m. of soil h ⁻¹)						
150	0.885 ^b	0.955 ^a	0.880 ^b	0.804 ^c	0.789 ^c	0.791 ^c
450	0.802 ^c	0.800 ^c	0.667 ^{fg}	0.776 ^c	0.806 ^c	0.681 ^{ef}
600	0.787 ^c	0.672 ^{ef}	0.602 ⁱ	0.637 ^{gh}	0.722 ^d	0.701 ^{de}
Average	0.824	0.809	0.717	0.739	0.772	0.724
Urease (mmol N-NH ₄ kg ⁻¹ d.m. of soil h ⁻¹)						
150	0.543 ^g	0.772 ^c	0.651 ^e	0.568 ^f	0.854 ^b	0.935 ^a
450	0.406 ⁱ	0.468 ^h	0.455 ^h	0.540 ^g	0.720 ^d	0.663 ^e
600	0.313 ^k	0.316 ^k	0.304 ^k	0.305 ^k	0.361 ^j	0.261 ^l
Average	0.421	0.518	0.470	0.471	0.645	0.619

Same letters within a given enzyme, both in columns and in rows, are assigned to homogenous groups.

yellow lupine, the values of this index were approximately the same, but in soil under spring rape, the RS of catalase reached as much as 0.955. Catalase was relatively highly resistant in soils polluted with 600 mg Zn²⁺ kg⁻¹, which was demonstrated by rather high values of the RS, within 0.602 to 0.787. The RS of catalase, in contrast to dehydrogenases, was not usually affected by the type of soil.

Urease (Table 3) was more sensitive to excessive amounts of zinc in soil than catalase but more resistant than dehydrogenases. This conclusion is supported by the intermediate values of the RS of urease, between the values obtained by dehydrogenases and catalase. In the loamy sand polluted by the smallest zinc rate (150 mg kg⁻¹), the RS value ranged from 0.543 to 0.722; in the analogous treatment on sandy loam, the range was from 0.568 to 0.935. In both types of soil, urease in soil under oat was the least resistant to the above contamination rate; it demonstrated the highest tolerance in loamy sand cropped with spring rape, while the highest RS value in sandy loam was determined under yellow lupine.

The highest dose of zinc (600 mg kg^{-1}) significantly decreased the RS values. Whereas in loamy sand, the RS index did not depend on the species of cultivated crop, it reached the highest value (0.361) in sandy loam cropped with spring rape, being the lowest in soil under yellow lupine. Within the zinc doses of $150 \text{ mg} - 450 \text{ mg Zn}^{2+} \text{ kg}^{-1}$ urease was more tolerant in sandy loam than in loamy sand, but this relationship disappeared when soil had been polluted with $600 \text{ mg Zn}^{2+} \text{ kg}^{-1}$. While analyzing the above results, we should remember that the activity of soil enzymes in soils unpolluted by heavy metals is higher when leguminous plants rather than cereals are grown (VELMOURUGANE et al. 2013).

With respect to the resistance to zinc contamination, the analyzed enzymes can be ordered as follows: catalase > urease > dehydrogenases. These results coincide with the ED_{20} for zinc (Table 4). The ED_{20} index had the highest values for catalase in loamy sand and sandy loam, while being the lowest for dehydrogenases, except in soil under oat. Regarding dehydrogenases and urease, higher values of the zinc ED_{20} index were recorded in sandy loam than in loamy sand. No such relationship was detected in the case of

Table 4

The dose of zinc ($\text{mg Zn}^{2+} \text{ kg}^{-1} \text{ d.m. of soil}$) decreases by 20% the activity of soil enzymes (ED_{20})*

Enzyme	Kind of soil					
	loamy sand			sandy loam		
	kind of plant					
	oat	spring rape	yellow lupine	oat	spring rape	yellow lupine
Dehydrogenases	169 ^c	116 ^d	96 ^e	209 ^b	243 ^a	171 ^c
Catalase	788 ^a	655 ^b	465 ^e	529 ^c	648 ^b	493 ^d
Urease	131 ^e	247 ^d	111 ^f	277 ^c	362 ^a	301 ^b

* Homogenous groups in rows labelled with identical letters.

catalase. However QU et al. (2013) found more inhibition of soil urease activity by zinc than dehydrogenase.

It is interesting to notice that enzymes of the same class, i.e. dehydrogenases and catalase, respond to zinc contamination. Catalase is more resistant than dehydrogenases, which may be a result of the specific nature of dehydrogenases. The disproportions between these enzymes could also be caused by the fact that dehydrogenases appear in soil as an integral part of intact cells and are an indicator of the rate of respiratory metabolism carried out by soil microorganisms (PRAVEEN-KUMAR, TARAFDAR 2003), whereas catalase, having been partially released from cells, shows some stability in soil owing to its sorption on the surface of loamy minerals or association with soil's organic colloid (CALAMAI et al. 2000).

Despite the higher activity of all the enzymes in sandy soil, that is soil with a higher sorption capacity, than in loamy sand, characterized by lower sorption, which is in accord with numerous studies (CALAMAI et al. 2000, KAR-

LEN et al. 2003, WYSZKOWSKA et al. 2010), dehydrogenases and urease were determined to be more resistant to zinc in sandy loam than in loamy sand, in contrast to catalase, which was more resistant in loamy sand. The fact that urease was more tolerant to the presence of zinc in sandy loam than in loamy sand could be attributed to the protective role played by organic and mineral colloids towards this enzyme (ABRAYMANA 1993) or to stronger sorption of zinc (ADAMOA et al. 2003, QU et al. 2013). And although the catalytic efficiency of enzymes associated with colloids is typically lower than enzymes alone, in the free state or inside cells, they are more resistant to periodic changes of the conditions in a given ecosystem, and they mostly decide about the directions of biochemical transformations in soil, thus shaping soil fertility (CALAMAI et al. 2000, PRAVEEN-KUMAR, TARAFDAR 2003). On the other hand, a higher resistance of dehydrogenases in sandy loam than in loamy sand can be a result of both stronger sorption of zinc in more compact soil and a higher number and diversity of microorganisms.

Resistance of plants

Large differences in the sensitivity response to excessive doses of zinc in soil were observed not only among enzymes but also among plant species (Table 3). Of the three crops grown on loamy sand, oat was most resistant while lupine was most sensitive to zinc. The RS of oat ranged from 0.825 to 0.891 and was not correlated with the degree of soil contamination with zinc; on the other hand, the RS of yellow lupine decreased from 0.575 (150 mg Zn²⁺ kg⁻¹) to 0.035 (600 mg Zn²⁺ kg⁻¹) and that of spring rape declined from 0.939 to 0.315, respectively. The least resistant to the influence of zinc was also yellow lupine grown on sandy loam, while the resistance of oat and spring rape grown in pots with 150 mg and 450 mg Zn²⁺ kg⁻¹ was similar. However, spring rape was more resistant to zinc than oat in treatments consisting of loam polluted with 600 mg Zn²⁺ kg⁻¹. Varied resistance of particular plant species to excessive quantities of zinc and other heavy metals in soil, mentioned in relevant references (KARLEN et al. 2003, HUA et al. 2008, ZALEWSKA 2012, NADGÓRSKA-SOCHA et al. 2013, TRAN, POPOVA 2013, WYSZKOWSKI, RADZIEMSKA 2013). Is attributed to the anatomical structure and physiological characteristics of plants, a thesis supported by the high resistance to zinc contamination demonstrated by oat, a plant which tolerates well stress conditions in the environment.

When soil was polluted with zinc, the dependence between yields of plants and activity of soil enzymes was not unambiguous (Table 5). The correlation between the activity of dehydrogenases and urease versus the yield of oat on loamy sand was negative, being positive on sandy loam. Positive correlation also occurred between the activity of all the tested enzymes in loamy sand and the yields of spring rape and yellow lupine. The same relationship was maintained between the enzymes in sandy loam and yields of yellow lupine. In contrast, the activity of dehydrogenases and catalase in sandy loam was negatively correlated with yields of spring rape.

Table 5

Index of plant resistance (RS) depending on zinc pollution

Dose of Zn ²⁺ (mg kg ⁻¹ soil)	Kind of soil					
	loamy sand			sandy loam		
	kind of plant					
	oat	spring rape	yellow lupine	oat	spring rape	yellow lupine
150	0.856 ^{bc}	0.939 ^a	0.575 ^f	0.719 ^{de}	0.685 ^e	0.190 ^h
450	0.825 ^c	0.341 ^g	0.041 ⁱ	0.702 ^{de}	0.734 ^{de}	0.066 ⁱ
600	0.891 ^{ab}	0.315 ^g	0.035 ⁱ	0.566 ^f	0.738 ^d	0.061 ⁱ
Average	0.858	0.531	0.217	0.662	0.719	0.105

Interactions of enzymes and plants with physicochemical properties of soil

The activity of enzymes was not univocally correlated with certain physicochemical properties of soil (Table 6). In loamy sand under all the tested crops, the activity of dehydrogenases, catalase and urease was positively correlated with pH and negatively with hydrolytic acidity, but in sandy loam, a significant positive correlation was found only between soil pH and urease in soil cropped with yellow lupine. Literature (WANG et al. 2006, ZABOROWSKA et al. 2006) suggests that in general there is positive correlation between enzymes and pH of soil, as observed in loamy sand.

The activity of catalase in sandy loam under oats and urease in soil under yellow lupine was positively correlated with hydrolytic acidity. An opposite correlation was determined between the activity of dehydrogenases, catalase and urease in loamy sand and in sandy loam, versus the content of organic carbon and total nitrogen or the base cations saturation. The content of C_{org} and N_{total} was negatively correlated with enzymes in sandy loam sown with spring rape and yellow lupine, while being positively correlated with enzymes in loamy sand under any of the tested crops. Furthermore, the degree of soil's saturation with base cations was positively correlated with the activity of enzymes in loamy sand, but in sandy loam, the correlation was univocally negative, not only in treatments with oat, as suggested by the negative values of TEB and CEC.

The physicochemical properties significantly shape the activity of soil enzymes (HINOJOSA et al. 2004, KUCHARSKI et al. 2011, WYSZKOWSKA et al. 2008), but differences in the correlations between the activity of dehydrogenases, catalase and urease versus some soil properties of loamy sand and sandy loam could be a result of the degree of buffering of these soils, a claim supported by the fact that the resistance of dehydrogenases and urease to zinc contamination was higher in sandy loam, that is in soil with higher sorption capacity, than in loam sand, characterized by lower sorption. Of cer-

Table 6

Pearson's correlation coefficients between enzymatic activity and plants yield and physicochemical properties of soil

Variable	Kind of plant								
	oat			spring rape			yellow lupine		
	Deh	Cat	Ure	Deh	Cat	Ure	Deh	Cat	Ure
Loamy sand									
Plants yield	-0.675*	-0.245	-0.771**	0.931**	0.942**	0.967**	0.963**	0.984**	0.968**
C _{organic}	-0.885**	-0.571*	-0.920**	-0.150	-0.556*	-0.408	-0.967**	-0.855**	-0.951**
N _{total}	-0.854**	-0.603*	-0.850**	-0.199	-0.536	-0.417	-0.967**	-0.913**	-0.973**
pH	0.984**	0.771**	0.999**	0.913**	0.962**	0.969**	0.999**	0.936**	0.989**
HAC	-0.972**	-0.961**	-0.900**	-0.990**	-0.904**	-0.965**	-0.987**	-0.898**	-0.975**
TEB	0.311	0.711**	0.098	-0.134	0.233	0.085	-0.426	-0.143	-0.342
CEC	-0.011	0.449	-0.228	-0.285	0.091	-0.064	-0.662**	-0.411	-0.592*
BS	0.920**	0.988**	0.815**	0.795**	0.902**	0.881**	0.986**	0.957**	0.994**
Sandy loam									
Plants yield	0.803**	0.499	0.928**	-0.787**	-0.899**	-0.217	0.944**	0.986**	0.634*
C _{organic}	0.801**	0.599*	0.608*	0.878**	0.642*	0.849**	0.966**	0.913**	0.937**
N _{total}	0.899**	0.931**	0.768**	0.750**	0.810**	0.897**	0.959**	0.898**	0.932**
pH	0.306	-0.071	0.530	-0.120	0.204	-0.597*	0.401	0.318	0.828**
HAC	0.336	0.697**	0.451	-0.171	-0.041	0.475	0.069	-0.094	0.564**
TEB	-0.986**	-0.727**	-0.934**	-0.112	-0.495	0.049	-0.531	-0.618*	0.036
CEC	-0.985**	-0.724**	-0.936**	-0.105	-0.486	-0.008	-0.490	-0.592*	0.085
BS	-0.967**	-0.766**	-0.860**	-0.136	-0.303	0.562*	0.257	0.410	-0.276

Explanations: Deh – dehydrogenases, Cat – catalase, Ure – urease, HAC – hydrolytic acidity, TEB – sum of exchangeable bases Ca⁺⁺, Mg⁺⁺, K⁺, and Na⁺, CEC – cation exchange capacity, BS – base saturation;

* significant for $P=0.05$, ** significant for $P=0.01$, $n=12$

tain importance are the interferences caused by zinc with the growth of the examined plant species, which are manifested by amounts of root excretions produced by plants, inducing a certain level of biochemical activity of soil (DIJKSTRA et al. 2006, PEREZ-DE-MORA et al. 2006, RENELLA et al. 2005, TEJADA et al. 2008).

CONCLUSIONS

1. Soil contamination with zinc within the range of doses 150 mg to 600 mg kg⁻¹ of soil significantly inhibits the activity of dehydrogenases, urease and catalase. With respect to their sensitivity to zinc, the analyzed enzymes can be ranked as follows: dehydrogenases > urease > catalase.

2. Dehydrogenases were most resistant to the negative influence of zinc in soil under oat; urease – in soil cropped with spring rape, and catalase – in soil sown with yellow lupine.

3. Dehydrogenases and urease were more resistant to the effect of zinc in sandy loam than in loamy sand, in contrast to catalase, which was more resistant in loamy sand than in sandy loam.

4. Tolerance of plants to zinc contamination of soil is a species-specific trait. Among the analyzed plants, yellow lupine is the most sensitive to the presence of zinc in soil, while oat seems to be most tolerant to this contaminant.

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NITROGEN FRACTIONS IN SPENT MUSHROOM SUBSTRATE

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Abstract

In the last decade, Poland has become the leader in mushroom production in Europe. Mushroom cultivation generates large amounts of spent substrate, which is a waste material and must be recycled. The substantial content of macrolelements, including nitrogen, prompts using this organic material as a soil fertilizer.

The objective of the paper was to determine the proportion of nitrogen in different forms extracted from spent mushroom substrates. The studies were aimed at determining the potential for the rational use of spent mushroom substrate in soil fertilization. The sequential extraction of nitrogen compounds was performed with a 0.25 M KCl solution (for the extraction of mineral nitrogen forms and the most labile organic nitrogen compounds) and with 0.25 M and 0.25 M H₂SO₄ (hot hydrolysis for sequencing of organic nitrogen linkage that are easily hydrolysing and difficult to hydrolyse). The prepared solutions were tested for the total content of nitrogen with the method by Kjeldahl and for carbon with the oxidative-potentiometric method. The samples were also subjected to extraction of nitrogen bound to humic substances. It was found that spent mushroom substrate had a substantial potential for enriching fertilized soil with organic nitrogen compounds, which are, to a large extent, potentially liable to degradation. In summary, approximately 2/3 of the total nitrogen pool in the organic complexes and about 1/3 of the carbon pool were extracted using sequential extraction with a neutral salt solution and acid hydrolysis at different concentrations of hydrogen ions. The calculated C/N values suggest that the extracted organic compounds are vulnerable to degradation and indicate the resistance of compounds present in post-extraction residues. While fractioning organic matter, it was found that a relatively large amount of nitrogen was sequenced at the stage of decalcification of the samples, and the proportion of humic substances equalled about 1/5 of the total content of this element.

Key words: spent mushroom substrate, nitrogen fractions, acid hydrolysis.

FRAKCJE AZOTU W PODŁOŻU POPIECZARKOWYM

Abstrakt

W ostatniej dekadzie Polska stała się liderem produkcji pieczarek w Europie. Podczas uprawy powstaje duża ilość zużytego podłoża, które jako odpad musi podlegać utylizacji. Znaczna zawartość makroelementów, w tym azotu, zachęca do wykorzystania tego organicznego materiału odpadowego do nawożenia gleb.

Celem pracy była ocena udziału różnych form azotu wydzielonych z podłoża po uprawie pieczarki. Badania prowadzono uwzględniając perspektywę racjonalnego wykorzystania zużytego podłoża do nawożenia gleb. Do sekwencyjnego wydzielenia związków azotu zastosowano roztwory 0,25 M KCl (do wyekstrahowania mineralnych oraz najbardziej labilnych organicznych form azotu) oraz 0,25 M i 2,5 M H_2SO_4 (hydroliza na gorąco – do wydzielenia organicznych połączeń azotu, łatwo i trudno ulegających hydrolizie). W uzyskanych roztworach oznaczono ogólną zawartość azotu metodą Kjeldahla, a węgla – metodą oksydacyjno-miareczkową. W pobranych próbkach wykonano także ekstrakcję azotu związanego z substancjami humusowymi. Stwierdzono duży potencjał podłoża pieczarkowego do wzbogacania nawożonych gleb w związki organiczne azotu, w znacznym stopniu potencjalnie podatnych na procesy rozkładu. W sumie, za pomocą ekstrakcji sekwencyjnej z wykorzystaniem roztworu soli obojętnej oraz w wyniku hydrolizy kwaśnej o różnym stężeniu jonów wodorowych wydzielono ok. 2/3 całkowitych zasobów azotu w połączeniach organicznych oraz ok. 1/3 zasobów węgla. Obliczone wartości C/N sugerują podatność na procesy rozkładu wydzielonych związków organicznych oraz odporność związków w poekstrakcyjnej pozostałości. Podczas frakcjonowania materii organicznej stwierdzono relatywnie dużą ilość azotu wydzielonego na etapie dekalcytacji próbek, a udział azotu substancji humusowych wyniósł ok. 1/5 całkowitej zawartości tego pierwiastka.

Słowa kluczowe: podłoże pieczarkowe, frakcje azotu, hydroliza kwaśna.

INTRODUCTION

In the last decade, following the introduction of modern cultivation systems and the concentration of large-scale industrial mushroom producing operations located mainly in the eastern part of the province of Mazowieckie, Poland has become the leader in mushroom production in Europe. According to the Polish regulations, spent mushroom substrate, as a by-product of mushroom cultivation, is regarded to be waste material (*Regulation ... 2001*). This has prompted a search for methods for its rational and judicious utilization. This organic waste material, which in modern cultivation techniques is disinfected (at 70°C) following mushroom collection, contains:

- substrate for mushroom cultivation (the most important quantitative component) made of straw and chicken manure (often with the addition of peat, urea, soy protein, dolomite or gypsum);
- surface cover made of valley peat with an alkalizing compound (which contains $CaCO_3$);
- remnant mycelium and unharvested mushroom fruiting bodies.

Numerous studies on the elementary composition of spent mushroom substrate have demonstrated a substantial amount of organic matter and

nutrients, including nitrogen (STEWART et al. 1998, ZMORA-NAHUM et al. 2007, JORDAN et al. 2008, KALEMBASA, MAJCHROWSKA-SAFARYAN 2009, MEDINA et al. 2009, RUTKOWSKA et al. 2009, KALEMBASA, BECHER 2011, 2012). The application of spent mushroom substrate as a soil fertilizer seems to be the most appropriate method for its utilization, as it improves the nitrogen balance in soil. It is particularly recommended for fertilization of soil with a low content of humus, where nitrogen is present at lower concentrations than required by plants, which reduces the profitability of plant production. The cultivated soil in the Siedlce Upland (Poland), where mushroom operations are concentrated, often has low humus and nitrogen reserves and good aerobic conditions, which makes them suitable for the recycling of spent mushroom substrate (BECHER, KALEMBASA 2011, PAKUŁA, KALEMBASA 2012, BECHER 2013).

The speciation analysis of nitrogen compounds found in the organic matter of environmental samples consists in their hydrolysis with mineral acid or base solutions and in the quantitative analysis of hydrolysates (SCHULTEN, SCHNITZER 1998). Until recently, the studies conducted in the Department of Soil Sciences and Agricultural Chemistry, University of Natural Sciences and Humanities in Siedlce (Poland), have demonstrated that over 97% of nitrogen reserves in spent mushroom substrate are enclosed in organic compounds with different resistance to degradation, of which 47% belong to the non-hydrolysing organic nitrogen pool (KALEMBASA, BECHER 2011).

The objective of the paper was to evaluate the proportion of nitrogen in the fractions sequenced from spent mushroom substrate. The study was encouraged by the need to rationalise applications of spent substrate in soil fertilization.

MATERIAL AND METHODS

The study was conducted in three modern mushroom producing operations located near Siedlce, Poland. The samples of spent substrate used for common mushroom, *Agaricus bisporus*, cultivation were collected from 8 cultivation rooms (with a shelf surface of 620 m²) following steam disinfection and immediately before the removal of substrate with mushrooms. In the selected rooms, the substrates for mushroom cultivation originated from different suppliers. The samples were collected from 23.10.2011 to 15.01.2012 with each being taken from five points. The averaged samples (DM, $n=8$) were dried at 40°C, comminuted in an agate mill to particles < 25 mm in diameter and chemically analysed (in three repetitions). The results were referred to the absolute dry matter of the tested material that was determined by weight after drying the substrate at 105°C. The total content of carbon (TC) and nitrogen (TN) were determined on a Series II 2400 auto-analyser (Perkin Elmer) equipped with a thermal conductivity detector and

acetanilide as the standard material. The sequential extraction of different nitrogen-containing compounds was performed by modifying the method described by KALEMBASA (1995). The following solutions were used (m/v = 1/25): 0.25 M KCl (extraction at room temperature for 3 hours); 0.25 M H₂SO₄ and 2.5 M H₂SO₄ (hydrolysis at boiling temperature of the mixture, under an aqueous reflux condenser for 3 hours); and concentrated H₂SO₄ (mineralization of organic compounds in residuum). Following the extraction and hydrolysis reactions, the solution was clarified by centrifugation (4000 rpm) and filtered through a 0.45 µm filter. After each stage of the extraction procedure, the tested material was washed three times with deionised water. The applied method of sequential fractioning allowed us to define soil nitrogen in mineral and organic forms (operational fractions) with different potential degradability in soil after fertilization (Table 1).

Table 1

Nitrogen forms and the methods of their recovery

Nitrogen form (operational)	Method of recovery
N-NH ₄ – ammonia form	distillation from 0.25 M KCl extract after alkalization with MgO
N-NO _x – nitrate forms (III and V)	distillation from 0.25 M KCl extract after distillation with N-NH ₄ and application of Devard's reductive mixture
N _{MIN} – nitrogen in mineral compounds	$N_{MIN} = N-NH_4 + N-NO_x$
N _{ORG} – nitrogen in organic compounds	$N_{ORG} = TN - N_{MIN}$
N _{KCL}	measured in 0.25 M KCl extract after mineralization of the solution
N _{OR} – soluble organic nitrogen	$N_{OR} = N_{KCL} - N_{MIN}$
N _{OLH} – easily hydrolysing organic nitrogen	measured after hydrolysis in 0.25 M H ₂ SO ₄ and mineralization of the solution
N _{OLH} -NH ₄ – ammonia form in easily hydrolysing fraction	nitrogen distilled off from the hydrolysate after alkalization with 10 M NaOH
N _{OTH} – organic nitrogen difficult to hydrolyse	measured after hydrolysis in 2.5 M H ₂ SO ₄ and mineralization of the solution
N _{ONH} – non-hydrolysing organic nitrogen	measured after post-extraction mineralization of the residues in concentrated H ₂ SO ₄

The total content of nitrogen was determined with the method by Kjeldahl. The mineralization of nitrogen incorporated in organic compounds present in the extraction solution was carried out in Kjeldahl's flasks, from which ammonium was distilled off immediately after mineralization.

The omission of dilution of the mineralized samples and the use of a micropipette for titration allowed for the determination of nitrogen in the samples with low concentrations of this element. The analytical fractioning procedure and determination of nitrogen in organic compounds were verified with acetanilide (the standard material, Perkin Elmer 0240-1121) (Table 2)

Table 2

Verification of the procedure of quantitative nitrogen analysis

Expected value (%)	Recorded value, mean for $n=5$ (%)	Recovery (%)
10.36	10.24	98.8

Table 3

Comparison of the summary nitrogen content in the sequenced fractions with
the total content of this element

Number of samples	8
Number of sequential extractions	24
Recovery (%) = $N_{KCL} + N_{OLH} + N_{OTH} + N_{ONH} / TN \cdot 100$	
Range	93.4 – 96.6
Mean	94.5
SD	1.06

SD – standard deviation

and by comparing the total amount of nitrogen in the selected fractions with the total content of this element in the sample (Table 3).

The solutions generated by fractioning were also tested for the content of carbon with the oxidative-potentiometric method (KALEMBASA 1991). The analogical fractions of organic carbon were identified: soluble (C_{OR}), easily hydrolysing (C_{OLH}), difficult to hydrolyse (C_{OTH}) and non-hydrolysing (C_{ONH}). The calculations of C_{OR} content included the amount of Cl^- ions (derived from KCl) that during mineralization of organic compounds cause the reduction of Cr^{6+} in $K_2Cr_2O_7$. The amount of C_{ONH} was calculated with the following formula: $C_{ONH} = TC - (C_{OR} + C_{OLH} + C_{OTH})$.

In addition, humic substances were extracted from the samples according to the following procedure: decalcification ($0.05\text{ M H}_2\text{SO}_4$) → extraction of humic substances (0.1 M NaOH) → separation into humic acids and fulvic acids by means of acidification – $pH = 1.5$ (DZIADOWIEC, GONET 1999). The extracted solutions were tested for the content of nitrogen with the method by Kjeldahl and the following fractions were obtained: N_{DEKA} – nitrogen in the fraction after decalcification; N_{SH} – nitrogen in the fraction of humic substances (sequenced with 0.1 M NaOH); N_{KH} – nitrogen in the fraction of humic acids; N_{KF} – nitrogen in the fraction of fulvic acids; N_{SNH} – nitrogen in non-humified substances: $N_{SNH} = TN - (N_{DEKA} + N_{SH})$.

RESULTS AND DISCUSSION

The contents of nitrogen and carbon (Table 4) in the spent mushroom substrate indicate considerable potential for enrichment of soil with these

Table 4

Selected parameters of the examined spent mushroom substrates

Sample	Dry matter (%)	TN	TC	TC/TN
		(g kg ⁻¹ DM)		
SMS-1	22.8	23.3	303	13.0
SMS-2	41.6	25.9	312	12.0
SMS-3	27.5	23.6	299	12.7
SMS-4	42.8	23.7	301	12.7
SMS-5	38.9	20.0	237	11.8
SMS-6	37.0	19.6	233	11.9
SMS-7	36.7	20.6	244	11.8
SMS-8	28.0	23.8	304	12.8
Mean				
Standard deviation	34.4	22.6	279	12.4
Relative standard deviation (%)	7.35	2.23	34.4	0.47
	21.4	9.88	12.3	3.93

elements. According to MAZUR and MOKRA (2009), the content of nitrogen in fresh biomass of the spent mushroom substrate approximates the content of this element in natural fertilizers. The recorded TC/TN values suggest a high degree of organic matter transformation in the examined samples. The low value of coefficient of variation indicates that this quotient is a very stable characteristic. The TC/TN values are comparable with the levels in the humus horizons of arable mineral soil with good conditions for mineralization of organic matter (KALEMBASA, BECHER 2009b, BECHER, KALEMBASA 2011). It is supposed that the application of spent mushroom substrate will not significantly influence a change in the ratio between these compounds in soil and will probably prompt intensification of the mineralization of organic nitrogen compounds. The results reported by other authors (ZMORA-NAHUM et al. 2007, JORDAN et al. 2008, KALEMBASA, MAJCHROWSKA-SAFARYAN 2009, RUTKOWSKA et al. 2009, KALEMBASA, BECHER 2011) have indicated some differences in the content of C and N and C/N ratio in spent mushroom substrates depending on the method of preparation, especially on the components and cultivation technology.

A characteristic feature of the tested spent mushroom substrates was considerable variation in the content and proportion of mineral nitrogen forms (Table 5) and a substantial advantage (app. 10-time) of reduced am-

Table 5

Content and proportion of mineral and organic nitrogen forms in the spent mushroom substrates

Parameter	N _{MIN}		N _{ORG}		N-NH ₄	N-NO _x
	(g kg ⁻¹ DM)	(%TN)	(g kg ⁻¹ DM)	(%TN)	(g kg ⁻¹ DM)	
Mean	1.02	4.65	21.5	95.3	0.94	0.09
Range	0.510-1.44	2.19-7.04	18.2-25.1	93.0-97.8	0.49-1.26	0.02-0.18
SD	0.323	1.73	2.42	1.73	0.28	0.05
Relative SD (%)	31.5	37.3	11.2	1.82	29.7	60.0

monia form (as an effect of ammonification) over oxidized nitrate forms. In other studies, it has been found that the proportion of mineral nitrogen forms was lower and amounted to, on average, 2.62% TN (KALEMBASA, BECHER 2011).

The organic compounds constituted over 95% of the total nitrogen content. The sequential extraction allowed for recovery of organic nitrogen compounds in different forms (Table 6, Figure 1). The use of extraction reagents with incremental “extraction potency” ($0.25 \text{ M KCl} < 0.25 \text{ M H}_2\text{SO}_4 < 2.5 \text{ M H}_2\text{SO}_4 < \text{concentrated H}_2\text{SO}_4$) permitted the separation of nitrogen fractions which represented the organic compounds with varied susceptibility to microbiological degradation. The probable susceptibility of extracted organic nitrogen compounds to degradation in soil (following the application with fertilization) is arranged in the following order: $N_{\text{OR}} > N_{\text{OLH}} > N_{\text{OTH}} > N_{\text{ONH}}$.

Thanks to the applied extraction procedure, app. 2/3 of the total nitrogen reserves in organic compounds was extracted. Assuming the proportion of nitrogen, the selected forms can be arranged in the following order of increasing quantitative importance: $N_{\text{OR}} < N_{\text{OTH}} < N_{\text{OLH}} < N_{\text{ONH}}$. In the case of carbon, the efficiency of extraction with the reagent was substantially lower as only 31.1 - 37.4% of the total carbon pool was extracted (Table 7, Figure 2).

Nitrogen and carbon which can be extracted with a neutral reagent at a low concentration (0.25 M KCl) are the components of organic compounds

Table 6
Content of organic nitrogen forms ($\text{g kg}^{-1} \text{ DM}$) in the spent mushroom substrates

Parameter	N_{OR}	N_{OLH}	$N_{\text{OLH}}\text{-NH}_4$	N_{OTH}	N_{ONH}
Mean	3.12	5.49	1.27	4.77	6.93
Range	2.50-3.99	4.59-6.18	1.05-1.75	3.86-5.69	5.43-8.50
SD	0.507	0.497	0.209	0.699	1.22
Relative SD (%)	16.2	9.06	16.4	14.6	17.5

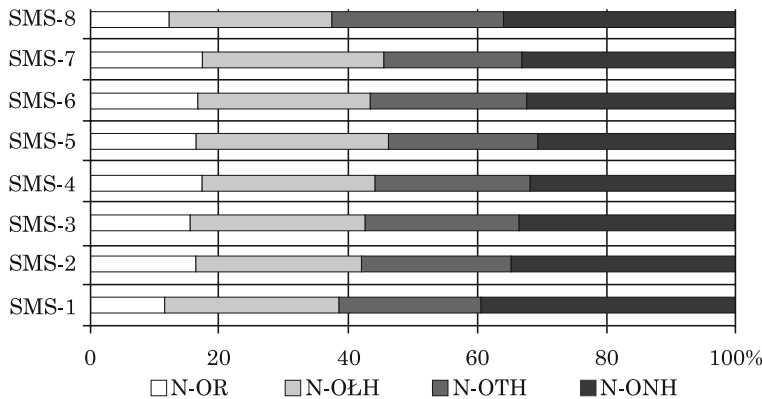


Fig. 1. Proportion of the selected nitrogen fractions in spent mushroom substrates ($\%N_{\text{ORG}}$)

Table 7
Content of organic carbon forms (g kg⁻¹ DM) in the spent mushroom substrates

Parameter	C _{OR}	C _{OLH}	C _{OTH}	C _{ONH}
Mean	23.3	44.4	29.2	182
Range	19.2-28.8	38.0-51.3	24.0-35.5	148-209
SD	3.10	4.50	4.00	26.8
Relative SD (%)	13.2	10.2	13.8	14.7

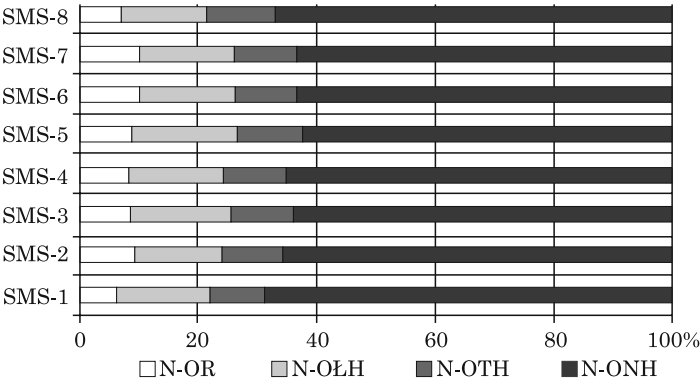


Fig. 2. Proportion of the selected carbon fractions in spent mushroom substrates (%TC)

that probably represent the so-called dissolved organic matter (DON – dissolved organic nitrogen, DOC – dissolved organic carbon). This is the most mobile fraction and may comprise carbohydrates, amino acids, hydrocarbons and their derivatives, low-molecular humic acid fractions and other simple organic compounds (MCDOWELL 2003, DEBSKA 2004, PAUL, WILLIAMS 2005).

Of the form extracted with acid hydrolysis, on average 47.7% of the total organic nitrogen was separated with a minor advantage of readily hydrolysing form (N_{OLH}) – Figure 1. Moreover, on average, 1.27 g kg⁻¹ N as ammonia form (N_{OLH}-NH₄) was detected, which constituted on average 5.90% of organic nitrogen in the tested material (Table 6).

During acid hydrolysis, nitrogen bound to the humic matter and nitrogen incorporated in microbial proteins is released into a solution and is partially hydrolysed to ammonium, amino acids, amino sugars and other organic compounds (KALEMBASA 1995).

While investigating different types of soil, SOWDEN et al. (1977) found that hydrolysing nitrogen constituted 84-89% of the total content of this element (following hydrolysis in 6 M HCl) with nitrogen in amino acids amounting to 33-42%, in amino sugars – 4.5-7.4%, and in ammonia form – 18-32%. The studies on organic soil (KALEMBASA, BECHER 2009a) have demonstrated (with the same method) that the proportion of hydrolysing nitrogen ranges from 43-64%.

Non-hydrolysing organic nitrogen constituted the largest pool (30.7-39.4%) of organic nitrogen reserves in the examined spent mushroom substrates (Figure 1). SCHULTEN and SCHNITZER (1998) reported that within the fractions of non-hydrolysing nitrogen, derivatives of aromatic and heterocyclic compounds were predominant. KNICKER et al. (1995) suggested that the organic matter in soil contained peptide structures resistant to microbiological degradation.

The application of the tested substrate for fertilization at the dose permitted by the Nitrates Directive – 170 kg N ha⁻¹ (Code ... 2004), i.e. app. 7.5 t DM ha⁻¹, will enrich soil in app. 31 kg N ha⁻¹ in mineral and soluble organic forms (that potentially mineralize very rapidly) and app. 77 kg N in hydrolysing forms, including 41 kg in easily hydrolysing form.

The determination of carbon content in the individual fractions allowed for calculation of carbon-to-nitrogen ratios which is the parameter commonly referred to in discussions on the vulnerability of organic compounds to mineralization. In general, the value of this quotient in the recovered fractions was characterized by minor variations in the recovered extracts (Table 8).

Table 8
Quotients of carbon and nitrogen contents in the selected fractions

Parameter	C _{OR} /N _{KCl}	C _{OLH} /N _{OLH}	C _{OTH} /N _{OTH}	C _{ONH} /N _{ONH}
Mean	5.66	8.09	6.14	26.4
Range	5.06-6.38	7.35-8.93	5.75-6.52	24.5-29.1
SD	0.469	0.477	0.263	1.50
Relative SD (%)	8.29	5.90	4.28	5.67

These values also confirm the qualitative changes of organic compounds found in the recovered extracts. The narrowest C/N ratios (<10) were detected in the solution after extraction of the substrate with potassium chloride and in both hydrolysates (0.25 and 2.5 M H₂SO₄). The lowest value of this ratio (5.66 on average) was measured in the extract with 0.25 M KCl, i.e. in the pool of soluble organic compounds (C_{OR}/N_{KCl}). It confirms the highest potential susceptibility of organic compounds which represent this fraction to degradation in soil. The narrower ratio was detected in the fraction that was difficult to hydrolyse (6.14 on average) in comparison with the easily hydrolysing fraction (8.09 on average). The value of this ratio demonstrates that organic compounds extracted with acid hydrolysis are probably susceptible to mineralization. The widest C/N value was determined in the non-hydrolysing fraction, which probably confirms the highest resistance of organic compounds that are left in the examined material after extraction procedures (residuum) to microbiological degradation. Following the application of substrate as a fertilizer, the group of these organic compounds will show the longest life in soil.

The content and proportion of nitrogen in the fraction generated with

the procedure of extraction of organic matter are presented in Table 9. A relatively large amount of nitrogen extracted at the stage of decalcification of the samples (N_{DEKA}) was detected. This fraction is probably represented mainly by simple organic compounds which are relatively low-molecular, loose and poorly bound to mineral components of the examined material and potentially most labile in soil after the application with fertilization (STEVENSON 1985). A minor fraction of nitrogen that passes into 0.05 M H_2SO_4 extract also represents the mineral forms of this element.

Table 9

Content (g kg^{-1} DM) and proportion of nitrogen in the fractions of organic matter in the spent mushroom substrates

Parameter	N_{DEKA}	N_{SH}	N_{KH}	N_{KF}	N_{SNH}	$N_{\text{KH}}/N_{\text{KF}}$
Mean	3.52	4.96	2.53	2.43	14.1	1.11
Range	2.12-4.32	3.36-7.59	1.68-3.44	1.50-4.15	11.9-16.5	0.83-1.59
SD	0.782	1.33	0.488	0.901	1.55	0.29
Relative SD (%)	22.2	26.8	19.3	37.1	11.0	26.0
% TN						
Mean	15.8	21.7	11.1	10.6	62.5	-
Range	9.10-20.0	16.8-29.3	8.40-13.3	7.65-16.0	57.5-70.6	

During alkaline extraction (with 0.1 M NaOH), slightly over 1/5 of the nitrogen pool was extracted (Table 9). The organic compounds in this fraction probably represent the part which is transformed as a result of humification. In the examined material, the proportion of fulvic acids was more variable. A minor advantage of nitrogen in the more stable fraction of humic acids was detected. Fulvic acids, as a fraction of the humus which is soluble in a wider array of reagents (e.g. in acidic solutions), were partially extracted in the first phase of the procedure, i.e. with 0.05 M H_2SO_4 . In the examined material, the residual fraction (N_{SNH}) which constituted the largest proportion should be largely categorized as non-humified organic matter and, to a minor extent, as humins. It probably contains difficult to degrade organic residues (lignin and cellulose) which are genetically associated with straw and peat (used in the production of mushroom substrate).

CONCLUSIONS

1. The mushroom substrates used to cultivate common mushroom, *Agaricus bisporus*, had considerable potential for enriching fertilized soil in mineral and organic nitrogen compounds which were substantially susceptible to degradation in soil.

2. With the sequential extraction, app. 2/3 of the total organic nitrogen pool and app. 1/3 of the carbon pool were extracted.

3. The proportion of nitrogen in humic substances constituted app. 1/5 of the total pool of this element.

4. The presented procedure of extraction may serve as a potential method of qualitative evaluation of organic nitrogen with a perspective for judicious use of waste organic materials in soil fertilization.

5. Further research is warranted in order to investigate the relations between the proportions of individual fractions and the release of nitrogen during mineralization and the possibility for its absorption by plants (in conjunction with time and soil conditions). Furthermore, it is recommended to conduct a qualitative analysis of organic nitrogen compounds which represent the individual fractions.

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CHANGES IN THE CHEMICAL COMPOSITION OF ORGANIC MEDIA USED IN CULTIVATION OF GARDEN HORNED VIOLET (*VIOLA CORNUTA* L.) FROM THE PATIOLA F1 GROUP

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Abstract

Compost substrates based on sewage deposits, tannery sludge as well as coconut fiber can become an alternative for peat substrates in the cultivation of ornamental plants. The use of municipal deposit composts to fertilise soils increases their organic mass content, which, as a result of the humidification processes, improves their physical properties, sorptive capacity, creates a lumpy structure and enhances the microbiological activity in soils. The aim of the research was to assess the use of nutrients contained in substrates produced with the use of municipal sewage deposits and coconut fibre in the cultivation of the horned violet. In 2005-2007, experiments were conducted using six horned violet cultivars (*Viola cornuta*) from the Patiola F1 group. Four organic substrates were used in the research: I - sphagnum peat, II – coconut fibre, III – peat and compost substrate 1 (1:1 v/v), IV – peat and compost substrate 2 (1:1 v/v). Compost 1 was prepared from municipal sewage sludge (35%), potato pulp (35%) and straw (30%). Compost 2 was made from municipal sewage sludge (35%), potato pulp (35%) and sawdust (30%). Both of them were composted for 10 months. The following chemical analyses of the composts, their components and substrates after plant cultivation were performed: pH; dry matter content, total nitrogen, total phosphorus, total potassium, calcium and total magnesium, cadmium, copper, manganese, nickel, lead, total zinc.

The compost substrates made from municipal sewage deposits were rich in nutrients and provided the plants with their appropriate amounts. The composts from municipal sewage waste also contained considerable amounts of heavy metals; however, their levels did not exceed the concentrations allowable in mineral soils intended for cultivations and lands.

Key words: coconut fibre, compost, heavy metals, sewage sludge, sphagnum peat.

ZMIANY SKŁADU CHEMICZNEGO PODŁOŻY ORGANICZNYCH ZASTOSOWANYCH W UPRAWIE FIOŁKA ROGATEGO (*VIOLA CORNUTA* L.) Z GRUPY PATIOLA F1

Abstrakt

W uprawie roślin ozdobnych alternatywą substratów torfowych mogą stać się podłoża kompostowe z osadów ściekowych, a także włókno kokosowe. Zastosowanie kompostów z osadu komunalnego do nawożenia gleb powoduje wzbogacenie ich w masę organiczną, która w następstwie procesów humifikacji poprawia właściwości fizyczne, pojemność sorpcyjną, tworzy strukturę gruzelkową i intensyfikuje aktywność mikrobiologiczną w glebach. Celem badań była ocena wykorzystania składników pokarmowych zawartych w podłożach z udziałem komunalnego osadu ściekowego, a także włókna kokosowego w uprawie bratka ogrodowego. W latach 2005-2007 badano 6 odmian bratka ogrodowego z grupy Patiola z zastosowaniem 4 podłoży organicznych: I – torfu wysokiego, II – podłoża z torfu i kompostu 1, III – podłoża z torfu i kompostu 2, IV – włókna kokosowego. Kompost 1 sporządzono z komunalnego osadu ściekowego (35%), wycierki ziemniaczanej (35%) i słomy (30%), kompost 2 – z komunalnego osadu ściekowego (35%), wycierki ziemniaczanej (35%) i trocin (30%). Do badań użyto kompostów po 12 miesiącach kompostowania.

Podłoża kompostowe z komunalnego osadu ściekowego były zasobne w składniki pokarmowe i zapewniały odpowiedni ich poziom uprawianym roślinom. Komposty z komunalnego osadu ściekowego zawierały także znaczne ilości metali ciężkich, jednak ich wartości nie przekraczały dopuszczalnych stężeń dla gleb mineralnych przeznaczonych pod uprawę oraz ziem.

Słowa kluczowe: kompost, metale ciężkie, osad ściekowy, torf wysoki, włókno kokosowe.

INTRODUCTION

Sphagnum peat is the basic substrate in the cultivation of ornamental plants and also the main component of garden substrates; however, other substrates which could at least partially replace it are being searched for (DOBROWOLSKA, STARTEK 2003, DOBROWOLSKA et al. 2007). Compost substrates based on sewage deposits, tannery sludge as well as coconut fibre can become an alternative to peat substrates in the cultivation of ornamental plants (CZUCHAJ, SZCZEPANIAK 2005, Krzywy et al. 2007, TUDUNWADA et al. 2007). The use of municipal deposit composts to fertilise soils increases their organic mass content, which, as a result of the humidification processes, improves their physical properties, sorptive capacity, creates a lumpy structure and enhances the microbiological activity in soils (KRZYWY et al. 2004, HARRISON et al. 2006). Sewage deposits are characterised by a high content of organic substances, owing to which soil properties can be improved, including its density, porosity or water and nutrient absorption capacity (KRZYWY et al. 2004). Research shows an advantageous plant response to the use of municipal deposits as a substrate or substrate component (ANDRE et al. 2002, NASCIMENTO et al. 2002). The most frequently occurring elements include nitrogen, phosphorus, magnesium, calcium and, to a lesser extent, potassium; therefore, if sewage deposits are used for substrate production, it is necessary to add potassium in the form of mineral fertilizers (MAĆKOWIAK 2001, KRZYWY et

al. 2000a). Toxic substances are a hazard when sewage deposit-based composts are used. Industrial waste and stormwater flowing down from streets or other hardened surfaces are often discharged into the municipal sewage system, which may result in numerous harmful substances entering the sewage, including detergents and crop protection products (SOMMERS et al. 1976).

Considerable diversity of sewage deposits produced at various wastewater treatment plants, which are used for compost production, makes it necessary to examine them thoroughly in respect of their chemical composition before they are introduced into the soil. Their characteristics depend on wastewater treatment processes and on deposit processing. Generally, sewage deposits are rich in organic substances; they contain macro- and micronutrients, trace amounts of metals, including heavy metals, as well as organic impurities, including microorganisms (KULLING 2001).

The aim of the research was to assess the use of nutrients contained in substrates produced with the use of municipal sewage deposits and coconut fibre in the cultivation of the horned violet.

MATERIAL AND METHODS

Research shows that organic substrates influence the growth and development of plants as well as causing changes in the chemical properties of substrates during plant cultivation. Experiments with horned violet cultivars (*Viola cornuta* L.) from the Patiola F1 group: Patiola Pure Yellow, Patiola Pure Violet, Patiola Violet with Yellow Face, Patiola Pure Light Blue, Patiola Pure Lemon Yellow and Patiola Tangerine (Syngenta Seeds), were conducted in 2005-2007. Four substrates were used in the experiment: I – sphagnum peat (control), II – substrate from peat and compost 1 (1:1 v/v), III – substrate from peat and compost 2 (1:1 v/v), IV – coconut fibre. Compost 1 was prepared using municipal sewage sludge (35%), potato pulp (35%) and straw (30%). Compost 2 was prepared using municipal sewage sludge (35%), potato pulp (35%) and sawdust (30%). After 10 months of composting, the composts were submitted to the research, and the methods of their preparation were described by KRZYWY et al. (2000). Osmocote Exact Hi-Start (15+10+10+) fertiliser in the amount of 5 g dm⁻³ was added to the substrates with peat and coconuts fibre. Substrates with the addition of compost were supplemented with ammonium nitrate (NH₄NO₃) and potassium sulphate (K₂SO₄), and nutrients were provided in amounts necessary to ensure their following levels in substrates: N – 250 mg dm⁻³ and K₂O – 300 mg dm⁻³.

From September 1 to September 10, pansy seeds were sown into a sphagnum substrate. They were sown spot-wise, at a distance of 2 cm x 0.5 cm. After the seeds were sown, the substrate was sprayed with a fungicidal agent Previcur 607 SL in the concentration 0.15%, covered with perforated

film to retain the moisture and placed in a greenhouse with moderately high air temperature 18-20°C. The plants were transplanted between October 11 and October 20, at a 2- 4-leaf phase, into 0.4 l pots filled with the tested substrates. The plants were sprayed with Previcur 607 SL (0.2%) to prevent disease, and then placed on tables in an unheated, plastic film tunnel at distances of 10 x 10 cm. From 11 to 20 April in all the years of the research, pansies from the pots were placed in a plastic tunnel on 120-cm wide beds, at distances of 20 x 15 cm. Regardless of the year, the experiments were terminated between 11 and 20 June.

Chemical analyses of compost from the municipal wastewater treatment plant in Stargard Szczeciński and substrates after plant cultivation included determinations of pH, dry matter content, total nitrogen, total phosphorus, total potassium, calcium and total magnesium, cadmium, copper, manganese, nickel, lead, total zinc. All determinations were performed in accordance with the Polish Standards using the methods specified below. The organic carbon content was determined using the Lichterfeld method as modified by Alten; the total nitrogen was assessed using the Kjeldahl method after wet mineralisation in concentrated H_2SO_4 and a selenium mixture; the total phosphorus was tested using the spectrometric method with ammonium molybdate (according to Barton); assimilable phosphorus was determined calorimetrically, while potassium, calcium and assimilable magnesium were determined using the atomic absorption spectroscopy procedure (ASA) after mineralisation in a mixture of perchloric and nitric acids at a 1:1 ratio; total sulphur was determined using the nephelometric method after mineralisation in a mixture of perchloric acid(VII) and nitric acid(V) at a 1:1 ratio.

The results of chemical composition determinations were analysed statistically using a one-factor and two-factor variance analysis in the Statistica 10 programme. The Tukey's test was used for the verification of statistical significance between the means at the significance level of $\alpha = 0.05$. In the tables, means marked with the same letter do not differ significantly. The results of chemical analyses of substrates used for planting are presented in Table 1. The content of macro- and microelements in sphagnum peat, coconut fibre and both composts 10 months after composting is presented in Table 2.

RESULT AND DISCUSSION

In cultivation of garden pansy and horned violet, seeds as well as properly prepared, nutrient-rich soil have great impact on the plant quality (ROSIŃSKA, HOŁUBOWICZ 2008). The nutritional requirements of ornamental plants are quite well known (STROJNY 1993); therefore, after performing chemical analyses of composts from municipal sewage deposits, potato pulp and bulk components (straw, sawdust), it was found that they could be used as

Table 1

Chemical composition of components of compost – in 2004-2006

Specifica- tion	2004				2005				2006				Aggregated for 2004-2006			
	components of composts															
	MSS*	PP	RS	S	MSS	PP	RS	S	MSS	PP	RS	S	MSS	PP	RS	S
Content of macroelements (g kg ⁻¹ d.m.)																
C org.	32.8c**	11.0a	29.4c	18.5b	26.7c	9.9a	33.4d	18.8b	29.5c	9.8a	34.2c	20.1b	29.7c	10.2a	32.3c	19.1b
N	36.54c	5.86b	3.45ab	1.97a	37.56c	5.66b	4.06ab	2.03a	40.05b	5.72a	3.53a	2.31a	38.05b	5.75a	3.68a	2.10a
P	20.16b	3.08a	2.98a	2.86a	23.98b	4.52a	3.45a	3.51a	23.36b	4.12a	3.65a	3.85a	22.50b	3.90a	3.36a	3.41a
K	6.24b	9.68c	7.89bc	1.24a	5.54b	12.42d	8.85c	1.56a	4.67b	10.91c	7.96bc	1.11a	5.48b	11.00c	8.23bc	1.30a
Ca	19.22c	5.47b	0.85a	0.34a	15.26c	7.43b	0.94a	0.53a	14.12c	6.48b	0.93a	0.94a	16.20c	6.46b	0.91a	0.60a
Mg	2.14c	1.02b	0.19a	0.08a	2.88c	1.25b	0.24a	0.06a	2.84b	0.88a	0.22a	0.18a	2.62c	1.05b	0.22a	0.11a
S	12.84c	1.26b	1.52b	0.21a	9.70c	1.76b	1.37b	0.33a	8.21c	1.42b	1.41b	0.29a	10.25c	1.48b	1.43b	0.28a
C:N	8.97	18.77	85.00	94.00	7.11	17.49	82.00	93.00	7.36	17.13	97.02	87.00	7.81	17.80	88.01	91.30
Content of microelements (mg kg ⁻¹ d.m.)																
Cd	5.34b	0.11a	0.12a	0.24a	4.23b	0.07a	0.09a	0.19a	4.96b	0.07a	0.09a	0.16a	4.84b	0.08a	0.10a	0.20a
Cu	51.5b	2.51a	4.98a	3.26a	42.6b	2.23a	4.52a	3.08a	36.4b	2.20a	4.38a	2.86a	43.50b	2.31a	4.63a	3.07a
Mn	345.1c	16.2a	72.3b	27.1a	270.5c	14.9a	65.4b	25.8b	234.8c	13.8a	60.5b	26.2b	283.5c	15.0a	66.1b	26.4a
Ni	28.6c	1.95b	1.21ab	0.78a	32.2c	1.68b	0.89a	0.65a	33.1c	1.61b	0.86a	0.60a	31.30c	1.75b	0.99a	0.68a
Pb	49.21c	3.58b	1.62a	2.01a	41.33b	3.32a	1.41a	1.88a	45.50c	3.41b	1.25a	1.76a	45.35c	3.44b	1.43a	1.89a
Zn	240.2c	12.8a	28.5ab	44.6b	202.4c	10.6a	25.4ab	42.3b	166.4c	12.3a	24.1ab	41.8b	203.0c	11.9a	26.0ab	42.9b

* MMS – municipal sewage sludge, PP – potato pulp, RS – rye straw, S – sawdust;

** Means marked with the same letter do not differ significantly at $\alpha = 0.05$ according to the Tukey's test.

Table 2
Content of macro- and microelements in sphagnum peat, coconut fibre and two composts after 10-month composting (years 2005-2007)

Specifica- tion	Components of composts and composts															
	2005				2006				2007				aggregated for 2005-2007			
	1*	2	3	4	1*	2	3	4	1*	2	3	4	1*	2	3	4
pH	3.74a**	6.02b	7.24c	7.03c	3.47a	5.93b	6.87bc	6.72c	3.51a	5.76b	6.96c	6.74c	3.57a	5.90b	7.02c	6.83c
	N	1.44a	6.06b	20.60d	17.20c	5.43b	17.40c	15.80c	1.18a	4.86b	16.60c	15.30c	1.31a	5.45b	18.20c	16.10c
P	1.69a	4.48b	15.90c	15.61c	1.56a	3.89b	15.46c	15.30c	1.64a	3.86b	15.71c	14.39c	1.63a	4.08b	15.70c	15.10c
K	1.26a	12.52c	10.42bc	7.98b	1.10a	11.66c	10.22c	7.82b	1.12a	11.23c	8.94b	7.24b	1.16a	11.80c	9.86bc	7.68b
Ca	3.02a	3.90a	7.42b	6.88b	2.88a	4.01a	6.85b	6.80b	2.58a	3.72a	6.76b	6.68b	2.83a	3.88a	7.01b	6.79b
Mg	0.33a	0.72b	1.66c	1.62c	0.42a	0.56a	1.52b	1.48b	0.29a	0.76b	1.55c	1.51c	0.35a	0.68b	1.58c	1.54c
Cd	0.11a	0.25a	2.04b	1.85b	0.14a	0.31b	1.68c	1.52c	0.10a	0.22a	1.76b	1.48b	0.12a	0.26a	1.83b	1.62b
Cu	1.28b	0.36a	34.20d	30.20c	1.47b	0.47a	31.60d	27.80c	1.24b	0.38a	30.09d	27.51c	1.33b	0.40a	32.00d	28.50c
Mn	17.1a	114.2b	201.6c	183.7c	14.6a	108.4b	162.4c	170.2c	15.1a	110.4b	170.1c	162.1c	15.6a	111.0b	178.0c	172.0c
Ni	1.74a	1.26a	18.60b	17.50b	2.11a	1.44a	15.50b	19.80c	1.62a	1.43a	16.60b	14.11b	1.82a	1.38a	16.90b	17.10b
Pb	1.36a	16.85b	23.24c	23.60c	2.32a	20.64b	21.03b	20.20b	2.21a	16.86b	21.72b	21.40b	1.96a	18.13b	22.00b	21.70b
Zn	9.58a	19.54b	240.7c	231.2c	7.34a	22.74b	230.2c	221.6c	9.78a	20.04b	183.2c	216.2d	8.90a	20.77b	218.0c	223.0c

* 1 – sphagnum peat, 2 – coconut fibre, 3 – compost 1 (municipal sewage sludge 35%, potato pulp 35%, sawdust from coniferous trees 30%);
sewage sludge 35%, potato pulp 35%, straw 30%), 4 – compost 2 (municipal

** Means marked with the same letter do not differ significantly at $\alpha = 0.05$ according to the Tukey's test.

garden substrates. In the authors' own research, municipal sewage deposit composts were mixed with sphagnum peat. Thus, it is possible to obtain substrates with optimal physicochemical properties for individual plant species (GOUIN 1992, KLOCK-MOORE 1999, ZUBILLAGA, LAVADO 2001). During the composting process, some organic substances undergo mineralisation to carbon dioxide, ammonia and water, while the remaining organic matter is transformed into humus substances, which are very similar to those present in the soil in terms of their structure (HERNANEZ-APAOLAZA et. al. 2000). The use of composts made from municipal sewage deposits as material appropriate for plant cultivation is also justified economically, as it lowers the substrate costs (INGELMO et al. 1998).

Research performed by some authors confirms the high content of N, P and even K in sewage deposits, which is even higher than in garden soil. This shows that they can be used as a source of nutrients for plants as well as in the stabilisation process of soil structural properties (KUMAR et al. 2008).

Before substrate preparation, chemical analyses of individual components used in the composts as well as analyses of peat, coconut fibre and both composts were performed (Tables 1, 2). It was shown that municipal sewage deposits contained large amounts of N, P and Ca and that it was characterised by a low potassium content. Potato pulp had a relatively high nitrogen and potassium content. Composts obtained after 10-month fermentation were also rich in N, P and Ca; however, before planting, the substrates had to be supplemented with N and K in the form of a mineral fertiliser. The pH of the sewage deposit was high (7.75), but when mixed it was with a bulk component and composted, its pH was reduced to neutral. Sewage deposits contain nitrogen and phosphorus, hence the incorporation of such deposits into the soil offers excellent fertilisation benefits, because the macronutrients they contain are necessary for plant cultivation. Substrates were prepared by mixing the composts obtained with acidic compost in appropriate proportions, which lowered the pH of the substrates to a slightly acidic level.

The analyses performed after the completion of the experiments showed that the substrates used for the cultivation of the horned violet differed in terms of chemical composition (Tables 2-6). The lowest total nitrogen content after the termination of the experiment was found in coconut fibre, especially if it was used for the cultivation of *Patiola Pure Lemon Yellow* horned violets. The highest amount of nitrogen after the experiment was found in the substrate with the addition of compost I. The P content also differed, depending on the substrate use; the highest amount of this nutrient after the experiment was found in the substrate with the addition of compost II, while it was the lowest in the coconut fibre. Coconut fibre was also relatively poor in calcium (Ca), which is confirmed by research results of STARTEK et al. (2006). Our study revealed a low potassium content in the peat substrate as well as in substrates with the addition of composts, with the largest amount

Table 3

Changes in the chemical composition of media used for cultivation horned violet from the Patiola F1 group after the end of plant cultivation in 2005

Medium		Cultivars from Patiola F1 group						Mean
		Pure Yellow	Violet with Yellow Face	Pure Light	Pure Violet	Tange-rine	Pure Lemon Yellow	
		content (g kg ⁻¹ d.m.)						
N	Sphagnum peat	9.84	10.25	6.12	8.55	7.36	3.69	7.64ab*
	Coconut fibre	7.42	5.54	6.52	5.69	7.68	4.52	6.23a
	Medium with compost 1	10.68	9.56	12.02	13.56	4.65	5.23	9.28c
	Medium with compost 2	9.24	9.88	8.96	10.14	5.74	4.97	8.16b
	Mean	9.30c	8.81c	8.41c	9.49c	6.36b	4.60a	
P	Sphagnum peat	4.56	5.23	2.44	2.16	2.28	1.95	3.10ab
	Coconut fibre	4.21	2.38	1.96	2.21	2.31	2.17	2.54a
	Medium with compost 1	5.26	0.84	5.21	4.56	4.01	3.62	3.92b
	Medium with compost 2	5.05	3.12	4.36	4.78	3.95	4.85	4.35c
	Mean	4.77b	2.89a	3.49ab	3.43ab	3.14a	3.15a	
K	Sphagnum peat	3.18	1.65	2.67	3.52	2.96	2.6	2.76ab
	Coconut fibre	5.16	5.68	4.21	4.26	5.54	5.03	4.98c
	Medium with compost 1	4.02	4.11	3.58	3.02	3.82	3.14	3.62b
	Medium with compost 2	1.42	5.11	1.35	1.62	2.03	1.78	2.22a
	Mean	3.45b	4.14c	2.95a	3.11ab	3.59b	3.14 ab	
Ca	Sphagnum peat	18.2	12.69	15.14	13.65	15.07	14.32	14.85b
	Coconut fibre	4.23	5.84	5.34	5.26	5.62	5.38	5.28a
	Medium with compost 1	15.88	16.52	16.47	15.01	16.11	13.22	15.54b
	Medium with compost 2	14.12	16.74	11.84	13.45	13.45	14.96	14.09b
	Mean	13.11b	12.95ab	12.20a	11.84a	12.56a	11.97a	
Mg	Sphagnum peat	0.64	0.36	0.72	0.42	0.63	0.48	0.54a
	Coconut fibre	0.52	0.42	0.49	0.66	0.51	0.42	0.50a
	Medium with compost 1	0.48	0.46	0.56	0.47	0.63	0.44	0.51a
	Medium with compost 2	0.51	0.48	0.51	0.52	0.52	0.47	0.50a
	Mean	0.54a	0.43a	0.57a	0.52a	0.57a	0.45a	
S	Sphagnum peat	2.46	2.54	2.13	2.14	1.15	1.16	1.93a
	Coconut fibre	2.98	1.86	1.88	2.12	1.89	2.11	2.14a
	Medium with compost 1	2.45	2.36	2.51	2.45	0.94	0.96	1.95a
	Medium with compost 2	2.65	3.05	1.97	1.85	1.67	1.11	2.05a
	Mean	2.64b	2.45b	2.12ab	2.14ab	1.41a	1.34a	
C org.	Sphagnum peat	364	241	215	295	255	152	254ab
	Coconut fibre	286	237	341	266	297	325	292b
	Medium with compost 1	252	265	361	296	120	145	240ab
	Medium with compost 2	202	315	221	210	144	135	205a
	Mean	276b	265ab	285b	267ab	204a	189a	

* Means marked with the same letter do not differ significantly at $\alpha = 0.05$ according to the Tukey's test.

Table 4

Changes in the chemical composition of media used for cultivation horned violet from the Patiola F1 group after the end of plant cultivation in 2006

Medium		Cultivars from Patiola F1 group						Mean
		Pure Yellow	Violet with Yellow Face	Pure Light	Pure Violet	Tange-rine	Pure Lemon Yellow	
N	Sphagnum peat	7.42	10.11	5.86	8.92	5.98	5.38	7.28ab*
	Coconut fibre	6.56	6.38	6.85	7.03	7.94	4.79	6.59a
	Medium with compost 1	8.72	10.11	11.47	12.48	4.42	5.11	8.72b
	Medium with compost 2	7.55	7.56	8.42	9.82	5.23	4.75	7.22ab
	Mean	7.56ab	8.54b	8.15ab	9.56c	5.89a	5.01a	
P	Sphagnum peat	4.06	4.86	2.16	2.31	2.46	2.04	2.98ab
	Coconut fibre	3.98	2.71	2.3	2.01	2.22	2.22	2.57a
	Medium with compost 1	4.68	0.75	4.63	4.26	3.78	3.41	3.59bc
	Medium with compost 2	4.89	2.81	3.65	4.66	3.87	4.61	4.08c
	Mean	4.40b	2.78a	3.19a	3.31a	3.08a	3.07a	
K	Sphagnum peat	3.33	1.94	2.88	3.11	3.52	2.55	2.89b
	Coconut fibre	6.47	6.5	4.86	4.62	4.86	4.56	5.31c
	Medium with compost 1	2.89	3.26	3.26	2.77	3.51	2.86	3.09b
	Medium with compost 2	1.31	4.38	1.17	1.53	1.84	1.58	1.97a
	Mean	3.50a	4.02c	3.04ab	3.01a	3.43b	2.89a	
Ca	Sphagnum peat	16.7	13.45	13.34	14.92	14.73	15.11	14.71b
	Coconut fibre	5.82	5.49	4.25	5.62	5.89	5.55	5.44a
	Medium with compost 1	15.03	15.93	15.62	14.22	15.21	12.54	14.76b
	Medium with compost 2	14.02	16.04	10.86	13.22	12.95	14.58	13.61a
	Mean	12.89a	12.73a	11.02a	12.00a	12.20a	11.95a	
Mg	Sphagnum peat	0.59	0.41	0.56	0.38	0.42	0.64	0.50a
	Coconut fibre	0.43	0.51	0.64	0.48	0.41	0.48	0.49a
	Medium with compost 1	0.44	0.49	0.47	0.42	0.47	0.43	0.45a
	Medium with compost 2	0.43	0.43	0.43	0.33	0.41	0.44	0.41a
	Mean	0.47a	0.46a	0.53a	0.40a	0.43a	0.50a	
S	Sphagnum peat	2.89	2.11	1.56	2.22	1.38	1.15	1.89a
	Coconut fibre	2.11	1.54	2.02	2.02	2.04	1.87	1.93a
	Medium with compost 1	2.11	2.02	1.82	2.21	0.78	0.74	1.61a
	Medium with compost 2	1.14	2.75	1.87	1.66	1.44	0.96	1.64a
	Mean	2.06ab	2.11b	1.82ab	2.03ab	1.41a	1.18a	
C org.	Sphagnum peat	298	224	234	315	221	168	243ab
	Coconut fibre	224	242	301	222	324	352	278b
	Medium with compost 1	234	242	298	324	146	120	227ab
	Medium with compost 2	184	289	182	236	137	140	195a
	Mean	235ab	249ab	254ab	274b	207a	195a	

* Means marked with the same letter do not differ significantly at $\alpha = 0.05$ according to the Tukey's test.

Table 5

Changes in the chemical composition of media using to cultivation horned violet from the Patiola F1 group after the end of plant cultivation in 2007

Medium		Cultivars from Patiola F1 group						Mean
		Pure Yellow	Violet with Yellow Face	Pure Light	Pure Violet	Tange-rine	Pure Lemon Yellow	
N	Sphagnum peat	8.68	9.29	5.92	6.71	6.91	4.86	7.06ab*
	Coconut fibre	6.35	5.84	6.76	5.42	8.18	4.68	6.21a
	Medium with compost 1	9.87	8.87	10.76	12.38	4.38	4.99	8.54b
	Medium with compost 2	6.77	7.94	7.65	9.37	4.96	4.66	6.89ab
	Mean	7.92c	7.99c	7.77c	8.47c	6.11b	4.80a	
P	Sphagnum peat	3.76	4.78	2.36	2.48	2.36	2.06	2.97ab
	Coconut fibre	4.38	2.02	1.82	1.99	2.16	2.08	2.41a
	Medium with compost 1	4.88	0.69	3.89	3.85	3.59	3.23	3.36ab
	Medium with compost 2	4.76	2.68	3.68	4.48	3.76	4.51	3.98b
	Mean	4.45b	2.54a	2.94a	3.20a	2.97a	2.97a	
K	Sphagnum peat	3.21	1.76	3.34	3.48	3.16	2.47	2.90ab
	Coconut fibre	5.72	6.12	4.58	4.75	5.16	5.01	5.22c
	Medium with compost 1	2.85	3.62	2.96	2.58	3.52	3.05	3.10b
	Medium with compost 2	1.04	4.24	0.96	1.32	1.79	1.53	1.81a
	Mean	3.21ab	3.94b	2.96a	3.03a	3.41ab	3.02a	
Ca	Sphagnum peat	16.1	13.72	13.68	14.79	14.36	14.43	14.51b
	Coconut fibre	5.11	5.54	5.21	5.33	5.57	5.44	5.37a
	Medium with compost 1	14.67	15.24	15.64	14.01	14.02	11.97	14.26b
	Medium with compost 2	13.01	16.69	10.61	12.95	12.86	14.56	13.45b
	Mean	12.22ab	12.80b	11.29a	11.77a	11.70a	11.60a	
Mg	Sphagnum peat	0.74	0.54	0.61	0.4	0.72	0.67	0.61a
	Coconut fibre	0.46	0.36	0.68	0.59	0.44	0.45	0.50a
	Medium with compost 1	0.46	0.49	0.46	0.45	0.32	0.41	0.43a
	Medium with compost 2	0.42	0.44	0.44	0.43	0.42	0.43	0.43a
	Mean	0.52a	0.46a	0.55a	0.47a	0.48a	0.49a	
S	Sphagnum peat	2.24	2.14	1.82	2.43	1.33	1.25	1.87a
	Coconut fibre	1.21	1.88	1.68	2.11	1.92	1.75	1.76a
	Medium with compost 1	1.34	1.57	1.68	2.04	0.75	0.65	1.34a
	Medium with compost 2	0.85	2.7	1.65	1.69	1.48	0.87	1.54a
	Mean	1.41a	2.07b	1.71ab	2.07b	1.37a	1.13a	
C org.	Sphagnum peat	328	218	208	291	237	178	243bc
	Coconut fibre	292	194	281	258	290	311	271c
	Medium with compost 1	246	237	286	288	101	151	218ab
	Medium with compost 2	170	306	167	217	125	106	182a
	Mean	259b	239ab	236ab	264b	188a	187a	

* Means marked with the same letter do not differ significantly at $\alpha = 0.05$ according to the Tukey's test.

Table 6

Changes in the chemical composition of media used for cultivation horned violet from Patiola F1 group after the end of plant cultivation (years 2005-2007)

Medium		Cultivars from Patiola F1 group						Mean
		Pure Yellow	Violet with Yellow Face	Pure Light	Pure Violet	Tange-rine	Pure Lemon Yellow	
N	Sphagnum peat	8.65	9.88	5.97	8.06	6.75	4.64	7.33ab*
	Coconut fibre	6.78	5.92	6.71	6.05	7.93	4.66	6.34a
	Medium with compost 1	9.76	9.51	11.4	12.8	4.48	5.11	8.84b
	Medium with compost 2	7.86	8.46	8.34	9.78	5.31	4.79	7.42ab
	Mean	8.26c	8.44c	8.11c	9.17c	6.12b	4.80a	
P	Sphagnum peat	4.13	4.96	2.32	2.32	2.37	2.02	3.02ab
	Coconut fibre	4.19	2.37	2.02	2.07	2.23	2.16	2.51a
	Medium with compost 1	4.94	0.76	4.58	4.22	3.79	3.42	3.62bc
	Medium with compost 2	4.90	2.87	3.90	4.64	3.86	4.66	4.14c
	Mean	4.54b	2.74a	3.21a	3.31a	3.06a	3.07a	
K	Sphagnum peat	3.24	1.78	2.96	3.37	3.21	2.54	2.85ab
	Coconut fibre	5.78	6.10	4.55	4.54	5.19	4.87	5.17c
	Medium with compost 1	3.25	3.66	3.27	2.79	3.62	3.02	3.27b
	Medium with compost 2	1.26	4.58	1.16	1.49	1.89	1.63	2.00a
	Mean	3.38ab	4.03b	2.99a	3.05ab	3.48ab	3.02a	
Ca	Sphagnum peat	17.0	13.3	14.05	14.5	14.7	14.6	14.69b
	Coconut fibre	5.05	5.62	4.93	5.40	5.69	5.46	5.36a
	Medium with compost 1	15.2	15.9	15.9	14.4	15.1	12.6	14.85b
	Medium with compost 2	13.7	16.5	11.1	13.2	13.1	14.7	13.70b
	Mean	12.7a	12.8a	11.5a	11.9a	12.1a	11.8a	
Mg	Sphagnum peat	0.66	0.44	0.63	0.40	0.59	0.60	0.55a
	Coconut fibre	0.47	0.43	0.60	0.58	0.45	0.45	0.50a
	Medium with compost 1	0.46	0.48	0.50	0.45	0.47	0.42	0.46a
	Medium with compost 2	0.45	0.45	0.46	0.43	0.45	0.45	0.45a
	Mean	0.51a	0.45a	0.55a	0.47a	0.49a	0.48a	
S	Sphagnum peat	2.53	2.26	1.84	2.26	1.29	1.19	1.90a
	Coconut fibre	2.10	1.76	1.86	2.08	1.95	1.90	1.94a
	Medium with compost 1	1.97	1.98	2.00	2.23	0.82	0.78	1.63a
	Medium with compost 2	1.55	2.83	1.83	1.73	1.53	0.98	1.74a
	Mean	2.04ab	2.21b	4.42c	4.47c	4.38c	1.21a	
C org.	Sphagnum peat	330	228	219	300	238	166	247bc
	Coconut fibre	267	224	308	249	304	329	280c
	Medium with compost 1	244	248	315	303	122	139	229ab
	Medium with compost 2	185	303	190	221	135	127	194a
	Mean	257b	251b	258b	268b	200a	190a	

* Means marked with the same letter do not differ significantly at $\alpha = 0.05$ according to the Tukey's test.

of this element found in coconut fibre (Table 2). As quoted in the literature, sewage deposits often contain small amounts of this element and potassium supplementation is required when they are used in substrate components (KRZYWY et al. 2000, MAĆKOWIAK 2001). The Mg content in all the analysed substrates was at a similar level, ranging from 0.45-0.55 g kg⁻¹ in dry matter. The highest S content, on the other hand, was found in the substrate with the addition of compost I; it was over three-fold higher than in the remaining substrates (Tables 2-6). All of the analysed substrates were organic substrates with a high organic carbon content. After the cultivation period, its amount was approximately 200-280 g kg⁻¹ in dry matter. Coconut fibre was characterised by the highest organic carbon content.

Research conducted by other authors also confirmed that composts made from municipal sewage deposits, which were used as a component of substrates for cultivation of bedding plants, had a positive influence on plants, provided that they were used in appropriate amounts for a given species (ANDRE et al. 2002, DOBROWOLSKA et al. 2007, VABRIT et al. 2007). CZYŻYK et al. (2002) showed that long-term composting of sewage deposits resulted in progressive mineralisation of the compost mass and a reduction in the content of nearly all mineral ingredients. However, despite nutrient depletion, composts made from sewage deposits maintain a high fertilising balance, even after two years of storage. Other authors claim (ZAWADZIŃSKA et al. 2009) that sewage deposit-based composts may have too low a content of nutrients available for plants, especially for plants with high nutritional requirements, such as New Guinea impatiens (STARTEK, DOBROWOLSKA 2002).

Numerous elements, such as boron, iron, copper, manganese, zinc, molybdenum and chlorine (B, Fe, Mn, Cu, Zn, Mo, Cl) are micronutrients necessary for the proper functioning of plants. Heavy metals in soils can be a potential hazard for plants and groundwater and, as a result, also for animals and people (KARCZEWSKA et al. 2008). Their elevated concentration can be frequently observed in sewage deposits (WARMAN, TERMEER 2005). Due to physicochemical processes occurring actively in sewage, heavy metals tend to accumulate in existing sewage and in its deposits. A high content of metals such as zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), lead (Pb), mercury (Hg) and chromium (Cr) is usually a factor limiting the use of sewage deposits for agricultural purposes (HSIAU, LO 1998). Heavy metal concentrations in sewage deposits can differ considerably, depending on the sources of contamination.

In the authors' own research, the presence of heavy metals was found in sewage sludge, in composts with the addition of sewage sludge and in substrates with the addition of composts made from municipal waste deposits (Tables 1, 2, 7, 8). The sewage sludge had a high content of Mn and Zn. A relatively high content of Mn was also found in rye straw and a high content of Zn appeared in sawdust (Table 1). Among the substrates used for the cultivation, coconut fiber was characterised by a high concentration of Mn (111 mg kg⁻¹ in dry matter) and Pb (18.13 mg kg⁻¹ in dry matter), which

accounted, respectively, for over 60% and 80% of the content of these elements in composts made from sewage sludge (Table 2). Regardless of the substrate used, after the end of plant cultivation, Zn (*ca* 250 mg kg⁻¹ in dry matter) and Mn (*ca* 140 mg kg⁻¹ in dry matter) and Pb (26-32 mg kg⁻¹ in dry matter) were found to be present in the largest amounts. Out of the heavy metals under analysis, Cd was observed in the lowest amount of Cd (0.35-0.36 mg kg⁻¹ in dry matter) was observed (Tables 7, 8).

However, despite the high accumulation of heavy metals, their content in the soil did not exceed the allowable concentrations (*Regulation ... 2002*).

The heavy metal content underwent significant changes both in the process of composting, and during plant cultivation (Figure 1). It was found

Table 7

Content of heavy metals in media with addition of composts after the end of plant cultivation in 2005-2007

Specification		Medium with compost 1						Medium with compost 2					
		Cd	Cu	Mn	Ni	Pb	Zn	Cd	Cu	Mn	Ni	Pb	Zn
		content (mg kg ⁻¹ d.m.)						content (mg kg ⁻¹ d.m.)					
2005	Pure Yellow	0.41	24.8	142	12.3	38.3	258	0.39	27.3	141	14.0	26.8	266
	Violet with Yellow Face	0.40	28.3	136	16.8	28.5	258	0.38	28.6	106	12.2	22.6	269
	Pure Light Blue	0.42	25.6	115	12.5	44.3	256	0.27	24.5	123	17.2	22.9	225
	Pure Violet	0.39	19.6	183	16.5	28.8	244	0.43	23.5	166	16.8	40.4	256
	Tangerine	0.44	15.2	171	13.1	32.6	302	0.37	13.9	189	17.3	25.6	294
	Pure Lemon Yellow	0.33	18.8	156	11.8	28.6	238	0.42	20.2	174	14.5	29.5	279
	Mean	0.40a*	22.1a	151a	13.8a	33.5b	259a	0.38a	23.0a	150a	15.3b	28.0a	265b
2006	Pure Yellow	0.37	20.6	121	11.7	36.7	284	0.39	24.1	129	12.3	24.3	248
	Violet with Yellow Face	0.37	27.1	128	15.3	26.3	243	0.36	28.2	101	10.7	22.1	245
	Pure Light Blue	0.36	23.2	99	11.3	41.7	231	0.22	24.1	108	17.1	20.6	216
	Pure Violet	0.36	17.5	159	15.8	27.6	226	0.39	22.2	152	16.5	37.2	248
	Tangerine	0.41	14.6	162	11.9	30.8	275	0.36	12.8	177	15.8	28.3	257
	Pure Lemon Yellow	0.32	18.6	151	11.3	24.3	223	0.44	18.6	158	14.0	25.2	280
	Mean	0.37a	20.3a	137a	12.9a	31.2b	247a	0.36a	21.7a	138a	14.4b	26.3a	249a
2007	Pure Yellow	0.31	19.6	117	10.5	37.6	204	0.34	23.7	121	10.7	20.3	240
	Violet with Yellow Face	0.35	23.9	126	16.2	25.6	261	0.31	24.6	101	9.1	20.7	223
	Pure Light Blue	0.34	22.6	94	10.9	37.2	226	0.25	23.1	114	14.3	21.6	184
	Pure Violet	0.34	17.9	165	15.7	23.2	235	0.32	20.1	139	15.1	37.9	262
	Tangerine	0.39	12.8	157	11.1	32.2	274	0.37	12.7	150	15.6	26.2	249
	Pure Lemon Yellow	0.27	18.6	138	10.3	26.2	202	0.35	17.6	165	12.8	23.6	254
	Mean	0.33a	19.2a	133a	12.5a	30.3b	234a	0.32a	20.3a	132a	12.9a	25.1a	235a

* Means marked with the same letter do not differ significantly at $\alpha = 0.05$ according to the Tukey's test.

Table 8

Content of heavy metals in media with addition of composts
after the end of plant cultivation (data aggregated for 2005-2007)

Media	Cultivars from Patiola F1 group	Cd	Cu	Mn	Ni	Pb	Zn
		content (mg kg ⁻¹ d.m.)					
Medium with compost 1	Pure Yellow	0.36	21.7	127	11.5	37.5	249
	Violet with Yellow Face	0.37	26.4	130	16.1	26.8	254
	Pure Light Blue	0.37	23.8	103	11.6	41.1	238
	Pure Violet	0.36	18.3	169	16.0	26.5	235
	Tangerine	0.41	14.2	163	12.0	31.9	284
	Pure Lemon Yellow	0.31	18.7	148	11.1	26.4	221
	Mean	0.36a*	20.5a	140a	13.1a	31.7b	245a
Medium with compost 2	Patiola Pure Yellow	0.37	25.0	130	12.3	23.8	251
	Patiola Violet with Yellow Face	0.35	27.1	103	10.7	21.8	246
	Patiola Pure Light Blue	0.25	23.9	115	16.2	21.7	208
	Patiola Pure Violet	0.38	21.9	152	16.1	38.5	255
	Patiola Tangerine	0.36	13.1	172	16.2	26.7	267
	Patiola Pure Lemon Yellow	0.40	18.8	166	13.8	26.1	271
	Mean	0.35a	21.6a	140a	14.2a	26.4a	249.4a

* Means marked with the same letter do not differ significantly at $\alpha = 0.05$ according to the Tukey's test.

that during the composting process of sewage sludge and straw, potato pulp or sawdust, the content of heavy metals – except Zn – decreased by 15-60%. The addition of peat and cultivation itself caused a further decline of these elements. Only the share of Pb and Zn grew larger in the media (Figure 1.)

The research performed by FUENTES et al. (2004) showed that the heavy metal content is often so low that it does not pose a threat to cultivated plants or other living organisms, regardless of the origin of sewage deposits or the method of their stabilisation. The results of this research also showed that all deposits under analysis could be used to improve soil properties, owing to their high content of organic substances and the content of N, P and K.

The disposal and subsequent use of sewage deposits have been discussed for years and the interest in this subject has been growing in Europe. However, skilful management of this waste is as important as convincing the public opinion that it is possible to use it in agriculture or horticulture (SINGH, AGRAWAL 2008).

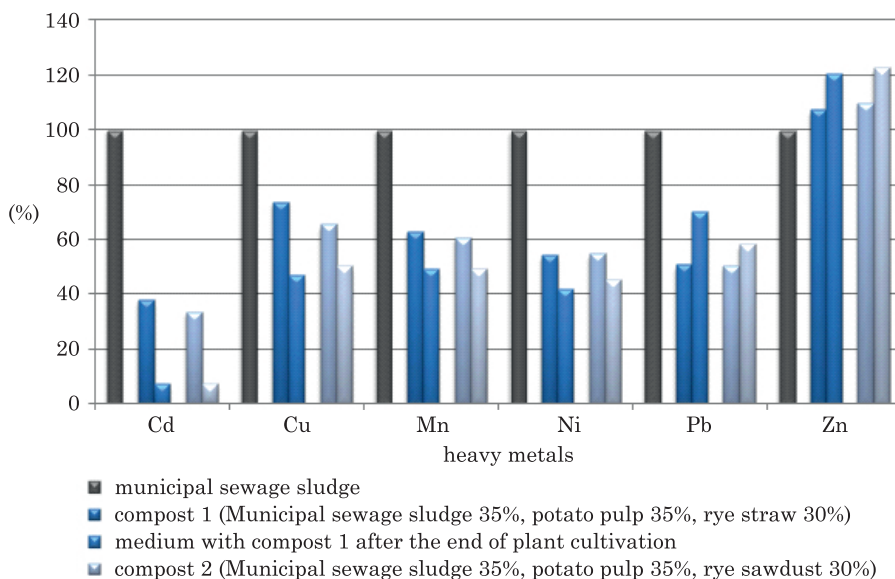


Fig. 1. Changes in the heavy metal content [%] in municipal sewage sludge, composts and media with addition of composts after the end of plant cultivation (2005-2007)

CONCLUSIONS

1. After a ten-month composting period, municipal waste deposits were a rich source of nitrogen, phosphorus and calcium; however, they required the supplementation of potassium in its mineral form.

2. Compost substrates made from municipal sewage deposits were rich in nutrients and provided plants with their appropriate levels.

3. After the completed plant growing season, peat substrates, even if previously supplemented with nutrients in the form of fertilisers, contained lower amounts of these elements, except K, than compost substrates.

4. Coconut fiber used as a substrate was characterised by the high content of K and microelements such as Pb and Mn.

5. Composts made from the municipal sewage waste contained considerable amounts of heavy metals; however, their values did not exceed the allowable concentrations for mineral soils intended for cultivations and lands.

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INFLUENCE OF COAL ASH FROM FLUIDIZED-BED COMBUSTION AND EM-1 PREPARATION ON THE CONTENT OF Fe AND Mn IN SPRING WHEAT AND BARLEY

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Abstract

The object of the research was to analyze the influence of coal ash from fluidized-bed combustion applied to light soil, and combined with the microbiological preparation Effective Microorganisms (EM-1), on changes of the iron and manganese content in cultivated plants of spring wheat and spring barley. Commissioned by the company Vattenfall, a field study on seven fertilizer variants was carried out in 2007-2009. The experiments were set up in Małyszyn near Gorzów Wielkopolski (Hodowla Roślin Strzelce sp. z o.o. Oddział Małyszyn) on light soil, soil valuation class IVb. Each plot covered 10 m². The research was carried out in completely randomized blocks with four replications. Plant samples (grain and straw) were mineralized in a solution of nitric acid(V) and chloric acid(VII); afterwards the total content of iron and manganese was assessed. Application of coal ash to the soil resulted in a decrease of iron and manganese in spring wheat and spring barley grain and straw, especially in the second and third year of the experiment. Additionally, the enrichment of soil with the microbiological preparation EM-1 decreased the concentrations of iron and manganese in the plants. It was noted that the quantities of iron and manganese in the grain and straw of both wheat and barley were decreasing in the subsequent years of the experiment, as a result of which the plant content of these microelements in individual years was varied. To some extent, the above relationship can be explained by the fact that in the last growing season was characterized by the least rainfall. No influence of the applied coal ash on the Fe:Mn weight ratio was determined in grain from cv. Nawra wheat or cv. Lot barley.

Key words: spring wheat, spring barley, coal ash from fluidized-bed combustion, effective microorganisms (EM-1), iron, manganese.

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WPLYW POPIÓŁÓW FLUIDALNYCH Z WĘGLA KAMIENNEGO I PREPARATU EM-1 NA ZAWARTOŚĆ Fe I Mn W PSZENICY I JĘCZMIENIU JARYM

Abstrakt

Analizowano wpływ popiołów fluidalnych z węgla kamiennego wprowadzonych do gleby lekkiej w połączeniu z preparatem mikrobiologicznym efektywne mikroorganizmy (EM-1) na zmiany zawartości żelaza i manganu w pszenicy jarej i jęczmieniu jarym. Doświadczenie zlokalizowano w Małyszynie k. Gorzowa Wielkopolskiego (Hodowla Roślin Strzelce sp. z o.o. Oddział Małyszyn) na glebie lekkiej, klasy bonitacyjnej IVb. Powierzchnia poszczególnych poletek wynosiła 10 m². Doświadczenie założono metodą bloków kompletnie zrandomizowanych w 4 powtórzeniach. W próbkach roślinnych (ziarno i słoma) po mineralizacji na mokro w mieszaninie kwasów azotowego(V) i chlorowego(VII) oznaczono ogólną zawartość żelaza i manganu. Wprowadzanie do gleby popiołów fluidalnych z węgla kamiennego spowodowało zmniejszenie ilości manganu w ziarnie i słomie pszenicy jarej oraz jęczmienia jarego. Szczególnie uwidocznienie tego wpływu można było zaobserwować w 2. i 3. roku doświadczenia. Ponadto zanotowano pewną tendencję zmniejszenia się ilości żelaza i manganu w badanych w roślinach w wyniku wprowadzania do gleby preparatu mikrobiologicznego EM-1. W kolejnych latach badań odnotowano zmniejszenie ilości żelaza i manganu w ziarnie i słomie zarówno pszenicy, jak i jęczmienia, co powodowało zróżnicowanie zawartości tych mikroskładników w roślinach w poszczególnych latach. W pewnym stopniu powyższą zależność można tłumaczyć tym, że w ostatnim roku badań okres wegetacyjny charakteryzował się najmniejszą ilością opadów. Nie stwierdzono jednoznacznego wpływu dodatkowych popiołów fluidalnych na wartość stosunku wagowego Fe:Mn w ziarnie pszenicy odmiany Nawra i jęczmienia odmiany Lot.

Słowa kluczowe: pszenica jara, jęczmień jary, popiół fluidalny, efektywne mikroorganizmy (EM-1), żelazo, mangan.

INTRODUCTION

Being a product of fluidized-bed coal combustion, coal ash is a skeleton-free material containing much iron and manganese. Crystalline grains of ash are built of ferrous phases (RATAJCZAK et al. 1999). Coal ash originating fluidized-bed combustion is nature friendly, hence its recycling has been broadly studied in Poland and abroad (NOWOSIELSKI, ORNOWSKI 1998, GODLEWSKA, KALEMBASA 2008, ANTONKIEWICZ 2009, KOVÁČIK et al. 2011).

Metals that perform the function of micronutrients are usually required in trace amounts specific for the species, variety, growth stage or body. Both their deficiency or excess may harm the plant organisms, thus the amounts should be appropriate and never exceed the standards (PISULEWSKA et al. 1998, HEBBERN et al. 2005).

Iron is a significant element of oxidation-reduction processes in plants. Its large quantities are needed in cytochromes, which play a fundamental role in electron transfer during photosynthesis. Iron is also needed to form magnesium and manganese ion bridges between enzymes and the substrate. Iron is particularly important as an activator in the processes of synthesis of chlorophyll and some proteins. Iron takes part in the reduction of nitrogen compounds and metabolism of fatty acids. Metal ions are directed for trans-

port in the xylem and may be complexed by nicotianamine (VON WIREN et al. 1999). JENTSCHKE and GOLDBOLD (2000) pay attention to the impact of fungi causing the release of metal chelating agents from roots into soil, and to the role of iron in maintaining the balance of minerals and hormones in plants.

Iron is a very mobile element, which - under unfavourable conditions - quickly migrates deep into the soil profile, to the detriment of the quantity of forms easily available to plants (JAROCIŃSKI 2005). Boganegra et al. (2004) indicate that humic complexes present in soil are a good source of iron for plants. Solubility of ferrous compounds depends on their oxidation-reductive potential (EL-FOULY et al. 2001, BECKER, ASCH 2005).

Manganese is a micronutrient necessary for life of plants; it regulates and stimulates their growth and is extremely important for all oxidation-reductive processes. Manganese forms unstable complexes with some enzymes or ATP, acting as an ion bridge between the substrate and enzymes; it takes part in photosynthesis, the metabolism of proteins, carbohydrates and lipids and the scavenging of free radicals (STADTMAN et al. 1990). Traditionally, it is assumed that Mn^{2+} can move freely in the xylem and be transported to the transpiration stream in leaves. DUCIC AND POLLE (2005) reported that manganese absorbed through a leaf can be transported back to the roots. In plants, the metal regulates the level of ferrous compounds and exerts influence on the reduction of nitrates(V). Basically, manganese exists in soils in the form of free oxides or silicates. During the weathering of silicates, Mn^{2+} ions are released into the soil solution. Such factors as the content of clayey minerals, pH value or oxidation-reductive potential of the soil play an important role in this process.

Deficits of iron are sometimes coincide with the symptoms of manganese toxicity. The iron to manganese ratio varies significantly in different species of plants.

Effective microorganisms are sold in Poland under the name EM-1 or as a concentrate containing more than 80 different microorganisms, such as lactic acid bacteria, photosynthetic bacteria, yeasts, actinomycetes, fermentations, etc. Professor Teruo Higa of the University of Agriculture in Ryukyus in Okinawa, Japan, is the creator of the technology of Effective Microorganisms. Microorganisms as an antioxidant agent affect soil and plants directly and indirectly. HIGA (2005) points out that controlling the soil microflora in order to reinforce the advantageous impact of beneficial and effective microorganisms can help to improve and sustain the chemical and physical properties of soil.

The aim of this study was to assess the suitability of coal ash obtained from fluidized-bed combustion for the de-acidification of mineral soil. The analysis was based on a comparison of its effect versus and the traditional calcium and magnesium fertilizers. The object was to examine the influence of coal ash applied to light soil, combined with the microbiological preparation Effective Microorganisms (EM-1), and the impact of the above on chan-

ges of the iron and manganese content in cultivated crops, such as spring wheat and spring barley.

MATERIAL AND METHODS

Experimental conditions

Commissioned by the company Vattenfall, a field study on seven fertilizer variants was conducted in 2007-2009 (Table 1). The experiments were set up in Małyszyn near Gorzów Wielkopolski (Hodowla Roślin Strzelce sp. z o.o. Oddział Małyszyn) on light soil, which belonged to soil valuation class IVb. The area of each plot was 10 m². The experiment was carried out in completely randomized blocks with four replications. Chemical and physicochemical parameters of the materials used in the experiment are given in Table 2. Dolomite calcium and ash were used once, in 2007, split into two doses equal 1.0 and 1.5 of hydrolytic acidity. Doses of calcium fertilizer and coal ash were established according to their content of calcium and magnesium. In order to neutralize the soil's acidity, each plot covering 10 m² was treated with two doses of 13.50 and 20.75 kg of coal ash. Dolomite lime in the doses of 4.74 and 7.11 kg was used to neutralize the hydrolytic acidity. Coal ashes from the Żerań Power Plant was supplied by Vatenffal and incorporated into the soil in the spring of 2007.

Table 1

The design of the experiment

The variant	The fertilization
1	control - soil
2	soil + 1 CaCO ₃ ×MgCO ₃
3	soil + 1,5 CaCO ₃ ×MgCO ₃
4	soil + 1 coal ash
5	soil + 1.5 coal ash
6	soil + 1 coal ash + effective microorganisms EM-1
7	soil + 1.5 coal ash + effective microorganisms EM-1

Table 2

Chemical and physicochemical parameters of the materials used in the experiment

Parameter	pH in KCl	pH in H ₂ O	Hydrolytic acidity	Iron (%)	Manganese (%)	Calcium (%)	Magnesium (%)
Soil at the A _p	4.30	5.13	32.0 mmol H ⁺ kg ⁻¹	0.4	0.02.	0.26	0.06
Coal ash	12.32	12.21	-	4.4	0.05	8.40	1.40
Dolomite lime			-	1.5	0.1	21.50	1.70

During the three-year long experiment, the following crops were grown: spring wheat cv. Nawra, spring barley cv. Lot and bean cv. Martin. However, only wheat and barley were submitted to analyses, as the bean crop was unrepresentative due to the drought which occurred at the flowering stage.

Phosphorus and potassium were applied in spring each year, in the form of Polifoska®5 NPK (Mg-S) 5-15-30-(2-7) dosed at 250 kg per ha. Nitrogen fertilizers were applied in three doses (before sowing, in the flowering stage and in the stem elongation), totalling 120 kg N ha⁻¹ each year. In each year, samples of plants were taken on the day of harvest.

In the experiment, the microbiological preparation Effective Microorganisms (EM-1) was used once, on May 15, during rainfall which supplied 15 dm³ of water ha⁻¹. Additionally, 600 dm³ ha⁻¹ of water was poured.

Methodology of chemical analyses

Plant samples (grain and straw) were first mineralized in a solution of nitric acid(V) and chloric acid(VII). Afterwards, the total content of iron and manganese was determined. The analyses were carried out on an atomic absorption spectrometer Solaar AA Series.

The concentrations of calcium, magnesium, iron and manganese in soil, dolomite lime and coal ash were additionally determined by spectrophotometry following wet mineralization. Soil pH was determined potentiometrically (pH_{H2O} and pH_{KCl}) according to the PN-ISO 10390/1997 standard. The soil hydrolytic acidity was determined using extraction by calcium acetate(II) (PN-R-04027/1997).

The results were analysed with one-way variance for a randomized block design and for values of Tukey's confidence half-intervals at the significance level of $\alpha=0.05$. An FR – Analwar 5 statistical software package was used for calculations as well as an original programme written by prof. Franciszek Rudnicki.

The meteorological data

The development of plants and uptake of macronutrients are largely dependent on the amount of precipitation, which should ensure adequate soil moisture. The normal plant growth during vegetation and intake of macroelements are to a large extent governed by the amount of precipitation which ought to maintain an adequate soil moisture content.

From the data provided by the Meteorological Station Plant Breeding, Strzelce Ltd. (<http://home20.static.kutno68.tnp.pl>), the plant growing period in 2007 was humid. For example, precipitations in May were much above the multi-year mean. The season in 2008 was dry due to relatively scanty rainfalls in May and June. In the last year, in 2009, the growing season was dry despite higher precipitation in May than in the multi-year period.

RESULTS AND DISCUSSION

The content of iron in wheat and barley

The content of iron in grain is important for two reasons: broad use of grains and significant role of iron in living organisms. However, the content of iron in plants should not exceed the set norms. In 2001-2003, SZTEKE et al. (2004) studied the content of iron in grain of wheat grown in Poland, and reported a wide range of results: from 18.7 to 167.0 mg Fe kg⁻¹ in dry mass.

In first year of our experiment, the content of iron in wheat grain was 46.0-50.4 mg Fe kg⁻¹ in dry mass, falling by half in the second year. During the first two years, no influence was observed of the applied liming agents or the microbiological preparation EM-1 on changes in the iron content in wheat grain. In the third year of the experiment, some influence of coal ash and microbiological preparation EM-1 was found, namely the iron content in wheat grain decreased (Table 3). Wheat grain harvested in the third year had the lowest content of iron. To a certain extent, this effect can be attributed to the fact that the plant growing season in that year was characterized by the lowest precipitation.

The composition of grain from wheat grown in this experiment corresponds to the data presented in the subject literature (JACKOWSKA, BORKOWSKA 2002, RACHOŃ, SZUMIŁO 2009, KOZŁOWSKA-STRAWSKA 2010, CIOŁEK et al. 2012, RACHOŃ et al. 2012).

The content of iron in the straw of spring wheat grown in the experiment ranged from 13.4 to 60.2 mg Fe kg⁻¹ dry mass, i.e. less than the one indicated by KOZŁOWSKA-STRAWSKA (2010), who received results ranging from 109.6 to 204.0 mg Fe kg⁻¹. The liming agents or the microbiological preparation EM-1 did not cause significant changes in the content of iron in spring wheat straw (Table 3).

Table 3

The content of iron in cv. Nawra spring wheat (mg Fe kg⁻¹ d.m.)

The variant	Years of experiment					
	2007		2008		2009	
	grain	straw	grain	straw	grain	straw
1	48,6	42.5	24,9	21.0	32,4	14.8
2	46,1	49.7	24,7	29.4	23.5	24.4
3	46.0	45.5	29.0	21.2	22.6	13.4
4	50.4	46.9	24.3	29.1	17.9	16.4
5	48.1	43.4	26.6	25.8	10.8	23.0
6	44.5	45.3	16.4	27.9	8.8	25.7
7	46.1	60.2	18.1	29.2	9.8	28.1
Average	47.1	47.6	23.4	26.2	18.0	20.8
LSD _{0.05}	n.s.	n.s.	n.s.	n.s.	7.46	n.s.

Table 4

The content of iron in cv. Lot spring barley (mg Fe kg⁻¹ d.m.)

The variant	Years of experiment					
	2007		2008		2009	
	grain	straw	grain	straw	grain	straw
1	62.9	60.1	31.4	28.7	30.0	26.8
2	57.6	60.8	31.4	34.3	24.1	32.7
3	57.2	59.5	29.4	34.8	25.0	43.1
4	64.3	72.9	33.8	16.3	17.2	38.6
5	69.4	52.4	31.8	15.8	23.6	30.6
6	55.2	53.4	30.4	28.5	18.8	24.3
7	64.1	52.3	33.6	24.4	21.5	28.1
Average	61.5	58.8	31.7	26.1	22.9	32.0
LSD _{0.05}	n.s.	n.s.	n.s.	13.25	11.60	18.72

Spring barley grain and straw contained a similar amount of iron: from 15.8 to 72.9 mg kg⁻¹ in dry mass. The results gathered in the experiment do not suggest that the applied liming agents or the microbiological preparation EM-1 affected changes in the iron content in barley grain and straw (Table 4).

It should be noted that in the subsequent years of the experiment the content of iron in grain and straw of spring wheat and barley was declining, which resulted in varied levels of this micronutrient in plants in individual years (Tables 3 and 4). The study by BORKOWSKA (2004) on the influence of nitrogen fertilization on the content of selected micronutrients in spring wheat grain also validates the findings concerning big differences in the content of copper, zinc, iron and manganese in wheat grain observed in individual years of the experiment.

The content of iron determined in this experiment was in the range of most frequently observed concentrations, being much lower than those regarded toxic to animals (WYSOKIŃSKI 2011).

The content of manganese in wheat and barley

Manganese takes part in physiological processes, mainly as the activator of enzymes regulating the metabolism of carbohydrates, lipids and proteins. SZTEKE et al. (2004), in their research carried out in 2001-2003 to determine the content of manganese in grain of wheat grown in Poland, obtained a wide range of results: from 9.2 to 69.1 mg Mn kg⁻¹ in dry mass.

In the first year of the experiment, the content of manganese in spring wheat grain was on the level of 81.0-93.9 mg Mn kg⁻¹ in dry mass. Particularly in the first year, the straw from spring wheat grown in ash fertilized soil with an addition of EM-1 preparation had about 30% lower content of manganese compared to the crops from the control (Table 5).

Table 5

The content of manganese in cv. Nawra spring wheat (mg Mn kg⁻¹ d.m.)

The variant	Years of experiment					
	2007		2008		2009	
	grain	straw	grain	straw	grain	straw
1	90.9	367	52.6	148	34.9	75.5
2	92.5	307	46.6	86	30.5	67.3
3	83.9	273	49.3	133	27.0	51.6
4	93.9	256	39.1	105	27.2	73.3
5	92.4	296	31.3	92	24.0	26.5
6	83.5	227	40.7	56	24.7	66.2
7	81.0	196	50.5	121	23.5	90.9
Average	88.3	274	44.3	106	27.4	64.5
LSD _{0.05}	n.s.	n.s.	n.s.	n.s.	3.18	23.06

In the following years of the experiment, a gradual decrease of the manganese content in spring wheat grain and straw was achieved; the minimum amount was 23.5 mg Mn kg⁻¹ in dry mass (Table 5). Similarly to the changes in the amount of iron in wheat grain, this relationship can be attributed to the fact that the last plant growing period had the smallest amount of atmospheric precipitation.

The content of manganese in spring barley grain grown in the experiment ranged from 14.6 to 80.5 mg Mn kg⁻¹ in dry mass. It has to be emphasized that barley straw was much richer in manganese and the determined amount of that element varied from 36.5 to 345 mg Mn kg⁻¹ in dry mass. BŁAZIAK (2007), while assessing changes of the microelemental composition of grain due to soil liming and magnesium enrichment of soil, reports analogous concentrations of microelements in grain and straw from cv. Aramir spring barley. The amount of manganese was between 24-60 mg Mn kg⁻¹ d.m. in grain and from 684 to 357 mg Mn kg⁻¹ d.m. in straw.

In the first year, no influence of the applied liming agents or the microbiological preparation EM-1 on changes of the content of manganese in barley grain and straw was observed (Table 6). The results gathered during the next two years indicate that the application of the microbiological preparation and coal ash causes a decrease of the manganese content in spring barley. Similar relations were observed by BŁAZIAK (2007), who concluded that liming and magnesium enrichment of soil led to a significant decrease of microelements, especially manganese (by 25–90%), in the aerial parts and roots of cereals.

Similarly to wheat, in the subsequent years of the trials, a decrease of the manganese content in spring barley grain and straw was noted, down to the lowest concentration in the third year (Tables 5 and 6).

Table 6

The content of manganese in cv. Lot spring barley (mg Mn kg⁻¹ d.m.)

The variant	Years of experiment					
	2007		2008		2009	
	grain	straw	grain	straw	grain	straw
1	54.7	268	19.1	224	19.3	82.3
2	58.2	323	20.4	114	16.2	49.2
3	52.8	220	17.6	80	14.6	60.2
4	64.3	318	19.9	166	14.9	40.9
5	80.5	345	18.7	140	16.0	36.5
6	65.7	317	16.8	111	15.6	58.7
7	61.7	318	24.5	100	16.8	54.3
Average	62.6	301	19.6	133	16.2	54.7
LSD _{0.05}	6.56	n.s.	1.98	13.58	2.59	15.35

Iron and manganese weight ratio in spring wheat and spring barley grain

Iron is present in plants in the oxidation state +2 and +3. Manganese occurs in plants in different oxidation states: from +2, through +3 and +4 up to +7. In certain conditions, the sum of cations in a plant changes insignificantly. An increase in absorption of one of the nutrients results in a decreased absorption of others. In this context, it is interesting that manganese and iron can act as biochemical antagonists and can compete with each other in terms of the absorption from soil (GUDMUNDSDÓTTIR et al. 2006). The discussed elements occur in different oxidation states in plants; to compare them, we can only use weight ratios, i.e. a simple quotient of their amounts in plants (Table 7).

The Fe:Mn weight ratio in cv. Nawra spring wheat grain ranged from 0.36 to 0.93, and its value indicates a higher concentration of manganese than iron in wheat grain (Table 7). The Fe:Mn ratio in cv. Lot spring barley grain ranged between 0.84-1.81, which means its value was near the lower limit considered optimal. In healthy fodder plants, the Fe:Mn ratio should range between 1.5-2.5. Below the value of 1.5, the symptoms of manganese toxicity and iron deficit are observed, whereas above 2.5 the excessive amount of iron becomes harmful and is followed by the symptoms of manganese deficit (MOTOWICKA-TERELAK 1978, MAZUR 1990, BŁAZIAK 2007).

The chemical composition of barley grain had an increasing value of the Fe:Mn weight ratio over time. The results indicate that the decrease of manganese in barley grain in the subsequent years of the experiment was more significant than that of iron.

The results of the experiment did not allow us to formulate an unequivocal conclusion on the influence of added coal ash on the Fe:Mn weight ratio.

Fe:Mn weight ratio in grain of wheat and barley

The variant	Years of experiment					
	2007	2008	2009	2007	2008	2009
	spring wheat			spring barley		
1	0.53	0.47	0.93	1.15	1.64	1.55
2	0.50	0.53	0.77	0.99	1.54	1.49
3	0.55	0.59	0.84	1.08	1.67	1.71
4	0.54	0.62	0.66	1.00	1.70	1.15
5	0.52	0.85	0.45	0.86	1.70	1.48
6	0.53	0.40	0.36	0.84	1.81	1.21
7	0.57	0.36	0.42	1.04	1.37	1.28
Average	0.53	0.55	0.63	0.99	1.63	1.41

CONCLUSIONS

Some influence of ash from fluidized-bed combustion of was observed only in the third year of the experiment. Namely, the amount of iron in cv. Nawra wheat grain decreased.

2. The results of the experiment do not indicate that coal ash from fluidized-bed combustion applied to soil exert influence on changes in the iron content in cv. Lot barley grain and straw. Some impact was observed only in the first year of experiment.

3. The application of coal ash to soil resulted in a decrease of iron and manganese in spring wheat and spring barley grain and straw, especially in the second and third year of the experiment.

4. When the microbiological preparation EM-1 was applied into soil, the levels of iron and manganese in the studied plants tended to decrease.

5. It should be emphasized that in the subsequent years of the research the concentrations of iron and manganese were decreasing in grain and straw of both wheat and barley, causing variation in the content of these micronutrients in plants between individual years. This trend was more evident in the case of manganese than iron.

6. No influence of the applied coal ash on the Fe:Mn weight ratio was determined in cv. Nawra wheat grain or cv. Lot barley grain.

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EFFECT OF SOIL POLLUTION WITH POLYCYCLIC AROMATIC HYDROCARBONS ON MAIZE BIOMASS YIELD AND ACCUMULATION OF SELECTED TRACE ELEMENTS*

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Abstract

The research was conducted to assess the effect of artificial soil pollution with polycyclic aromatic hydrocarbons on the amount of produced maize biomass and the accumulation of selected trace elements. Benzo(a)pyrene (BaP), chrysene (Ch) and fluorene (Fl) were added to soil in the liquid form (dissolved in dichloromethane – DCM) in doses of 0.1 mg kg⁻¹ d.m. and 10 mg kg⁻¹ d.m. The experiment comprised: the control (C) – soil with the natural content of the studied PAHs and without a mineral salt supplement; object 0 – soil with the natural content of PAHs and a mineral salt (NPK) supplement, object I – soil with a DCM and mineral salt supplement, object II – soil with a supplement of 0.3 mg PAHs per kg of soil d.m. (0.1 mg BaP + 0.1 mg Ch + 0.1 mg Fl) + mineral salts, the amount of introduced PAHs was equivalent to an elevated content; object III – soil with an addition of 30 mg PAHs per kg of soil d.m. (10 mg BaP + 10 mg Ch + 10 mg Fl) + mineral salts, the quantity of PAHs was equivalent to very strong pollution. The test plant was cv. San maize. The dried biomass was crushed in a laboratory mill and mineralized in a chamber furnace (450°C, 5 h). The residue was dissolved in diluted nitric acid 1:2 (v/v). The content of the trace elements (Zn, Cu) in the solutions was assessed with the ICP-AES method on a JY 238 Ultrac apparatus. The quantity of absorbed trace elements was derived from the biomass amount and the content of these elements in the biomass. On the basis of the total maize biomass (shoots and roots), the tolerance coefficient was computed as a ratio of the yield of the plant dry mass in objects C, I, II and III to the yield in the object where NPK medium was introduced to the unpolluted soil (object 0). The pollution coefficient was calculated from concentrations of the elements in the plant shoots and as a ratio of the elemental content in plants from objects C, I, II and III to the content in object 0. The translocation coefficient was calculated as a ratio of the element content in plant shoots to the content in

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roots. Soil pollution with the analyzed aromatic hydrocarbons did not inhibit either the growth or the development of maize roots or shoots. The biggest amount of biomass was obtained in the object where the soil was characterized by an elevated content of the analyzed aromatic hydrocarbons. The value of the tolerance index in the objects where the stressor had been introduced was above one, which indicates no effect of soil pollution with PAHs on the plant biomass quantity. The value of the tolerance index below one was achieved only in the control biomass. A significantly higher content of Cu and more of this element absorbed by maize shoots were determined in the objects where dichloromethane and polycyclic aromatic hydrocarbons had been introduced to the soil in comparison with the unpolluted objects. The values of maize shoot biomass contamination with Zn and Cu were visibly higher in the objects where the soil was polluted with aromatic hydrocarbons in comparison to the values obtained in the object where only the mineral medium was supplied to the soil. A similar dependency pertained to the translocation coefficient of zinc and copper.

Key words: soil pollution, PAHs, zinc, copper, maize.

WPŁYW ZANIECZYSZCZENIA GŁĘBY WIELOPIERŚCIENIOWYMI WĘGLOWODORAMI AROMATYCZNYMI NA IŁOŚĆ BIOMASY KUKURYDZY ORAZ AKUMULACJĘ WYBRANYCH PIERWIASTKÓW ŚLADOWYCH

Abstrakt

Celem badań była ocena wpływu sztucznego zanieczyszczenia gleby wielopierścieniowymi węglowodorami aromatycznymi na ilość wytworzonej biomasy kukurydzy oraz akumulację wybranych pierwiastków śladowych. Benzo(a)piren (BaP), chryzen (Ch) i fluoren (Fl) dodawano do gleby w postaci roztworu w ilościach $0,1 \text{ mg kg}^{-1}$ i 10 mg kg^{-1} . Odpowiednią ilość WWA rozpuszczono w dichlorometanie. Badania obejmowały: obiekt kontrolny (K) – gleba o naturalnej zawartości badanych WWA i bez dodatku soli mineralnych, obiekt (0) – gleba o naturalnej zawartości badanych WWA z dodatkiem soli mineralnych, obiekt (I) – gleba z dodatkiem dichlorometanu oraz soli mineralnych, obiekt (II) – gleba z dodatkiem $0,3 \text{ mg WWA kg}^{-1}$ s.m. gleby ($0,1 \text{ mg BaP} + 0,1 \text{ mg Ch} + 0,1 \text{ mg Fl}$) + sole mineralne – ilość WWA wprowadzona do gleby w tym obiekcie odpowiadała podwyższonej zawartości, obiekt (III) – gleba z dodatkiem $30 \text{ mg WWA kg}^{-1}$ s.m. gleby ($10 \text{ mg BaP} + 10 \text{ mg Ch} + 10 \text{ mg Fl}$) + sole mineralne – ilość WWA wprowadzona do gleby w tym obiekcie odpowiadała bardzo silnemu zanieczyszczeniu.

Rośliną testową była kukurydza odmiany San. Następnie wysuszoną biomasę rozdrobniono w młynku laboratoryjnym i mineralizowano w piecu komorowym (temp. 450°C , 5 h). Pozostałość rozpuszczono w rozcieńczonym kwasie azotowym 1:2 (v/v). W tak przygotowanych roztworach zawartość badanych pierwiastków śladowych oznaczono metodą ICP-AES w aparacie JY 238 Ultrace. Ilość pobranych pierwiastków śladowych obliczono na podstawie ilości biomasy i zawartości składnika w biomasie. Na podstawie sumarycznej ilości biomasy kukurydzy (części nadziemne i korzenie) wyliczono wskaźnik tolerancji jako iloraz suchej masy plonu roślin w obiektach I, II i III oraz obiekcie, w którym do gleby niezanieczyszczonej wprowadzono pożywkę mineralną (obiekt 0). Wskaźnik stopnia zanieczyszczenia wyliczono na podstawie zawartości pierwiastka w częściach nadziemnych roślin jako iloraz zawartości pierwiastka w roślinie z obiektów K, I, II i III i z obiektu, w którym do gleby niezanieczyszczonej wprowadzono pożywkę mineralną (obiekt 0). Wskaźnik translokacji obliczono jako iloczyn zawartości pierwiastka w częściach nadziemnych i w korzeniach roślin. Zanieczyszczenie gleby badanymi węglowodorami aromatycznymi nie hamowało wzrostu i rozwoju części nadziemnych i korzeni kukurydzy. Największą ilość biomasy uzyskano w obiekcie, w którym gleba zawierała zwiększoną ilość badanych węglowodorów aromatycznych. Wartość wskaźnika tolerancji w obiektach, w których wprowadzono czynnik stresowy, kształtowała się powyżej jedności, co wskazuje na brak wpływu zanieczyszczenia gleby WWA na ilość biomasy roślin. Wartość wskaźnika tolerancji poniżej jedności dotyczyła jedynie biomasy z obiektu kontrolnego. Istotnie większą zawartość Cu oraz

większą ilość tego pierwiastka pobraną przez części nadziemne kukurydzy stwierdzono w obiektach, w których do gleby wprowadzono dichlorometan i wielopierścieniowe węglowodory aromatyczne, w porównaniu z obiektami niezanieczyszczonymi. Wartości wskaźnika zanieczyszczenia biomasy części nadziemnych kukurydzy Zn i Cu były wyraźnie większe w obiektach, w których glebę zanieczyszczono węglowodorami aromatycznymi, w porównaniu z wartościami uzyskanymi w obiekcie, w którym do gleby wprowadzono tylko pożywkę mineralną. Podobna zależność dotyczyła wskaźnika translokacji cynku i miedzi.

Słowa kluczowe: zanieczyszczenie gleby, wielopierścieniowe węglowodory aromatyczne, cynk, miedź, kukurydza.

INTRODUCTION

Excessive levels of polycyclic aromatic hydrocarbons (PAHs) in soil constitutes a hazard to both the human health and all biotic elements of a soil ecosystem (COUSINS et al. 1997, MALISZEWSKA-KORDYBACH, SMRECZAK 1999). PAHs form a numerous group of cyclic compounds whose various structural forms are characterized by different reciprocal positions of benzene rings, which makes these compounds particularly dangerous. Moreover, their transformation may lead to the formation of intermediate products, characterized by considerably higher toxicity than the primary forms (MARR et al. 2006).

Apart from fuel burning, industrial processes or transport, application of organic waste materials in agriculture and processes connected with transformation of organic matter contained in soil may be the source of PAHs (MARR et al. 2006, STAHL et al. 2004).

Soil pollution with PAHs leads to changes in soil chemical properties and modifies the composition of microorganism populations (GONDEK et al. 2008). Quantitative and qualitative changes of soil microflora directly affect most of biochemical processes occurring in soil – apart from degradation of pollutant substances, also the processes involved in trace element mobility (LIN et al. 2008). A change in the trace element availability may have a serious influence on plants, particularly in the early stages of development (MALISZEWSKA-KORDYBACH, SMRECZAK 2003). Bioavailability of trace elements is of key importance for stimulation or inhibition of plant growth and development in later stages, and consequently leads to changes in the biological value of biomass, thereby restricting its use.

The research was conducted to assess the effect of artificial soil pollution with PAHs on the amount of maize biomass produced and the accumulation of trace elements (Zn, Cu) in the biomass.

MATERIAL AND METHODS

The investigations were conducted as a pot experiment on soil material collected from the Ap (0-20 cm) layer of an arable field. The soil contained 26% of the < 0.02 mm fraction and had the particle-size distribution and texture of sandy loams (IUSS Working Group WRB, 2007). The soil revealed a slightly acid reaction ($\text{pH H}_2\text{O} = 6.27$). Hydrolytic acidity assessed after extraction with $1 \text{ mol dm}^{-3} \text{ CH}_3\text{COONa}$ was $23.9 \text{ mmol}(+) \text{ kg}^{-1} \text{ d.m.}$ the organic carbon content was $15.99 \text{ g kg}^{-1} \text{ d.m.}$ and total nitrogen was $1.54 \text{ g kg}^{-1} \text{ d.m.}$ The total zinc content was $104 \text{ mg kg}^{-1} \text{ d.m.}$ and total copper was $8.01 \text{ mg kg}^{-1} \text{ d.m.}$

The soil was polluted with three hydrocarbons from the PAHs group: benzo(a)pyrene (BaP), chrysene (Ch) and fluorene (Fl), which differ in physicochemical properties. Because a soil environment is never polluted with a single PAH, mixtures of these compounds were used in the experiment (COUSINS et al. 1997, MALISZEWSKA-KORDYBACH, SMRECZAK 1998). BaP, Ch and Fl were added to the soil in the liquid form (dissolved in dichloromethane – DCM) in doses of $0.1 \text{ mg kg}^{-1} \text{ d.m.}$ and $10 \text{ mg kg}^{-1} \text{ d.m.}$ The experiment comprised the control (C) – soil with the natural content of the three PAHs and without a mineral salt supplement; object 0 – soil with the natural content of the PAHs and a mineral salt (NPK) supplement, object I – soil with a DCM and a mineral salt supplement, object II – soil with a supplement of $0.3 \text{ mg PAHs per kg of soil d.m.}$ ($0.1 \text{ mg BaP} + 0.1 \text{ mg Ch} + 0.1 \text{ mg Fl}$) + mineral salts, the amount of introduced PAHs was equivalent to an elevated content; object III – soil with an addition of $30 \text{ mg PAHs per kg of soil d.m.}$ ($10 \text{ mg BaP} + 10 \text{ mg Ch} + 10 \text{ mg Fl}$) + mineral salts, the quantity of PAHs equivalent to a very strong pollution (KABATA-PENDIAS et al. 1995).

The pot experiment was conducted in PVC containers, each filleed with 8.6 kg of air-dry soil. In order to meet the plant's nutritional requirements, the soil in all objects except the control (C) received nitrogen, phosphorus and potassium in the form of chemically pure salts. The quantities of nutrients introduced per 1 kg of soil were: $0.12 \text{ g N (NH}_4\text{NO}_3)$; $0.06 \text{ g P (Ca(H}_2\text{PO}_4)_2 \text{H}_2\text{O)}$; 0.19 g K (KCl) . The research was conducted in 4 replications; the soil moisture during the plant growing period was maintained at the level of 60% soil water capacity. The test plant was cv. San maize, and 5 plants per pot kept until harvest, which was conducted at the stage of 7-9 leaves. Following the harvest of maize shoots, the roots were taken from the soil and washed.

The plant material was dried until constant weight in an airflow dryer (70°C) so as to determine the dry weight. The dried biomass was crushed in a laboratory mill and mineralized in a chamber furnace (450°C , 5 h). The residue was dissolved in diluted nitric acid 1:2 (v/v) (OSTROWSKA et al. 1991). The content of the trace elements (Zn, Cu) in the solutions was assessed with the ICP-AES method on a JY 238 Ultrace apparatus. The quantity of

absorbed trace elements was computed from the volume of biomass and the content of elements in it. On the basis of the total maize biomass yield (shoots and roots), a tolerance coefficient was computed as a ratio of the plant dry mass yield in objects C, I, II and III to the analogous yield in the object where the NPK medium was introduced to unpolluted soil (object 0). The pollution coefficient was calculated from concentrations of the elements in the plant shoots and as a ratio of the content of the elements in plants from objects C, I, II and III to the content in object 0. The translocation coefficient was calculated as a ratio of the elemental content in plant shoots to the content in roots (KOPCEWICZ, LEWAK 1998).

All plant material analyses were conducted in 4 replications. Precision of the assessments was determined using reference material NCS DC733448 (China National Analysis Center for Iron & Steel). The data concerning precision and accuracy of the assessments (FUENTES et al. 2004) are presented in Table 1.

Table 1

Amounts of metals released from material NCS DC733448 (mean \pm SD)
and data for analytical precision and accuracy

Metal	Experimental value (mg kg ⁻¹ d.m.)	Recommended value (mg kg ⁻¹ d.m.)	Precision	Accuracy
Zn	21.4 \pm 1.0	20.6 \pm 2.2	4.71	3.88
Cu	5.3 \pm 0.1	5.2 \pm 0.5	1.88	1.92

The results were elaborated statistically according to a constant model, in which the PAH pollution level was a factor. The statistical computations included a one-way Anova and the significance of differences between arithmetic means was estimated by means of the t-Tukey test at the significance level $\alpha < 0.05$ (STANISZ 1998). StatSoft, Inc. (2011) Statistica version 10 was the software used.

RESULTS AND DISCUSSION

The effect of soil pollution with PAHs on the plant growth and development depends not only on the species but also – as observed by MALISZEWSKA-KORDYBACH and SMRECZAK (1999) – on soil properties, especially the organic matter content. In the present experiment, the amount of maize biomass in the objects where the soil was polluted with PAHs was bigger than in the control (object C – unpolluted soil without the NPK medium) – Table 2. Therefore, it may be stated that the soil pollution did not inhibit the maize growth or development. The analysis of the total maize biomass (shoots and roots) revealed the highest amount in object II, where the soil was characterized by a lower level of pollution. MALISZEWSKA-KORDYBACH and SMRECZAK

Table 2

Amounts of maize dry biomass (g d.m. pot⁻¹ ± SD) and values of tolerance coefficient

Object	Shoots	Roots	Total biomass	Tolerance coefficient
C	72.2 ^a ±4.0	12.6 ^a ±2.4	84.8 ^a ±6.3	0.58 ^a ±0.04
0	127.9 ^b ±4.1	17.6 ^{ab} ±1.1	145.5 ^b ±4.3	1.00*
I	135.3 ^b ±5.5	19.1 ^{ab} ±3.7	154.4 ^b ±9.1	1.06 ^b ±0.07
II	140.0 ^b ±7.0	20.4 ^b ±3.5	160.4 ^b ±10.0	1.10 ^b ±0.07
III	133.3 ^b ±1.9	17.8 ^{ab} ±0.9	151.2 ^b ±2.5	1.04 ^b ±0.03

* Object 0 = 1.00;

Means followed by the same letters in columns did not differ significantly at $\alpha < 0.05$ according to the *t*-Tukey test.

(1999) also described a stimulating effect of PAHs on plant yield at the level of these substances in soil below or slightly exceeding 1 mg kg⁻¹ d.m. Similar results were presented by KRZEBIETKE and SIENKIEWICZ (2010b) while studying the effect of foliar application of anthracene and pyrene (PAHs) on yields and chemical composition of butterhead lettuce (*Lactuca sativa* L.) grown under varied abundance of substrate in nutrients. KUMMEROVA et al. (1995) demonstrated that a PAHs concentration no more than 10 mg dm⁻³ in a solution might intensify the plant biomass increment.

Apart from the assessment of plant biomass volume in the presence of an increased content of stressor in soil, various other indices demonstrating more specifically the impact of a stress agent were tested as well. The value of the tolerance coefficient in the objects where the stressor was introduced (objects II and III) exceeded one (Table 2), which indicates the absence of any effect of soil pollution with PAHs on the biomass amount. The value of that coefficient lower than one was found only in the biomass from the control (object C – the soil with the natural PAHs content and no NPK medium), which may be associated with a deficiency of available nitrogen, phosphorus and potassium forms.

The zinc content in the maize shoots did not exceed 30 mg kg⁻¹ d.m. (Table 3) and was within the normal content range (GORLACH, GAMBUS 2000). The shoot biomass contained significantly the lowest Zn quantity in the control (object C – soil with natural PAHs content and without NPK medium). The level of soil load with PAHs did not cause any significant changes in the Zn content in the shoots. In the roots, this element was more abundant than in the shoots. A larger difference was noticed between the Zn content in the roots from the control (object C) and from the other objects than in the case of the shoots, which was due to Zn accumulating in a relatively smaller biomass amount.

The quantity of Zn absorbed by the shoots was the biggest in object I, where the NPK medium and DCM were introduced. Comparable amounts of Zn taken up by the shoots were registered in the objects where PAHs were

Table 3

Content of trace elements in maize biomass (mg kg^{-1} d.m. \pm SD), and uptake of trace elements by maize ($\text{mg pot}^{-1} \pm$ SD)

Object	Content of trace elements			
	shoots	roots	shoots	roots
	Zn		Cu	
C	19.0 ^a \pm 2.2	82.2 ^b \pm 5.7	1.71 ^a \pm 0.24	7.24 ^b \pm 1.01
0	25.7 ^b \pm 1.6	56.6 ^a \pm 1.4	1.93 ^a \pm 0.05	4.33 ^a \pm 0.68
I	29.1 ^b \pm 0.9	54.3 ^a \pm 3.3	2.42 ^b \pm 0.11	3.83 ^a \pm 0.37
II	25.7 ^b \pm 2.3	54.3 ^a \pm 9.6	2.24 ^b \pm 0.22	3.83 ^a \pm 0.45
III	28.7 ^b \pm 0.6	47.9 ^a \pm 5.5	2.38 ^b \pm 0.28	3.23 ^a \pm 0.42
Object	Uptake of trace elements			
	shoots	roots	shoots	roots
	Zn		Cu	
C	1.36 ^a \pm 0.10	1.03 ^a \pm 0.20	0.12 ^a \pm 0.01	0.091 ^a \pm 0.02
0	3.29 ^b \pm 0.27	1.00 ^a \pm 0.05	0.25 ^b \pm 0.01	0.077 ^a \pm 0.02
I	3.93 ^a \pm 0.21	1.05 ^a \pm 0.25	0.33 ^a \pm 0.01	0.074 ^a \pm 0.02
II	3.59 ^{bc} \pm 0.32	1.10 ^a \pm 0.26	0.31 ^a \pm 0.02	0.078 ^a \pm 0.02
III	3.82 ^{bc} \pm 0.11	0.85 ^a \pm 0.07	0.32 ^a \pm 0.04	0.057 ^a \pm 0.01

Means followed by the same letters in columns did not differ significantly at $\alpha < 0.05$ according to the *t*-Tukey test.

added to the soil, irrespective of the amount (Table 3). The quantities of Zn absorbed by the roots, regardless of the object, were lower and non-significantly diversified among the objects.

The coefficient of shoot biomass pollution with Zn, except the control (object C), was higher than one, which evidences a bigger Zn content in the biomass from the objects in which PAHs were introduced to the soil (objects II and III) and from the object where DCM was introduced to the soil (object I), in relation to the biomass from the object in which NPK medium was used (object 0) – Table 4. Zinc translocation from roots to shoots, described by the translocation coefficient, was significantly bigger in the objects where the DCM (object I) and PAHs (objects II and III) were added to the soil than the object where mineral salts were used (object 0) or in the control (object C).

More copper was found in the maize root system than in the shoots (Table 3). The shoot biomass from the objects where the DCM and PAHs were introduced (objects I, II and III) contained significantly more Cu. Cu concentrations in the roots were on average over twice as high as in the shoots. The highest levels of this element were determined in the root system of the control plants (object C).

The quantities of Cu taken up by the shoots were the highest in the objects where the DCM and PAHs were introduced to the soil (objects I, II

Table 4

Values of pollution and translocation coefficients (mean \pm SD)

Object	Pollution coefficient		Translocation coefficient	
	Zn	Cu	Zn	Cu
C	0.42 ^a \pm 0.06	0.50 ^a \pm 0.07	0.23 ^a \pm 0.02	0.24 ^a \pm 0.04
0	1.00*	1.00*	0.46 ^b \pm 0.03	0.46 ^b \pm 0.06
I	1.21 ^b \pm 0.15	1.33 ^b \pm 0.08	0.53 ^c \pm 0.04	0.64 ^c \pm 0.08
II	1.10 ^b \pm 0.15	1.27 ^b \pm 0.14	0.48 ^c \pm 0.06	0.59 ^c \pm 0.09
III	1.17 ^b \pm 0.11	2.51 ^c \pm 0.34	0.61 ^c \pm 0.06	0.74 ^c \pm 0.06

* Object 0 = 1.00

Means followed by the same letters in columns did not differ significantly at $\alpha < 0.05$ according to the *t*-Tukey test.

and III), which resulted from a higher content of this element in the biomass (Table 3). The amount of Cu absorbed by the root system was much smaller, which correlates with the volume of biomass amassed in this part of the plant. No significant diversification was noted in the quantity of Cu absorbed by the roots.

The values of the coefficient of maize shoot pollution with Cu were significantly higher in the objects where the DCM and PAHs were introduced to the soil (objects I, II and III) than the same coefficient computed for the control (object C) – Table 4. The values of the translocation coefficient calculated for Cu turned out to be less than one, although in objects I, II and III, where the DCM and PAHs were introduced to the soil, these parameter values were significantly higher than the ones computed for the control (object C) and object 0, in which mineral salts were added to the soil.

Discussion of the research results of heavy metal content in maize in conditions of soil pollution with PAHs is difficult due to the shortage of references addressing the presented issue. The problem of heavy metal bioavailability is widely discussed in the aspect of the effect of these elements on biochemical processes occurring in plants, in the context of remediation of chemically polluted lands, but also of environmental application of waste materials (GONDEK et al. 2010, KHAN et al. 2000). Plants growing in a polluted environment may accumulate considerable amounts of toxic trace elements or change the proportions of macronutrients, which poses a serious threat to animals and people (OKORONKOWO et al. 2005, KRZEBIETKE, SIENKIEWICZ 2010a).

Heavy metal detoxification mechanisms developed by plants, which are activated after absorbing metals from the soil solution, enable these organisms to function in a polluted environment without any visible symptoms of phytotoxicity (LIN et al. 2008). Investigations conducted by other authors demonstrated considerable differences in trace element uptake depending on soil particle-size distribution and texture, pH, organic matter content or sorption capacity, although a plant species is not without importance

either (BASTA et al. 2005). Increasing the soil pH value and organic matter content results in diminishing trace element availability (ROSSELLI et al. 2003). However, the trace element content in plants may be also significantly influenced by soil pollution, caused for example by PAHs (KHAN et al. 2011). The investigations presented above justify the statement that the trace elements were accumulated mainly in the maize roots. Investigations conducted by MACNICOL and BECKETT (1985) confirm that roots constitute the first barrier restricting trace element translocation to shoots, irrespectively of the stressor, although – as stated by BATTY and ANSLOW (2008) – there are plants which accumulate more trace elements in shoots. Barriers against the transport of trace elements from roots to shoots generally act effectively towards copper in all plants. On the other hand, considering zinc transport, it obviously depends on a cultivated plant species. According to CHU and WONG (1987) and GALLER (1992), such barriers inhibit also the transport of metals within shoots.

In the conducted experiment, the Cu content in the maize shoots was higher in the objects where the DCM and PAHs were introduced to the soil. According to LIN et al. (2008), a high content of pyrene in soil may inhibit the Cu uptake by plants. No such dependence was observed in the present experiment, in which the soil material was polluted with benzo(a)pyrene, chrysene and fluorene. BATTY and ANSLOW (2008) revealed that soil contamination with pyrene did not reduce the Zn uptake, nevertheless Zn and pyrene application to the soil significantly decreased the growth of *Brassica juncea*. In the authors' own research, the introduction of hydrocarbons did not reduce significantly the Zn uptake by the maize shoots, nor did it affect negatively the amount of maize biomass.

CONCLUSIONS

1. Soil pollution with the analyzed PAHs did not inhibit the growth and development of the maize roots or shoots. The greatest amount of the biomass was obtained in the object where the soil was characterized by an elevated content of PAHs. The value of the tolerance coefficient in the objects where the stressor was introduced was above one, which indicates the lack of any effect of soil pollution on plant biomassyield.

2. In comparison with the unpolluted objects, significantly higher Cu content of Cu and the amounts of this element up taken up by the shoots were determined in the objects in where DCM and PAHs were introduced to the soil. The values of the coefficient of maize shoot biomass pollution with Zn and Cu were distinctly higher in the objects where the the soil was polluted with PAHs in comparison with the values obtained in the object where only NPK medium was introduced to the soil. A similar dependency pertained to the translocation coefficient.

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MICROELEMENTS IN SOILS AND IN LEAVES OF SELECTED TREE SPECIES IN AN INDUSTRIAL URBAN AREA

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Abstract

Some adverse effects of human activities on the environment are observed with the development of civilization. Urban centers have a well-developed industrial infrastructure and transportation, hence the presence of numerous and diverse sources of environmental pollution.

The aim of the study was to determine the general content of Mn, Zn, Cu, Cr, and Ni in soils and in leaves of some tree species grown in Stalowa Wola, and to determine the trends in their spatial diversity.

Soil samples (from the layers 0-25 cm and 26-50 cm deep) were collected at sites located 0.5, 1, 2, 3, 4, 5 and 15 km (control) from the center point (the main gate of the Stalowa Wola Steelworks) to the east, west, north and south. Plant material (tree leaves) was collected from the vicinity of the soil sampling points.

The soils were characterized by strong acidity and the prevalence of the sand fraction: on average above 70% in both layers (0-25 cm and 26-50 cm). The content of microelements in soils from the town was within a wide range: $8 \div 1778 \text{ mg Mn kg}^{-1}$, $2.1 \div 1090 \text{ mg Zn kg}^{-1}$, $0.9 \div 61.5 \text{ mg Cu kg}^{-1}$, $3.8 \div 77.1 \text{ mg Cr kg}^{-1}$, $1.2 \div 56.5 \text{ mg Ni kg}^{-1}$. The arithmetic mean content of all the investigated metals in soils from Stalowa Wola was more than twice as high (more than 3-fold in the case of Cr and Ni) as the corresponding average values for the checkpoints. There were no clear patterns in the distribution of the analyzed elements depending on the depth. Except for one sample (the Zn content), no excess of heavy metals in the analyzed soils above the permissible levels was recorded. However, according to the IUNG criteria, only 50% of soil samples were characterized by the natural content of heavy metals (0 degree of contamination).

Leaves of some tree species were analyzed. The highest accumulation of manganese, an average of $640 \text{ mg kg}^{-1} \text{ DM}$, was found in oak leaves, while the lowest, an average of $4.3 \text{ mg kg}^{-1} \text{ DM}$, occurred in leaves of black locust. Linden leaves were distinguished by the tendency to accumulate chromium (mean 12.3 mg kg^{-1}), while ash leaves had a particularly high zinc content (mean 98.0 mg kg^{-1}). Concentrations of the analyzed elements in oak tree leaves from the north-south transect did not present any regular trend in the spatial differentiation.

Key words: microelements, environmental contamination, urban environment.

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MIKROELEMENTY W GLEBACH I LIŚCIACH WYBRANYCH GATUNKÓW DRZEW Z TERENU MIEJSKO-PRZEMYSŁOWEGO

Abstrakt

Wraz z rozwojem cywilizacji obserwuje się nasilenie negatywnego wpływu działalności człowieka na środowisko. Ośrodki miejskie charakteryzują się rozwiniętą infrastrukturą przemysłową i komunikacyjną, a więc występowaniem licznych i różnorodnych źródeł zanieczyszczeń środowiska. Celem badań było określenie ogólnej zawartości Mn, Zn, Cu, Cr i Ni w glebie i liściach wybranych gatunków drzew z terenu miasta Stalowa Wola i ustalenie tendencji w ich przestrzennym zróżnicowaniu.

Próby glebowe (z głębokości 0-25 cm i 26-50 cm) pobierano w miejscach zlokalizowanych w odległości 0,5; 1; 2; 3; 4; 5 i 15 km (kontrola) od punktu centralnego (rejonu bramy głównej Huty Stalowa Wola) w kierunku wschodnim, zachodnim, północnym i południowym. W okolicy punktów poboru prób glebowych pobierano także próby materiału roślinnego – liście drzew. Ogólną zawartość badanych metali oznaczono metodą płomieniową AAS po uprzedniej mineralizacji mikrofalowej próbek glebowych w mieszaninie stężonych kwasów HCl i HNO₃ (3:1), a materiału roślinnego – w stężonym HNO₃ z dodatkiem 30% H₂O₂.

Badane gleby charakteryzowały się silnym zakwaszeniem oraz zdecydowaną przewagą frakcji piasku, średnio w przypadku każdej z badanych warstw (0-25 cm i 26-50 cm) powyżej 70%. Stwierdzono, że zawartość mikroelementów mieściła się w szerokim zakresie: 8÷1778 mg Mn kg⁻¹, 2,1÷1090 mg Zn kg⁻¹, 0,9÷61,5 mg Cu kg⁻¹, 3,8÷77,1 mg Cr kg⁻¹, 1,2÷56,5 mg Ni kg⁻¹. Średnia arytmetyczna zawartość wszystkich badanych metali w glebach z terenu miasta Stalowa Wola była ponad 2-krotnie (w przypadku Cr i Ni ponad 3-krotnie) wyższa, w porównaniu z kontrolą. Nie stwierdzono wyraźnych prawidłowości w rozmieszczeniu badanych pierwiastków w zależności od głębokości. Nie stwierdzono przekroczenia dopuszczalnej zawartości analizowanych metali ciężkich w badanych glebach (z wyjątkiem Zn w 1 próbie glebowej). Jednakże uwzględniając kryteria IUNG, jedynie w 50% badanych prób glebowych stwierdzono ich naturalną zawartość (0 stopień zanieczyszczenia). Wykazano, że spośród badanych liści niektórych gatunków drzew, liście dębu akumulowały najwięcej manganu – średnio 640 mg kg⁻¹ s.m., najmniej – liście robinii akacjowej, średnio 4,3 mg kg⁻¹ s.m. Liście lipy miały tendencję do kumulacji chromu (średnio 12,3 mg kg⁻¹), a liście jesionu zawierały szczególnie dużo cynku (średnio 98,0 mg kg⁻¹). Analizując zawartość badanych pierwiastków w liściach dębu z transektu północ-południe, nie stwierdzono jednoznacznej tendencji w przestrzennym zróżnicowaniu wyników.

Słowa kluczowe: mikroelementy, zanieczyszczenie środowiska, środowisko miejskie.

INTRODUCTION

Some adverse effects of human activities on the environment are observed together with the development of civilization (PACYNA, PACYNA 2001, NORRA, STÜBEN 2003, GAN-LIN et al. 2005, WÓJCIKOWSKA-KAPUSTA, MAKUCH 2007, SZYŃKOWSKA et al. 2009). The civilization growth is intrinsically linked to growing human populations in urban agglomerations. So far, urban areas and populations have shown an upward trend. According to the data of the Central Statistical Office (GUS 2011), nearly 61% of the total Polish population in 2009 lived in 897 cities and towns, which covered just 1,572,402 hectares of land, i.e. about 5% of Poland. Urban centers are particularly vulnerable to environmental contamination. They are characterized by well-de-

veloped industrial infrastructure and transportation, hence the presence of numerous and diverse sources of environmental pollution. Particularly large contribution to the soil pollution is attributed to mining, metallurgy, metal industry, fuel combustion and transportation (KABATA-PENDIAS, PENDIAS 1993, PACYNA, PACYNA 2001, SALVAGIO MANTA et al. 2002, SZERSZEŃ et al. 2004, SZYŃKOWSKA et al. 2009, WEI, YANG 2010). Different types of activities generate different emission of heavy metals into the environment, e.g. road transport emits mainly Pb, Cd, Ni, Cu, Zn, fuel combustion is the major source of V, Ni, Hg, Se, Sn, and metallurgy is responsible for remarkable emission of As, Cd, Cu, and Zn (KARCZEWSKA 2003, MACIEJEWSKA, OCIEPA 2003). Green areas (parks, gardens, woodlands, allotments and gardens) are that part of an urban landscape which, among various functions, plays the role of natural filters of contaminants, able to accumulate significant amounts of metallic elements (KARCZEWSKA 2003, MACIEJEWSKA, OCIEPA 2003, PICZAK et al. 2003, BARAN et al. 2007, STRZYSZCZ, RACHWAŁ 2010). An elevated content of metallic elements, including Cu, Zn, Ni, and Cr, has been recorded in many urban soils worldwide (KOVAL'CHUK et al. 2002, SALVAGIO MANTA et al. 2002, NORRA, STÜBEN 2003, GAN-LIN et al. 2005, NORRA et al. 2006, KRMČOWA et al. 2009, WEI, YANG 2010).

The Subcarpathian Province (*województwo podkarpackie*) is a typical agricultural region. Stalowa Wola is one of the major urban and industrial centers in this part of Poland. The aim of the study was to determine the general content of Mn, Zn, Cu, Cr, and Ni in soils and leaves of some tree species grown in Stalowa Wola, and to determine the trends in their spatial diversity.

MATERIAL AND METHODS

The analyzed area lies in Stalowa Wola (Figure 1), a town located in the Sandomierz Basin, on the edge of Sandomierz Forest. It covers an area of 8240 hectares and has a population of over 70,000. Geologically, the area lies within the Carpathian Foredeep filled with sediments of the Miocene Sea. Stalowa Wola is a relatively young town. Plans to create the Central Industrial District were approved in 1937, and the construction of the Southern Works started in 1938, in a village called Pławo. Some of the factory buildings and a residential area named Stalowa Wola were founded before the outbreak of World War Two. In 1945, Stalowa Wola became a chartered town and in 1948 the Southern Works located in the southern part of the town were named Huta Stalowa Wola. The steel and defense industry continued to develop throughout the early 1990s. Today, after the restructuring, the former steelworks function as several companies, mainly representing the steel and metal industry. The second largest facility is the Stalowa Wola Power Plant, localized in the eastern part of the town. The total number of

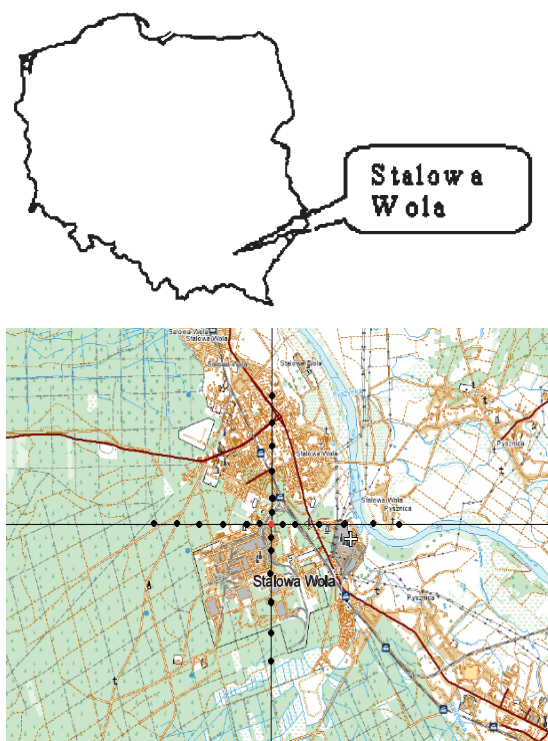


Fig. 1. Localization of the studied area (black dots indicate the sampling points)

business entities registered in the town is about 550. In the Subcarpathian Province, Stalowa Wola is a town with high emission of pollutants into the environment. Annual emissions of particulate pollutants in 2003-2005 was in the range of 523-714 million tons (*Strategy ...* 2007). In July 2009, soil samples were collected from the woodland area located at distances of 0.5, 1, 2, 3, 4, 5 km from the center point (the main gate of Huta Stalowa Wola) towards the east, west, north and south. Three soil samples were collected from each point (separately from the depth of 0-25 cm and 26-50 cm), which were subject to prepare a total of 50 average laboratory samples.

For comparison, soil samples were also collected at checkpoints (K) situated outside the town, in each of the geographical directions within 15 km from the center (8 samples).

Soil samples were air-dried, ground and passed through a sieve with 2 mm mesh. Tree leaf samples were air-dried and milled in a cutter mill. Analyses of soil material were carried out by means of the following methods (OSTROWSKA et al. 1991):

- grain size composition of soil – by the Bouyoucos-Casagrande aerometric method with modifications by Prószyński;

- soil reaction in 1 mol KCl dm⁻³ solution (pH_{KCl}) – by potentiometry;
- hydrolytic acidity (H_h) – by the Kappen method;
- total exchangeable bases (TEB) and cation exchange capacity (CEC) – by the Kappen method;
- base saturation percentage (BS) – from the formula BS=TEB·100/CEC (%);
- organic carbon content in soil (Corg.) – by the Tiurin method;
- total contents of Mn, Zn, Cu, Cr, and Ni – by atomic absorption spectrophotometry (AAS, Hitachi Z-2000) in acetylene-air flame after microwaving the soil samples (Mars Xtraction, CEM) in a mixture of concentrated HCl and HNO₃ at the 3:1 ratio (GRZEBISZ et al. 2002).

The plant material (tree leaves) was collected within a radius of 100 m around the sampling points from the following tree species: maple (*Acer* sp.), oak (*Quercus* sp.), black locust (*Robinia pseudoacacia*), lofty ash (*Fraxinus excelsior*), and linden (*Tilia* sp.). About 1 kg FW was gathered. In order to determine the effect of winds on the pollution of the town with microelements, their content in oak leaves was analyzed along the north-south transect, chosen because of the prevailing southerly and west-southerly winds in the region. The content of microelements in the plant material was determined by the FAAS method after digestion in a microwave system in concentrated HNO₃ with 30% H₂O₂ (BUSZEWSKI et al. 2000).

The results were statistically processed applying the Excell and Statistica software. The means, medians and coefficients of variation were determined. Correlation analysis was performed for total trace elements and some properties of examined soil to determine the relationships between these parameters. Differences of the metal content in soil between Stalowa Wola and the control area were demonstrated by Anova. The means for the different sites were separated by the Tukey's test, using the significance level of $\alpha=0.05$.

RESULTS AND DISCUSSION

Considering the particle size distribution, most of the soils were characterized by the predominant sand fraction: on average above 70% in each of the two layers. The average content of the clay fraction (below 0.002 mm) was 9%, in the 0-25 cm layer and 7% in the deeper layer. The soils contained an average of 18.2 g kg⁻¹ (0-25 cm layer) and 11.3 g kg⁻¹ (26-50 cm layer) of organic carbon. In terms of reaction, most of the soils were classified as acidic or very acidic (Table 1). They varied in the cation exchange capacity (5-48 cmol (+) kg⁻¹).

The arithmetic means of the content of the tested microelements in soils in Stalowa Wola can be put in the following order: Mn (283 mg kg⁻¹ from 0-25 cm depth and 305 mg kg⁻¹ from 26-50 cm depth), Zn (115.3 and 56.6 mg

Table1

General statistical parameters for properties of soil from Stalowa Wola

Parameter		pH _{KCL}	<i>H_h</i>	<i>TEB</i>	<i>CEC</i>	<i>BS</i>	Corg.	<0.002 mm
			(cmol(+) kg ⁻¹)				(g kg ⁻¹)	(%)
City soils								
Arithmetic mean	A	5.20	3.77	18.5	22.3	81.0	18.2	9
	B	5.08	3.26	19.1	22.4	82.8	11.3	7
Geometric mean	A	5.06	2.58	14.4	19.4	74.1	13.7	5
	B	4.94	2.31	15.9	20.4	77.9	10.3	4
Median	A	5.30	2.00	15.8	17.6	84.5	17.5	5
	B	5.04	2.10	16.2	19.6	88.8	10.6	6
Minimum	A	2.89	0.50	0.5	5.0	4.3	0.5	1
	B	2.95	0.40	0.8	8.6	9.3	2.5	1
Maximum	A	7.59	11.20	42.5	48.0	97.4	36.3	30
	B	7.91	10.50	39.5	45.1	98.0	21.7	20
Coefficient of variation (%)	A	23.3	86.6	60.5	54.0	23.9	569.1	100.6
	B	24.4	79.1	52.8	45.6	22.9	395.6	73.7
Control								
Arithmetic mean	A	4.00	4.45	21.8	26.2	82.1	18.4	10
	B	3.95	3.93	28.6	32.5	87.4	9.0	7
Geometric mean	A	3.99	4.41	20.9	25.5	82.0	16.8	9
	B	4.94	2.31	15.9	20.4	77.9	10.3	4
Median	A	4.02	4.15	18.6	23.5	80.9	14.3	10
	B	3.97	4.25	28.6	32.1	89.3	9.5	6
Minimum	A	3.78	4.00	16.8	20.8	77.4	11.9	4
	B	3.25	2.20	18.8	22.4	80.7	4.4	3
Maximum	A	4.17	5.50	33.1	37.1	89.2	33.2	16
	B	4.59	5.00	38.5	43.5	90.2	12.7	14
Coefficient of variation (%)	A	4.1	16.0	34.9	28.2	6.1	541.9	51.9
	B	14.0	31.1	36.9	34.5	5.2	382.7	66.0

A – soil from 0-25 cm depth
 B – soil from 26-50 cm depth

kg⁻¹ respectively), Cr (27.6 and 24.8 mg kg⁻¹), Cu (15.7 and 16.2 mg kg⁻¹), Ni (13.7 and 13.3 mg kg⁻¹). HAJDUK et al. (2012), when examining soils in the vicinity of the Stalowa Wola Power Plant, recorded slightly lower arithmetic means for Cu and Zn, but higher ones for chromium, which may indicate slightly different participation of emitters in the contamination of soils. The metal concentrations determined in the present study are much lower than those reported for the soils of the Upper and Lower Silesia (KARCZEWSKA

2003, SZERSZEŃ et al. 2004, BARAN et al. 2007, STRZYSZCZ, RACHWAŁ 2010), and in some urban soils worldwide (KOVAL'CHUK et al. 2002, SALVAGIO MANTA et al. 2002, BURGOS et al. 2008, KRMČOWA et al. 2009).

The fact that the geometric means of the content of all the metals were significantly lower than the arithmetic mean values, and than the median values of Mn, Zn, and Cu seems to be very interesting (Table 2). This indicates an upward skew distribution of the results for elements in the

Table 2

General statistical parameters for microelement content in soils from Stalowa Wola (mg kg⁻¹)

Parameter		Mn	Zn	Cu	Cr	Ni
City soils (<i>n</i> =25)						
Arithmetic mean	A	283 <i>a</i> *	115.3 <i>c</i>	15.7 <i>e</i>	27.6 <i>g</i>	13.7 <i>i</i>
	B	305 <i>b</i>	56.6 <i>d</i>	16.2 <i>f</i>	24.8 <i>h</i>	13.3 <i>j</i>
Geometric mean	A	179	59.0	10.5	20.9	9.4
	B	144	37.6	9.8	16.5	8.5
Median	A	221	64.1	12.6	20.3	9.1
	B	189	53.8	13.8	17.6	8.2
Minimum	A	25	2.1	0.9	6.2	1.5
	B	9	2.1	0.9	3.8	1.2
Maximum	A	1231	1091.2	46.8	77.1	42.1
	B	1778	189.7	61.5	74.7	56.5
Coefficient of variation (%)	A	98.3	183.2	82.1	80.2	82.5
	B	123.3	81.7	93.8	91.6	97.3
Control (<i>n</i> =4)						
Arithmetic mean	A	126 <i>a</i>	33.1 <i>c</i>	7.9 <i>e</i>	9.0 <i>g</i>	3.8 <i>i</i>
	B	113 <i>b</i>	17.5 <i>d</i>	6.0 <i>f</i>	7.4 <i>h</i>	3.5 <i>j</i>
Geometric mean	A	117	26.1	6.0	8.5	3.5
	B	144	37.6	9.8	16.5	8.5
Median	A	116	24.0	4.4	8.4	3.1
	B	109	15.9	4.9	7.6	3.5
Minimum	A	73	12.6	3.5	5.3	2.4
	B	90	12.4	2.6	6.2	1.8
Maximum	A	198	71.8	19.1	14.1	6.8
	B	143	25.9	11.8	7.9	5.0
Coefficient of variation (%)	A	43.6	81.9	95.7	42.4	52.2
	B	19.9	33.4	67.2	10.8	43.6

A – soil from 0-25 cm depth

B – soil from 26-50 cm depth

* The same letters indicate arithmetic means not showing statistically significant differences according to Tukey's test ($\alpha=0.05$) (homogeneous groups).

soil, which in turn may suggest the presence of a number of points with abnormally high levels of pollutants (due to anthropogenic contamination). This is confirmed by the high values of variation coefficients (Table 2). The range of Zn, Cu, and Ni levels in the examined soils falls within the brackets expected for the humus layer of arable soils in the Subcarpathian Province (TERELAK, TUJAKA 2003), or in whole Poland (OLESZEK et al. 2003). Similarly, the geometric mean values for these elements are higher than those reported by OLESZEK et al. (2003), TERELAK, TUJAKA (2003).

Arithmetic means of all the tested metals in soils from Stalowa Wola were more than double (even over 3-fold higher for Cr and Ni) the corresponding averages for the control points, but the analysis of variance and the Tukey's test did not verify statistical significance of these differences ($\alpha=0.05$). This finding, as well as the variation coefficients significantly lower in soils from the checkpoints than from the whole study area, also implies that anthropogenic sources emitting these elements were present in Stalowa Wola.

In the light of the current legal regulations in Poland (*Regulation...* 2002), the permissible levels of heavy metals were not exceeded in soils of Stalowa Wola, except for the Zn content in a single sample. However, according to the criteria set by the IUNG (KABATA-PENDIAS et al. 1993), only 50% of the soil samples had the natural content of heavy metals (0 level of contamination), 31% showed an elevated content (I level), another 15.5% of soil samples had weak contamination (II level), and 1.7% represented moderate (III level) and strong pollution (IV level) – Figure 2.

The tendency to accumulate metallic elements in upper soil levels has been observed by many authors (MACIEJEWSKA, OCIEPA 2003, BIENIEK, ŁACHACZ 2003, BURGOS et al. 2008, JAWORSKA, DĄBKOWSKA-NASKRĘT 2012, MEDYŃSKA-JURASZEK, KABAŁA 2012, LOURENÇO et al. 2014). The studied microelements from the checkpoints localized south and (except for Mn) east of Stalowa

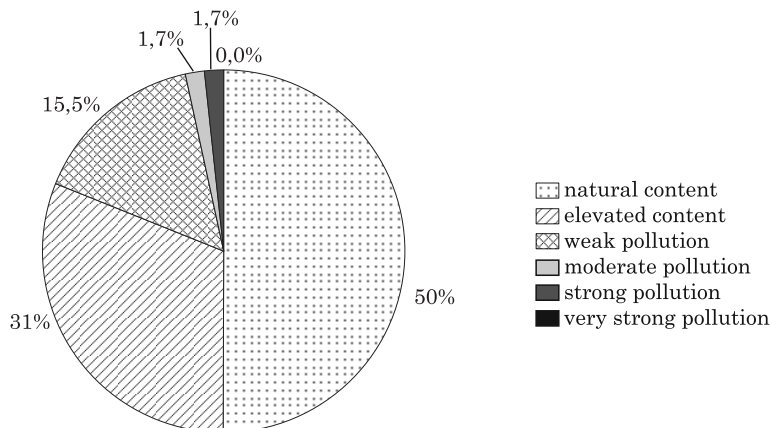


Fig. 2. Total percentage of soils in particular categories of copper, zinc, nickel, and chromium contamination according to the IUNG criteria (KABATA-PENDIAS et al. 1993)

Wola were usually subject to accumulation in the surface soil layer. Such trends were not observed at other points in the other directions. No regular distribution of the concentrations of the elements in the vertical pattern of urban soils was found, partly because of their specific characteristics such as mixing the layers or addition of sand and gravel during engineering works (NORRA et al. 2006). Another reason could be the significant reduction in industrial emissions into the environment after 1990, which – combined with strong soil acidification and high mobility of metal compounds – could have led to their substantial transfer in the soil profile. MERCIK et al. (2003) or KIEPUL and GEDIGA (2009), based on their own and other authors' research, emphasized the increased solubility of microelements in acidic environment. Recently, these authors have stressed that in soil weakly contaminated with zinc, the metal accumulates in the surface soil layer and does not penetrate down the profile, although its transport depends on the flow of groundwater. It should be noted that the analyzed area is covered predominantly by sandy soils, through which water percolates easily. Furthermore, the presence of different man-made facilities, ongoing industrial activities, but also the share of green areas including forests, are likely to interfere with the direction of winds, causing variable immissions of dust settling on the soil surface. Especially tall trees in urban areas may stop falling dust particles containing heavy metals.

The results also indicate that the average content of Mn, Zn, Cu, Cr, and Ni was higher in soils from the north than from the south direction (Table 3). Also, the corresponding average values for soils from the eastern part of the city were higher than those from the western side (Table 3). It should be emphasized that southerly and south-westerly winds prevail in this part of Poland (SUCHY 2008), and such a distribution of the analyzed metals in soils can be explained by the increased accumulation of contaminants due to large point sources of emissions in the town (the Stalowa Wola Steelworks, Stalowa Wola Power Plant) on the leeward side.

Taking into account the content of microelements in soils at different distances from the “central point” (the main gate of the Stalowa Wola Steelworks), quantities of all metals higher than average can be observed in points 3 and 4 km from the centre in the east-west transect. However, as observed a steady increase was observed along the perpendicular direction in the content of all elements in soils from of the southern outskirts of the town up to the central point of the transect, after which their concentrations significantly decreased in the northern direction, finally showing an upward tendency, peaking at 3 km from the center. Urban soils are typically characterized by a higher concentration of anthropogenic contaminants in the city centre (ALEXANDROVSKAYA, ALEXANDROVSKIY 2000, GĄSIÓREK, NIEMYSKA-ŁUKASZUK 2004, BIELIŃSKA et al. 2011) and that trend was noted in our study. PENG et al. (2013) examined soils of Beijing (China) and found that the content of heavy metals correlated not only with the distance from the center, but also

Table 3

The content of microelements in soils of Stalowa Wola at different distances from the central point – the main gate of the Stalowa Wola Steelwork

Distance (km)	Mn		Zn		Cu		Cr		Ni	
	A	B	A	B	A	B	A	B	A	B
0	463	441	213.2	70.0	39.7	15.3	75.3	27.6	22.1	7.6
East										
0.5	221	359	33.8	57.9	12.4	17.9	21.2	28.8	12.9	20.9
1	115	88	62.9	35.0	10.6	8.5	14.1	10.9	6.5	6.2
2	92	41	25.9	13.8	12.6	3.8	13.5	7.1	5.6	3.8
3	297	568	110.6	100.0	35.3	21.2	77.1	71.5	34.4	15.9
4	327	519	150.0	150.0	46.8	42.4	76.8	72.9	31.5	36.5
5	25	25	2.1	2.1	1.8	0.9	6.2	4.1	1.5	3.8
15 K	198	113	71.8	25.9	19.1	5.9	14.1	7.6	6.8	1.8
West										
0.5	403	83	62.9	57.4	14.4	8.5	25.0	15.0	17.6	7.4
1	45	16	70.9	21.8	2.9	1.5	10.0	6.2	2.9	2.1
2	59	122	25.3	37.1	5.0	8.5	9.7	10.0	4.7	3.8
3	728	635	68.5	70.6	23.5	24.4	34.7	38.5	24.7	24.7
4	72	246	22.4	189.7	4.1	28.8	8.8	62.6	4.4	21.2
5	469	476	55.6	53.8	16.2	15.3	26.2	25.6	22.1	20.9
15 K	95	90	12.6	15.3	5.0	3.8	9.7	7.6	2.4	5.0
North										
0.5	101	97	39.1	38.8	9.7	8.8	13.5	15.9	3.5	8.2
1	147	21	1091.2	10.9	19.7	61.5	20.3	3.8	11.8	3.8
2	482	500	65.9	59.4	21.2	21.5	33.5	34.1	22.9	24.1
3	1230	1780	105.9	132.4	38.2	46.8	53.2	74.7	42.1	56.5
4	403	462	32.4	42.6	7.9	11.5	12.9	19.7	6.2	10.3
5	601	593	65.6	54.4	17.9	17.9	37.6	29.7	21.8	20.0
15 K	138	143	31.8	16.5	3.8	11.8	5.3	7.9	3.2	4.4
South										
0.5	245	189	183.8	72.1	24.7	15.3	47.4	18.5	17.6	10.6
1	248	194	198.5	71.8	13.2	5.9	22.6	17.6	7.1	2.4
2	46	9	64.1	5.6	0.9	13.8	18.2	3.8	9.1	1.2
3	58	24	23.8	8.8	6.2	1.5	11.8	4.7	2.9	5.6
4	101	64	93.8	44.7	3.8	2.4	11.2	7.9	4.7	3.5
5	89	64	14.7	13.8	3.5	1.5	7.9	8.2	2.9	2.6
15 K	73	104	16.2	12.4	3.5	2.6	7.1	6.2	2.8	2.6

K – control point

A – soil from 0-25 cm depth

B – soil from 26-50 cm depth

with the population density, density of the road grid and sometimes even the age of buildings. However, simple correlation coefficients between the distance from the central point and the content of microelements in soils calculated in the current study were statistically insignificant ($\alpha=0.05$).

The calculated simple correlation coefficients (Table 4) indicate the presence of statistically significant positive dependencies between the content of Mn, Cu, Cr, and Ni in the soil versus the content of <0.02 mm fraction and the amount of organic carbon. The content of Cr was also dependent on soil pH_{KCl} . In contrast, the zinc content in the topsoil was not statistically associated with any of the analyzed soil characteristics, which may indicate its

Table 4
Simple correlation coefficients among microelements in soils of Stalowa Wola
and soil properties ($n=25$)

Parameter		Mn	Zn	Cu	Cr	Ni
<0.02 mm	A	0.8952***	ns	0.4480*	ns	0.6807***
	B	0.8317***	ns	0.4104*	0.5094**	0.7875***
<0.002 mm	A	0.7375***	ns	0.4070*	ns	0.5448**
	B	ns	ns	ns	ns	ns
pH_{KCl}	A	ns	ns	ns	0.4282*	ns
	B	ns	0.4365*	ns	0.4839*	ns
Hydrolytic acidity	A	ns	ns	ns	ns	ns
	B	ns	ns	ns	ns	ns
TEB	A	-0.6694***	ns	-0.5538**	-0.4675*	-0.7004***
	B	-0.5112**	ns	-0.4185*	-0.4288*	-0.5828**
CEC	A	-0.5413**	ns	-0.5333**	-0.4708*	-0.6560***
	B	-0.4555*	ns	-0.4460*	-0.4629*	-0.5714**
BS	A	-0.8117***	ns	ns	ns	-0.4798*
	B	-0.7000***	ns	ns	ns	-0.5433**
Corg.	A	0.4226*	ns	0.5277**	0.4770*	0.4086*
	B	0.5002*	ns	0.4736*	ns	0.4749*
Mn	A		ns	0.6282***	0.5346**	0.8185***
	B		0.5726**	0.5307**	0.7548***	0.9173***
Zn	A			ns	ns	ns
	B			0.5061**	0.8984***	0.7211***
Cu	A				0.9587***	0.8930***
	B				0.5812**	0.6281***
Cr	A					0.8585***
	B					0.8485***

* significant at $\alpha=0.05$, ** significant at $\alpha=0.01$, ***significant at $\alpha=0.001$

A – soil from 0-25 cm depth, B – soil from 26-50 cm depth, ns – insignificant correlation

anthropogenic origin. SALVAGIO MANTA et al. (2002) did not find statistically significant correlation coefficients between heavy metals and pH, CEC or organic matter in the soils of Palermo. These authors, after performing the hierarchical cluster analysis, distinguished two groups of metals: I – strongly correlating with the content of the alumino-silicate phase due to their natural content, and II – not having this characteristics, including metals from anthropogenic sources (Cu, Zn, Sb, Pb, Hg).

Plants have different adaptability to soil pollution with heavy metals (ZACARIAS et al. 2012). Among the examined tree species, oak leaves presented the highest accumulation of manganese (an average of $640 \text{ mg kg}^{-1} \text{ DM}$), while black locust leaves accumulated the least of this metal (an average of $4.3 \text{ mg kg}^{-1} \text{ DM}$) – Figure 3. Linden leaves tended to accumulate chromium (mean 12.3 mg kg^{-1}), whereas leaves of ash trees had a particularly high zinc content (mean 98.0 mg kg^{-1}). Average amounts of Cu and Ni determined in the plant material were similar, although ash leaves were characterized by the smallest quantities of these elements.

The arithmetic mean content of the microelements in the dry matter of oak

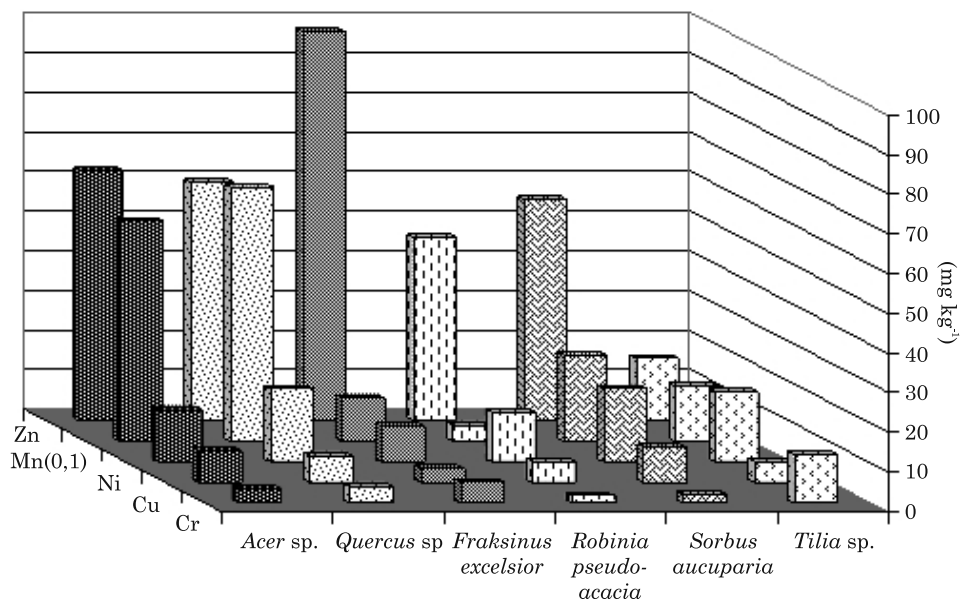


Fig. 3. Content of microelements in tree leaves from Stalowa Wola

leaves are following: Mn – 640, Zn – 60, Cu – 6.9, Cr – 4.3, Ni – 18.4 $\text{mg kg}^{-1} \text{ DM}$ (Table 5). Variability coefficients for the manganese and chromium content were much larger than for the other elements. The medians of the Mn and Cr content were approximately 2-fold lower than the arithmetic average values. Analysis of the distribution of the analyzed elements in oak leaves from the north-south transect revealed that high levels of Ni were found in

Table 5

General statistical parameters for microelement content in oak leaves from Stalowa Wola
(mg kg^{-1} DM)

Parameter	Mn	Zn	Cu	Cr	Ni
Arithmetic mean	640	60	6.9	4.3	18
Geometric mean	410	58	6.6	2.3	17
Median	332	64	7.1	1.8	18
Minimum	62	30	3.6	0.8	5
Maximum	2145	98	10.6	19.6	32
Coefficient of variable (%)	104	26	33	129	35

samples collected from the surroundings of the main gate of Huta Stalowa Wola, and that leaves accumulated smaller amounts of this metal at a larger distance (Figure 4). This did not correspond with the content of Ni in soils of the northern part of the studied area (Table 3), where high amounts of Ni

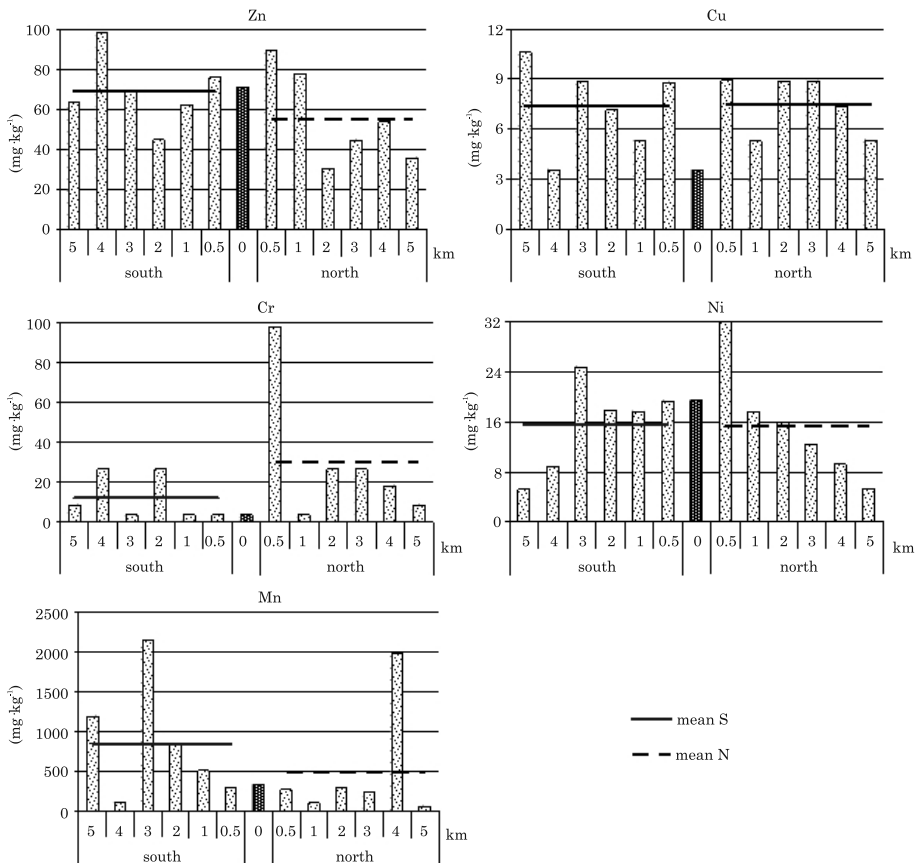


Fig. 4. Content of microelements in oak leaves from Stalowa Wola in the north-south transect at different distances from the central point

and other metals were observed in a 3 km zone. In general, higher amounts of Mn and Zn were observed in leaves from the southern part of the city. It was only chromium that was determined in higher amounts, in the northern part of the town, which was in accordance with the direction of prevailing winds. It can be assumed that the atmospheric pollution with microelements due to large sources emission (such as Huta Stalowa Wola) did not influence significantly the diversity of the content of elements in oak leaves, although it might have contributed in the past to their remarkable accumulation in soils of the northern part of the town. It should be emphasized that the emission of pollutants into the environment from industrial sources in Stalowa Wola was substantially reduced in the 1990s, but compared to other towns in the same region, high levels of dust and gas emissions into the atmosphere of the town continue to be recorded (*Strategy ...* 2007, SUCHY 2008).

Tree leaves are often used to diagnose the environmental pollution (SAWIDIS et al. 2001, PICZAK et al. 2003, ROSSELLI et al. 2003, CICEK, KOPARAL 2004, MADEJON et al. 2004, AKSOY, DEMIREZEN 2006, MASSADEH et al. 2009, SAMECKA-CYMERMAN et al. 2009). The chemical composition of leaves also indicates whether they can be composted. MADEJ et al. 2010). The averaged values of the Cu, Zn, Cr content in tree leaves from Warsaw reported by the above authors were similar to those obtained in the present study, although values for Ni were slightly lower. HAJDUK et al. (2006) found significantly lower concentrations of nickel in leaves of trees located south of Rzeszów and in its surroundings, while noticing higher nickel amounts in the soil. SAMECKA-CYMERMAN et al. (2009), when examining the content of heavy metals in leaves of black locust from the Oleśnica environs, found zinc and copper levels similar to those obtained in the present studies, but a lower content of manganese, nickel, and chromium. CICEK, KOPARAL (2004) determined much higher amounts of Cr, Ni, Cu, and Zn in leaves of various tree species growing near a thermal power plant, but the soils were much more abundant of the analyzed elements. However, high content of microelements in soil does not always translate into their higher accumulation in tree leaves, for example AKSOY and DEMIREZEN (2006) reported amounts of nickel and chromium in leaves of ash tree from Turkey similar to those found in leaves of trees from Stalowa Wola, with a much higher content of these metals in soils. It should be emphasized that the accumulation of metallic elements in plant tissues depends not only on the plant species, organ and plant growth phase, but also on their content in soils (in particular available forms), on soil properties (including pH, redox potential, organic matter content, and other elements contents), and on air pollution (KABATA-PENDIAS, PENDIAS 1993, SAWIDIS et al. 2001, KARCZEWSKA 2003, KABATA-PENDIAS 2004, MADEJON et al. 2004, MASSADEH et al. 2009, MADEJ et al. 2010). The low pH of the soils in the studied area is worth mentioning, as it means better bioavailability of the analyzed micronutrients. However, except for Mn, there was no statistically significant correlation between the content of microelements in tree leaves and the pH or acidity of soils. The content of all the analyzed elements in

tree leaves was not statistically significantly ($\alpha=0.05$) related to their content in the soil, soil texture, total exchangeable bases, cation exchange capacity, or (except for Zn in the 26-50 cm layer) the organic carbon content.

Absence of any statistically significant relationship between the content of the metals in leaves of trees and their content in soil, as well as the soil's physicochemical properties in part may be due to the existence of active barriers protecting plants against the uptake of toxic quantities of various substances from soil (RASCIO, NAVARI-IZZO 2011), but it can also be directly caused by the leaf uptake of elements from atmospheric dust settling on their surface. This seems to be particularly important in the case of nickel and chromium, as the levels of these two metals in leaves of the tree species exceeded values reported by KABATA-PENDIAS and PENDIAS (1993) as normal (physiological) for plants with moderate sensitivity (Ni 5 mg kg⁻¹, 0.5 mg Cr kg⁻¹). Furthermore, 30% of leaf samples contained more than 300 mg Mn kg⁻¹, which is the upper limit given by those authors as typical for plants.

It should be emphasized that Ni, Cr, and Mn were classified by KABATA-PENDIAS and PENDIAS (1993) as elements with a low degree of accumulation at plants, for which the accumulation index, calculated from the ratio of the content of these elements in the plant to their concentrations in the soil, is in the range of 0.01-0.1. In contrast, calculations made on the basis of our results indicate that the plant-soil accumulation ratios exceeded the upper limit of the range in over 90% of cases for Mn and Ni and about 60% for Cr. In general, the highest values of these coefficients were recorded in the southern part of Stalowa Wola, where industrial facilities, including Huta Stalowa Wola, are located. It can be concluded that although the permissible thresholds of heavy metals in soil were not exceeded, there is a risk of environmental pollution (especially with nickel, chromium, and manganese) as a result of particulate emissions, rich in these elements, from a large number of industrial facilities in the southern part of the town (including the steel industry and metallurgy). These dusts are largely retained by woody vegetation, and some microelements are accumulated in leaves.

CONCLUSIONS

1. Light soils prevailed in the area of Stalowa Wola city, with an average content of the fraction < 0.002 mm: 9% in the 0-25 cm layer and 7% in 26-50 cm layer. They were usually strongly acidic soils with varied cation exchange capacity (5-48 cmol(+) kg⁻¹).

2. The content of microelements in the urban soils was in a wide range: 8 to 1778 mg Mn kg⁻¹, 2.1÷1090 mg Zn kg⁻¹, 0.9÷61.5 mg Cu kg⁻¹, 3, 8÷77.1 mg Cr kg⁻¹, 1.2÷56.5 mg Ni kg⁻¹. The arithmetic means of the content of all the investigated metals in soils from Stalowa Wola were over two-fold (more than 3-fold for Cr and Ni) higher than the corresponding average values for

the control points, which may indicate a large impact of anthropogenic factors on soil contamination. There were no clear patterns in the distribution of the analyzed elements depending on the depth.

3. The levels of the analyzed heavy metals in the soils did not exceed the legal norms in Poland (except for Zn in one soil sample). However, according to the IUNG criteria, only 50% of soil samples were characterized by the natural content of heavy metals (0 degree of contamination).

4. Most Mn, Zn, Cu, and Ni were found in soils in the northern part of the town (Cr in the eastern part). The lowest average content of these elements was in the western and southern parts of the town. Such a distribution of elements in urban soils may be the result of atmospheric transport of pollutants by the southerly and south-westerly winds, prevailing in this region.

5. The zinc content in the topsoil was not correlated with any of the analyzed soil characteristics. Some positive correlations were recorded between concentrations of Mn, Cu, Cr, and Ni in the soil and the content of <0.02 mm fraction and the amount of organic carbon; statistically significant albeit negative correlation coefficients were obtained between the soil content of the analyzed elements and the total exchangeable bases and cation exchange capacity.

6. It was found that oak leaves showed the highest manganese accumulation (average 640 mg kg⁻¹ DM) while the least manganese accumulated in leaves of black locust (average 4.3 mg kg⁻¹ DM). Linden leaves tended to accumulate chromium (mean 12.3 mg kg⁻¹), while leaves of ash had a particularly high content of zinc (average 98.0 mg kg⁻¹). When analyzing the content of the elements in oak leaves from the north-south transect, no regular trends in the spatial differentiation of the results were observed.

7. Numerous cases of exceeding the natural concentration limits for Cr, Ni, and Mn in leaves of the tree species and high values of the soil-plant accumulation coefficients indicate the risk of pollution by these metals as a result of particulate pollutant emissions from industrial plants localized in the southern part of the town.

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EFFECT OF SEWAGE SLUDGE APPLICATION ON THE GROWTH, YIELD AND CHEMICAL COMPOSITION OF PRAIRIE CORDGRASS (*SPARTINA PECTINATA* LINK.)

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Abstract

Prairie cordgrass is a perennial C4 grass characterized by high adaptability to different habitats. The highest yields are obtained on sites with sufficient water supply, on soils of coarse or medium texture and with a wide range of the C:N ratio. Sewage sludge may be the source of nutrients for the crops grown for renewable energy purposes.

The objective of the research was to evaluate the effect of sewage sludge fertilization on the growth and yield of prairie cordgrass and the content of crude ash, macronutrients and heavy metals in the plant biomass in the first three years of cultivation.

The experiments were conducted at the Pawlowice Agricultural Experimental Station near Wrocław, in 2008-2010. Their aim was to investigate the impact of differentiated sewage sludge applications and harvest periods on the growth, yield formation and chemical composition of prairie cordgrass. Two-factor experiments in a split-plot design were conducted. The first factor was a dose of sewage sludge: 0, 1.4, 2.8 and 4.2 t ha⁻¹ DM, corresponding to the nitrogen fertilization of 0, 50, 100, 150 kg ha⁻¹, and the second factor was the harvest time: autumn and winter.

The sewage sludge dose of 2.8 t ha⁻¹ DM, compared to the control, significantly increased the plant height by 4%, one shoot mass by 11%, the number of shoots per 1 m² by 14% and the dry mass yield by 22%, but it showed no influence on the yield structure formation and the content of macronutrients and heavy metals. The postponement of the harvesting time from autumn to winter resulted in drier plants, lower dry mass yield and less crude ash, K, Mg and S.

Key words: prairie cordgrass, sewage sludge, harvest period, crude ash, macronutrients, heavy metals.

WPLYW NAWOŻENIA OSADEM ŚCIEKOWYM NA ROZWÓJ, PLONOWANIE I SKŁAD CHEMICZNY SPARTINY PRERIOWEJ (*SPARTINA PECTINATA* LINK.)

Abstrakt

Spartina preriowa jest wieloletnią trawą o cyklu fotosyntezy C-4, charakteryzującą się dużą zdolnością adaptacyjną do różnych siedlisk. Najlepiej plonuje na stanowiskach o dostatecznej ilości wody, na glebie o gruboziarnistej lub średniej teksturze i szerokim stosunku C do N. Źródłem składników pokarmowych dla roślin uprawianych na cele energetyczne mogą być osady ściekowe.

Celem badań było określenie wpływu nawożenia osadem ściekowym na rozwój i plonowanie spartiny preriowej oraz kształtowanie zawartości popiołu surowego, makroskładników i metali ciężkich w biomase w pierwszych trzech latach uprawy.

W latach 2008-2010 w Rolniczym Zakładzie Doświadczalnym Pawłowice k. Wrocławia badano wpływ zróżnicowanego nawożenia osadami ściekowymi i terminu zbioru na rozwój, kształtowanie plonu i skład chemiczny spartiny preriowej. Dwuczynnikowe doświadczenie założono w układzie „split-plot”. Pierwszym czynnikiem była dawka osadu ściekowego: 0, 1,4, 2,8 i 4,2 t ha⁻¹ s.m., co odpowiadało nawożeniu azotem: 0, 50, 100, 150 kg ha⁻¹, a drugim – terminy zbioru: jesienny i zimowy.

Osad ściekowy w dawce 2,8 t ha⁻¹ s.m., w porównaniu z kontrolą, zwiększał istotnie wysokość roślin o 4%, masę 1 pędu o 11%, liczbę pędów na 1 m² o 14% oraz plon suchej masy o 22%, natomiast nie miał wpływu na kształtowanie struktury plonu oraz zawartość makroskładników i metali ciężkich. Przesunięcie terminu zbioru z jesiennego na zimowy skutkowało mniejszym uwilgotnieniem roślin, wpłynęło na zmniejszenie plonu suchej masy oraz zawartości popiołu surowego, K, Mg i S.

Słowa kluczowe: spartina preriowa, osad ściekowy, termin zbioru, popiół surowy, makroskładniki, metale ciężkie.

INTRODUCTION

Spartina pectinata (prairie cordgrass) is a perennial C4 grass. It forms loose clumps of 0.90 to 2.40 m tall plants with densely attached leaves up to 0.80-0.90 m long and 3 to 13 mm wide. The grass develops a strong and deep root system of 1.5 to 3 m long, which allows the plant to endure short-term droughts. In mid-summer, the plants grow 0.30 m long panicle-type inflorescences. Generative shoots are hollow (MAJTKOWSKI 2006). The number of culms per 1 m² ranges from 100 to 300 (FRASER, KINDSCHER 2005), exceeding 1000 culms under favourable conditions (BOE et al. 2009, POTTER et al. 1995).

Prairie cordgrass shows high adaptability, growing well in different habitats, which supports its widespread occurrence, from Newfoundland to Canada. Although it flourishes in *hygrophilous plant communities* and on dry prairies (MAJTKOWSKI 2006), the highest yield is obtained on sites with adequate quantities of water, on coarse or medium texture soils with a wide range of C:N ratio. The grass is well adapted to soils with variable, seasonably excessively high water tables (BONILLA-WARDFORD, ZEDLER 2002, MOBBERLEY 1956, WHO et al. 2012). An optimum soil pH is 6.0 to 8.5, but *Spartina*

pectinata also tolerates strong acidification. Its performance is poor on arid lands and heavy soils, but it tolerates grass burning (JOHNSON, KNAPP 1993). It performs well under adverse conditions that occur in the vicinity of nitrogen production plants and coal mining dumps (MAJTKOWSKI 1997). WEISS et al. (2006) showed a high content of heavy metals, particularly Pb, in aerial parts of prairie cordgrass, which indicates its suitability for degraded land reclamation. The thermal needs of prairie cordgrass are not high. During winter dormancy, it withstands temperatures down to -39°C, and during the growing season it requires at least 110 days with temperatures above 0°C. Thus, it grows well throughout Poland and has gained great popularity in the Nordic countries. Despite its hardiness, prairie grass does not tolerate shady sites. The minimum depth of the root system is about 0.5 m. Its fertilization requirements are moderate.

In Poland, *Spartina pectinata* produces seeds, but because their germination capacity declines rapidly, vegetative propagation through division of the rhizomes in the spring is recommended (MAJTKOWSKI 2006). Prior to establishing a plantation, special attention should be paid to the careful weeding of a field, preferably several times. Usually, the weed control is necessary not only in the first, but also in the second year of cultivation. With the vigorous root system, prairie cordgrass does not require deep aeration of the soil. Organic fertilization is unnecessary. In Poland, prairie cordgrass is planted at 1.8-3.0 m inter-row and 0.5-0.75 m intra-row spacing (MAJTKOWSKI 2006). However, a higher density of planting, i.e. 0.9 x 0.35 m, is possible (BOE, LEE 2007).

Sewage sludge is a source of organic matter, which in the process of mineralization releases mainly N and P, and therefore may be recognized as an organic fertilizer with a strong, favourable impact on the balance of organic matter in the soil (DUSZA et al. 2009, ŻUKOWSKA et al. 2002). Types of sewage sludge differ in terms of fertilization values since their effect may be similar to that of mineral fertilizers, and sometimes close to manure (GAMBUŚ, WIECZOREK 2003). The presence of heavy metals in sewage sludge poses a risk of soil contamination with these elements and their uptake by plants (SZATANIK-KLOC 2004). This is why sewage sludge may be a source of nutrients for crops not intended for human or animal consumption (MERICIK et al. 2003). SIENKIEWICZ and CZARNECKA (2012) demonstrated that an application of 280 Mg ha⁻¹ of sewage sludge to alkaline soil elevated the content of available forms of Cu, Zn and Mn. However, it did not pose a threat to the environment. Instead, it improved the nutrition of plants with these elements. Fertilization with high doses of sewage sludge has a long-lasting effect on the microbial activity of the soil accumulation layer (JONIEC, FURCZAK 2008). In the fourth year after the application of sewage sludge, evident stimulation of protease activity and nitrification process as well as slight inhibition of ammonification were observed (JONIEC, FURCZAK 2008).

In the second year of cultivation, *Spartina pectinata* favourably responds

to fertilization with mineral fertilizers in doses of 60-110 kg ha⁻¹ N. The amounts of P and K should be determined based on soil fertility (MAJTKOWSKI 2006). The N: P: K ratio is recommended to be 1:0.4:0.5. Some studies have indicated that fertilization may be discontinued for a few years after establishing a plantation (POTTER et al. 1995).

KALEMBASA and MALINOWSKA (2010) found that fertilization of sugar miscanthus with 30 t ha⁻¹ of sewage sludge, regardless of the age of a plantation, significantly increased the content of heavy metals in the plant biomass compared to the control, and their uptake can be arranged in the following series with decreasing values: Zn > Cd > Ni > Cu > Pb > Cr > Co. The ash and sulphur content in the biomass of prairie cordgrass is influenced by nitrogen fertilization and the number of cuts (KOWALCZYK-JUŚKO 2010). Plants harvested once in winter contain less ash and sulphur compared to those harvested twice. The ash of prairie cordgrass contains a prevalent quantity silica and smaller amounts of Ca, K, S, P and Mg oxides (KOWALCZYK-JUŚKO 2009).

The objective of our study was to assess the effect of fertilization with sewage sludge on the growth and yield of prairie cordgrass and amounts of crude ash, macronutrients and heavy metals in the plant biomass in the first three years of cultivation.

MATERIAL AND METHODS

The field experiment was set up in a two-factor split-plot design. The first factor was a dose of sewage sludge: 0, 1.4, 2.8 and 4.2 t ha⁻¹ DM, which corresponded to nitrogen fertilization doses of: 0, 50, 100, 150 kg ha⁻¹. The second factor was the harvest period: autumn and winter. The area of the plots was 3.5 m².

The experiment was established in alluvial soil, very light, on loose sand and sandy gravel, classified to the soil category V in the Polish soil valuation system. The content of available macroelements in the soil (mg kg⁻¹) was as follows: P (86-111) – high to very high; K (65-81) – low; Mg (27-45) – very low to low. The soil pH in 1 M KCl in the first and second year of the experiment was acidic (4.9-5.4) and slightly acidic (5.7-5.9) in the third year.

The experiment was set up on 11 June 2008 and prairie cordgrass was planted in the following spacing: 0.35x0.35 m (82 thousand seedlings ha⁻¹). *Spartina pectinata* was planted on the site where white mustard had been previously grown for seeds. During the growing season, the height of the stand was measured at two-week intervals. Prior to the harvest, the number of fully developed shoots (culms) per 1 m² was counted. Major morphological features were recorded on 10 shoots, i.e.: diameter at 0.10 m above the ground, the height of the plants before harvest and the number of leaves on

the culm. In addition, the content of water in the leaves, in the culm and in the whole plant was measured by oven-drying method. Moreover, the yield structure of the aboveground parts of the plant and the yield of fresh and dry mass were determined.

The chemical analysis, performed in the laboratories of the Crop Production Department and Plant Nutrition Department at the Wrocław University of Environmental and Life Sciences, focused on:

- dry mass (oven-drying) – drying shredded plants for 4 hrs at the temp. of 105°C;
- crude ash – burning the plant material in an electric furnace at the temp. of 600°C;
- general nitrogen – the Kjeldahl's method;
- P and Mg – calorimetric method;
- K and Ca – flame photometry;
- S – the Barclay's method;
- Mn, Fe, Cu, Cd, Pb, Ni and Zn – flame atomic absorption spectroscopy (Varian 220 FS AA Spectrometer).

The chemical analysis of the sewage sludge was typical for that from municipal sewage treatment plants (Table 1). The sewage sludge was applied on 05.06.2008, 31.03.2009 and 25.03.2010. Apart from the doses of nitrogen introduced to the soil, according to the methodology, the sewage sludge contributed macro- and microelements as well as toxic elements to plants, whose weight was proportional to the dose of sewage sludge (Table 2). The data were statistically analyzed and their significance assessed at $\alpha=0.05$. The AWA programme was used for data computation (BARTKOWIAK 1978).

Table 1

Chemical composition of sewage sludge from the Wrocław Sewage Treatment Plant, Janówek

Specifi- cation	Dry mass	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn	Ni	Pb	Cd
Unit	(g kg ⁻¹ DM)						(mg kg ⁻¹ DM)						
Content	200	35.7	12.9	1.8	19.5	7.0	412	2385	464	1260	47.6	36.4	2.46

Table 2

Macro- and microelements and heavy metals in a sewage sludge dose

DM sewage sludge dose (t ha ⁻¹)	(kg ha ⁻¹)									(g ha ⁻¹)		
	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn	Ni	Pb	Cd
1.4	50	18.1	2.5	27.1	9.8	0.57	3.33	0.64	1.76	66.6	51.0	3.4
2.8	100	36.2	5.0	54.3	19.6	1.15	6.66	1.29	3.53	133.3	102.0	6.8
4.2	150	54.3	7.5	81.5	29.3	1.72	10.00	1.93	5.29	199.9	153.0	10.4

RESULTS AND DISCUSSION

Despite the precipitation deficit during the planting (Table 3), no loss of plants occurred in the first year of cultivation, and some plants started blooming on July 30. The plant growth was inhibited on 15.11.2008. In the second and third year of the study, *Spartina pectinata* began to grow on 2.04.2009, and 6.04.2010, respectively. The precipitation during the growing season was higher than the long-term average. The onset of flowering was on 23 July 2009 and 25 July 2010, and the growth inhibition took place at the end of November.

Table 3

Weather conditions in 2008-2010
Data collected at the Wrocław-Swojec weather station

Month	Temperature (°C)				Precipitation (mm)			
	2008	2009	2010	average 1976-2005	2008	2009	2010	average 1976-2005
I	2.9	-2.3	-5.9	-1.0	56.7	34.6	40.6	31.9
II	3.9	0.2	-1.1	0.1	20.4	46.8	11.0	26.7
III	4.6	4.6	4.2	3.7	33.0	48.3	44.9	31.7
IV	8.9	12.0	9.3	8.3	87.1	30.9	45.4	30.5
V	14.3	14.2	12.7	14.1	37.3	67.6	140.7	51.3
VI	18.8	15.8	17.9	16.9	36.5	141.7	32.9	59.5
VII	19.8	19.5	21.4	18.7	65.6	134.2	78.6	78.9
VIII	18.8	19.3	18.9	17.9	94.0	53.5	61.5	61.7
IX	13.2	15.4	12.5	13.3	27.9	12.0	134.1	45.3
X	9.6	7.9	7.0	9.2	41.1	76.0	5.7	32.3
XI	6.1	6.8	6.5	3.7	29.6	32.5	66.4	36.6
XII	2.1	-0.4	5.3	0.2	20.5	51.9	63.2	37.4
Average temperature or total precipitation	10.3	9.42	8.2	8.8	549.7	730.0	772.6	527.4

In the first year of the study, prairie cordgrass did not lodge. The measurements of the standing crop's height showed that plants from the sites fertilized with sewage sludge at 4.2 t ha⁻¹ DM were the highest (Figure 1).

Regardless of the dose of sewage sludge, *Spartina pectinata* was lodged in the second and third year of cultivation. In the second year, the beginning of lodging took place in mid-August, and at the end of July in the third year (Figures 2, 3).

The experimental factors did not affect the diameter of culms or the

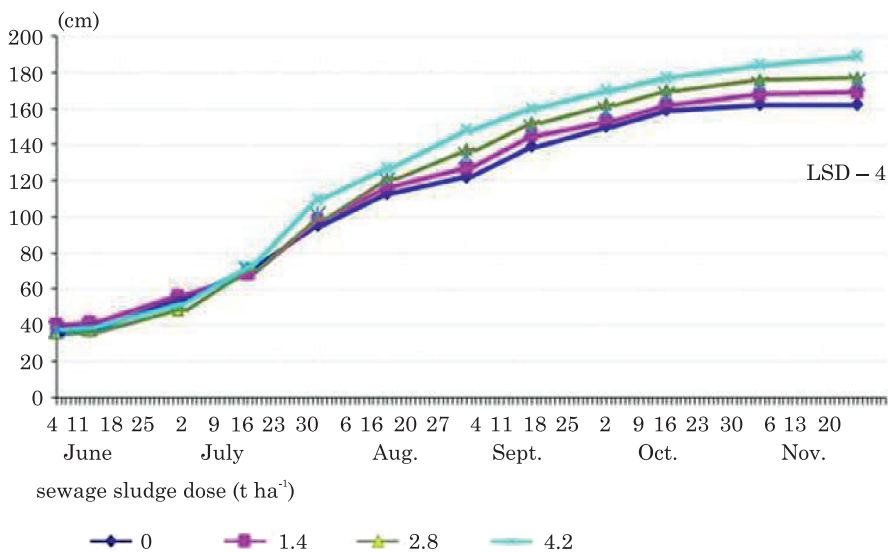


Fig. 1. Prairie cordgrass growth dynamics – 2008

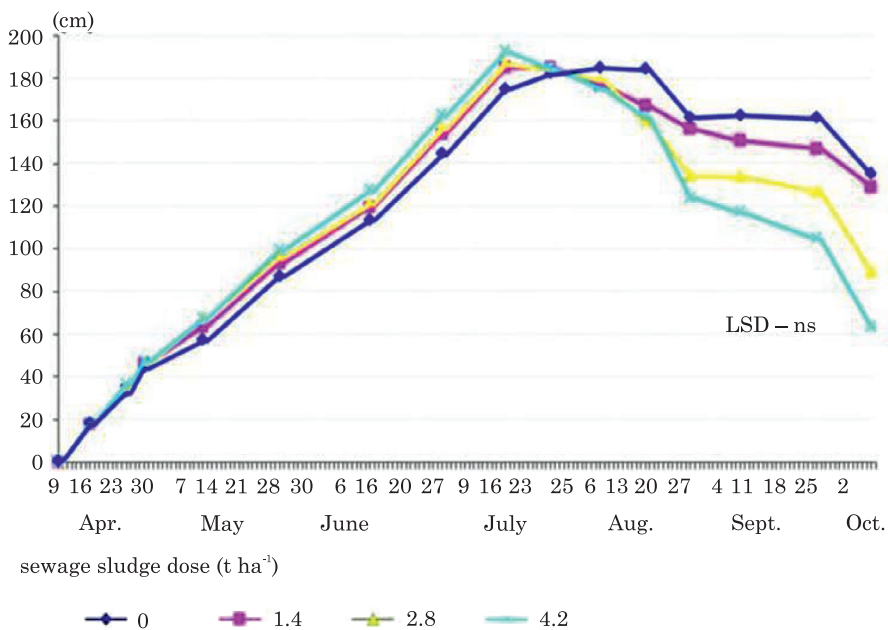


Fig. 2. Prairie cordgrass growth dynamics – 2009

ns – non-significant difference

number of leaves on one shoot (Table 4). As compared to the control, the dose of 4.2 t ha^{-1} DM of sewage sludge increased the height of shoots by 6%. The winter harvest, as compared to the autumn one, reduced the plant height by

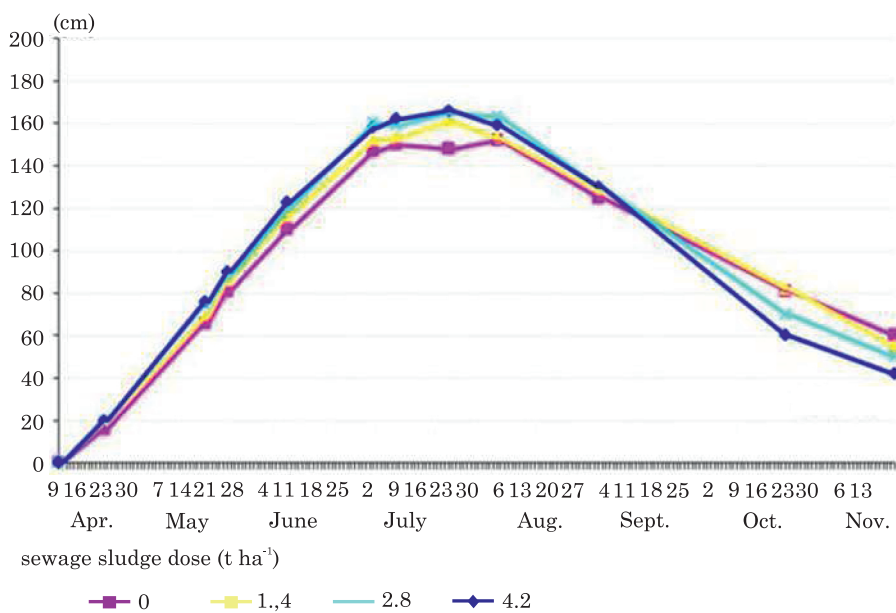


Fig. 3. Prairie cordgrass growth dynamics - 2010
ns - non-significant difference

Table 4
Morphological traits of prairie cordgrass shoots (means by factors and years)

DM sewage sludge dose (t ha ⁻¹)	Harvest time	Years	Diameter (mm)	Height before harvesting (cm)	No. of leaves per shoot
0			5.3	182	6.9
1.4			5.1	183	7.0
2.8			5.5	189	7.2
4.2			5.4	194	7.1
LSD ($\alpha=0.05$)			ns	6	ns
	autumn		5.4	193	7.1
	winter		5.2	181	7.0
LSD ($\alpha=0.05$)			ns	4	ns
		2008	5.5	169	6.7
		2009	5.2	182	7.3
		2010	5.2	209	7.3
LSD ($\alpha=0.05$)			0.2	5	0.2

6% due to breaking of the plant tops. The weather conditions in the years of the experiment combined with the plantation age had a significant impact on morphological traits. The plants of 3-year old, compared to one-year old, were higher by 24%, produced more leaves, and the diameter of the culm was smaller because of light competing. This favored earlier plant lodging. The findings of KOWALCZYK-JUŚKO and KOŚCIK (2004) indicate that the height of prairie cordgrass in the fifth year of vegetation depended largely on the soil compactness. Plants on loamy and sandy soils were shorter than those on clay soils, where the height reached 1.69 m. On the other hand, MAJTKOWSKI (1998) found that *Spartina pectinata* reached the height of 2.30 m in the fourth year of growth. BOE et al. (2009) showed that the number of leaves on a shoot was from 4.4 to 4.8, and it was higher than that in plants reproduced generatively.

The experimental factors affected the culm's dry weight and its water content. The dose of 2.8 t ha⁻¹ DM sewage sludge, compared to the control, increased the weight of culms by 8%, and that of a culm with leaves by 12% (Table 5). The winter harvest, compared to the autumn one, significantly reduced the weight of the culm leaves, the weight of culms and the weight of the entire aboveground part. The water content in the leaves, culms and culms with leaves increased as a result of the sewage sludge fertilization. The highest amount of water was observed in leafless culms, then in leafy culms and the lowest one was in leaves, which were losing water most quickly. A delay in harvesting resulted in lower moistening of leaves, culms and

Table 5

Prairie cordgrass dry mass structure and shoot moist content (means by factors and years)

DM sewage sludge dose (t ha ⁻¹)	Harvest time	Years	1 shoot dry mass (g)			Percentage in shoot mass (%)		Water content (g kg ⁻¹)		
			leaves	culms	total	leaves	culms	leave- se	culms	above gro- und mass
0			5.4	4.9	10.3	52.1	47.9	296	508	406
1.4			5.8	4.8	10.6	55.2	44.8	314	506	420
2.8			6.2	5.3	11.5	52.8	47.2	334	543	444
4.2			6.4	5.2	11.6	54.7	45.3	344	555	459
LSD ($\alpha=0.05$)			ns	0.4	0.9	ns	ns	ns	24	26
	autumn		6.6	5.5	12.1	54.1	45.9	373	544	466
	winter		5.3	4.6	9.9	53.3	46.7	271	513	400
LSD ($\alpha=0.05$)			0.6	0.3	0.7	ns	ns	ns	12	1.1
		2008	5.1	4.0	9.1	56.0	44.0	429	610	530
		2009	6.3	6.1	12.4	50.9	49.1	234	481	360
		2010	6.3	5.1	11.4	54.3	45.7	302	494	406
LSD ($\alpha=0.05$)			0.6	0.4	0.8	2.9	2.9	2.9	21	22

culms with leaves. The two-year old plants, compared to one-year old, had a higher culm weight and a lower water content.

The number of shoots per 1 m² increased in the subsequent years of the study and under the influence of sewage sludge fertilization (Table 6). In the third year of the experiment, as compared to the first year, the number of culms per 1 m² was over six-fold higher. The compactness of prairie cordgrass in the first years of cultivation depended primarily on the number of seedlings per 1 m², which are most often planted at 1.80-3.00 m inter-row and 0.50-0.75 m intra-row spacing. With the spacing as above, the plant population per one hectare ranges from 4444 to 11111. However, a higher density planting is possible, up to 0.25x0.25 m spacing between crops (POTTER et al. 1995), 0.90x0.35 m (BOE, LEE 2007) or 1.00x0.50 m (KOWALCZYK-JUŠKO 2013). In our study, the plant stance was 0.35x0.35 m, which corresponded to 82 thousand plants per 1 ha. A large number of plants meant strong compactness of the stand in the second year of the experiment and a total lack of weeds, which in the absence of registered chemical plant protection products becomes an important issue on the plantations with a small number of plants. POTTER et al. (1995) demonstrated that in the natural conditions of East England, the number of shoots of prairie cordgrass per 1 m² steadily increased for four consecutive years, but in the 5th and 6th year it significantly dropped.

The yield of fresh and dry mass of prairie cordgrass was closely related to the number of shoots per 1 m² and affected by the factors examined and plantation age (Table 6). As compared to the control, the dose of 2.8 t ha⁻¹ of sewage sludge increased one shoot weight by 11%, the number of shoots per 1 m² by 14% and the dry mass yield by 22%. The dry mass yield of the

Table 6
Number of shoots per 1 m² and biomass yield (means by factors and years)

DM sewage sludge dose (t ha ⁻¹)	Harvest time	Years	Number of shoots per 1 m ² before harvesting (pcs)	Yield (t ha ⁻¹)	
				fresh mass	dry mass
0			119	13.1	7.74
1.4			133	14.4	8.58
2.8			136	17.1	9.48
4.2			138	17.9	9.83
LSD ($\alpha=0.05$)			13	1.7	0.85
	autumn		132	19.1	9.89
	winter		131	12.2	7.93
LSD ($\alpha=0.05$)			ns	1.4	0.66
		2008	40	5.6	3.60
		2009	96	13.0	8.00
		2010	259	28.3	15.13
LSD ($\alpha=0.05$)			11	1.50	0.74

winter harvest, compared to that in autumn, was lower by 21% as a result of leaf breaking and leaf and culm rotting. KOWALCZYK-JUŚKO and KOŚCIK (2004) showed that the yield of prairie cordgrass in the fifth year of cultivation depended on the soil type and ranged from 10 to 16 t ha⁻¹. In other studies, KOWALCZYK-JUŚKO (2013) found that the dry mass yield of one-year old prairie cordgrass did not exceed 1 t ha⁻¹, and that in the third year was 11 t ha⁻¹, with 180 shoots per 1 m². Based on the research conducted in South Dakota, BOE et al. (2009) found that the biomass yield of prairie cordgrass depended on the type of propagation. The yield of plants grown from seeds was 11.7 t ha⁻¹, and the yield of vegetatively propagated plants reached 14.6 t ha⁻¹. It should be stressed that the rhizome mass and that of underground shoots to a depth of 0.25 m in generatively reproduced plants was 20.6 t ha⁻¹ and that in vegetatively propagated plants was 21.8 t ha⁻¹. Under the conditions of East England, POTTER et al. (1995) found a wide variability in the prairie cordgrass yields between years of the study and a low correlation between the number of shoots and dry mass yield.

The doses of sewage sludge had no significant effect on the crude ash content and macronutrients in biomass of prairie cordgrass (Table 7). KOWALCZYK-JUŚKO (2010) showed that the crude ash content in biomass of prairie cordgrass was on average 48 g kg⁻¹ and that of sulphur was 1.1 g kg⁻¹. In our study, the content of these components was 37.7 and 1.84 g kg⁻¹, respectively.

Table 7

Crude ash and macronutrients (g kg⁻¹) in prairie cordgrass dry mass
(means by factors and years)

DM sewage sludge dose (t ha ⁻¹)	Harvest time	Years	Crude ash	N	P	K	Ca	Mg	S
0			38.0	2.73	0.45	3.70	2.78	0.22	1.68
1.4			36.2	2.83	0.43	3.75	2.67	0.22	1.70
2.8			36.9	2.73	0.42	3.65	2.63	0.25	1.82
4.2			39.9	2.93	0.48	3.82	3.05	0.33	2.17
LSD ($\alpha=0.05$)			ns	ns	ns	ns	ns	ns	ns
	autumn		40.4	2.85	0.43	5.02	2.74	0.31	2.14
	winter		35.1	2.77	0.46	2.44	2.83	0.20	1.54
LSD ($\alpha=0.05$)			2.9	ns	ns	0.13	ns	0.07	0.27
		2008	43.0	3.09	0.39	3.80	2.94	0.24	2.26
		2009	35.6	2.68	0.44	3.81	2.68	0.19	1.56
		2010	34.7	2.66	0.51	3.58	2.74	0.34	1.70
LSD ($\alpha=0.05$)			3.5	ns	ns	0.17	ns	0.09	0.33

The winter harvest of prairie cordgrass, due to breaking, caused a decrease of the crude ash content by 13%, K by 51%, Mg by 35% and S by 28%. Similar results were obtained by KOTECKI et al. (2010) for *Miscanthus giganteus*, where the winter harvest decreased the dry mass yield by 9.5%, water content by 16.8 % and crude ash by 26%.

In the first year of study, *Spartina pectinata* contained more crude ash, K and S in the biomass than in the third year.

The accumulation of crude ash and macronutrients in prairie cordgrass biomass is a function of the yield and content of individual components. Therefore, in the absence of differences in the crude ash content and macronutrients, the uptake per area unit is proportional to the dry mass yield. As compared to the control, sewage sludge fertilization at 4.2 t ha⁻¹ DM raised the accumulation of crude ash and uptake of all the macronutrients (Table 8).

The biomass of prairie cordgrass harvested in winter, as compared to the autumn harvest, accumulated less macroelements: N – by 25%, P – by 13%, K – by 58%, Ca – by 12% Mg by 50%, S – by 33%. The crude ash accumulation was lower by 38%. The highest uptake of macronutrients and ash accumulation were recorded in the third year of the study.

Five heavy metals (Mn, Fe, Cu, Ni and Zn) are microelements necessary for the growth and development of plants, and two (Cd and Pb) are toxic elements. The doses of sewage sludge had no significant effect on the heavy

Table 8

Crude ash and macronutrients (kg ha⁻¹) in harvested prairie cordgrass
(means by factors and years)

DM sewage sludge dose (t ha ⁻¹)	Harvest time	Years	Crude ash	N	P	K	Ca	Mg	S
0			279	19.9	3.8	28.7	20.5	1.9	12.3
1.4			303	24.6	4.1	32.9	23.2	2.1	13.6
2.8			329	25.5	3.9	34.7	24.1	2.3	15.3
4.2			383	27.5	4.9	38.6	30.1	3.9	21.9
LSD ($\alpha=0.05$)			30	2.4	0.4	4.0	2.2	0.4	1.5
	autumn		375	27.8	4.5	47.6	25.9	3.4	18.9
	winter		272	20.9	3.9	19.9	22.9	1.7	12.7
LSD ($\alpha=0.05$)			24	1.9	0.4	3.2	1.8	0.3	1.2
		2008	157	11.1	1.4	14.5	10.6	0.9	8.5
		2009	288	21.5	3.5	30.9	21.5	1.6	12.8
		2010	526	40.6	7.7	55.8	41.3	5.3	26.1
LSD ($\alpha=0.05$)			46	3.6	0.6	5.8	3.4	0.5	2.3

Table 9

Heavy metals (mg kg⁻¹) in prairie cordgrass dry mass (means by factors and years)

DM sewage sludge dose (t ha ⁻¹)	Harvest time	Years	Mn	Fe	Cu	Cd	Pb	Ni	Zn
0			233	45.7	2.39	0.26	3.84	1.41	32.9
1.4			240	37.9	2.31	0.31	4.21	1.40	33.2
2.8			234	37.8	2.32	0.34	3.29	1.54	34.0
4.2			234	51.3	2.48	0.26	3.76	1.55	34.5
LSD ($\alpha=0.05$)			ns	ns	ns	ns	ns	ns	ns
	autumn		242	28.6	2.06	0.27	2.80	1.28	34.0
	winter		228	57.8	2.69	0.32	4.75	1.67	33.3
LSD ($\alpha=0.05$)			ns	12.6	0.15	ns	0.54	0.14	ns
		2008	268	48.9	3.22	0.45	2.51	1.78	47.5
		2009	201	35.6	1.91	0.22	2.58	1.17	24.8
		2010	236	45.0	1.99	0.21	6.24	1.47	28.7
LSD ($\alpha=0.05$)			43	ns	0.19	0.10	0.66	0.17	5.75

metal content in the dry mass of prairie cordgrass (Table 9). Similar results were obtained by OCIEPA (2013). The postponement of harvest from autumn to winter resulted in an increase in the content of Fe, Cu, Pb, and Ni in biomass. Prairie cordgrass contained more Mn, Fe, Cu, Cd, Ni and Zn in the first year of cultivation than in the third year.

KUZIEMSKA et al. (2011) showed that orchard grass grown on soils contaminated with Ni, limed and fertilized with sewage sludge, contained more Fe, Cu and Ni in the first year than in the third year. Fertilization of sugar miscanthus resulted in more heavy metals in the third year biomass than in the fourth year (KALEMBASA, MALINOWSKA 2010).

The uptake of heavy metals by *Spartina pectinata* was in the following series of decreasing values: Mn > Fe > Zn > Pb > Cu > Ni > Cd (Table 10). In the case of Mn, Fe, Pb, Ni and Zn, a correlation was observed between the doses of sewage sludge and the harvest periods. As compared to the control, on the plots fertilized with sewage sludge at 4.2 t ha⁻¹ DM, an increase was recorded in the uptake of Mn by 27%, Fe (45%), Cu (30%), Pb (26%), Ni (37%) and Zn (23%). The plants harvested in winter, compared to those collected in autumn, accumulated less Mn, Cu, Cd, Ni and Zn. In the third year of cultivation, the uptake of heavy metals by prairie cordgrass was the highest.

Table 10

Heavy metals (g ha⁻¹) in harvested prairie cordgrass

DM sewage sludge dose (t ha ⁻¹)	Harvest time	Years	Mn	Fe	Cu	Cd	Pb	Ni	Zn
0	autumn		2093	271	17.0	1.8	22.9	10.2	269
	winter		1605	456	16.8	1.5	47.9	11.4	210
1.4	autumn		2326	192	17.6	2.4	35.7	13.1	306
	winter		1591	404	17.5	2.0	51.7	11.4	207
2.8	autumn		2546	203	19.1	2.7	20.0	12.2	318
	winter		1654	389	19.0	2.4	51.7	13.0	253
4.2	autumn		3094	594	23.7	2.6	31.3	16.9	364
	winter		1618	460	20.1	1.9	57.9	12.7	226
LSD ($\alpha=0.05$)			349	47	ns	ns	5.8	1.9	38
Means by factors and years									
0			1849	363	16.9	1.7	35.4	10.8	240
1.4			1958	298	17.5	2.2	43.7	12.2	257
2.8			2100	296	19.1	2.6	35.9	12.6	286
4.2			2356	527	21.9	2.2	44.6	14.8	295
LSD ($\alpha=0.05$)			207	31	1.4	0.2	3.2	1.2	24
	autumn		2515	315	19.4	2.4	27.5	13.1	314
	winter		1617	427	18.4	2.0	52.3	12.1	224
LSD ($\alpha=0.05$)			175	25	ns	0.2	2.9	ns	21
		2008	948	165	11.3	1.6	9.2	6.2	170
		2009	1647	279	15.4	1.8	20.7	9.4	203
		2010	3603	668	29.9	3.1	89.9	22.2	434
LSD ($\alpha=0.05$)			345	59	2.5	0.3	5.4	2.0	39

CONCLUSIONS

1. Fertilization with sewage sludge at a dose of 2.8 t ha⁻¹ DM, compared to the control, significantly increased the plant height, weight of one shoot, number of shoots per 1 m² and dry mass yield, but had no significant effect on the yield structure and content of macroelements and heavy metals.

2. Winter harvest resulted in collecting drier plants, with less crude ash and inhibited uptake of all macroelements as well as Mn, Fe, Cd and Zn, which is favourable in the use of biomass for renewable energy.

3. Compared to the first year of cultivation, the third-year plants formed more shoots per 1 m², were higher and more leafy, the uptake of macroelements and heavy metals was higher, and the dry matter yield was over four-fold higher.

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EFFECT OF LAND USE ON THE CARBON AND NITROGEN FORMS IN HUMIC HORIZONS OF STAGNIC LUVISOLS

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Abstract

The aim of the study was to assess the effect of land use on the forms of carbon and nitrogen in humic horizons of Stagnic Luvisols located in the area of Sławieńska Plain (northern Poland). Soil samples were collected from six stands with different land uses located in the same soil complex: more than 100-year-old beech-oak forest (BOF), meadow (M), arable field (AF), fallow (F), secondary afforestation with 15-year-old birch trees (SAB) and secondary afforestation with 30-year-old alder trees (SAA). In every stand, soils were sampled in five replications and analyzed with standard methods used in soil science. The content of different forms of carbon and nitrogen was analyzed after sequential extraction in 0.25 mol KCl dm⁻³, 0.25 mol H₂SO₄ dm⁻³ and 2.5 mol H₂SO₄ dm⁻³ (BECHER, KALEMBASA 2011). Different land uses were reflected in the properties of humic horizon of the investigated soils. Particularly large differences were observed between the forest soils never used for agriculture, and arable or post-arable soils. Tillage caused the increase of the A horizon depth from 9.2 to 26.4–35.6 cm, and the increase of its volumetric density. The highest content of soil organic matter (SOM), total organic carbon (TOC) and total nitrogen (TN) as well as the highest TOC:TN ratio were observed in BOF stand. Statistically significant differences were observed between the stands in the content of carbon forms. Nonhydrolyzable carbon was dominant in the soils (55.98–68.11% of TOC), and dissolved organic carbon (DOC) had the smallest contribution (1.43–3.70% in TOC). In general, higher contribution of DOC in TOC was observed in arable and post-arable soils than in soils under forest. The content of mineral nitrogen (NO₃-N + NH₄-N) in the studied soils ranged from 0.028 to 0.053 g kg⁻¹, and NH₄-N dominated in the pool. The lowest concentration of mineral nitrogen was observed in arable soils, and significantly higher in the soils under forest and fallow. Easily hydrolyzable nitrogen (EHN), weakly hydrolyzable nitrogen (WHN) and nonhydrolyzable nitrogen (NHN) were the main forms of the element in the studied soils and their content was closely related to the content of SOM. The highest concentration of the forms was noticed in BOF stand. There were differences between the stands in DOC:DON, EHC:EHN, WHC:WHN and NHC:NHN ratios, which indicate varied biological activity of the soils under different uses.

Key words: carbon forms, nitrogen forms, Stagnic Luvisols, arable soils, forest soils, Sławieńska Plain.

WPLYW SPOSOBU UŻYTKOWANIA NA FORMY WĘGLA I AZOTU W POZIOMIE PRÓCHNICZNYM GLEB PŁOWYCH STAGNOGLEJOWYCH

Abstrakt

Celem pracy była ocena wpływu sposobu użytkowania na zawartość i udział form węgla i azotu w poziomie próchnicznym gleb płowych stagnoglejowych położonych na obszarze Równiny Sławieńskiej (północna Polska). Próbkę gleb pobrano z 6 stanowisk użytkowanych w różny sposób, położonych w obrębie tego samego kompleksu gleb. Stanowiska obejmowały: ponad 100-letni las bukowo-dębowy (BOF), łąkę (M), pole uprawne (AF), ugor (F), oraz 15-letnie zalesienie brzozą (SAB) i 30-letnie zalesienie olchą (SAA) na gruntach porolnych. Próbkę gleb na każdym stanowisku pobrano w 5 powtórzeniach. Oznaczono w nich właściwości fizyczne i chemiczne, stosując metody powszechnie stosowane w gleboznawstwie, oraz zawartość form węgla i azotu w roztworach po sekwencyjnej ekstrakcji z zastosowaniem 0,25 mol KCl dm⁻³, 0,25 mol H₂SO₄ dm⁻³ i 2,5 mol H₂SO₄ dm⁻³ (BECHER, KALEMBASA 2011). Stwierdzono istotny wpływ sposobu użytkowania gleb na właściwości poziomu próchnicznego badanych gleb. Szczególnie duże różnice notowano między nigdy nie użytkowanymi rolniczo glebami leśnymi a glebami rolnymi i porolnymi. Użytkowanie rolnicze gleb spowodowało wzrost miąższości poziomu A z ok. 9,2 cm do 26,4-35,6 cm oraz zwiększenie gęstości objętościowej w tym poziomie. W glebach leśnych obserwowano największą zawartość materii organicznej (SOM), węgla organicznego (TOC) i azotu ogółem (TN), a także najszerzy stosunek TOC: TN. Stwierdzono istotne statystycznie różnice między glebami na badanych stanowiskach pod względem zawartości różnych form węgla. Dominującą frakcją był węgiel nie ulegający hydrolizie (55,98-68,11% TOC), najmniejszy zaś udział miał rozpuszczalny węgiel organiczny (DOC) – 1,43-3,70% TOC. Na ogół większy udział DOC w TOC notowano w glebach rolnych i porolnych w porównaniu z glebami leśnymi. Zawartość azotu mineralnego (NO₃-N + NH₄-N) wynosiła 0,028-0,053 g kg⁻¹. W tej puli dominowała forma amonowa. Najniższe stężenie azotu mineralnego odnotowano w glebach ornych i istotnie wyższe w glebach leśnych oraz ugorowanych. W badanych glebach dominowały formy azotu łatwo hydrolizująca (EHN), trudno hydrolizująca (WHN) i niehydrolizująca (NHN), występując w zbliżonych proporcjach. Ich zawartość była pozytywnie skorelowana z zawartością materii organicznej, więc największe stężenie tych form występowało w glebach na stanowisku BOF. Stwierdzono różnice między stanowiskami pod względem stosunków DOC:DON, EHC:EHN, WHC:WHN i NHC:NHN, co świadczy o zróżnicowanej aktywności biologicznej gleb użytkowanych w różny sposób.

Słowa kluczowe: formy węgla, formy azotu, gleby płowe stagnoglejowe, gleby uprawne, gleby leśne, Równina Sławieńska.

INTRODUCTION

The quantity and quality of SOM varies across space and time and is a result of the influence of many environmental and anthropogenic factors (cf. CHERTOV, KOMAROV 1997, PULLEMAN et al. 2000, KUŽEL et al. 2001, KWIATKOWSKA, MACIEJEWSKA 2003, BEJGER, GOŁĘBOWSKA 2005, DZIADOWIEC, LUTOWSKA 2005, DZIĄMSKI et al. 2005, SZOMBATHOVA et al. 2005, TOBIAŠOVA et al. 2005, GONDEK 2007, GONET, DĘBSKA 2007, SIMANSKY 2007, ŁABAZ et al. 2011, KALEMBASA, BECHER 2012). SOM represent different forms – from fresh litter fall, through humic substances to easily soluble organic components, like amino acids or carbohydrates. Microorganisms play an important role in the processes of SOM transformation, and dissolved organic matter (DOM) is a product of their activity. The biological activity of soil and the intensity of DOM

production are strongly affected by soil properties (especially pH, moisture, the content of available forms of nutrients, pollution with heavy metals), weather conditions and other factors (HUE et al 1986, QUALLS, HAINES 1991, COTRUFO et al 1995, CHRIST, DAVID 1996, MURPHY et al. 2000, ANDERSON et al. 2002, SMOLANDER, KITUNEN 2002, FRÖBERG et al. 2005, DAWSON et al. 2008, SCHMIDT, GLASER 2011). Carbon, hydrogen, oxygen and nitrogen are the main components of SOM. The percentage of carbon in SOM is always about 50%, but the content of nitrogen is varied, which is reflected in differentiated C:N ratios. The type of land use is one of the most important factors affecting the content and contribution of different forms of carbon and nitrogen in the soil, as well as intensity of leaching of their labile forms (LIPIEC, STĘPNIEWSKI 1995, MURPHY et al 2000, JONCZAK, PARZYCH 2012).

The aim of the study was to assess the influence of land use type (from beech-oak forest, through meadow, arable field, fallow to secondary afforestation with birch and alder) on carbon and nitrogen forms in humic horizons of Stagnic Luvisols.

MATERIAL AND METHODS

The studies were conducted in northern Poland, in the area of Sławieńska Plain, near Stary Kraków (54°26'N; 16°36'E). The investigated Stagnic Luvisols were formed from glacial till of the Pomeranian phase of Baltic glaciation. Spatial variability in the character of land use type was observed within the investigated soil complex. Soil samples were collected in April 2012 from six stands with different land uses: more than 100-year-old beech-oak forest (BOF), meadow (M), arable field (AF), fallow (F), post-arable afforestation with 15-year-old birch (SAB) and post-arable afforestation with 30-year-old alder (SAA). In every stand, soil was sampled in five replications as monoliths from the A horizons and as volumetric samples using 100 cm³ steel rings from central parts of the horizon. The samples were dried at 40°C, passed through a 2 mm sieve and analyzed. The following soil properties were analyzed:

- texture with mixed pipette and sieve methods (textural fractions and groups were taken after classification of Polish Society of Soil Science 2008);
- volumetric density with gravimetric method using 100 cm³ steel rings;
- the content of water (% v/v) in volumetric samples with gravimetric method;
- pH potentiometrically (Elmetron CP-401) in water and 1 mol KCl dm⁻³ in a 1:2.5 ratio;
- the content of soil organic matter (SOM) as loss on ignition at 550°C;
- the content of total organic carbon (TOC) with the Tiurin method;

- the content of total nitrogen (TN) with the Kjeldahl method using a VELP UDK-127 distilling unit;
- the content of carbon and nitrogen forms after sequential extraction in 0.25 mol KCl dm⁻³, 0.25 mol H₂SO₄ dm⁻³ and 2.5 mol H₂SO₄ dm⁻³ (BECHER, KALEMBASA 2011).

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

- dissolved organic carbon (DOC) – after extraction with 0.25 mol KCl dm⁻³;
- easy hydrolyzable carbon (EHC) – after extraction with 0.25 mol H₂SO₄ dm⁻³;
- weakly hydrolyzable carbon (WHC) – after extraction with 2.5 mol H₂SO₄ dm⁻³;
- nonhydrolyzable carbon (NHC) – was calculated as TOC-DOC-EHC-WHC;
- nitrate nitrogen (NO₃-N) – after extraction with 0.25 mol KCl dm⁻³;
- ammonium nitrogen (NH₄-N) – after extraction with 0.25 mol KCl dm⁻³;
- dissolved organic nitrogen (DON) – the content of the Kjeldahl nitrogen after extraction with 0.25 mol KCl dm⁻³ – NH₄N;
- easy hydrolyzable nitrogen (EHN) – after extraction with 0.25 mol H₂SO₄ dm⁻³;
- weakly hydrolyzable nitrogen (WHN) – after extraction with 2.5 mol H₂SO₄ dm⁻³;
- nonhydrolyzable nitrogen (NHN) – was calculated as TN-NH₄-N – DON – EHN – WHN.

The content of carbon in the extracts was analyzed with the Tiurin method after the evaporation of a sample, NO₃-N – colorimetrically with sodium salicylate, NH₄-N – by distillation using a VELP UDK-127 distilling unit, and organic nitrogen – by the Kjeldahl's method using a VELP UDK-127 distilling unit.

Mean concentrations of analyzed components were calculated for each stand and differences between the stands were tested with the *T*-Student test. Correlation coefficients between the content of the forms of carbon and nitrogen and some properties of the soils were calculated.

RESULTS AND DISCUSSION

The results of many studies show that a land use type in one of the most important factors affecting soil properties, especially stocks and properties of SOM, and nutrient cycling in ecosystems (CHESHIRE et al. 1992, HU et al. 1997, MANDER et al. 2000, NIEDER, RICHTER 2000, KLIMOWICZ, UZIAK 2001, SIMANSKY 2007, SIENKIEWICZ et al. 2009, DOMAGAŁA-ŚWIĄTKIEWICZ, SADY 2011). The results of the current studies show changes in some properties of the A

horizons of Stagnic Luvisols under different land uses – from forest soils never used for agriculture (BOF), through arable soils (M, AF) and fallow (F) to the secondary afforestation with birch (SAB) and alder (SAA). Different land uses were reflected in the physical and chemical properties of the A horizons of the soils, as well as in the content and contribution of the forms of carbon and nitrogen. The soils in BOF stand were characterized by the smallest thickness of the horizon (9.2 cm) and its volumetric density (0.84 g cm^{-3}). The A horizon thickness in the remaining stands was affected by tillage and ranged from 26.4 cm to 35.6 cm (Table 1). The highest volumetric density was observed in the soils of AF (1.59 g cm^{-3}) and SAB stands (1.53 g cm^{-3}), slightly lower in M (1.38 g cm^{-3}) and F (1.41 g cm^{-3}) and significantly lower in SAA stand (1.17 g cm^{-3}). Volumetric density can strongly influence the uptake and loss of the elements, especially nitrogen (LIPIEC, STĘPNIEWSKI 1995). The soils in general were acid, but the pH varied from 4.05 in BOF stand to 6.25 in M stand. The higher values of pH observed in arable soils are the result of fertilization. The soils of BOF stand were the richest in SOM (107.3 g kg^{-1}), TOC (51.2 g cm^{-3}) and TN (3.05 g cm^{-3}). The content of SOM in the remaining stands was $26.3\text{--}48.9 \text{ g cm}^{-3}$, TOC $9.2\text{--}21.4 \text{ g cm}^{-3}$ and TN $0.82\text{--}1.60 \text{ g cm}^{-3}$. TOC:TN ratios ranged from 11.3:1 in SAB stand to 16.8:1 in BOF stand (Table 1). The observed TOC:TN ratios indicate relatively good conditions of soil organic matter mineralization.

Labile forms of organic carbon, whose content is a good indicator of soil quality as influenced by changes in management practices (LAIK et al. 2009),

Table 1

Selected properties of the A horizon of the soils (mean \pm SD)

Soil properties	BOF	M	AF	F	SAB	SAA
Thickness of A horizon (cm)	9.2 \pm 0.8	31.6 \pm 3.2	26.4 \pm 5.8	30.4 \pm 3.7	35.6 \pm 2.6	28.4 \pm 2.5
Soil texture group	sand	sandy loam	sandy loam	sandy loam	sandy loam	sandy loam
Sand (%)	83.9	61.0	50.9	64.0	66.3	66.0
Silt (%)	16.1	36.9	43.4	34.8	30.8	33.3
Clay (%)	0.0	2.1	5.7	1.2	2.9	0.7
Volumetric density (g cm^{-3})	0.84 \pm 0.09	1.38 \pm 0.02	1.59 \pm 0.06	1.41 \pm 0.17	1.53 \pm 0.07	1.17 \pm 0.10
Water content (% v/v)	31.8 \pm 2.2	34.6 \pm 2.1	28.2 \pm 1.6	27.6 \pm 3.1	25.0 \pm 1.4	38.4 \pm 5.0
pH _{H2O}	4.05 \pm 0.17	6.25 \pm 0.12	6.01 \pm 0.18	5.62 \pm 0.40	5.12 \pm 0.17	4.23 \pm 0.11
SOM (g kg^{-1})	107.3 \pm 31.3	33.7 \pm 2.1	43.3 \pm 3.3	36.3 \pm 0.7	26.3 \pm 1.8	48.9 \pm 9.0
TOC (g kg^{-1})	51.2 \pm 13.5	13.2 \pm 0.6	16.0 \pm 2.7	12.8 \pm 0.7	9.2 \pm 0.8	21.4 \pm 5.1
TN (g kg^{-1})	3.05 \pm 0.82	1.12 \pm 0.02	1.22 \pm 0.10	1.12 \pm 0.12	0.82 \pm 0.11	1.60 \pm 0.28
TOC:TN	16.8 \pm 0.6	11.8 \pm 0.6	13.0 \pm 1.5	11.4 \pm 1.0	11.3 \pm 0.9	13.4 \pm 1.3

Table 2

The content of carbon forms in soil under different land use

Carbon form		BOF	M	AF	F	SAB	SAA
DOC	g kg ⁻¹	0.71	0.34	0.33	0.33	0.34	0.40
	% in TOC	1.43	2.57	2.08	2.57	3.70	1.89
EHC	g kg ⁻¹	11.33	3.05	3.48	2.82	2.17	5.00
	% in TOC	22.65	23.13	21.81	22.04	23.49	23.67
WHC	g kg ⁻¹	3.99	1.79	2.57	2.44	1.54	2.19
	% in TOC	7.81	13.58	17.02	19.31	16.84	10.44
NHC	g kg ⁻¹	35.16	8.03	9.61	7.17	5.17	13.85
	% in TOC	68.11	60.71	59.09	56.08	55.98	64.01

Table 3

The content of nitrogen forms in soil under different land use

Carbon form		BOF	M	AF	F	SAB	SAA
NO ₃ -N	g kg ⁻¹	0.005	0.003	0.008	0.004	0.005	0.011
	% in TN	0.17	0.30	0.68	0.35	0.56	0.70
NH ₄ -N	g kg ⁻¹	0.048	0.034	0.020	0.044	0.047	0.038
	% in TN	1.77	3.06	1.62	3.91	5.78	2.47
DON	g kg ⁻¹	0.047	0.024	0.044	0.015	0.024	0.034
	% in TN	1.55	2.09	3.60	1.63	2.85	2.13
EHN	g kg ⁻¹	0.874	0.339	0.338	0.383	0.280	0.436
	% in TN	29.72	30.23	27.40	34.27	34.26	27.44
WHN	g kg ⁻¹	0.911	0.370	0.413	0.348	0.184	0.542
	% in TN	30.41	32.90	33.84	31.36	22.69	34.32
NHN	g kg ⁻¹	1.161	0.353	0.397	0.330	0.285	0.534
	% in TN	36.38	31.42	32.88	28.75	33.86	32.94

Table 4

Values of the DOC:DON, EHC:EHN, WHC:WHN and NHC:NHN ratios

Ratio	BOF	M	AF	F	SAB	SAA
DOC:DON	16.6	18.6	7.5	19.2	18.2	11.7
EHC:EHN	12.9	9.1	10.6	7.4	7.7	11.5
WHC:WHN	4.4	4.9	6.4	7.0	8.3	4.1
NHC:NHN	33.0	23.6	25.2	24.4	19.4	28.0

play an important role in the functioning of natural and modified ecosystems. Mobile in soils, they are a carrier of nutrients and energy compounds (QUALLS, HAINES 1991), at the same time playing an important role in some pedogenetic processes (HAYES, MOORE 1992, JONCZAK 2012). The sizes of DOC pools and their contribution to the total soil carbon pool vary among different

ecosystems (HU et al. 1997). The intensity of DOC production and leaching is strongly affected by soil temperature and moisture (CHRIST, DAVID 1996), and in forest ecosystems by tree species composition and its age (REMĚŠ, KULHAVÝ 2009, JONCZAK, PARZYCH 2012). In the studied soils, the highest content of DOC was observed in BOF stand (0.71 g kg^{-1}), where it was significantly higher than the one observed in remaining stands ($0.33\text{-}0.40 \text{ g kg}^{-1}$) – Tables 2, 5. The distribution of DOC in BOF, SAB and SAA stands has been, in general, consistent with the data presented by REMĚŠ and KULHAVÝ (2009), who observed the influence of tree-stand age on the concentration of DOC in lysimetric waters.

Large differences between the stands were observed in the content of EHC – from 2.17 g kg^{-1} in SAB stand to 11.33 g kg^{-1} in BOF stand, but the contribution of EHC in TOC was comparable in each stand (21.81-23.67%) and with the data of BECHER AND KALEMBASA (2011), who observed 16.9-30.6% in arable Cambisols and Luvisols of Siedlce Upland. WHC contributed be-

Table 5

Statistical significance of differences between the soils in the content of different forms of carbon and nitrogen and C:N ratios

Specification	BOF vs M	BOF vs AF	BOF vs F	BOF vs SAB	BOF vs SAA	M vs AF	M vs F	M vs SAB	M vs SAA	AF vs F	AF vs SAB	AF vs SAA	F vs SAB	F vs SAA	SAB vs SAA
DOC	++	++	++	++	++	-	-	-	-	-	-	-	-	-	-
EHC	++	++	++	++	++	-	-	++	++	+	++	++	+	++	++
WHC	++	+	++	++	++	++	++	-	+	-	++	-	++	-	+
NHC	++	++	++	++	++	-	+	++	++	+	++	+	++	++	++
TOC	++	++	++	++	++	+	-	++	++	+	++	+	++	++	++
$\text{NO}_3\text{-N}$	++	-	+	-	++	+	-	++	++	+	-	-	+	++	++
$\text{NH}_4\text{-N}$	-	++	-	-	-	++	+	++	-	++	++	++	-	+	++
DON	+	-	++	+	-	+	-	-	-	++	++	+	-	++	-
EHN	++	++	++	++	++	-	-	+	+	-	-	+	++	-	++
WHN	++	++	++	++	++	-	-	++	++	+	++	+	++	++	++
NHN	++	++	++	++	+	-	-	-	+	-	+	-	-	+	+
TN	++	++	++	++	++	+	-	++	++	-	++	+	++	++	++
DOC:DON	-	++	-	-	-	+	-	-	-	+	+	++	-	-	-
EHC:EHN	++	+	++	++	+	-	+	+	++	++	++	-	-	++	++
WHC:WHN	-	+	++	++	-	-	+	++	+	-	-	+	+	++	++
NHC:NHN	+	-	-	++	-	-	-	-	-	-	-	-	-	-	-
TOC:TN	++	++	++	++	++	-	-	-	+	+	+	-	-	+	+

++ differences statistically significant at $p < 0.01$; + differences statistically significant at $p < 0.05$; - differences not significant statistically

tween 7.81-19.31% in TOC with concentrations from 1.54-3.99 g kg⁻¹. The above values were slightly lower than those quoted by BECHER and KALEMBASA (2011).

In each stand, the main form of carbon was NHC (55.98-68.11% in TOC), whose concentration was 5.17-35.16 g kg⁻¹. In most cases, the differences in the content of EHC, WHC, NHC and TOC observed between the stands were statistically significant (Table 5). In general, the highest content of TOC, as well as its different forms, was observed in BOF stand. However, the percentage of DOC in TOC was the lowest in BOF stand and the highest in SAB stand. Moreover, the lowest contribution of WHC and the highest of NHC were observed in BOF stand. The contribution of EHC in TOC was comparable in every stand and ranged from 21.81 to 23.67%. The content of each studied form of carbon was positively related to the content of SOM and TOC:TN ratio and negatively to volumetric density and pH (Table 6).

The content of NO₃-N was very small in the studied soils and ranged from 0.003 to 0.011 g kg⁻¹ (0.17-0.70% in TN) – Table 3. The highest concentrations were observed in SAA and AF stands and the lowest in M stand. Low pH, as a limiting factor for nitrification, is probably the reason of the observed low concentrations of nitrates. The content of NO₃-N was negatively related to volumetric density, which is overall consistent with the data quoted by HAUNZ et al. (1992). The differences in NO₃-N concentrations observed between the stands could be partially a result of different intensity of nitrogen leaching in the soils with different compaction and under different uses (SHIPITALO, EDWARDS 1993). The content of NH₄-N was higher than NO₃-N, and ranged from 0.020 g kg⁻¹ in AF stand to 0.048 g kg⁻¹ in BOF stand, being comparable to the one observed by BECHER, KALEMBASA (2011) of 0.017-0.039 g kg⁻¹. The contribution of NH₄-N form in TN ranged from 1.62 to 5.78%. DON contributed in TN in 1.55-3.60% with concentrations 0.015-0.047 g kg⁻¹.

Hydrolyzable and nonhydrolyzable forms always predominate in the soil total nitrogen, but proportions between the forms are varied in different soil types and under different uses (eg. HERSEMAN 1987, SHARPLEY, SMITH 1995, SULCE et al 1996). EHN, WHN and NHN were also the main forms of nitrogen in the investigated soils. There were no large differences between the stands in contribution of the forms in TN. Such regularities were also

Table 6

Correlation coefficients between the content (g kg⁻¹) of carbon and nitrogen forms and some properties of the soils

Specification	DOC	EHC	WHC	NHC	NO ₃ -N	NH ₄ -N	DON	EHN	WHN	NHN
Volumetric density	-0.782*	-0.871	-0.621	-0.842	-0.381	-0.067	-0.340	-0.868	-0.825	-0.785
pH	-0.645	-0.691	-0.450	-0.658	-0.424	-0.348	-0.373	-0.649	-0.617	-0.615
SOM	0.888	0.978	0.886	0.987	0.098	0.110	0.648	0.948	0.951	0.949
TOC:TN	0.738	0.869	0.644	0.858	0.117	0.162	0.571	0.863	0.840	0.660

* in bold – correlations statistically significant at $p < 0.05$

observed by BECHER and KALEMBASA (2011) in arable Cabbisols and Luvisols, and by the same authors in peat-muck soils (KALEMBASA, BECHER 2009), as well as by CZEKAŁA (2010) in Luvisols under different uses. The contribution of EHN in TN was 27.40-34.27%, and WHN – 22.69-34.32%. These percentages of NHN (28.75-36.38%) were comparable to the ones observed by SULCE et al. (1996) in Cambisols (34.5%), as well as by SHARPLEY and SMITH (1995) in different soil types (30.4-34.8%). The content of organic forms of nitrogen (DON, EHN, WHN and NHN) in the investigated soils was positively correlated to the content of SOM and TOC:TN ratio, and negatively to the volumetric density and soil pH (Table 6).

The C:N ratio is a good parameter in the assessment of the impact of different factors on soil's biological activity and the intensity of nutrient cycling (GUNDERSEN et al. 1998, SMOLANDER, KITUNEN 2002, AKSELSSON et al. 2005, VESTERDAL et al. 2008). The lowest DOC:DON ratio was observed in AF stand (7.5:1) and a slightly higher in SAA stand (11.7:1). In the remaining stands, the ratios were 16.6:1-18.6:1. High differences were observed between the stands in the DOC:DON ratio (7.5:1-18.6:1), which indicates varied biological activity of the investigated soils. The observed EHC:EHN ratios (7.4:1 to 12.9:1) were comparable or slightly higher than the ones given by BECHER and KALEMBASA (2011). The lowest values of the C:N ratio were observed in the weakly hydrolyzing (4.1:1-8.3:1) and the highest ones - in the nonhydrolyzable fraction (19.4:1-33.0:1). The lowest C:N ratios observed in the weakly hydrolyzing fraction could be a result of the hydrolysis of bodies of microorganisms, relatively resistant to reagents, which accumulate a significant share of soil nitrogen. Most nitrogen was released after extraction with 2.5 mol H₂SO₄ dm⁻³.

CONCLUSIONS

Different land uses were significantly reflected in the properties of the A horizon of the investigated Stagnic Luvisols. Particularly high differences were observed between the forest soils, never used for agriculture, and the arable or post-arable soils. Tillage caused the increase of the A horizon depth from 9.2 to 26.4-35.6 cm, which was a result of the mixing of the primary A horizon with E horizon, and the increase of its volumetric density. The highest content of SOM, TOC and TN as well as the TOC:TN ratio were observed in forest soils.

Statistically significant differences between the stands in the content of different forms of carbon were identified in most cases. The content of every form was positively correlated with the content of SOM and the TOC:TN ratio, but negatively with volumetric density and pH. The nonhydrolyzable form of carbon dominated in the soils (55.98-68.11% of TOC), and DOC had the lowest contribution (1.43-3.70% in TOC). In general, higher contribution

of DOC in TOC was observed in arable and post-arable soils in relation to forest soils. The observed content and contributions of different forms of carbon in TOC were comparable to the ones observed by other authors in similar soil types.

The content of mineral nitrogen ($\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$) ranged from 0.028 to 0.053 g kg⁻¹, and $\text{NH}_4\text{-N}$ dominated in the pool. The lowest concentration of mineral nitrogen was observed in arable soils, and significantly higher in forest and fallow soils. EHN, WHN and NHN were the main forms of nitrogen. As the content of the forms was positively correlated to the content of SOM, their highest concentration was observed in BOF stand.

The differences in the DOC:DON, EHC:EHN, WHC:WHN and NHC:NHN ratios observed between the stands suggest varied biological activity of the soil under different uses. The lowest C:N ratio values were observed in the weakly hydrolyzable (4.1:1-8.3:1) and the highest – in the nonhydrolyzable fraction (19.4:1-33.0:1).

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USEFULNESS OF CLINICAL, HISTOPATHOLOGICAL AND SOME BIOCHEMICAL AND MINERAL INVESTIGATIONS IN DIAGNOSIS OF BOVINE HYPERKERATOSIS IN DAIRY CATTLE

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Abstract

Bovine hyperkeratosis is a polyetiologic disease that is increasingly widespread at high milk yielding dairy farms. Clinical manifestation is characterized by focal skin lesions with distinct borders. A clinical study and observation were carried out on 26 Holstein-Friesian dairy cows. During the initial phase of the condition, the skin of the affected cows was itchy, hence the animals tended to lick the skin lesions, or else rub against surrounding objects, with the resultant formation of single spots of raised coat and skin flaking resembling dandruff. These changes appeared on the posterolateral upper sides of the pelvic limbs and around the vulva. Distinct thickening of the wrinkled skin was observed as a result of excessive growth of the epidermis. The superficial part of the skin on major portions of the lesions was dry, corrugated and covered with numerous scales. The epidermis was dry, thickened and rough, with cracks showing the reddened dermal layer. When touched, the animals reacted as if in pain. The disease generally progressed into a chronic condition. In the studied cases, histopathological examination confirmed hyperkeratosis with widened hair follicle infundibulums filled with keratin, the swelling of sweat glands, epithelial atrophy of sweat glands, infiltration of inflammatory cells between and around blood vessels, and massive expansion of keratinized layers of the epidermis.

The content of both calcium and magnesium as well as copper, zinc, iron and manganese in grain, roughage, mineral mixtures and in other feeds met the requirements of dairy cows. Blood biochemistry profiles revealed only slightly lower serum calcium values, while zinc values were

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within the reference range. However, the zinc concentration in skeletal muscles and in the skin was reduced. The mean serum alkaline phosphatase (ALP) activity in the tested animals was also somewhat decreased. Adverse environmental factors such as direct skin contact with faeces and urine as well as zinc deficiency in the cows' tissues were significant factors in the formation of skin lesions characteristic for hyperkeratosis.

Key words: dairy cattle, the environment, zinc, hyperkeratosis.

PRZYDATNOŚĆ BADAŃ KLINICZNYCH, HISTOPATOLOGICZNYCH I NIEKTÓRYCH BIOCHEMICZNYCH ORAZ MINERALNYCH W DIAGNOSTYCE HIPERKERATOZY U BYDŁA MLECZNEGO

Abstrakt

Hiperkeratoza bydła jest chorobą polietiologiczną, występującą coraz częściej w gospodarstwach bydła mlecznego o wysokiej wydajności. Objawy kliniczne zlokalizowane na skórze mają ograniczony charakter. Badania i obserwacje przeprowadzono na 26 krowach mlecznych, rasy czarno-białej. Na początku rozwoju choroby zaobserwowano świąd, intensywne oblizywanie się i lekkie łzawienie oraz ocieranie o otaczające przedmioty, co powodowało powstawanie pojedynczych ognisk nastroszenia sierści oraz łuszczenia się naskórka, przypominającego łupież. Zmiany pojawiały się na tylnogórnej stronie kończyn miednicznych i wokół sromu. W wyniku nadmiernego rozrostu naskórka rogowego obserwowano wyraźne zgrubienie pomarszczonej skóry. Skóra na znacznej powierzchni ogniska była sucha, pofałdowana, pokryta licznymi łuskami. Naskórek był suchy, zgrubiały, szorstki, popękany aż do widocznej czerwono zabarwionej skóry właściwej. Podczas dotyku zwierzęta odczuwały bolesność. Przebieg choroby miał charakter przewlekły. Badaniem histopatologicznym potwierdzono hiperkeratozę. Zaobserwowano poszerzone lejki mieszków włosowych wypełnionych keratyną, poszerzenie gruczołów potowych, zanik nabłonka gruczołów potowych, nacieki komórek zapalnych między naczyńkami krwionośnymi, nacieki komórek zapalnych wokół naczyń krwionośnych oraz masywne poszerzenie warstwy rogowaciejącej naskórka.

Zawartość zarówno wapnia i magnezu oraz miedzi, cynku, żelaza i manganu w granulowanej paszy treściwej oraz w mieszanke mineralnej, jak i w pozostałych paszach stosowanych w żywieniu krów pokrywała ich zapotrzebowanie. Jednak wyniki badań biochemicznych wykazały, że stężenie wapnia w surowicy krów było zaniżone, zaś cynku mieściło się w granicach wartości referencyjnych. Natomiast stężenie cynku w mięśniach szkieletowych i w skórze było obniżone. Średnia aktywność ALP w surowicy badanych krów była także dość niska.

Niekorzystne czynniki środowiskowe (kontakt bezpośredni skóry z kałem i moczem) oraz niedobór cynku w tkankach krów stanowiły istotną przyczynę powstawania zmian chorobowych skóry, charakterystycznych dla hiperkeratozy.

Słowa kluczowe: bydło mleczne, środowisko, cynk, hiperkeratoza.

INTRODUCTION

Skin disease can be looked upon as a manifestation of the impact of environmental factors and nutrition (especially mineral nutrition) on dairy cattle. Hyperkeratosis is counted as one of major diseases in cattle. Initially, the aetiology of the disease was unknown and that is why parakeratosis was called the X-disease (HAGAN 1950). Later, many studies showed that the disease can be induced by the prolonged feeding of cows with alfalfa hay,

containing calcium phosphate $\text{Ca}(\text{HPO}_4)_2$. Subsequently, parakeratosis was observed in cattle as a result of long-term nourishment with commercial foodstuffs and feeds with an experimentally reduced fatty acid content. At that time, decreased concentrations of vitamin A were also reported in blood samples from cattle. Then, general hyperkeratosis was recognized in Germany, in areas where highly chlorinated naphthalenes were used in industry. Among highly chlorinated naphthalenes, tetrapenta-hepta-chloronaphthalene can be found in wood protectants, some motor oils and commercial feeds contaminated with industrial lubricants (GREGORY et al. 1954). These naphthalenes can evoke the disease even several years after wood impregnation. Similar effects have been attributed to some insecticides and iodine preparations (FORET 2005). Exposing cattle to elevated emissions of arsenic can also lead to general hyperkeratosis. Causes of local hyperkeratosis include persistent pressure on specific body areas like joints or keeping animals on hard or damp surfaces for long periods of time. Typical hyperkeratotic lesions can also accompany a hereditary "fish-scale disease of cattle" (ichthyosis), also called "calf armour", and fetal hyperkeratosis (FI 2003, TESTONI 2006, JONG KI et al. 2007). Although many reports have appeared on hyperkeratosis, the aetiology and pathogenesis are not completely understood yet. It is believed that, like other skin diseases, it is a result of many exogenous and endogenous factors, including viruses and free radicals (YUKIKO et al. 2007). It should be emphasized that the immune and antioxidant systems especially make use of the germicidal properties of oxidants by producing oxidative forms of compounds, which is an essential step in the oxidative burst mechanism. Phagocytes activated in this way produce both reactive oxygen species (ROS) and reactive nitrogen species (RNS), including superoxides ($\cdot\text{O}_2^-$), nitric oxides ($\text{NO}\cdot$) and nitric ions ($\text{OONO}-$). These highly reactive compounds are involved in the cytotoxic response of phagocytes and, despite being obviously advantageous, they can also lead to cell and tissue damage in cattle. It is this nonspecific character of oxidants that enables them to destroy almost every cell, which is an effect that may be seen in hyperkeratosis. The consequences of the condition primarily include abnormal protein metabolism and excessive production of keratin or excessive keratin retention (ichthyosis). Initially, hyperkeratosis is characterized by the reddening of the affected area. Later, the skin thickens due to increased proliferation of keratinocytes, causing hypertrophy of the epidermal stratum corneum (O'TOOLE, FOX 2003).

In the early stage of the condition, cows can be seen intensely licking and slightly tearing the skin. In some cases, loss of appetite may occur. The disease progresses with a slow weight loss, diarrhoea, reddening of the oral mucosa and nose, followed by the appearance of small, round ulcerations. If the disease does not progress into a systemic form, the body temperature remains between 37.5 and 39.5°C. Otherwise, the temperature rises and roughly thickened and keratinized lesions appear, with a tendency to spread around the back, ears, sides of the thorax, and on the medial sides of the

pelvic limbs. Sometimes these lesions resemble warts, then change gradually to scale-like growths. Excessive epidermal keratinization causes substantial thickening of the wrinkled skin, with formation of cracks, sometimes giving the skin surface a reticular character. Lesions occur on the surface of the skin. The illness follows a more severe course in heifers than in adult cattle, with subsequent reproduction problems, including miscarriages, and reduced milk yield.

Bovine ichthyosis (also called “calf armour” and fetal hyperkeratosis) can occur in two forms. The fetal form, which leads to spontaneous abortions or damage to the reproductive system of the mother, and the congenital form, mostly affecting bulls, with lesions only on certain parts of the body. The affected fetus may be aborted, may be born less viable or dead, or may die within two weeks of birth. The skin is dry, thickened and rough, and cracked to the extent that the red-colored dermal layer is visible. The presence of numerous cracks gives the skin a “fish scale” appearance, and when the animals are touched it looks as if they feel pain. The mucous membranes are bright red, the borders between the skin and mucous membranes are curled outwards, and the ears seem to be shorter and thickened. Lesions are most often diffuse, but single focal lesions should not be ruled out. Sometimes, gestation is prolonged.

In general, the course of hyperkeratosis is chronic, although this is also dependent on the age of cattle. In younger animals, the disease is more severe, with a possible fatal outcome, while in older animals, the condition is milder with possible spontaneous healing of the skin.

The nature of anatomo-pathological changes depends on the duration of the disease. Inflammation is observed first, followed by hyperkeratosis of the skin and mucous membranes of the mouth, oesophagus, rumen, reticulum, bile ducts, gall bladder and the vagina. Histopathologic changes of various types can also occur in the testes and epididymides. Keratosis can reach the subcutaneous tissue. In addition, we can observe interstitial glomerulonephritis, cirrhosis of the liver, pancreatic degeneration, keratosis of hair follicles, brittle hair and hair loss. Large areas of the skin surface becomes dry, wrinkled and covered with numerous scales.

As the disease progresses, excessive and incomplete keratinization of epidermal cells occurs as well as the formation of intercellular bridges through excessive thickening of the stratum corneum. Hyperkeratosis may take on the orthokeratotic form, where the cells are devoid of nuclei, or the parakeratotic form, with the presence of nucleated cells (JELINEK, TACHEZY 2005). If present, the nuclei of the stratum corneum cells are shrunken and may take the form of rods. In the course of chronic dermatoses, the aforementioned forms of parakeratosis are not generally diagnosed. In the “fish scale” form, only the previously described clinical signs can be seen, and no other changes have been identified. However, in histopathological examination, other signs can be recognized, including distinct keratosis of the superficial

epidermal layers, the skin glands and hair follicles as well as papillary formations on the dermis.

The diagnosis is based on history, clinical examination and laboratory tests, which mainly include histopathological examinations; in cases of secondary infections, microbiological tests are also made, including isolation and identification of both bacteria and fungi. The differential diagnosis should focus mainly on foot-and-mouth disease, parakeratosis and dandruff. However, in the differential diagnosis of parakeratotic disseminated hyperkeratosis, seborrhoea, dermatitis caused by zinc and vitamin A deficiency and thallium poisoning should be considered.

The prognosis may be favourable if treatment is initiated in the early stages of the disease, and unfavourable when the disease process is well underway. The prognosis for the “fish scale” disease is unfavorable. For disease prevention, it is very important to monitor constantly a cows’ herd with respect to general welfare, including proper nutrition, and to eliminate exposure to highly chlorinated naphthalenes and arsenic compounds. In addition, it is vital to eliminate from the herd individuals afflicted with the hereditary bovine “fish scale” disease (JONG-KI et al. 2007).

Before treatment, the affected area should be washed with soap and warm water and then thoroughly dried. Substances with keratolytic properties (containing salicylic acid), anti-inflammatory, antibacterial, antifungal and astringent products as well as vitamin A are used. Farmers should be advised to change the animals’ diet by reducing the use of commercial feed and increasing homegrown feeds rich in vitamins and minerals (antioxidants). The “fish scale” disease is very difficult to treat and generally has an unfavourable outcome (WRIGHT, SPEARS 2004).

The purpose of this article is to present a review of the general concept of skin hyperkeratosis formation in dairy cattle as influenced by environmental factors, the concept of clinical pathology with respect of aetiological diagnosis, as well as mineral nutrition.

MATERIAL AND METHODS

The study involved 26 Holstein-Friesian (HF) dairy cows aged 3-6 years. The cows were in a transition period (21 days before to 21 days after parturition). The cows were divided into 2 groups (experimental-1 and control-2). The average annual yield of the cows was 6200 ± 100 kg of milk per year, with a milk fat content of 3.39%. The body condition score (BCS) ranged from 3 to 3.2 before parturition to 2.9 to 3.1 afterwards. In the spring (April), the breeder reported to the veterinarian that during the previous 10 months some animals had been observed to manifest progressive changes of the skin around the back, ears, sides of the chest, around the base of the tail

and around the vulva, with concomitant weight loss and reduced appetite. These changes had been preceded by the occurrence of itching, intense licking and rubbing against surrounding objects, as well as slight tearing. Each of the 26 cows was individually examined clinically, with particular attention paid to the skin and according to generally accepted standards. No obvious lesions were found in 23 cows (group II), although three cows (group I) had macroscopically visible lesions previously described by the breeder

These lesions were of a chronic nature. Analysis of the cows' feeding routine showed that they were fed according to the accepted NRC standards. A daily ration was balanced with respect to protein and energy content. In the winter season, daily rations consisted of homegrown maize silage and hay, while in the summer cows ate pasture grass. During both seasons, the roughage was supplemented with granules containing a mineral mixture. Based on data regarding the history of potential occurrence of environmental toxic agents, including chlorinated naphthalenes, such a threat was excluded. Samples were taken from group I cows, from the periphery of skin lesions, for further parasitological, bacteriological and mycological studies. These examinations were conducted in accordance with the relevant standards. Full thickness skin biopsy samples, approximately 1 cm², were taken from the gluteal region of cows in both group 1 (experimental – 3 cows) and 2 (control – 23 cows). These samples were divided into three parts. One was fixed in 10% neutralized formalin, embedded in paraffin and sliced with a Zeiss Hyrax M55 rotary microtome into 4µm sections. The sliced specimens were placed on glass slides and stained with hematoxylin-eosin stain. Another part of the full-thickness skin biopsies was used for determinations of the calcium, magnesium, copper, zinc and iron content. The remaining part of the biopsies was weighed and dried for 72 hours at 37°C. Subsequently, the concentration of the aforementioned elements was determined in the tissue dry weight. For 7 days after calving, each morning before the morning feeding, blood samples for biochemical and mineral analysis were drawn from the external jugular vein from all cows in groups I and II. Blood samples were collected into tubes without an anticoagulant to obtain serum. The concentrations of calcium, magnesium, copper, zinc and iron in the blood, in the full thickness skin biopsies and in the roughage (mineral mixture containing granules) were made using the flame atomic absorption method (Perkin-Elmer 1100) after prior mineralization in an Ethos 900 microwave system (Milestone). The ALP in serum was determined by spectrophotometry using Point equipment.

After the testing period, the barn conditions were improved through daily use of dry bedding consisting of rye and oat straw. Also, for 6 months, a higher dose of micronutrients was introduced in the form of a supplement (KenoBiotyn) in a therapeutic dose.

The results were statistically analyzed using Student *t*-test.

RESULTS

Clinical examination results

The results of the clinical examination, biochemical and histopathological findings are presented in Figures 1-5 and Tables 1-2.

Of the 26 dairy cows in the transition period (from 3 weeks before to 3 weeks after parturition), 11.5% had clinically evident skin lesions around the back, ears, the sides of the chest, the pelvic limbs, around the vulva and at the base of the tail. The hair was raised, and there were small white scales and scaly skin evident (Figure 1). No scratching was seen in the examined



Fig. 1. A clinical picture of a cow with hyperkeratosis

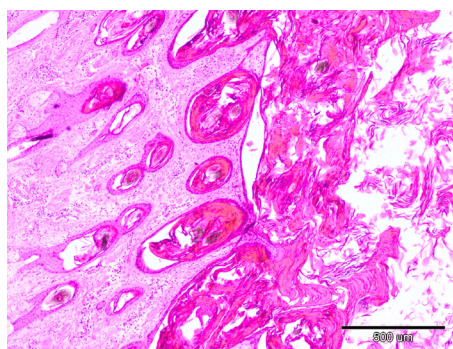


Fig. 2. Massive distension of the follicular infundibula with keratyn, hyperkeratosis

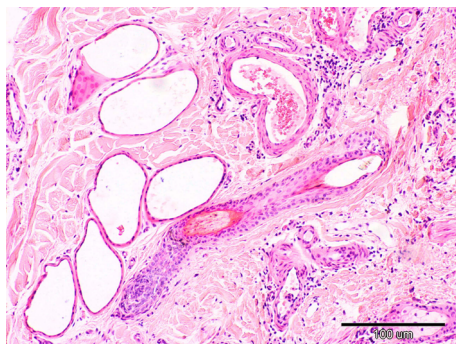


Fig. 3. Significant dilatation of sweat gland, atrophic epithelial cells layer of sweat gland. Inflammatory infiltration among blood vessels

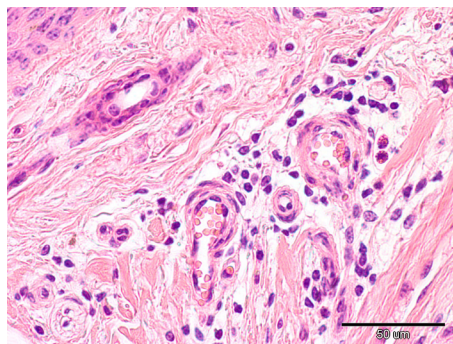


Fig. 4. Inflammation infiltration among blood vessels, composed mainly of mononuclear cells

animals, even though this sign was reported during the interview with the breeder. Vital signs were normal in both groups, cows with no skin lesions (group II) and with lesions (group I). The average body temperature of the animals was $38.5 \pm 0.5^\circ\text{C}$, the average heart beat rate was of $55.0 \pm 5.8 \text{ min}^{-1}$, and the average respiratory rate was $28.5 \pm 3.1 \text{ min}^{-1}$. The accessible lymph

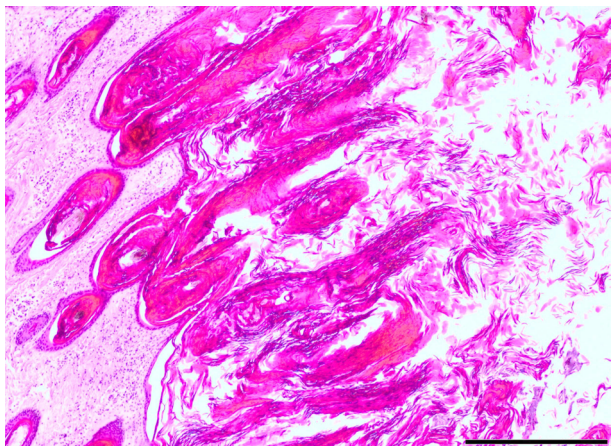


Fig. 5. Massive distension of the keratinized epithelium layer

nodes on both sides of each animal were of normal size, consistency and temperature upon palpation. The mucous membranes and conjunctiva were of normal colour with no signs of dehydration.

The concentration of macro- and microelements in feed

The concentration of calcium, magnesium, copper, zinc and iron in the feed is shown in Table 1.

Table 1

Mineral content in feed for dairy cows

Type of feed	Calcium	Magnesium	Copper	Zinc	Iron
	(g kg ⁻¹ d.m.)		(mg kg ⁻¹ d.m.)		
Granules	8.37±0.15	3.89±0.06	15±2.35	212.0±18.7	210.0±18.7
Mineral mixture	12.6±0.8	7.8±0.72	560.5±76.9	4393.4±495	7043.4±179.0

Key: \bar{x} – means , \pm SD – standard deviation (15 samples).

Parasitological and microbiological examination

Parasitological examination of the cows with and without skin lesions did not reveal the presence of ectoparasites. Likewise, bacteriological and mycological examinations did not reveal the presence of pathogenic bacteria, or any dermatophytes or their spores, in direct smears. The microbial flora of predominantly Gram positive bacteria was seen in the samples, consisting of bacilli and spherical forms. Haemolytic *Streptococcus* sp. L, coagulase-negative *Staphylococcus* and *Bacillus* sp. were grown on bacteriological substrates.

Histopathological examination of the skin samples

The microscopic structure of full-thickness skin samples taken from the cows in group II with no macroscopically visible lesions was normal, including the epidermis, dermis, hair follicles and sweat glands. Conversely, the microscopic structure of full-thickness skin samples taken from the cows in group I, with macroscopic skin lesions, revealed massive areas of keratinized epidermis and widening of hair follicle infundibulums, which were filled with keratin. There was significant widening of the sweat glands as well as atrophy of the epithelium of these glands. Infiltration of primarily mononuclear inflammatory cells around the blood vessels was also noted (Figures 2-5).

Blood microelements and biochemistry results

There were statistically significant ($P \leq 0.05$) lower levels of serum calcium (by 192%), magnesium (20%) and zinc (27.7%), as well as higher concentrations of serum iron (by 48.5%) in the cows with skin lesions compared to the cows without dermatological changes. No statistically significant differences were noted in the serum concentrations of copper in the cows from groups I and II ($P > 0.05$) – Table 2. Alkaline phosphatase activity in blood samples taken from the cows with skin lesions (group I) was slightly lower than in samples taken from the healthy animals, but the difference was not statistically significant.

Concentration of microelements in skin biopsy samples

In cows with dermatological changes (group I), significantly lower ($P \leq 0.01$) concentrations of calcium, magnesium and zinc in full-thickness skin biopsies were seen compared to the concentrations of these elements in skin samples taken from the animals without skin lesions (group II). The concentrations of calcium, magnesium, copper and zinc in the skin of animals in group I were lower than in group II by 287.7 to 14.9%. The concentration of iron in the full-thickness skin biopsies from the animals with dermatological changes was statistically significantly higher ($P \leq 0.05$) by about 62% compared to the concentration of this element in the skin taken from the animals without dermatological lesions (Table 2).

DISCUSSION

Rapid increase of milk yield in dairy cows that has occurred during the last 20 years has also contributed to negative trends in the health of these animals. Therefore, monitoring levels of mineral concentrations in animal tissues is important for assessing the effect of contamination on animal health and safety of products of animal origin in human nutrition. Unfavorable changes can occur especially during the transition period, covering a period

Table 2
Characteristics of selected indicators of metabolism in biological material derived from dairy cows

Type of biological materials	ALP (U l ⁻¹)		Ca (g kg ⁻¹ d.m.)		Mg (mmol l ⁻¹) serum		Cu (mg kg ⁻¹ d.m.)		Zn (μmol l ⁻¹) serum		Fe	
	(U l ⁻¹)		(g kg ⁻¹ d.m.)		(mmol l ⁻¹)		(mg kg ⁻¹ d.m.)		(μmol l ⁻¹)		(mg kg ⁻¹ d.m.)	
	groups I and II											
	experi- mental (n-3)	control (n-23)	experi- mental (n-3)	control (n-23)	experi- mental (n-3)	control (n-23)	experi- mental (n-3)	control (n-23)	experi- mental (n-3)	control (n-23)	experi- mental (n-3)	control (n-23)
Serum	36.8±2.8	38.5±10.1	0.66**±0.14	1.93±0.23	0.90*±0.28	1.08±0.23	15.53±2.28	16.4±1.25	15.58*±1.33	19.9±3.4	35.5*±5.9	23.9±5.7
Muscles	-	-	16.1*±4.2	21.5±3.5	30.0**±4.2	37.8±3.4	0.412*±0.11	0.9.4±0.236	29.5***±6.8	42.4±30.4	42.2*±5.8	35.6±5.6
Skin	-	-	26.5*±3.1	35.3 ±26.5	16.1*±1.7	21.5±2.5	1.94±0.98	2.13±0.41	23.6***±7.98	1.56±13.2	17.2*±1.3	27.9±2.59

Key: \bar{x} – means, \pm SD – standard deviation, * statistically significant differences ($P \leq 0.05$) between control and experimental group, ** statistically significant differences ($P \leq 0.01$) between control and experimental group, ***statistically significant differences ($P \leq 0.001$) between control and experimental group.

of three weeks before parturition to about three weeks post-parturition (KANDYLIS 1999, NOWAK et al. 2009). These changes include mainly a reduction in oxidative stress resistance and immunity (BREEN et al. 2006) in dairy cows, increased predisposition to dermatological, infectious and invasive diseases, as well as metabolic disorders in relation to reproduction, an increased number of somatic cells in milk, changes in the chemical composition of milk, mastitis, and foot diseases (ERDMAN 1993, CISOWSKI 2001, BERNABUCCI et al. 2005). The final result of a higher disease incidence in a herd is a shortened lifespan of cows (up to 2-3 lactations), increased mortality and premature culling of breeding cows. This in turn decreases the profitability of milk production and discourages farmers from breeding dairy cattle (AFZAAL et al. 2004, GAËTAN et al. 2006). Proper nutrition during the drying-up period determines the profitability of milk production in subsequent cycles. This is particularly important as the maintenance of cows during the antenatal period has not been researched as thoroughly as in the postpartum period. The drying-up period is a very difficult time in the production cycle of an animal due to some enormous hormonal and metabolic changes. These changes are aimed at supplying intermediate transitional minerals needed to meet the new requirements during the postpartum period, especially with respect to energy management (GOFF, HORST 1993, GOFF et al. 1995, TWARDON et al. 2006, JONES et al. 2009, WÓCIK et al. 2009). The metabolic and hormonal changes that occur during the transition period prepare the body for both the labour and lactation (CASTILLO et al. 2005, FÜRLI et al. 1996). In terms of both nutrition and well-being, the decisive factor is the reduction of the risk of many bovine diseases, including hyperkeratosis, reinforcement of the immune status, increased resistance to oxidative stress and improved profitability of milk production.

Observations have shown that the stalls in which the cows were kept were too long, resulting in the accumulation of faeces in the furthest area. Each day during the long hours of rest, the cows were forced to have the most caudal portions of their bodies in direct contact with faeces and urine. That situation would last from the beginning of the cowshed period (late autumn) to spring. As a result of persisting high humidity and direct exposure of the skin to faeces and urine, 8 of the animals exhibited syndromes of itching. Intense licking and slight tearing heralded the disease. Rubbing against surrounding objects resulted in the formation of focal areas of raised hair and skin flaking. Small white scales composed of horny skin cells were observed on the surface of the skin. This initial stage of the disease looked like dandruff. The internal body temperature was in the range of 37.5 to 39.5°C. Rough, keratinized calluses, showing a tendency to spread, appeared on the posterior side of the upper portion of the pelvic limbs and around the vulva. Sometimes the lesions resembled scales. As a result of the excessive growth of the epithelial stratum corneum, there was marked thickening of the wrinkled skin as well as formation of cracks that sometimes gave the skin surface a reticular-like nature. These lesions were localized on the sur-

face of the skin. The skin on most of the surface of the lesions was dry, wrinkled, and covered with numerous scales. The epidermis was dry, thickened, rough, with deep fissures showing the underlying red-colored dermal layer. When touched, the animals exhibited reactions as if in pain. The disease had a chronic character, although it was somewhat dependent on the age of a cow. In younger animals, the disease was slightly more acute, whereas in older animals it was milder. There were even some instances of spontaneous healing of the skin. All of the environmental factors mentioned above, in which the cows were housed, adversely affected their health, causing skin damage. Similar observations have also been made by other authors (HRISTOV et al. 2008, ALAM et al. 2010).

Macro- and microminerals play important roles in bodily functions. Cattle require 10 microminerals. The requirements for seven of the 10 microminerals, including copper, zinc, iron, manganese, selenium, cobalt and iodine, have been established. Table 1 and the estimated values it comprises show that the content of calcium, magnesium, copper, zinc, iron and manganese in granulated feeds, mineral mixtures as well as in other feeds supplied to the cattle met their requirements. In other words, absolute mineral deficiencies, which quite frequently lead to skin changes in cows in Poland, were not a direct cause of hyperkeratosis in that case. However, the results in Table 2 indicate that the concentration of serum calcium in cows was somewhat decreased at 0.66 mmol l^{-1} , and was in the upper range of values characteristic for cows with postpartum shock ($0.62\text{-}1.75 \text{ mmol l}^{-1}$) (SOBIECH et al. 2010). This may indicate an excessively alkaline diet caused by high levels of strong cations. Regardless of calcium metabolism disturbances, alkaline diet may predispose to skin disease. A recent study has also shown that “anionic salts” had a positive effect on mineral metabolism and antioxidant status of dairy cows (KLECZKOWSKI et al. 2011). The microbiological examination revealed normal body surface and environmental flora.

In this study, magnesium could affect the prooxidative-antioxidative status in dairy cows as well as their health, which was demonstrated by the earlier study. The average serum concentration of magnesium in cows was 0.90 mmol l^{-1} . Variations in Mg concentrations can be observed during different months throughout the year. The highest concentration of about 0.9 mmol l^{-1} is seen from January to April and from July to December, while the lowest one occurs from May to June. The serum concentration of Mg in the studied cows was typical for April and did not differ from the reference values.

Copper is an important micromineral of many enzyme systems essential for the normal prooxidative-antioxidative status, immunity and health of skin. In addition, copper affects the activity of lysyl oxidase, which in turn depends on the biosynthesis of collagen and elastin present in the skin (BARSZCZ et al. 2009). Tables 1 and 2 show that the serum copper concentrations ($15.53 \text{ mmol l}^{-1}$) were within the reference values ($10.2\text{-}17.3 \text{ mmol l}^{-1}$). The concentration of this trace element was similar (0.412 mg kg^{-1}) in skele-

tal muscles compared to reference values (about 0.64 mg kg^{-1}) as well as in the skin (1.94 mg kg^{-1}) compared to the reference values (about 2.0 mg kg^{-1}) (KLECZKOWSKI et al. 2011).

Zinc is required for the function of a large number of enzymes, therefore its nutritional deficiency contributes to the formation of numerous clinical symptoms. Among the many factors that predispose ruminants to zinc deficiency are an increased calcium and phosphorus intake, a diet rich in legumes, high phosphorus grain supplements without increased microminerals, and increased soil fertilizers, especially those containing nitrogen and phosphorus. The most important symptoms include skin damage around the nostrils, eyes, lips, anus, vulva, and at the base of the ears, around the back of the larynx, neck, outer thighs, on the lower limbs and folds of the joints, the skin of the udder and scrotum. Initially, one can observe skin peeling as well as raised and clumped-up hair. Pruritus causes the animal to lick and intensely rub at surrounding objects, leading to the formation of wounds and infection. The hair falls out and there is inflammation. Fissures and wounds form as a consequence of the numerous skin lumps and cracks, sometimes limiting the ability of the animals to move. There is an inflammation of the oral and nasal mucous membranes, swelling of the gums and drooling. Sometimes the only lesion seen in cows is eczema at the base of the tail. There may be a seasonal pattern in the clinical signs, particularly during the drying-up period. Sometimes ulcers of the abomasum and alopecia occur. Histologically, hyperkeratosis of the epidermis was observed with multiple deformities, loss of granulosa cells and hypertrophy of basal layer of the epidermis. The results obtained show that the serum concentrations of zinc in cows ($15.58 \text{ mmol l}^{-1}$) were within the reference values ($10.8\text{--}20.0 \text{ mmol l}^{-1}$). However, the zinc concentration in skeletal muscle was 29.5 mg kg^{-1} (reference value of about 70 mg kg^{-1}). This shows that the content of this trace element in bovine tissues was reduced. Serum zinc concentrations of $4.9\text{--}6.1 \text{ mg l}^{-1}$ were reported in sheep and goats in a herd where anorexia, wool eating, alopecia, hyperkeratosis and parakeratosis were observed. This demonstrates the strong homeostatic mechanisms that work in order to maintain at all costs an appropriate level of this micronutrient in the blood. However, the distribution of zinc in tissues differed from adequate values. Perhaps some of the reasons of reduced zinc levels in diseased tissues were undesirable environmental conditions, including long-term local pressure on some body parts as well as contact with urine and feces. Also, complex interactions between zinc and iron, and between zinc and copper, which may alter the distribution of zinc in tissues, must be kept in mind (AL-SAAD et al. 2010). Hyperkeratosis is primarily characterized by the presence of damaged skin cells. Histopathological changes also confirm the occurrence of lesions typical for parakeratosis (Figures 2-5). This results from the fact that zinc is involved in numerous biochemical life processes as well as in the replication of skin cells. In addition, zinc is responsible for cell oxidation and sequestration of free radicals (WRIGHTT, SPEARS 2004, MARKIEWICZ et al. 2007).

Stress is thought to increase susceptibility to skin diseases, which in part is due to increased endogenous glucocorticosteroid concentrations. Glucocorticosteroids have an immunosuppressive effect resulting from their interference with interleukin-1.4, leading to a blunted acute phase of inflammation. Hyperferremia, also induced by glucocorticosteroids, may as well contribute to the mechanism of stress-induced susceptibility to infection. Exogenous glucocorticosteroids in cattle have been related to increased serum iron concentrations, therefore it may be assumed that hyperferremia contributes to bacterial infections in this species (MUELLER, PAHI 2000, TAIRA et al. 2008, SORDILLO, AITKEN 2009). Oxidative stress is a major factor in inflammatory, malignant and skin diseases in cattle. The cellular and total body iron status influences oxidative stress-mediated cell damage as a result of an excess iron (Fe^{2+}) pool, which produces harmful free radicals (hydroxyls) via the Fenton reaction (KAPLOVITZ 2000, KOICHI, KIYOTAKA 2008).

The serum iron concentration in the cows was 35.5 mmol l^{-1} (reference range $30.67\text{-}36.1 \text{ mmol l}^{-1}$), whereas the muscle iron concentration was 42.2 mg kg^{-1} (reference range of about 56 mg kg^{-1}) and the skin iron concentration was 17.2 mg kg^{-1} . These results indicate that the iron serum concentrations were within the reference values.

Alkaline phosphatase (ALP) is a zinc-dependent enzyme located in many cell membranes, where it is involved in the transport of metabolites across cell membranes. A particularly high content of alkaline phosphatase is found in osteoblasts, which explains its increased activity in young, growing animals. High levels of alkaline phosphatase are also present in hepatocytes, renal tubules and intestinal epithelial cells. The enzyme is excreted into bile, therefore its concentration increases in cases of obstruction of bile outflow from the liver to the intestines as a result of urolithiasis or cancer. Blood ALP activity in cattle ranges from 41.0 to 116.0 U l^{-1} . The bone fraction makes up 50-60% of the total enzyme activity, the liver fraction - 10-20% of the total enzyme activity, and the intestinal fraction – 30% of the total enzyme activity. The average serum AP activity of the tested cattle was rather low, at 36.8 U l^{-1} , which might have resulted from an inadequate level of animal welfare and nutritional conditions (ALAM et al. 2010).

CONCLUSIONS

Deficiency of dietary zinc and adverse environmental factors had recognizable influence on the cows, which exhibited different clinical signs. Significant changes were noticed in blood biochemical values and biochemical and histopathological results of tissue studies that were characteristic for hyperkeratosis. After diagnostic tests revealed these problems, the animal welfare was improved through everyday use of a desiccant bedding, consisting of rye and oat straw, in individual stalls.

An increased supply of micronutrients (KenoBiotyn) was also given for a period of 6 months in a preparation which consisted of 25 000 mg of zinc, 2 500 mg of copper, 12 500 mg of manganese in the form of a chelate and 1 000 mg of biotin. Yeast (*Saccharomyces cerevisiae*) was used as a medium. The KenoBiotyn preparation was used for 6 months in a therapeutic dose of 100 g/cow.

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THE EFFECT OF TYTANIT APPLICATION ON THE CONTENT OF SELECTED MICROELEMENTS AND THE BIOLOGICAL VALUE OF TOMATO FRUITS

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Abstract

Titanium is an element exhibiting bio-stimulatory properties. The aim of the following investigations was to assess the effect of Tytanit application on the content of micronutrients in leaves and fruits, as well as the biological value of fruits from tomatoes grown in rockwool. The following levels of titanium side dressing were used: the control (no titanium applied), Ti-I (corresponding to the annual dose of 80 g Ti ha⁻¹), Ti-II (240 g Ti ha⁻¹), Ti-III (480 g Ti ha⁻¹) and Ti-IV (960 g Ti ha⁻¹). Tytanit at the level Ti-I had significant impact on the iron and manganese content in indicator parts of plants. A significant effect of Ti application on the zinc content in indicator parts of plants was observed at the Ti-II treatment in comparison with the control and other treatments. The application of Ti did not have any significant effect on the Cu content in indicator parts, except for the Ti-IV treatment. Increasing Ti doses caused a significant reduction of the copper content in fruits except for Ti-II and Ti-III. The application of Ti was shown to influence total acidity of tomato fruits. The highest content of nitrates was recorded in the combination Ti-III (30.03 mg kg⁻¹). No effect of Ti on the nitrate content was observed in the other combinations. A significant increase was found for the lycopene content in fruits when applying Ti-I (46.11 mg kg⁻¹) in relation to the other doses.

Key words: titanium, nitrates, lycopene, index parts, fruits.

WPLYW STOSOWANIA TYTANITU NA ZAWARTOŚĆ WYBRANYCH MIKROELEMENTÓW I WARTOŚĆ BIOLOGICZNĄ OWOCÓW POMIDORA

Abstrakt

Tytan jest pierwiastkiem o właściwościach biostymulujących. Celem badań była ocena wpływu stosowania Tytanitu na zawartość mikrośladników w liściach i owocach oraz wartość

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biologiczną owoców pomidora uprawianego w welnie mineralnej. Badano następujące poziomy dokorzeniowego stosowania tytanu wynoszące odpowiednio: kontrola (bez stosowania tytanu), Ti-I (co odpowiada dawce rocznej 80 g Ti ha⁻¹), Ti-II (240 g Ti ha⁻¹), Ti-III (480 g Ti ha⁻¹), Ti-IV (960 g Ti ha⁻¹). Wykazano istotny wpływ Tytanitu w dawce Ti-I na zawartości żelaza i manganu w częściach wskaźnikowych roślin. Stwierdzono istotny wpływ Ti na zawartość cynku w częściach wskaźnikowych roślin w kombinacji Ti-II w porównaniu z kontrolą i innymi kombinacjami. Stosowanie Ti nie miało istotnego wpływu na zawartość Cu w częściach wskaźnikowych z wyjątkiem kombinacji Ti-IV. Wzrastające dawki Ti miały istotny wpływ na zmniejszenie zawartości miedzi w owocach z wyjątkiem kombinacji Ti-II i Ti-III. Wykazano wpływ Ti na kwasowość ogólną owoców pomidora. Największą zawartość azotanów stwierdzono w kombinacji Ti-III (30,03 mg kg⁻¹). Nie stwierdzono wpływu Ti na zawartość azotanów w pozostałych kombinacjach. Wykazano istotny wzrost zawartości likopenu w owocach w przypadku dawki Ti-I (46,11 mg kg⁻¹) w stosunku do innych kombinacji.

Słowa kluczowe: tytan, azotany, likopen, części wskaźnikowe, owoce.

INTRODUCTION

Titanium is an element exhibiting bio-stimulatory properties (MICHALSKI 2008). Its effect consists, for example, in the stimulation of activity of certain enzymes, such as lipogenases (DAOOD 1998). One of the commercially available fertilizers containing titanium is Tytanit (0.8% Ti, i.e. 8.5 g Ti in the chelated form per 1 l stimulant). According to WÓJCIK and WÓJCIK (2001), Tytanit application has a positive effect on plant growth and development. The application of Tytanit showed an advantageous effect on the yielding of plants from the family of *Solanaceae* (JANAS et al. 2002). A positive effect of titanium application was also reported in the culture of orchard plants such as apples (WÓJCIK 2002). This element also reduces plant damage caused by heavy metals (LESKÓ et al. 2002).

The aim of the investigations was to assess the effect of Tytanit application on the content of selected micronutrients in tomato leaves and fruits as well as on the biological value of fruits in tomato grown in rockwool.

MATERIAL AND METHODS

Plant-growing experiments were conducted in 2010 - 2011. The effect of Tytanit fertigation on the content of microelements in leaves and fruits as well as on the biological values of fruits was investigated in tomato grown in rockwool. The experiments were run in a greenhouse dedicated to growing plant cultures, equipped with a modern climate control system. Climate parameters (temperature, CO₂ content, % RH) were recorded using the Synopta software. The greenhouse facilities comprised a modern, computer-controlled fertigation system and energy-conservation curtains. Plants were grown at a density of 2.7 plants m⁻². The following doses of titanium side dressing were

tested, i.e. the control (no titanium applied), Ti-I (corresponding to an annual dose of 80 g Ti ha⁻¹), Ti-II (240 g Ti ha⁻¹), Ti-III (480 g Ti ha⁻¹) and Ti-IV (960 g Ti ha⁻¹). Seeds were sown to culture plugs in the 1st half of March each year. After 2 weeks, seedlings were transplanted to rockwool cubes (10x10x10 cm). Transplants were moved to permanent beds on 15 April (2010) and 19 April (2011). The experiment was concluded on the 30th of September each year of the study. The experiment was conducted on the ISI 68249 tomato variety. Plants were grown in standard rockwool (density of 60 kg m⁻³, mats of 100x15x7.5 cm). A one-factor experiment was established in a completely randomized system, with six replications, two plants in each. Biological plant protection was used. All cultivation measures were performed in accordance with the current recommendations for tomato growing (ADAMICKI et al. 2005). Plant fertigation was applied using a standard nutrient solution containing (in mg dm⁻³): N-NH₄ – 2.0, N-NO₃ – 225, P-PO₄ – 50, K – 445, Ca – 150, Mg – 60, S-SO₄ – 115, Fe – 4.7, Mn – 0.3, Zn – 1.648, B – 0.40, Cu – 0.05, Mo – 0.08, at pH 5.50 and EC 3.00 mS cm⁻¹. Titanit fertilizer (by Intermag Olkusz), containing 8.5 g Ti in 1 dm³, was the source of titanium. The nutrient solution dose depended on the development phase of the plants and climatic conditions. In the period of intensive plant yielding and high temperatures (the months of June-July), 3.0-3.5 dm³ nutrient solution per plant was applied daily, in 15-20 single doses at 20-30% drip from mats.

Leaf samples for chemical analyses were collected on 15 June, 15 July and 16 August each year of the study. Indicator parts comprised 8th-9th leaves counting from the plant apex. One bulk sample consisted of 12 leaves collected from plants within a given combination. Representative fruit samples were collected in the 2nd half of August each year of the study. The collected plant material was dried at 45-50°C and ground. In order to determine the total content of iron, manganese, zinc and copper, the plant material was mineralized in the mixture of acetic and perchloric acids (3:1 v/v). After mineralization Fe, Mn, Zn and Cu were determined according to ASA. Biological parameters of fruits were determined using the following methods: nitrates by colorimetry, lycopene by HPLC, total acid by PN-90 A 75101/04. Results of chemical analyses were analyzed statistically using the Duncan test with inference at $\alpha=0.05$.

RESULTS AND DISCUSSION

Micronutrient content in leaves

A significant effect of Ti application on changes in the content of metallic microelements was observed in indicator parts of plants (Table 1). The highest content of iron and manganese was determined at Ti-I in comparison to the control as well as Ti-II – Ti-IV. According to MALINOWSKA and KALEM-

BASA (2012), titanium has a significant effect on the reduction of bioaccumulation of iron and manganese. Irrespective of a titanium level in this study, a higher mean content of iron in leaves was recorded than reported by CHOHURA and KOMOSA (2003), KOMOSA et al. (2011) and KLEIBER et al. (2012). The detected manganese content was also higher than recorded by KLEIBER et al. (2012), but lower than given by CHOHURA and KOMOSA (2003) and KOMOSA et al. (2011). Significantly the highest zinc content was recorded at the application of Ti-II (59.1 mg Zn kg⁻¹ d.m.). The significantly lowest copper content (13.3 mg Cu kg⁻¹ d.m.) was found at Ti-IV, with a simultaneous lack of impact at Ti-I – Ti-III. The reported copper content in leaves was lower than recorded by KOMOSA et al. (2011) and KLEIBER et al. (2012), but higher than given by CHOHURA and KOMOSA (2003). The content of iron, manganese, copper and zinc found in this study (except for Ti-I) in indicator parts fell within the ranges optimal for tomato (CAMPBELL 2000).

Table 1

The effect of Tytanit on the mean content (mg kg⁻¹ d.m.) of metallic microelements in tomato leaves (means from 2010-2011)

Treatment	Fe	Mn	Zn	Cu
Control	168.4ab	198.1a	39.70c	16.20b
Ti-I	175.4b	241.9b	14.90a	15.10b
Ti-II	163.6a	199.1a	59.10d	16.20b
Ti-III	149.6a	198.8a	24.80b	15.40b
Ti-IV	149.8a	181.3a	30.30b	13.30a

Values designated same letters do not differ significantly at $\alpha=0.05$.

Microelement content in fruits

A multifaceted effect was caused by Ti application in the nutrient solution applied in fertigation on the content of iron and zinc in tomato fruits (Table 2). The significantly higher content of iron and zinc was detected in the combination of Ti-IV (respectively 72.0 mg Fe kg⁻¹ d.m., 19.8 mg Zn kg⁻¹ d.m.) than in the Ti-II treatment (70.0 mg Fe kg⁻¹ d.m., 19.5 mg Zn kg⁻¹ d.m.). The lowest content of iron and zinc was found in the combination Ti-I (57.4 mg Fe kg⁻¹ d.m. and 13.5 mg Zn kg⁻¹ d.m.). The iron content detected herein in tomato fruits was lower than recorded by CHOHURA et al. (2009) and KLEIBER et al. (2012), but higher than demonstrated by KOMOSA et al. (2011) and JAROSZ et al. (2012). The application of increasing Tytanit doses in the nutrient solution had no effect on the manganese content in tomato fruits, which ranged from 12.8 mg Mn kg⁻¹ d.m. (Ti-IV) to 14.9 mg Mn kg⁻¹ d.m. (Ti-I). The content of manganese recorded in this study in tomato fruits was higher than given by KLEIBER et al. (2012), but lower than reported by KOMOSA et al. (2011) or JAROSZ et al. (2012). A significant effect of titanium fertigation on reducing the copper content in tomato fruits was found for combinations Ti-I

(13.2 mg Cu kg⁻¹ d.m.) – Ti-IV (4.0 mg Cu kg⁻¹ d.m.). The copper content determined in the control as well as combinations Ti-II – Ti-IV was lower than the levels given by KOMOSA et al. (2011) or KLEIBER et al. (2012). The copper content in tomato fruits was higher (except for combination Ti-IV) than the one reported by JAROSZ et al. (2012).

Table 2

The effect of Tytanit on the mean content (mg kg⁻¹ d.m.) of metallic microelements in tomato fruits (means from 2010-2011)

Treatment	Fe	Mn	Zn	Cu
Control	65.50 <i>b</i>	13.60 <i>a</i>	14.60 <i>a</i>	7.800 <i>b</i>
Ti-I	57.40 <i>a</i>	14.90 <i>a</i>	13.50 <i>a</i>	13.20 <i>c</i>
Ti-II	70.00 <i>c</i>	14.30 <i>a</i>	19.50 <i>b</i>	8.600 <i>b</i>
Ti-III	64.40 <i>b</i>	14.10 <i>a</i>	15.60 <i>a</i>	7.900 <i>b</i>
Ti-IV	72.00 <i>c</i>	12.80 <i>a</i>	19.80 <i>b</i>	4.000 <i>a</i>

Values designated the same letters do not differ significantly at $\alpha=0.05$.

Biological parameters of fruits

Analyses showed the impact of Tytanit application on the total acidity of tomato fruits (Table 3). The highest content of citric acid was recorded in combination Ti-IV (0.33%), while the lowest one was in combination Ti-I (0.22%). No significant differences were found in the total acidity between the control (0.27%) and combinations Ti-II (0.26%) and Ti-III (0.28%). The detected acidity was lower than observed in studies by HALLMANN and REMBIAŁOWSKA (2007) and MAJKOWSKA-GADOMSKA et al. (2008). The highest nitrate content was observed in combination Ti-III (30.03 mg kg⁻¹). No effect of Tytanit on the nitrate content was found in the control combinations Ti-I and Ti-II as well as Ti-IV. The nitrate content found in fruits in this study was greater than the levels reported by KOMOSA et al. (2011); at the same time, it was lower than recorded in a field culture by MAJKOWSKA-GADOMSKA et al. (2009) and GAJC-WOLSKA et al. (2010).

Tomato fruits are an important source of antioxidants (CROZIER et al. 1997, FANASCA et al. 2006). Lycopene, a carotenoid, is the primary pigment responsible for the red colour of fruits (DAVIS et al. 2003). Major sources of lycopene in the human diet are fruits of tomato, red peppers and red grapefruits (HAKALA, HEINONEN 1994). In the present study, a significant increase was recorded in the lycopene content in fruits when applying Ti-I (46.11 mg kg⁻¹) in relation to the control and treatments Ti-II – Ti-III. The application of the highest titanium concentration (Ti-IV) significantly reduced the lycopene content in fruits. In this study, we found a higher lycopene content than recorded by HALLMANN and REMBIAŁOWSKA (2007), although it was lower than the data given by BRANDT et al. (2006) and GALPAZ et al. (2008). According to SHI (2000), lycopene accounts for 80-90% of all carotenoids in

ripe tomato fruits. The carotenoid content depends on the cultivar, environmental conditions and the ripeness of fruits (SHI 2000). Temperature is a factor influencing the lycopene content. Tomato fruits in the summer contain 30% less lycopene than those in a winter culture (TOOR et al. 2006).

Table 3

The effect of Tytanit on selected biological parameters of tomato fruits
(% means from 2010-2011)

Treatment	Total acid (% citric acid)	Nitrates (mg kg ⁻¹)	Lycopene (mg kg ⁻¹)
Control	0.270 <i>b</i>	23.44 <i>a</i>	34.26 <i>b</i>
Ti-I	0.220 <i>a</i>	26.10 <i>a</i>	46.11 <i>c</i>
Ti-II	0.260 <i>b</i>	24.66 <i>a</i>	34.30 <i>b</i>
Ti-III	0.280 <i>b</i>	30.03 <i>b</i>	32.70 <i>b</i>
Ti-IV	0.330 <i>c</i>	24.35 <i>a</i>	18.01 <i>a</i>

Values designated the same letters do not differ significantly at $\alpha=0.05$.

CONCLUSIONS

1. Tytanit application had a significant effect on the increase of the iron and manganese content in indicator parts (the highest Fe and Mn content at Ti-I) as well as zinc (the highest content at Ti-II).

2. No significant differences were found in the copper content in tomato leaves between the control and combinations Ti-I – Ti-III.

3. The multifaceted effect of titanium application was observed for the iron and zinc content in tomato fruits. For the copper content, a significant reduction was found at Ti-IV in comparison to the other combinations. The manganese content in fruits did not differ significantly.

4. A significant effect of Tytanit on the biological value of tomato fruits was observed in this study. The lycopene content was the highest in the case of Ti-I (46.11 mg kg⁻¹), whereas the Ti-IV treatment induced a significant increase in total acidity of fruits. The nitrate content did not differ significantly (except for Ti-III).

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**DESCRIPTION AND ASSESSMENT
OF CHEMICAL PROPERTIES OF FRUITS
OF THE CHOCOLATE VINE (FIVE-LEAF
AKEBIA) *AKEBIA QUINATA* (HOUTT.)
DECNE AND DEAD MAN'S FINGERS
DECAISNEA INSIGNIS (GRIFF.) HOKK. F.
& THOMSON, GROWN IN SZCZECIN
AND IN THE ARBORETUM IN GLINNA
(NORTHWESTERN POLAND)**

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Abstract

Originating from East Asia, the five-leaf akebia *Akebia quinata* and dead man's fingers *Decaisnea insignis* are rarely cultivated in Poland. In China, the fruits of these shrubs are a delicacy, and their skins, rich in saponins and flavonoids; are used in folk medicine. The natural range of occurrence of *Akebia quinata* and *Decaisnea insignis* is in East Asia, and the earliest mention of their cultivation in Europe dates back to 1945. Fully ripe fruits for the study were collected in September and October. The determinations included the size, color, reflectance and chemical composition (fresh berries - soluble solids, titratable acidity, pH, total polyphenol, L-ascorbic acid, nitrates and nitrites; frozen berries - phenolics and mineral composition) of fruits harvested from shrubs grown in Szczecin and in the Arboretum in Glinna near Szczecin.

In the climatic conditions of Szczecin, these species can flourish and produce ripe fruit. *Akebia quinata* bears significantly heavier fruits (72.1 g) than *Decaisnea insignis* fruits (31 g), despite similar length of fruits from both species. The fruits of *Decaisnea insignis* are more acidic (0.72 g 100 mL⁻¹) and have a higher content of polyphenols (567 mg 100 mL⁻¹), while the ones of *Akebia quinata* contain more extract (16.8%) and L-ascorbic acid (37.5 mg 100 mL⁻¹). *Deca-*

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isnea insignis have darker colour of the fruit surface (L^* 18.92) and fruit flesh (L^* 46.92) than fruits of *Akebia quinata* (respectively L^* 25.49 and 59.93). The former species accumulated more polyphenols (*D. insignis* 567 mg, *A. quinata* 382 mg 100 mL⁻¹) in the outer parts of fruits than in the flesh (respectively 34 mg and 11 mg 100 mL⁻¹). The five-leaf *Akebia quinata* and dead man's fingers *Decaisnea insignis* are woody, ornamental plants, which can also be grown for consumption and medicinal uses.

Key words: color, fruit size, mineral composition, phenolics, reflectance.

**CHARAKTERYSTYKA I OCENA WŁAŚCIWOŚCI CHEMICZNYCH OWOCÓW
AKEBII PIĘCIOLISTKOWEJ *AKEBIA QUINATA* (HOUTT.) DECNE
I PALECNIA CHIŃSKIEGO (P. FARGESA) *DECAISNEA INSIGNIS* (GRIFF.)
HOKK. F. & THOMSON UPRAWIANYCH W SZCZECINIE I W ARBORETUM
W GLINNEJ (PÓŁNOCNO-ZACHODNIA POLSKA)**

Abstrakt

Pochodzące z Azji Wschodniej akebia pięciolistkowa i palecznik chiński są w Polsce rzadko uprawiane. W Chinach owoce tych krzewów stanowią przysmak, bogate w saponiny oraz flawonoidy skórki (osłonki) są wykorzystywane w medycynie ludowej. Naturalne stanowiska gatunków *Akebia quinata* i *Decaisnea insignis* znajdują się w Azji Wschodniej, a pierwsze wzmianki o uprawie w Europie pochodzą z 1945 roku. W pełni dojrzałe owoce do badania zebrano w trzeciej dekadzie września. W badaniach określono wielkość, barwę i refraktancję oraz skład chemiczny owoców (w świeżych – ekstrakt, kwasowość, pH, sumę polifenoli i kwasu L-askorbinowego, azotany i azotyny; w mrożonych-związki polifenolowe oraz makro- i mikroelementy), zebranych z krzewów uprawianych w Szczecinie oraz w Arboretum w Glinnej k. Szczecina. Omawiane gatunki w warunkach klimatycznych Szczecina kwitły oraz wytwarzały dojrzałe owoce. Owoce akebii chińskiej są znacznie cięższe (72,1 g) niż owoce palecznika chińskiego (31 g), pomimo zbliżonej długości. Owoce palecznika miały wyższą kwasowość (0,72 g 100 mL⁻¹) oraz zawartość polifenoli (567 mg 100 mL⁻¹), natomiast owoce akebii zawierały więcej ekstraktu (16,8%) i kwasu L-askorbinowego (37,5 mg 100 mL⁻¹). Owoce palecznika miały ciemniejszą skórkę (L^* 18,92) i miąższ (L^* 46,92) niż owoce akebii (odpowiednio L^* 25,49 i 59,93). Badane gatunki akumulowały kilkukrotnie więcej polifenoli (palecznik 567 mg, akebia 382 mg 100 mL⁻¹) w zewnętrznej ciemniejszej części owoców niż w miąższu (odpowiednio 34 mg i 11 mg 100 mL⁻¹). Akebia pięciolistkowa i palecznik Fargesa, drzewiaste rośliny ozdobne, mogą być również uprawiane na potrzeby konsumpcyjno-lecznicze.

Słowa kluczowe: kolor, polifenole, refraktancja, składniki mineralne, wielkość owoców.

INTRODUCTION

The five-leaf akebia [(*Akebia quinata* (Houtt.) Decne)] and dead man's fingers *Decaisnea insignis* (Griff.) Hokk. f. & Thomson are Asian shrub species within the family *Lardizabalaceae* Decne (*Delectis Florae* ...). The natural range of occurrence of the five-leaf akebia is in central China and Japan, where it is a common plant, and on the Korean Peninsula (KRÜSSMANN 1960, 1962, SENETA 1991), while the earliest mention of its cultivation in Europe (in England) dates back to 1945. It is a clockwise climbing creeper with

evergreen, palmate compound leaves, consisting of five entire margin leaflets. The flowers of *Akebia* are dioecious, monoecious, with a single perianth; masculine with pink perianth sepals; feminine with twice as big, reddish-purple and darker sepals (Photo 1). It usually blooms at the end of April. Several fleshy, purple when ripe, bursting, fleshy follicles (MABBERLEY 2008), and – according to SENETA (1991) – oblong berries, 10 cm long, create the fruit complex (syncarpium) – Photo 2. Under good conditions on plantations in China, it is possible to collect up to 30 tons of fruit in the first fruit bearing year,



Phot. 1. Male and female flowers (larger) of *Akebia quinata* (phot. M. Kubus)



Phot. 2. Aggregate fruit of *Akebia quinata* (phot. M. Kubus)

and the yield can double in subsequent years – 3 to 4 years after planting (ZHONG et al. 2006). Seeds are numerous, black, immersed in white, jelly-like, sweet pulp. The seeds contain a large amount of fatty acids, mainly oleic (47.63%), palmitic (20.14%), and linoleic acid (27.05%) (BAI 2007). The fruits are edible, with chocolate-like flavor (akebia is commonly known as the ‘chocolate vine’), although the skin is bitter and unpalatable. In China, culinary uses are common, e.g. unripe and grated fruits are a spice and stuffing for meat, and ripe ones are a dessert. In medicine, their diuretic, anti-inflammatory and analgesic features are valued. Dried fruits are commonly used in acupuncture (Internet 1). They contain a large amount of potassium salts and saponins and flavonoids (MADHURI, PANDEY 2009). The fruits are also characterized by high antioxidant activity (KIM et al. 1994). *Akebia quinata* is listed in the Chinese Pharmacopoeia (Pharmacopoeia Commission of PRC 2005). According to SENETA (1991), in Poland, the five-leaf *Akebii quinata*

specimens are rare. During severe winters the plant freezes up to the snow line, but regenerates well. Fruits, even in areas of Poland with a mild climate, are rare.

Decaisnea insignis grows naturally in western China, in areas located in the upper reaches of the Yangtze River to the Himalayas. It is a shrub with apinnate, compound, 12-25 leaflet leaves; its leaves are entire margin ones, pointed at the distal end, bluish on the bottom. Flowers from hanging clusters of *Decaisnea insignis* are polygamous, no-leaflet ones, greenish, in the companulate form (Photo 3). The plant flowers in May and June. The fruit – fleshy, cylindrical, blue follicle – contains black seeds (SENETA 1996) – Photo 4. White, milky and viscous substance oozes from a cut fruit, whereas



Phot. 3. Flowers of *Decaisnea insignis*
(phot. M. Kubus)



Phot. 4. Leaves and fruits of *Decaisnea insignis* (phot. M. Kubus)

overripe fruits disintegrate into gelatinous mass. Sweet fruits are a delicacy in China, often used to garnish desserts. In Poland, *Decaisnea insignis* is rarely grown outside botanical gardens and arboreta.

The aim of this study was to determine the quality and chemical composition of *Akebia quinata* and *Decaisnea insignis* fruits obtained from shrubs grown in northwestern Poland.

MATERIAL AND METHODS

The fruits of *Akebia quinata* came from a plant grown in a private garden on Zbójnicka Street in Szczecin, which was planted in 2001 and creeps up a wall of a three-story building with southern exposure. Since 2002, that chocolate vine plant produced a number of flowers and fruit buds (10-12 mm in diameter), which were dropped prematurely in the early years of the plant's growth. Fully ripe fruits for the study were collected in the third decade of September. It was observed that the earliest fruit buds appeared abundantly, but were shed off. It was the following, less abundant flowering period that led to the closing of the *Akebia*'s generative development cycle, producing fully developed and ripe fruits (Photo 2).

Fully ripe *Decaisnea insignis* fruits were collected in early October from several shrubs growing for over ten years in the Arboretum in Glinna near Szczecin. The plants were propagated from seeds obtained from maternal specimens growing in the Rogów Arboretum of Warsaw University of Life Sciences, in central Poland (TUMIŁOWICZ 2005). They were planted in Glinna in 2001 and began bearing fruit in the sixth year (TUMIŁOWICZ 2013).

Physical features of fruits and soluble solids, titratable acidity, pH, total polyphenol, L-ascorbic acid, nitrates and nitrites were measured on fresh berries immediately after the harvest. The composition of phenolics and minerals was determined on samples which were kept frozen (-32°C) in polyethylene bags (2 x 500 g) until analysed.

The fruit weight was measured on a RADWAG WPX 4500 electronic scales (0.01 g accuracy). Fruit color and reflectance were measured in a transmitted mode on a Konica Minolta CM-700d spectrophotometer. Measurements were conducted in the CIE L*a*b* system through a 10° observer type and D65 illuminant, with the aperture diameter measuring 3 mm. The full nomenclature is in the 1976 CIE L*a*b* Space, International Commission on Illumination in Vienna: L* white (100) black (0), a* green (-100) red (+100), b* blue (-100) yellow (+100) (Hunterlab 2012). After harvest, the fruits were de-stemmed and crushed. Next, the colour of the pulp was determined (OCHMIAN et al. 2012a). To make juice, berries were macerated for 60 min. at 50°C, with the PT 400 Pektopol enzyme added in a dose of 400 mg per kg of fruits. After the enzymatic processing finished, the pulp was pressed in a hydraulic press at a pressure of 3 Mpa, after which the colour of the juice was determined (OSZMIANSKI, WOJDYŁO 2005).

The content of soluble solids was determined with a digital refractometer PAL-1 (Atago, Japan). Titratable acidity was determined by titration of a water extract with 0.1 N NaOH to the end point of pH 8.1 (measured with a multimeter Elmetron CX-732) according to PN-90/A-75101/04. L-ascorbic acid, NO₃ and NO₂ were determined with aRQflex 10 reflectometer Merck (OCHMIAN et al. 2012a). The total polyphenol content was estimated in methanol (70%) extracts according to SINGLETON and ROSSI (1965) with the Folin-

-Ciocalteu reagent. The data are expressed as mg of gallic acid equivalents (GAE) per 100 g of fruit tissue. 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 the atomic emission spectrometry, whereas the Mg content was checked with flame atomic absorption spectroscopy using an SAA Solaar device. The phosphorus content was determined with the Barton method at 470 nm wavelength, whereas the sulphur content was assayed with the turbidimetric method at 490 nm wavelength with a Marcel s 330 PRO spectrophotometer. The content of microelements (Cu, Zn, Mn, Fe), after mineralization in HClO_4 and HNO_3 , was measured with flame atomic absorption spectroscopy using an SAA Solaar (IUNG 1972).

The values were evaluated by the Tukey's test and the differences at $p < 0.05$ were considered significant. The statistical analyses were performed using the Statistica 10.0 software (Statsoft, Poland).

RESULTS AND DISCUSSION

Ripe fruits of the Chinese *Akebia quinata* were more than twice as heavy (the weight of 1 fruit – 72.1 g) as the fruits of *Decaisnea insignis* (the weight of one fruit – 31 g). Fruits of a similar length characterized both studied species, but the diameter of open follicles of *Akebia quinata* was more than twice the length of the diameter of the *Decaisnea insignis* fruits (39.2 mm vs 18.4 mm). The dimensions of *Akebia quinata* fruit collected in Szczecin are typical for this species (Li et al. 2010). The edible parts of fruits are both the skin, used mainly for pharmaceutical purposes, and the sweet pulp. The flesh of a *Decaisnea insignis* fruit made up 48.5% of the whole fruit weight. In *Akebia quinata*, the analogous percentage was 30.2%. The skin contributed as much as 62.9% to the weight of fruit, and the seeds corresponded to 6.9% of the whole fruit weight. In the wild forms of *Akebia quinata*, the skin and seeds can constitute up to 80% of the whole fruit weight (ZHONG et al. 2006). In the fruits of *Decaisnea insignis*, the seeds constituted 15.6% of the total weight. The fruit of *Akebia quinata* has a delicate and sweet flavour and soft juicy texture, tasting like a mixture of banana, litchi, and passion fruit. However, the flavour varies – some fruits are fairly bland, others have more complex flavour profiles. *Akebia quinata* fruits should be harvested at optimum maturity (Li et al. 2010).

The analyses showed different content of organic and mineral components in the skins and fruit pulp of the two species (Tables 1 and 2). The percentage of extracts from *Akebia quinata* and *Decaisnea insignis* skins was similar: 9.8% and 8.3%, respectively, being significantly lower than in the fruit pulp: 13.6 and 16.8%, respectively. Pulp sugars include fructose – 4.10, glucose – 2.78, and sucrose 1.57; all given in g in 100 g (Li, Li 1991).

Table 1

Some characteristics of the analysed fruits

Testfeature	Species			
	<i>Decaisnea insignis</i>		<i>Akebia quinata</i>	
Physical characteristics				
Mass of 1 fruit (g)	31.0 <i>a</i>		72.1 <i>b</i>	
Fruit length (mm)	111 <i>a</i>		104 <i>a</i>	
Fruit diameter (mm)	18.4 <i>a</i>		39.2 <i>b</i>	
Share of skins in the fruit weight (%)	35.9 <i>a</i>		62.9 <i>b</i>	
Share of fruit pulp in the mass (%)	48.5 <i>b</i>		30.2 <i>a</i>	
Weight of seeds per fruit weight (%)	15.6 a		6.9 b	
Chemical composition				
	skin	flesh	skin	flesh
Soluble solids (%)	8.33 <i>a</i>	13.61 <i>b</i>	9.82 <i>a</i>	16.80 <i>c</i>
Juice pH	5.03 <i>a</i>	5.21 <i>b</i>	5.86 <i>c</i>	5.82 <i>c</i>
Titrateable acidity (g 100 mL ⁻¹)	0.72 <i>b</i>	0.64 <i>b</i>	0.34 <i>a</i>	0.28 <i>a</i>
L-ascorbic acid (mg 100 mL ⁻¹)	29.55 <i>a</i>	24.90 <i>a</i>	44.20 <i>c</i>	37.55 <i>b</i>
Total polyphenol (mg 100 mL ⁻¹)	567 <i>c</i>	34 <i>a</i>	382 <i>b</i>	11 <i>a</i>
Nitrates - NO ₃ (mg 1000 mL ⁻¹)	65.62 <i>d</i>	34.23 <i>c</i>	24.30 <i>b</i>	11.81 <i>a</i>
Nitrites - NO ₂ (mg 1000 mL ⁻¹)	0.72 <i>c</i>	0.51 <i>b</i>	0.38 <i>a</i>	0.33 <i>a</i>

* Means marked in row with the same letter do not differ significantly at $p < 0.05$ according to the Tukey's test.

The fruits were characterized by a high pH and low content of organic acids. In 100 grams of *Akebia quinata* fruits, an average of 0.31 g of acid was found, which was half the amount found in 100 g of fruit harvested from the shrubs of *Decaisnea insignis*. The acidity of fruit flesh was generally low, which results in a high sugar-to-acid ratio (Li, Li 1991). The organic acid content in lowbush blueberry, chokeberries and blue honeysuckle ranges from 0.8 g to 1.4 g in 100 g of fruits (OCHMIAN et al. 2009, 2012a).

Fruits of neither species were rich in L-ascorbic acid. *Decaisnea insignis* had a similar content of L-ascorbic acid in its skin (29.5 mg 100 mL⁻¹) and pulp (24.9 mg 100 mL⁻¹), whereas in the *Akebia quinata* fruits, the level of L-ascorbic acid was significantly higher, especially in the skins (44.2 mg 100 mL⁻¹). Fruits of various species of *Akebia quinata*, according to Li et al. (2010), are very rich in this vitamin, containing up to 930 mg 1000 mL⁻¹. The skins of both species, especially *Decaisnea insignis* (567 mg 100 mL⁻¹), were

Table 2

The content of macro-and micronutrients in fruits of *Decaisnea insignis* and *Akebia quinata*

Mineral components		Species			
		<i>Decaisnea insignis</i>		<i>Akebia quinata</i>	
		skin	flesh	skin	flesh
Content (g 100 g ⁻¹ f.w.)	N	2.34 ^c	1.60 ^b	1.77 ^b	1.32 ^a
	P	0.42 ^b	0.33 ^a	0.49 ^c	0.45 ^{bc}
	K	1.58 ^c	1.45 ^{bc}	1.31 ^b	1.13 ^a
	Ca	0.17 ^c	0.13 ^{bc}	0.11 ^{ab}	0.09 ^a
	Mg	0.12 ^c	0.09 ^b	0.06 ^a	0.05 ^a
Content (mg 100 g ⁻¹ f.w.)	Cu	12.55 ^c	9.46 ^b	8.21 ^b	5.37 ^a
	Zn	11.34 ^c	7.21 ^b	7.89 ^b	4.69 ^a
	Mn	27.42 ^b	30.13 ^b	14.60 ^a	10.14 ^a
	Fe	25.26 ^b	9.32 ^a	31.55 ^b	8.64 ^a

* Means marked in rowwith the same letter do not differ significantly at $p<0.05$ according to the Tukey's test.

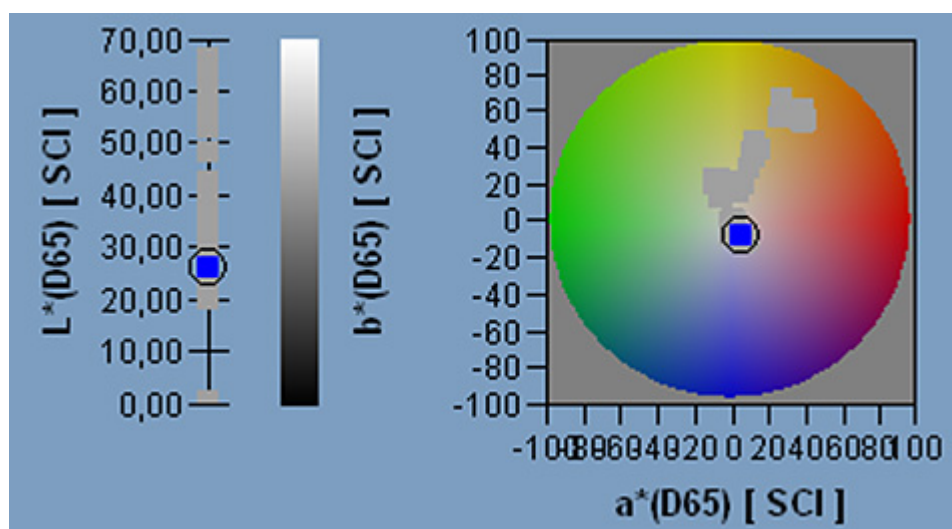
rich in polyphenolic compounds (Table 1), while the pulp was practically void of polyphenolics (*Akebia quinata* - 11, *Decaisnea insignis* 34 mg 100 mL⁻¹). This is manifested by the colour of the fruit parts as polyphenolic compounds, particularly anthocyanins, give fruit blue tinge. The skins of the analysed fruits are much darker than the pulp, as indicated by parameter L* (Table 3). Measurements also showed significant differences in the values of parameters a* and b*. They demonstrate that the skins were red-blue (Figure 1). The surface of *Akebia quinata* fruits can go from green through purple to brown in colour (Li et al. 2010). The skins of *Decaisnea insignis* fruits were darker (L* 18.92) than the skins of *Akebia quinata* fruits

Table 3

The flesh and fruit surface color of tested species

Colour CIE L*a*b*		Species	
		<i>Decaisnea insignis</i>	<i>Akebia quinata</i>
Color of the fruit surface	L*	18.92 ^b	25.49 ^a
	a*	16.02 ^a	21.72 ^b
	b*	-24.56 ^a	-25.72 ^a
Color offlesh	L*	46.92 ^b	59.83 ^a
	a*	0.33 ^a	-3.82 ^b
	b*	5.95 ^a	14.41 ^b

* Means marked in rowwith the same letter do not differ significantly at $p<0.05$ according to the Tukey's test.



L* (100 white, 0 black) a* (-100 green, +100 red) b* (-100 blue, +100 yellow)

Fig. 1. CIE L*a*b* chromaticity diagram

(L^* 25.49), but contained less of the compounds which give a red tinge (parameter a^* 16.02). Parameter b^* was at a similar level: 24.56 for *Decaisnea insignis* and 25.72 for *Akebia quinata*. Also, the pulp of *Decaisnea insignis* fruits (L^* 46.92) was much darker than the pulp of *Akebia quinata* fruits (L^* 59.83). Parameter b^* measured for the pulp of *Akebia quinata* was much higher than for the pulp of *Decaisnea insignis*. This has also been confirmed by the refractive index of individual wavelengths shown in Figure 2. Parameters L and b determined for the analysed species showed similar values to the ones reported for blue honeysuckle berries, L^* 21.08; b^* -25.40 (OCHMIAN et al. 2012b), and parameter L was similar in value to the one determined for fruits of the genus *Amelanchier* (OCHMIAN et al. 2013).

The analyses have shown that more nitrates were accumulated in the skins of both species (*Decaisnea insignis* – 47.9%, *Akebia quinata* – 52.4%) than in the pulp. Nitrites were also more abundant in the skins of *Decaisnea insignis*, while the levels of harmful nitrites in the *Akebia quinata* fruits were similar in both the skin and pulp. Generally, *Akebia quinata* fruits accumulated significantly less nitrates and nitrites than fruits of *Decaisnea insignis*. In Poland, and in many other countries, there are no regulations on a permissible nitrate content in fruits.

The analysis of the mineral content showed that in most cases the skin of the analyzed species contained more macro- and micronutrients (Table 2). The *Decaisnea insignis* fruits also contained more minerals (N, K, Ca, Mg, Cu, Zn, Mn) compared to the fruits of *Akebiaquinata*.

In the above study, the fruits of *Akebia quinata* contained much more macronutrients. In another study, by ZHANG et al. (2003), the level of potas-

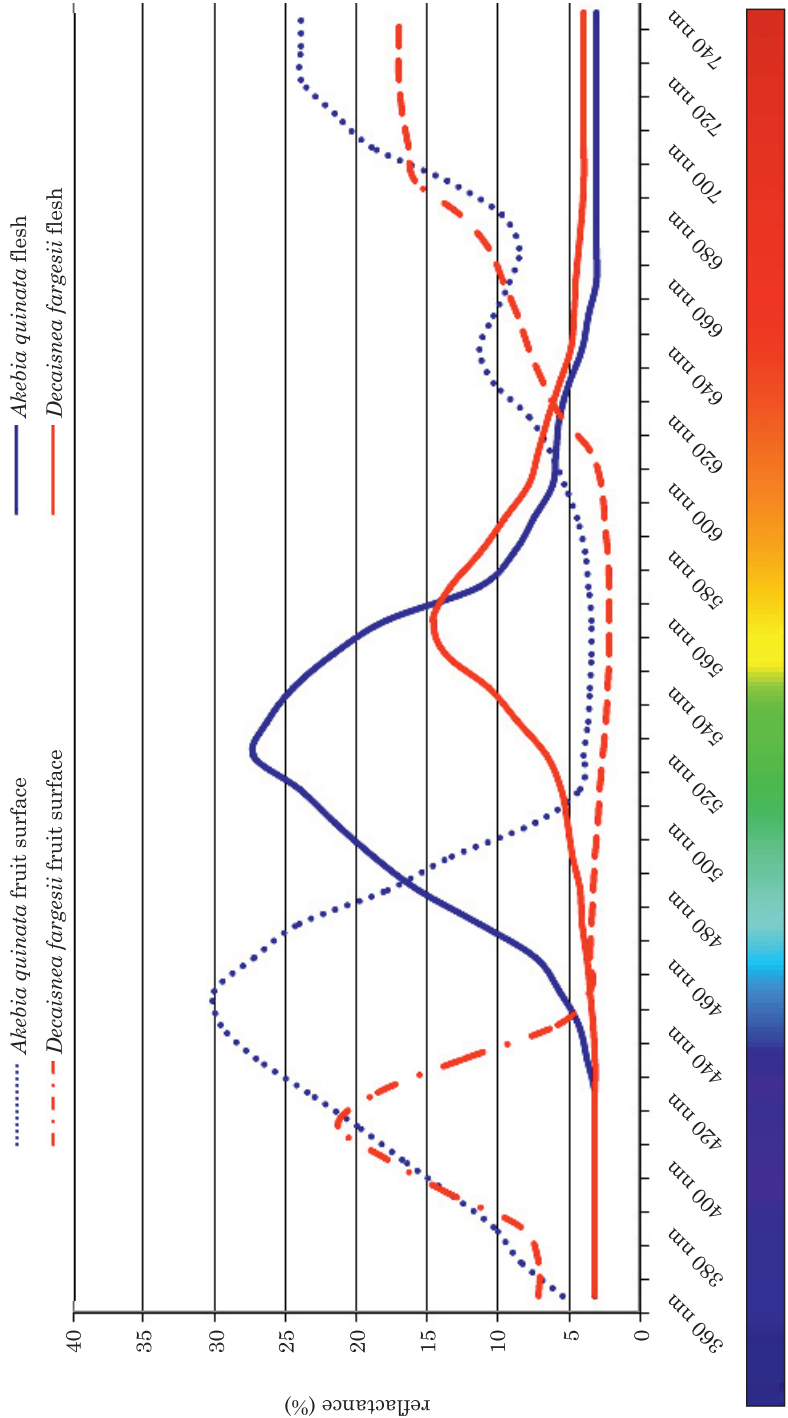


Fig. 2. Reflectance of the fruits

sium was more than double (3.21 to 4.96 g per 100 g of fresh fruit) that *Akebia quinata* fruits (1.31 and 1.13 g 100 g⁻¹). The levels of magnesium and calcium were also significantly lower. In general, concentrations of potassium, magnesium, zinc, iron, and manganese in the *Akebia quinata* species are higher than in other major dry fruits, such as apples, pears, oranges, etc. (ZHANG et al. 2003). The content of macronutrients and iron in the analysed fruits was at the same level as the content of copper, while manganese was at a higher level than in fruits of several varieties of plum trees (MILOŠEVIĆ, MILOŠEVIĆ 2012).

CONCLUSIONS

1. The five-leaf akebia *Akebia quinata* and dead man's fingers *Decaisnea insignis* can complete the full generative phase in the climatic conditions of Szczecin, producing flowers, and then fully developed and ripe fruit.

2. The morphological traits of the analysed fruits of *Akebia quinata* and *Decaisnea insignis* correspond to the ones of the above species growing in natural conditions.

3. *Decaisnea insignis* fruits are characterized by a higher content of organic acids and polyphenols, while fruits of *Akebia quinata* contain more extract and L-ascorbic acid. In both plants, a higher content of most of these organic compounds was found in the skin or outer part of the fruit than in the pulp.

4. The skins and pulp of *Decaisnea insignis* were darker, and fruits of *Akebia quinata* contained more of the compounds, which give fruit a red and blue tinge.

5. Fruits of *Decaisnea insignis*, especially their skins, are characterized by a higher content of macro-and micronutrients, except phosphorus.

6. The five-leaf akebia *Akebia quinata* and dead man's fingers *Decaisnea insignis*, rarely grown in Western Pomerania as woody, ornamental plants, can also be cultivated for consumption and medicinal uses.

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ESTIMATION OF CATION EXCHANGE CAPACITY AND CATION SATURATION OF LUVISOLS DEVELOPED FROM LOESS

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Abstract

The cation exchange capacity and saturation of the sorptive complex with base cations are very important indicators for quality assessment of soils, because they define soil fertility and resistance to chemical degradation. The objective of the following study was to estimate the cation exchange capacity and saturation with exchangeable cations of Luvisols developed from loess, under agricultural use. The study comprised 12 pedons under winter wheat culture, classified into various complexes of soil suitability. Exchangeable cations of alkaline character were assayed with the Pallmann method in 1 mol $\text{NH}_4\text{Cl dm}^{-3}$ extract with pH 8.2. The content of cations Ca^{2+} , K^+ and Na^+ was determined with a flame photometer, while that of Mg^{2+} was measured with the AAS method. In the Ap horizons of the analyzed soils, the quantitative sequence of base cations was most frequently $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+ > \text{Na}^+$. The total exchange bases (TEB) was favourable in most of the soils (3.8-16.9 cmol(+) kg^{-1}), and the total acidity (TA) was varied (0.5-3.7 cmol(+) kg^{-1}) but generally low. The cation exchange capacity (CEC) in the Ap, Et, EB, Bt and BC horizons of the soils was assessed as medium or high (5.2-17.9 cmol(+) kg^{-1}). Only the C horizon, containing calcium carbonate, was characterised by very high CEC (76.2 cmol(+) kg^{-1}). The base saturation (BS) was highly favourable in most of the soils (62.9-99.4%) and increased deeper into the pedon. The soils were characterised by generally favourable shares of particular cations: the percentage of Ca^{2+} was 51.5-95.1%, most often higher than the optimum, while the shares of Mg^{2+} and K^+ were slightly below the optimum. The calculated quantitative ratios between the cations showed very strong variation of values among particular pedons. The content of Mg^{2+} cations and the values of TEB and CEC in the Ap horizons of soils from the very good wheat complex were lower than in the Ap horizons of soils from the good wheat complex and the deficient wheat complex, whereas the levels of Ca^{2+} , K^+ , Na^+ and H^+ as well as BS displayed only minor differences.

Key words: Luvisols developed from loess, cation exchange capacity, exchangeable cations.

OCENA POJEMNOŚCI WYMIANY KATIONÓW I WYSYCENIA KATIONAMI GLEB PŁOWYCH WYTWORZONYCH Z LESSU

Abstrakt

Pojemność wymiany kationów i wysycenie kompleksu sorpcyjnego kationami zasadowymi są bardzo ważnymi wskaźnikami jakości gleb, ponieważ decydują o ich żyzności i odporności na degradację chemiczną. Celem pracy była ocena pojemności wymiany kationów i wysycenia kationami wymiennymi intensywnie użytkowanych rolniczo gleb płowych wytworzonych z lessu. Badaniem objęto 12 pedonów pod uprawą pszenicy ozimej, zaliczanych do różnych kompleksów przydatności rolniczej. Kationy wymienne o charakterze zasadowym oznaczono metodą Pallmana w wyciągu 1 mol NH_4Cl dm^{-3} o pH 8,2. Zawartość kationów Ca^{2+} , K^+ i Na^+ oznaczono na fotometrze płomieniowym, natomiast zawartość kationów Mg^{2+} – metodą AAS. W poziomach Ap badanych gleb szereg ilościowy kationów zasadowych kształtował się najczęściej jako $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+ > \text{Na}^+$. Suma kationów zasadowych była w większości gleb korzystna (3,8-16,9 $\text{cmol}(+) \text{ kg}^{-1}$), a kwasowość hydrolityczna zróżnicowana (0,5-3,7 $\text{cmol}(+) \text{ kg}^{-1}$), ale najczęściej mała. Pojemność wymiany kationów w poziomach Ap, Et, EB, Bt i BC badanych gleb oceniono jako średnią lub dużą (5,2-17,9 $\text{cmol}(+) \text{ kg}^{-1}$). Tylko w poziomie C zawierającym węglan wapnia wykazano bardzo dużą pojemność (76,2 $\text{cmol}(+) \text{ kg}^{-1}$). Stopień wysycenia kationami zasadowymi był w większości badanych gleb bardzo korzystny (62,9-99,4%) i wzrastał wraz z głębokością pedonu. W badanych glebach stwierdzono ogólnie korzystny udział poszczególnych kationów: udział Ca^{2+} wynosił 51,5-95,1% i był najczęściej większy od optymalnego, natomiast udział Mg^{2+} i K^+ był nieco mniejszy od optymalnego. Obliczone stosunki ilościowe między kationami wykazywały bardzo duże zróżnicowanie wartości w poszczególnych pedonach. Zawartość kationów Mg^{2+} , suma kationów zasadowych i pojemność wymiany kationów w poziomach Ap gleb kompleksu pszennego bardzo dobrego była mniejsza niż w poziomach Ap gleb kompleksu pszennego dobrego i pszennego wadliwego, natomiast zawartość Ca^{2+} , K^+ , Na^+ i H^+ oraz wysycenie zasadami wykazywały tylko nieznaczne różnice.

Słowa kluczowe: gleby płowe wytworzone z lessu, pojemność wymiany kationów, kationy wymienne.

INTRODUCTION

Soil ability of exchangeable adsorption of cations, the measure of which is the cation exchange capacity (CEC), is a very important indicator for the estimation of soil quality. The value of CEC is determined by the sum of cations which neutralise negative charges on the surface of soil colloids, and by the soil reaction (PEINEMANN et al. 2000, KAISER 2008, RASHIDI, SEILSEPOUR 2008). An equally important indicator of soil quality is its degree of saturation with base cations: Ca^{2+} , Mg^{2+} , K^+ and Na^+ . The higher the level of saturation with exchangeable bases, the lower the share of cations H^+ and Al^{3+} determining the total acidity of the soil. The level of saturation of the sorptive complex with base cations determines the fertility of soils and their resistance to chemical degradation. Depending on the soil reaction and relative shares and kinds of dominant mineral and organic colloids, various quantitative ratios appear in the soil among the particular cations taking part in the ionic exchange (STEVENS et al. 2005, KOPITKE, MENZIES 2007, SZYMAŃSKA et al. 2007). The content of base and acidic exchangeable cations in the sorptive

complex of soil has an effect on plant nutrition and on the status of ionic balance in plants. Cations adsorbed in soil colloids constitute a pool of nutrients for plants.

Particular types and kinds of soils differ in the degree to which the cation exchange capacity is utilized by base and acidic cations (DROZD et al. 2007, SZAFRANEK et al. 2007, KALEMBASA et al. 2011, KOBIERSKI et al. 2011). Intensive agricultural use of soils may cause both enrichment and impoverishment of the sorptive complex in base cations (STOJEK 2005, KOĆMIT et al. 2008, RYCHCIK et al. 2008). The objective of the study was to estimate the cation exchange capacity and the saturation with exchangeable cations of Luvisols developed from loess, under intensive agricultural use, classified to various complexes of agricultural suitability.

MATERIAL AND METHODS

Field studies on Luvisols developed from loess were conducted on 4 selected pedons each, classified into three complexes of agricultural suitability of arable soils: the very good wheat complex (1), the good wheat complex (2) and the deficient wheat complex (3). In total, the study comprised 12 Luvisols under winter wheat cultivation, situated in various mesoregions of the Lublin Upland and the Volyn Upland (KONDRACKI 2002) – Figure 1:

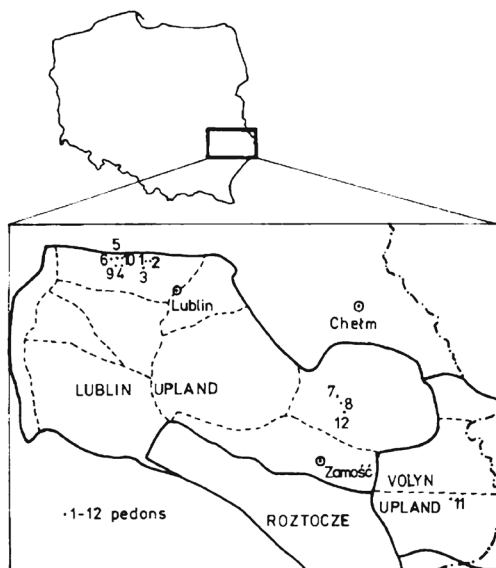


Fig. 1. Map of the location of the pedons

a) Luvisols classified into the very good wheat (1) complex of agricultural suitability and II valuation class, No.: 1 – Piotrawin, 51°19'39"N, 22°26'4"E, 2 – Jastków Kolonia, 51°19'40"N, 22°26'28"E, 3 – Tomaszowice, 51°17'9"N, 22°26'10"E, 4 – Ługów, 51°19'25"N, 22°18'33"E (Nałęczów Plateau);

b) Luvisols classified into the good wheat (2) complex of agricultural suitability and IIIa-IIIb valuation class, No.: 5 – Gutanów, 51°20'45"N, 22°17'42"E, 6 – Ludwinów, 51°20'15"N, 22°16'55"E (Nałęczów Plateau), 7 – Wólka Kraśniczyńska, 50°55'19"N, 23°22'19"E, 8 – Drewniki 50°54'26"N, 23°22'53"E (Grabowiec Plateau);

c) Luvisols classified into the deficient wheat (3) complex of agricultural suitability and IVa-IVb valuation class, No.: 9 – Kolonia Ługów, 51°19'31"N, 22°18'37"E, 10 – Kolonia Gutanów, 51°21'4"N, 22°18'37"E (Nałęczów Plateau), 11 – Poturzyn, 50°33'56"N, 23°54'55"E (Sokal Plateau), 12 – Majdan Skierbieszowski, 50°53'20"N, 23°23'16"E (Grabowiec Plateau).

The soils selected for the study lay in individual farms, where the share of cereals in crop rotation was most often 75%. The soils were characterised by varied, but generally fairly low, levels of organic fertilisation, consisting only of straw plough-over, and less frequently the application of farmyard manure. The level of mineral fertilisation of the soils was higher than that of organic fertilisation, but it was also varied and biased towards nitrogen fertilisers. Most of the soils studied were limed too rarely, and on some (pedons Nos 4, 5 and 12) no calcium fertilisers had been applied for twenty years.

Soil samples for laboratory analyses were taken in August, when wheat was in the phase of full ripeness or shortly after its harvest, from four layers of the pedons with depths of: 0-25 cm (from Ap horizon), 25-50 cm (from Et, EB, Bt1 or Bt2 horizons), 50-75 cm (from Bt1, Bt2 or BC horizons) and 75-100 cm (from Bt2, BC or C horizons). In total, 48 soil samples were taken and subjected to laboratory analyses.

The texture of the soils was determined with the Casagrande areometric method modified by Prószyński, separating the sand sub-fraction on sieves with mesh sizes of 1, 0.5, 0.25 and 0.1 mm. The particle-size groups were determined in accordance with the classification of the Polish Society of Soil Science of 2008 (5th Commission ... 2011). The content of total organic carbon (TOC) was assayed at the IUNG Central Laboratory of Chemical Analyses in Puławy, using an analyser Vario Max CNS Elementar. Soil pH in 1 mol KCl dm⁻³ was measured potentiometrically with a combined electrode.

Total acidity (TA) in cmol H⁺ kg⁻¹ was assayed with the Kappen method in 1 mol CH₃COONa dm⁻³. The level of base exchangeable cations in cmol(+) kg⁻¹ was assayed with the Pallmann method in 1 mol NH₄Cl dm⁻³ extract with pH 8.2. The content of exchangeable cations Ca²⁺, K⁺ and Na⁺ was determined on a flame photometer, and the content of cations Mg²⁺ – with the method of atomic absorption spectroscopy (AAS). Based on those assays, the following were calculated: sum of base cations (TEB) and cation exchange

capacity (CEC) in $\text{cmol}(+) \text{ kg}^{-1}$, degree of saturation of the sorptive complex with base cations (BS) in percentages, shares of individual cations in the sorptive complex of soil, and their molar ratios.

The results were subjected to statistical analysis. Calculations were made of the coefficients of simple correlation (r) between the content of particle-size fractions, content of TOC and physicochemical properties of all soil horizons, using the program Statistica 7.

RESULTS AND DISCUSSION

The Luvisols developed from loess classified into the very good wheat complex contained 13-14% of sand fraction (2-0.05 mm), 78-79% of silt (0.05-0.002 mm) and 7-8% of clay (<0.002 mm) in the Ap horizon (0-25 cm) – Table 1. Similar texture was found in the Et horizons, while the EB and Bt horizons had a higher content of clay (14-24%) and lower of silt (63-71%). Soils from the good wheat complex and deficient wheat complex had a higher content of clay (8-19%) and a lower level of silt (70-75%) in their Ap horizons. Only soil No. 7, in the 0-80 cm horizon, had a lower content of silt (52-62%) and a higher of sand (29-30%) compared to the other soils. In terms of their particle-size distribution, the soils were most frequently loamy silts or clayey silts.

The content of total organic carbon (TOC) in the Ap horizons of the first complex soils was 6.36-9.48 g kg^{-1} and it was only slightly higher than in soils from complexes two and three (4.68-7.50 g kg^{-1}). The subsurface horizons Et, Bt, BC and C contained only 0.72-3.36 g kg^{-1} of TOC (Table 1). The reaction of the Ap horizons of the examined soils was most often weakly acidic (pH 5.6-6.2), less frequently acidic (pH 4.7-5.5) or strongly acidic (pH 4.1-4.5). The reaction of the Et, Bt and BC horizons was most often acidic, less frequently weakly acidic or strongly acidic. Only the C horizon, containing calcium carbonate, was alkaline in reaction (pH 7.3). Acidification of the soils from complex 1 was slightly greater than of those from complexes 2 and 3.

Total acidity (TA) in the Ap horizons of the soils was 0.8-3.7 $\text{cmol}(+) \text{ kg}^{-1}$, and 0.5-3.0 $\text{cmol}(+) \text{ kg}^{-1}$ in the subsurface horizons. Differences in TA among the soils classified into various soil suitability complexes were small (Table 2). TA did not display any significant relation to the content of the particle-size fractions and of TOC, but it was closely negatively correlated with the value of pH ($r=-0.73$) – Table 3.

The composition of exchangeable cations of the soils was dominated by calcium cations (Table 2). In the Ap horizons of soils from complex 1, the content of cations Ca^{2+} was 3.1-10.4 $\text{cmol}(+) \text{ kg}^{-1}$ and it was slightly lower than in the Ap horizons of soils from complexes 2 (3.0-10.9 $\text{cmol}(+) \text{ kg}^{-1}$) and

Table 1

Texture and selected properties of the soils

Soil complex	Pedon No.	Horizon	Depth (cm)	Sampling depth (cm)	Particle size fraction (%) in mm			TOC (g kg ⁻¹)	pH KCl
					2-0.05	0.05-0.002	<0.002		
1 Very good wheat	1	Ap	0-30	5-15	14	78	8	9.48	4.7
		EB	30-40	30-40	13	68	19	3.36	4.6
		Bt1	40-67	55-65	16	63	21	1.74	4.5
		Bt2	67-102	80-90	14	68	18	1.44	4.6
	2	Ap	0-30	5-15	13	79	8	7.56	5.3
		EB	30-35	30-35	16	66	18	1.80	5.1
		Bt1	35-63	55-60	16	65	19	0.84	5.3
		Bt2	63-100	80-90	16	70	14	1.32	5.4
	3	Ap	0-30	5-15	14	78	8	8.34	6.2
		Et	30-48	30-40	13	76	11	3.30	5.8
		Bt1	48-75	55-65	12	64	24	1.68	5.2
		Bt2	75-113	80-90	12	68	20	0.90	5.2
	4	Ap	0-30	5-15	14	79	7	6.36	4.3
		Et	30-43	30-40	15	73	12	0.90	4.5
		Bt1	43-65	55-65	16	66	18	1.98	4.6
		Bt2	65-105	80-90	12	71	17	1.86	4.8
2 Good wheat	5	Ap	0-24	5-15	16	75	9	6.12	4.5
		EB	24-38	30-40	15	71	14	1.92	5.2
		Bt1	38-75	55-65	14	63	23	1.08	5.4
		Bt2	75-103	80-90	15	67	18	0.90	4.6
	6	Ap	0-27	5-15	14	70	16	6.60	6.2
		Bt1	27-49	30-40	15	63	22	1.92	5.4
		Bt2	49-78	55-65	13	71	16	1.32	5.8
		BC	78-101	80-90	14	73	13	0.72	6.1
	7	Ap	0-25	5-15	30	62	8	6.90	5.5
		EB	25-42	30-40	30	53	17	2.52	5.0
		Bt1	42-80	55-65	29	52	19	0.72	5.7
		Bt2	80-108	80-90	18	58	24	0.90	5.8
	8	Ap	0-24	5-15	14	70	16	6.48	5.8
		Bt1	24-60	30-40	12	61	27	1.44	5.4
		Bt2	60-80	60-70	13	62	25	1.14	4.3
		BC	80-111	80-90	29	51	20	0.72	4.2
3 Deficient wheat	9	Ap	0-25	5-15	14	71	15	7.14	6.1
		Bt2	25-40	30-40	16	70	14	1.20	5.9
		BC	40-75	55-65	17	70	13	1.38	6.1
		Cca	>75	80-90	16	73	11	1.08	7.3
	10	Ap	0-26	5-15	13	71	16	7.50	5.8
		Bt1	26-45	30-40	13	71	16	2.52	5.8
		Bt2	45-70	55-65	16	70	14	1.32	6.1
		BC	70-104	80-90	15	71	14	0.84	5.9
	11	Ap	0-25	5-15	11	70	19	4.68	5.6
		Bt1	25-46	30-40	13	66	21	2.40	5.2
		Bt2	46-65	55-65	13	70	17	2.16	5.2
		BC	65-100	80-90	11	74	15	0.72	5.4
	12	Ap	0-18	5-15	14	68	18	6.18	4.1
		Bt1	18-45	30-40	13	67	20	1.80	4.3
		Bt2	45-80	55-65	12	71	17	1.44	4.4
		BC	80-115	80-90	15	69	16	2.40	4.5

3 (6.8-13.0 cmol(+) kg⁻¹). The content of calcium cations increased slightly in the Bt horizons (5.5-14.1 cmol(+) kg⁻¹), while the highest values were attained in the C horizon – 72.4 cmol(+) kg⁻¹. Within the soil pedons studied, calcium cations correlated closely and positively with the pH value of the soils ($r=0.53$) – Table 3.

The content of Mg²⁺ cations in the Ap horizons of soils from the very good wheat complex was 0.5-1.0 cmol(+) kg⁻¹ and it was somewhat lower than in the Ap horizons of soils from the good wheat complex (0.5-1.3 cmol(+) kg⁻¹) and deficient wheat complex (1.4-1.7 cmol(+) kg⁻¹). The content of magnesium cations increased slightly with depth, and in the Bt, BC and C horizons it equalled 1.0-2.8 cmol(+) kg⁻¹ (Table 2). Magnesium cations correlated closely and positively with the content of clay ($r=0.72$) and weakly with the content of cations Ca²⁺ ($r=0.29$) – Table 3.

The content of K⁺ cations in the soils was slightly varied and equalled 0.1-0.6 cmol(+) kg⁻¹ (Table 2). The highest values of 0.5-0.6 cmol(+) kg⁻¹ in the Ap horizons of soils assigned numbers 2, 7 and 8 were caused by the application of potassium fertilisers. The content of exchangeable cations Na⁺ was most frequently at the level of 0.1-0.5 cmol(+) kg⁻¹, with the exception of the C horizon, where it reached 1.4 cmol(+) kg⁻¹. Potassium cations correlated closely and positively with the content of TOC ($r=0.40$) and with hydrolytic acidity ($r=0.43$), while sodium cations correlated with the value of pH ($r=0.48$), calcium cations ($r=0.97$) and magnesium cations ($r=0.40$) – Table 3.

In the Ap horizons of the soils, the quantitative sequence of base cations was most often Ca²⁺>Mg²⁺>K⁺>Na⁺, which is characteristic for most mineral soils of Poland (JAWORSKA et al. 2008, KALEMBASA et al. 2011, KOBIERSKI et al. 2011). Whereas, in the subsurface horizons of the soils, the sequence of Ca²⁺>Mg²⁺>Na⁺>K⁺ was frequently found. Especially in loess rock, sodium cations tend to appear in larger amounts than potassium cations (UZIĄK et al. 2004).

Total exchange bases (TEB) in the Ap horizons of soils from complex 1 was 3.9-11.8 cmol(+) kg⁻¹ and it was only slightly lower than in soils from complexes 2 (3.8-12.6 cmol(+) kg⁻¹) and 3 (8.8-14.9 cmol(+) kg⁻¹). In the Bt and BC horizons, the total bases ranged from 7.2 to 16.9 cmol(+) kg⁻¹, and in the C horizon it increased to 75.7 cmol(+) kg⁻¹ (Table 2). TEB was closely and positively correlated with the value of pH ($r=0.52$), calcium cations ($r=0.99$) and sodium cations ($r=0.97$), and weakly positively correlated with magnesium cations ($r=0.34$) – Table 3.

Cation exchange capacity (CEC) in the Ap horizons of soils from the very good wheat complex was in the range of 6.1-12.7 cmol(+) kg⁻¹, and it was only slightly lower than in soils from the good wheat complex (5.2-13.4 cmol(+) kg⁻¹) and deficient wheat complex (11.1-15.7 cmol(+) kg⁻¹). In the Bt and BC horizons, CEC was 8.2-17.9 cmol(+) kg⁻¹, and in the parent rock C it increased to 76.7 cmol(+) kg⁻¹ (Table 2). CEC displayed the same correlations as TEB, namely it was closely and positively correlated with pH ($r=0.47$), content of

Table 2

Content of exchangeable cations and sorptive capacity parameters

Soil complex	Pedon No.	Hori- zon	TA	Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	TEB	CEC	BS (%)	TA	Ca ²⁺	Mg ²⁺	K ⁺	
			(emol(+) kg ⁻¹)								(%)				
1 Very good wheat	1	Ap	1.5	4.3	0.5	0.3	0.1	5.2	6.7	77.4	22.6	65.0	6.8	4.1	
		EB	1.9	6.8	0.7	0.2	0.2	7.8	9.7	80.7	19.3	69.6	6.8	2.5	
		Bt1	1.8	7.0	1.3	0.3	0.2	8.8	10.6	82.9	17.1	66.1	12.5	2.7	
		Bt2	1.5	6.2	1.3	0.3	0.1	7.9	9.4	84.0	16.0	65.8	13.6	2.9	
	2	Ap	1.4	5.1	0.5	0.6	0.1	6.3	7.7	81.6	18.4	66.6	5.9	7.4	
		EB	1.3	7.4	0.8	0.3	0.2	8.7	10.0	87.1	12.9	74.2	7.9	3.1	
		Bt1	1.4	8.3	1.4	0.2	0.2	10.1	11.5	88.3	11.7	72.6	12.2	1.9	
		Bt2	1.0	6.0	1.3	0.2	0.1	7.6	8.6	87.8	12.2	69.6	14.8	1.8	
	3	Ap	0.9	10.4	1.0	0.2	0.2	11.8	12.7	92.9	7.1	82.0	7.8	1.2	
		Et	1.0	6.0	0.5	0.1	0.2	6.8	7.8	86.6	13.4	76.8	6.3	1.2	
		Bt1	0.8	10.3	1.3	0.2	0.3	12.1	12.9	93.6	6.4	79.9	9.9	1.7	
		Bt2	1.3	7.7	1.4	0.2	0.2	9.5	10.8	88.2	11.8	71.3	13.3	1.8	
	4	Ap	2.2	3.1	0.5	0.2	0.1	3.9	6.1	62.9	37.1	51.5	7.4	2.6	
		Et	1.5	4.3	0.6	0.1	0.1	5.1	6.6	77.4	22.6	65.2	8.4	2.0	
		Bt1	1.4	6.8	1.4	0.2	0.2	8.6	10.0	86.5	13.5	68.3	14.4	2.0	
		Bt2	0.9	5.5	1.4	0.2	0.1	7.2	8.1	88.9	11.1	67.8	17.3	2.1	
2 Good wheat	5	Ap	1.4	3.0	0.5	0.2	0.1	3.8	5.2	74.0	26.0	58.3	9.5	4.5	
		EB	1.3	6.2	0.7	0.2	0.3	7.4	8.7	85.1	14.9	72.3	7.7	1.8	
		Bt1	1.3	9.4	1.2	0.2	0.2	11.0	12.3	89.6	10.4	76.1	10.0	1.6	
		Bt2	1.0	6.8	1.2	0.2	0.2	8.4	9.4	89.5	10.5	73.3	12.4	1.8	
	6	Ap	0.8	10.9	1.2	0.2	0.3	12.6	13.4	94.4	5.6	81.4	9.2	1.8	
		Bt1	1.1	10.2	1.4	0.2	0.3	12.1	13.2	91.5	8.5	77.3	10.6	1.7	
		Bt2	1.0	7.7	1.2	0.2	0.2	9.3	10.3	90.5	9.5	75.0	12.0	1.7	
		BC	0.6	6.3	1.0	0.1	0.2	7.6	8.2	92.7	7.3	76.7	12.5	1.6	
	7	Ap	1.8	4.8	0.8	0.5	0.2	6.3	8.1	77.6	22.4	59.4	10.3	5.7	
		EB	2.3	7.4	1.1	0.3	0.2	9.0	11.3	80.1	19.9	65.4	9.8	2.7	
		Bt1	1.1	9.7	1.1	0.2	0.3	11.3	12.4	91.5	8.5	78.2	8.8	1.9	
		Bt2	1.0	14.1	2.1	0.2	0.5	16.9	17.9	94.1	5.9	78.4	11.5	1.3	
	8	Ap	1.7	8.3	1.3	0.5	0.2	10.3	12.0	86.2	13.8	69.6	11.0	3.7	
		Bt1	1.7	11.4	2.7	0.4	0.3	14.8	16.5	90.0	10.0	69.3	16.4	2.4	
		Bt2	2.3	9.3	2.8	0.3	0.3	12.7	15.0	84.6	15.4	62.0	18.6	1.9	
		BC	2.2	7.8	2.3	0.3	0.3	10.7	12.9	82.5	17.5	60.6	17.9	2.0	
3 Deficient wheat	9	Ap	0.8	13.0	1.4	0.2	0.3	14.9	15.7	94.8	5.2	82.4	8.9	1.6	
		Bt2	1.0	7.3	1.0	0.2	0.2	8.7	9.7	89.9	10.1	75.8	10.6	1.4	
		BC	0.8	13.0	1.6	0.2	0.3	15.1	15.9	94.8	5.2	81.6	10.1	1.0	
		Cca	0.5	72.4	1.7	0.2	1.4	75.7	76.2	99.4	0.6	95.1	2.3	0.2	
	10	Ap	1.0	8.3	1.4	0.2	0.2	10.1	11.1	91.2	8.8	75.2	12.2	1.8	
		Bt1	0.6	8.3	1.0	0.2	0.2	9.7	10.3	94.2	5.8	80.9	9.6	1.5	
		Bt2	0.8	6.9	1.0	0.1	0.2	8.2	9.0	91.6	8.4	77.3	11.0	1.5	
		BC	0.8	6.4	1.2	0.1	0.2	7.9	8.7	90.6	9.4	73.5	13.7	1.7	
	11	Ap	1.5	11.0	1.7	0.3	0.3	13.3	14.8	89.9	10.1	74.4	11.1	2.3	
		Bt1	1.2	11.5	1.9	0.3	0.4	14.1	15.3	92.1	7.9	75.6	12.1	1.9	
		Bt2	1.0	8.9	1.4	0.3	0.3	10.9	11.9	91.2	8.8	75.0	11.4	2.1	
		BC	0.7	8.3	1.3	0.3	0.3	10.2	10.9	93.7	6.3	77.0	11.8	2.3	
	12	Ap	3.7	6.8	1.5	0.3	0.2	8.8	12.5	70.6	29.4	54.3	11.8	2.7	
		Bt1	3.0	8.3	1.6	0.3	0.3	10.5	13.5	77.8	22.2	61.6	12.2	2.1	
		Bt2	2.2	7.2	1.5	0.3	0.2	9.2	11.4	80.9	19.1	63.2	13.3	2.2	
		BC	1.8	6.3	1.4	0.3	0.2	8.2	10.0	81.9	18.1	63.3	14.0	2.3	

Table 3

Correlations coefficients between investigated properties of soils ($n=48$)

Variable	TA	Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	TEB	CEC	BS
2-0.05	0.17	0.00	-0.01	0.15	0.05	0.00	0.01	-0.15
0.05-0.002	-0.26	0.01	-0.53**	-0.19	-0.12	-0.02	-0.04	-0.10
<0.002	0.17	-0.02	0.72**	0.11	0.10	0.03	0.04	0.28
TOC	0.15	-0.14	-0.38**	0.40**	-0.20	-0.15	-0.15	-0.33*
pH KCl	-0.73**	0.53**	-0.08	-0.19	0.48**	0.52**	0.47**	0.74**
TA	1	-0.26	0.18	0.43**	-0.21	-0.25	-0.19	-0.79**
Ca ²⁺		1	0.29*	-0.09	0.97**	0.99**	0.99**	0.42**
Mg ²⁺			1	0.16	0.40**	0.34*	0.35*	0.33*
K ⁺				1	-0.04	-0.07	-0.04	-0.26
Na ⁺					1	0.97**	0.97**	0.45**
TEB						1	0.99**	0.43**
CEC							1	0.39**
BS								1

* significance level 0.05, ** significance level 0.01

cations Ca²⁺ ($r=0.99$), Na⁺ ($r=0.97$), and weakly correlated with cations Mg²⁺ ($r=0.35$) – Table 3. CEC values in the Ap horizons of soils classified in complexes 1 and 2 were most frequently assessed as medium or high, and most often as high in the Ap horizons of soils from complex 3 and in the Bt and BC horizons. In the soils from complex 3 (pedons 9-12), situated on loess slopes with considerable inclination, the Ap horizons developed as a result of erosion from the illuvial Bt horizon, and therefore had a higher content of clay and higher CEC. It was only the C horizon, containing calcium carbonate, that was characterised by very high CEC.

The Luvisols developed from loess were characterised by higher CEC compared to Luvisols developed from loamy sands and to Brunic Arenosols and Podzols soils developed from weakly loamy sands (STOJEK 2005). CEC values similar to those of the soils under study were found for Luvisols developed from boulder loams (SZAFRANEK et al. 2007, KOBIERSKI et al. 2011). Compared to Chernozems developed from loess (DROZD et al. 2007), Phaeozems developed from loams (KOĆMIT et al. 2008) and alluvial soils (BARTKOWIAK, DŁUGOSZ 2010), the CEC values of the soils studied were lower.

The degree of saturation of the sorptive complex with base cations (BS) in the Ap horizons of the analyzed soils was varied (62.9-94.8%). BS increased with depth and reached 77.8-94.1%, in the Bt horizons, 81.9-94.8% in the BC horizons and 99.4% in the C horizon (Table 2). BS did not display any variation with relation to the soil suitability complex. The BS was closely and positively correlated with the pH value of the soils ($r=0.74$), cations Ca²⁺ ($r=0.42$), Na⁺ ($r=0.45$), TEB ($r=0.43$) and CEC ($r=0.39$), and weakly corre-

lated with cations Mg^{2+} ($r=0.33$) – Table 3. BS was highly favourable in most of the studied Luvisols, despite their acidic or strongly acidic reaction. This indicates very good buffering properties of Luvisols developed from loess. The lowest values of TEB, CEC and BS noted in pedons 3, 6 and 9 result from the application of calcium fertilisers. Whereas, the lowest degree of BS in the Ap horizons of pedons 4, 5 and 12 was due to the lack of liming and strongly acidic reaction of those soils.

The Luvisols developed from loess were characterised by considerable variation in contributions of particular cations to the saturation of the sorptive complex (Table 2). Saturation with cations Ca^{2+} in the Ap horizons was 51.5-82.4%, in the Et, EB, Bt and BC horizons – 61.6-81.6%, and in the C horizon – as much as 95.1% (Table 4). The share of cations Mg^{2+} in the Ap horizons varied within the range of 5.9-12.2%, while in the subsurface horizons it was 2.3-18.6%. Saturation with cations K^+ in the Ap horizons was 1.2-7.4%, and in the subsurface horizons – 0.2-3.1%. Saturation with sodium cations constituted 1.4-2.2% of the sorptive complex in the Ap horizons of the soils, and 1.6-3.2% in the deeper horizons. The share of total acidity (cations H^+) in the sorptive complex of the Ap horizons had a wider range: 5.2-37.1%. Most frequently, the share of cations H^+ decreased with the depth in the pedons to the level of 8.7-12.9% in the BC horizon and 0.6% in the C horizon. No significant differences were noted in the saturation with particular cations between pedons classified in different soil suitability complexes.

The percentages of particular cations are important for an assessment of the capability of soil to supply plants with nutrients and to provide optimum conditions for their growth. The concept of optimum saturation of soils with cations, generally accepted for a long time, presumes that their respective shares should be *ca.* 65% Ca^{2+} , 15% Mg^{2+} , 5% K^+ and no more than 15-20% H^+ . However, numerous studies indicate that the concept does not apply to all soils and all crops (STEVENS et al. 2005, KOPITTKE, MENZIES 2007). In the Ap and Et horizons of the studied Luvisols, the share of magnesium was lower than the optimum, unlike the share of calcium cations, which was higher. Despite those differences, most of the studied Luvisols were characterised by favourable shares of particular cations, irrespective of the soil suitability complex. The notable level of cations H^+ in the Ap horizons of pedons 4, 5 and 12 (25.0-37.1%) and their strongly acidic reaction resulted from long-term absence of liming, and indicated very weak chemical degradation of those soils. Nitrogen fertilisation is an additional factor contributing to the acidification of soils and causing a reduction in the shares of cations Ca^{2+} and Mg^{2+} (SZYMAŃSKA et al. 2007).

The differences in the per cent shares of particular cations resulted in different molar ratios between them. The ratios between the following cations: $Ca^{2+}+Mg^{2+}/K^++Na^+$, Ca^{2+}/Mg^{2+} , Ca^{2+}/K^+ and Mg^{2+}/K^+ are used as criteria in estimation of the sorptive complex quality of soils (STEVENS et al. 2005, SZYMAŃSKA et al. 2007, BARTKOWIAK, DŁUGOSZ 2010, KALEMBASA et al. 2011). The ratios calculated herein indicated very strong differentiation of values

Table 4

Basic cation saturation ratios in the sorption complex of soils

Horizon	$\text{Ca}^{2+}+\text{Mg}^{2+}/\text{K}^{+}+\text{Na}^{+}$	$\text{Ca}^{2+}/\text{Mg}^{2+}$	$\text{Ca}^{2+}/\text{K}^{+}$	$\text{Mg}^{2+}/\text{K}^{+}$
Ap	17.2* 8.0-28.5**	7.7 4.6-11.4	28.7 9.0-66.0	3.8 0.8-6.9
Et	21.5 18.8-24.1	10.0 7.8-12.2	47.9 31.8-63.9	4.7 4.1-5.3
EB	16.3 15.6-17.7	8.9 6.6-10.3	28.9 23.9-39.4	3.3 2.5-4.2
Bt1	21.6 18.1-24.5	6.5 4.2-8.9	39.1 24.6-53.0	6.1 4.6-7.4
Bt2	22.0 17.5-26.5	5.6 3.3-7.1	40.0 22.8-58.1	7.3 4.7-9.6
BC	22.5 16.9-29.3	5.7 3.4-8.1	44.3 27.8-82.2	7.8 5.0-10.2
Cca	48.5	42.0	425.3	10.1

* mean values, ** range

among the particular pedons (Table 4). The ratio of bivalent cations to monovalent cations in the Ap horizons was, on average, 17.2, and increased gradually with depth into the soils, up to 48.5 in the C horizon. A broad ratio of $\text{Ca}^{2+}+\text{Mg}^{2+}/\text{K}^{+}+\text{Na}^{+}$ is a natural feature of soils developed from loess. The ratio of cations $\text{Ca}^{2+}/\text{Mg}^{2+}$ in the Ap horizons was, on average, 7.7, and tended to vary: it narrowed down to 5.6 in the Bt2 horizon but broadened to 42.0 in the C horizon. For plant nutrition, the ratio of 5.0-7.5 is considered to be favourable (SZYMAŃSKA et al. 2007). The average ratio of cations $\text{Ca}^{2+}/\text{K}^{+}$ was 9-66 in the Ap horizons, 22.8-82.2 in the deeper horizons, and 425.3 in the parent rock. The value of the ratio considered to be the most suitable is 10-15 (KOPITKE, MENZIES 2007, SZYMAŃSKA et al. 2007). The broader ratio in the Ap horizons of most of the soils indicates a certain potassium deficit, resulting from insufficient application of potassium fertilisers. Due to the antagonistic effect of K^{+} towards Mg^{2+} , the ratio of magnesium cations to potassium cations is an important indicator of quality of the sorptive complex. In the Ap horizons, it was 3.8, on average, increasing deeper into the profile, thus attaining the highest value in the parent rock (10.1) – Table 4. Since the optimum value of the ratio is assumed to be 3.0 (KOPITKE, MENZIES 2007), that indicator verifies the deficit of potassium in the Ap horizons of some of the soils under study.

Summing up the discussion, it should be highlighted that the sorptive properties of the studied Luvisols developed from loess were determined primarily by their genetic traits: content and kind of clay minerals, content of organic matter, and pH (KOPITKE, MENZIES 2007, KAISER et al. 2008, RASHIDI et al. 2008). Clay minerals in Luvisols developed from loess are the primary

sorbents. According to UZIĄK et al. (2004), the composition of clay minerals in loess soils is dominated by expanding mineral, mixed-packet smectite/illite, and, additionally, some illite and kaolinite, sometimes also chlorite, vermiculite or smectite. Soil humus has the highest sorptive capabilities owing to carboxyl, hydroxyl and amine functional groups (PEINEMANN et al. 2000, KAISER et al. 2008, RASHIDI et al. 2008). However, Luvisols are characterised by a low content of humus, and fresh organic matter rapidly undergoes microbiological mineralization (STOJEK 2005, SZYMAŃSKA et al. 2007, RYCHCIK et al. 2008). Therefore, under the conditions of intensive cultivation with an excessive share of cereals (75% and more), regular organic fertilisation is highly important to prevent further impoverishment of the soils in TOC. Luvisols, as a result of the processes of leaching and leaching, are often characterised by acidic reaction and migration of clayey minerals, together with base cations, downwards into the pedon. As a result of the depletion of base components by the crop yields, their supplementation is required through liming and organic fertilisation in order to satisfy the needs of plants (SZYMAŃSKA et al. 2007, KOĆMIT et al. 2008). The results of the study presented here demonstrated also that the classification of Luvisols developed from loess into various agricultural suitability complexes, based on the morphological features of pedons, affected very slightly the levels of CEC and the degree of saturation of soil sorbents with cations.

CONCLUSIONS

1. In the Ap horizons of the Luvisols developed from loess, the quantitative sequence of base cations was most frequently $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+ > \text{Na}^+$, while in the subsurface horizons another sequence such as $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{Na}^+ > \text{K}^+$ was determined. The composition of base cations was dominated by calcium cations, and the content of magnesium, potassium and sodium cations was weakly diversified. TEB was favourable in the majority of the soils, and TA (content of H^+) was varied, but most often low.

2. CEC in the Ap, Et, EB, Bt and BC horizons of the soils was assessed to be medium or high. It was only the C horizon, containing calcium carbonate, that was characterised by very high CEC.

3. Despite the acidic or strongly acidic reaction, the degree of BS of the sorptive complex was highly favourable in most of the soils, and increased with the depth in a pedon.

4. The soils were generally characterised by favourable shares of particular cations. The percentage of calcium cations was most frequently higher than that accepted as the optimum, while the shares of magnesium and potassium cations were somewhat lower than the optimum.

5. The calculated quantitative ratios among the cations revealed big dif-

ferentiation of the values among the particular pedons. High values of the ratio of Mg^{2+}/K^+ in the Ap horizons of most of the soils indicated a slight deficit of potassium resulting from the application of small doses of potassium fertilisers.

6. The content of cations Mg^{2+} , TEB and CEC in the Ap horizons of soils from the very good wheat complex were lower than in the Ap horizons of soils from the good wheat complex and the deficient wheat complex. However, the differences in the content of Ca^{2+} , K^+ , Na^+ and H^+ and in the BS among soils classified in the different soil suitability complexes were very small.

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CONTENT OF PHOSPHORUS AND SELECTED METALS IN BOTTOM SEDIMENTS OF STARZYC LAKE UNDER CONDITIONS OF PULVERIZING WATER AERATION

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Abstract

Artificial aeration of benthic water is a method promoting more intensive phosphorus bonding in bottom sediments. The purpose of this paper was to evaluate changes in the content of phosphorus and some selected metals in the bottom sediments of Starzyc Lake in relation to changes occurring in some quality parameters for the benthic water. During the research, conducted in 2005, 2006 and 2009, the lake water was aerated with a pulverizing aerator. Water and the bottom sediment were sampled in seven sites distributed uniformly throughout the water surface, the inflow area and the area of the lake water outflow to the Krapiel River. The bottom sediments were analyzed for the total content of P, Ca, Mg and K, while the water samples were tested for pH and the levels of phosphate phosphorus (P-PO_4).

In the period covered by the research, the TP content in the bottom sediments of Starzyc Lake decreased only in the inflow area (by a mean of 43%). Additionally, a gradual decrease of the P-PO_4 concentration (on average by 42%) in the benthic water was recorded in that period as well as a decrease in the content of Ca, Mg and K in the bottom sediments. The results from the analysed metal content show that the Ca content was most stable, decreasing by an average 21%, whereas the difference for the Mg and K content was 64% and 56%, respectively. The horizontal variability in the content of the analysed elements was found in the bottom sediments collected from various zones of the lake. The lowest content was found in the sediment material collected from the outflow zone of water to the Krapiel River, and the highest one was recorded in the watercourse inflow zone. The highest variability was observed for the content of Ca and K, which on average was six times higher in the water inflow zone from drainage ditches than in the outflow zone. Throughout the research period, the bottom sediments comprised an indigenous source of phosphorus for the Starzyc Lake waters.

Key words: lake eutrophication, bottom sediment, benthic water, phosphorus, artificial aeration.

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ZAWARTOŚĆ FOSFORU I WYBRANYCH METALI W OSADACH DENNYCH JEZIORA STARZYC W WARUNKACH PULWERYZACYJNEGO NAPOWIETRZANIA WODY

Abstrakt

Jedną z metod intensyfikacji wiązania fosforu w osadach dennych jest sztuczne napowietrzanie wód strefy naddanej. Celem pracy była ocena zmian zawartości fosforu i wybranych metali w osadach dennych jeziora Starzyc, którego wody napowietrzano za pomocą aeratora pulweryzacyjnego, na tle zmian wybranych wskaźników jakości wody strefy przydennej. Badania prowadzono w ciągu 3 sezonów wegetacyjnych, w latach 2005, 2006 oraz 2009. Próbkę wody i osadu dennego pobrano w 7 punktach rozmieszczonych równomiernie na całej powierzchni jeziora oraz w strefie zasilania i w strefie odpływu wód jeziornych do rzeki Krapiel. W osadach dennych oznaczono zawartość ogólną: TP, Ca, Mg i K, a w próbkach wody – pH oraz stężenie fosforu fosforanowego ($P-PO_4$).

W okresie badań zawartość TP w osadach dennych jeziora Starzyc zmniejszyła się istotnie tylko w strefie dopływu (średnio o 43%). Stwierdzono także sukcesywne zmniejszanie się (średnio o 42%) stężenia $P-PO_4$ w wodzie przydennej oraz zawartości Ca, Mg i K w osadach dennych. Spośród badanych metali najbardziej stabilna była zawartość wapnia, która zmniejszyła się średnio o 21%, a w przypadku Mg i K różnice te wynosiły odpowiednio 64% i 56%. Stwierdzono horyzontalną zmienność zawartości badanych pierwiastków w osadach dennych pobranych w różnych strefach jeziora. Najmniejsze z tych zawartości oznaczono w materiale dennym pochodzącym ze strefy odpływu wód do rzeki Krapiel, a największe – w strefie dopływu cieków wodnych. Największą zmienność stwierdzono w przypadku zawartości Ca i K, była ona średnio 6-krotnie większa w strefie dopływu wód z rowów melioracyjnych w porównaniu z zawartością w strefie odpływu. Przez cały okres badań osady denne jeziora Starzyc stanowiły autochtoniczne źródło fosforu.

Słowa kluczowe: eutrofizacja jezior, osad denny, woda przydenna, fosfor, sztuczne napowietrzanie.

INTRODUCTION

Studies on the causes and rates of increased lake eutrophication have shown that the phosphorus content is one of the main factors controlling the development of phytoplankton. Excess phosphorus compounds in aquatic ecosystems are withdrawn by sedimentation mainly to bottom sediments, which constitute an almost inexhaustible source of phosphorus in a water body (KOC, SKWIERAWSKI 2003).

One of the methods for decreasing the rate of eutrophication of lakes is artificial aeration of benthic waters, which allows quick improvement of aerobic conditions in near-bottom water layers and limits the release of phosphate ions from bottom sediments to water (GAWROŃSKA, LOSSOW 2003, OSUCH, PODSIADŁOWSKI 2012). The efficiency of lake reclamation by artificial aeration is to a large extent dependent on the sorptive capacity of bottom sediments (GAWROŃSKA, BRZOZOWSKA 2005). It can be enhanced with chemical methods, which are more often being used along with the artificial aeration of water (OSUCH, PODSIADŁOWSKI 2012). Water can be aerated using pneumatic aerators, which inject air into water, or pulverizing aerators, which spray

water into the air. The latter solution is used more often in lake reclamation. Pulverizing aerators have high specific capacity and their construction allows the use of renewable energy sources. The use of wind-driven pulverizing aerators is the most popular method of lake reclamation in Poland. A pulverizing aerator is characterised by a straightforward design, invulnerability to changing wind direction and high resistance to water pollution (KONIECZNY, PIECZYŃSKI 2006, OSUCH, PODSIADŁOWSKI 2012).

The purpose of this paper was to evaluate changes in the content of phosphorus and some selected metals in the bottom sediments of Starzyc Lake in relation to changes occurring in some selected quality parameters for the benthic water. During the research, the lake water was aerated with a pulverizing aerator.

MATERIAL AND METHODS

The object of study

Starzyc Lake is a natural lake located in Ińsko Lakeland (Chociwel commune) in West Pomeranian Region. The lake belongs to the Ińsko Nature Refuge (PLB320008) and has the following morphometric parameters:

- water table - 59.2 ha,
- mean depth - 2.7 m,
- maximum depth - 6.1 m,
- maximum length - 1,960 m,
- maximum width - 370 m,
- shoreline length - 5,175 m.

Starzyc Lake is a polymictic lake supplied with influent water from drainage ditches, while the Krapiel is its effluent river (Figure 1). The ecological state assessment by the Regional Inspectorate of Environmental Protection in Szczecin shows that – according to abiotic typology – Starzyc Lake belongs to category 3b, meaning it is a large, unstratified aquatic ecosystem with a high calcium content ($>25 \text{ mg Ca dm}^{-3}$), highly influenced by the basin Shindler coefficient >2 (BAJKIEWICZ-GRABOWSKA 2010). The assessment of the lake's susceptibility to degradation shows that this lake falls outside any category, i.e. it is characterised by the lack of any resistance to degradation. The town of Chociwel located on the northwest shore of the lake has been operating its own mechanical-biological wastewater treatment plant since 1990. However, in some parts of the town, a combined sewage system is still in use. Both the treatment plant and the combined sewage system need to be modernised and expanded.

The first attempt to rehabilitate Starzyc Lake using a pulverizing aerator with the capacity of $200\text{--}800 \text{ m}^3 \text{ day}^{-1}$ was made in 2003, and in May 2004, the first dose of coagulant was used as an experiment (KONIECZNY,

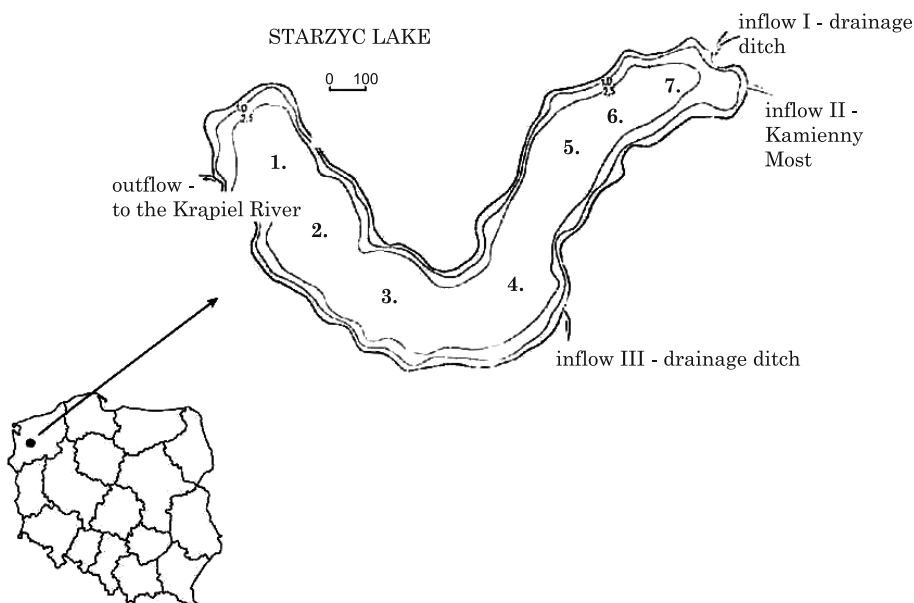


Fig. 1. Arranging of the research points on the Lake Starzyc

PIECZYŃSKI 2006). From 2008 to 2009, PIX1 13 coagulant was used over the whole area of the lake (WESOŁOWSKI et al. 2011).

Field research and chemical analysis

The study was carried out in 2005, 2006 and 2009, and included taking three samples of bottom sediments and near-bottom water each autumn. The measuring stations were located in the zone of the water supply from drainage ditches (point 7), t five sites were evenly distributed in the lake (points 2-6), and one site was located in the zone of effluent waters of the lake to the Krapiel River (point 1) – Figure 1. The samples of water were obtained with the use of a submersible Gigant pump (Geomor-Technik). The bottom sediment samples were taken using a universal probe, Multisampler (Eijkell-kamp), which enables obtaining the maximum 30 cm core sediment sample of intact structure. The sample cores were then split into layers of 10 cm.

The air-dry sediment samples were ground, sifted on a 1-mm mesh size sieve, mineralized in acid mixture of HNO_3 and HClO_4 and analysed. The total content of Ca, Mg and K in the prepared samples was determined with the use of atomic absorption spectrometry, while total phosphorus (TP) was assayed with spectrophotometric methods (GOŁACHOWSKA 1977). A hand-held pH-meter CP-401 (Elmetron) was used to measure pH of the samples, and the concentration of phosphate phosphorus (P-PO_4) was determined with the use of a single parameter photometer PC compact (WTW).

Statistical analysis

The results were submitted to statistical analysis. The differences between the mean content of the analysed elements in the layers of core sediment samples and in bottom sediments taken from various zones of the lake were analysed with the use of one-way analysis of variance, following an assessment of the distribution of the analysed content against normal distribution (the Shapiro-Wilk's test) and the test for homogeneity of variance (the Levene's test). The results gave no ground for excluding the hypothesis on normality and homogeneity of the analysed data. The significance of the differences between mean content of the analysed elements was assessed with the Tukey's post-hoc test. The selected parameters of water quality and sediment underwent linear regression analysis; the significance of the Pearson's linear correlation coefficients was assessed with the t test. All statistical processing was conducted at the significance level $\alpha=0.05$.

RESULTS AND DISCUSSION

The mean TP concentration in the analysed profiles of bottom sediments ranged from 0.97 to 3.43 mg P g⁻¹ d.m. (Table 1). The results concerning the content of this element were within the range of concentrations found in bottom sediments of mesotrophic and eutrophic lakes, i.e. from 0.55 to 7.00 mg P g⁻¹ d.m. and from 0.63 to 4.72 mg P g⁻¹ d.m (NÜRNBERG 1988). The mean content of the selected metals in bottom sediments of Starzyc Lake during the analysed period amounted to 60.02 mg Ca g⁻¹ d.m., 2.39 mg Mg g⁻¹ d.m. and 1.88 mg K g⁻¹ d.m. These values were within the range of the geochemical background of these elements in the Baltic Sea drainage basin, which is from 0.20 to 110.3 mg Ca g⁻¹ d.m., from 0.10 to 21.30 mg Mg g⁻¹ d.m. and from 1.90 to 22.50 mg K g⁻¹ d.m. The comparison of the mean content of the analysed elements in 10-cm layers of bottom sediment profiles shows no statistically significant differences (Table 2).

In the analysed profiles of bottom sediments, the relative content of the selected metals can be expressed as Ca>K>Mg. The bottom sediment profiles taken from the effluent zone were an exception, with the concentrations in the order of Ca>Mg>K, and the Mg content was on average four times higher than that of K. The K compounds are characterized by higher solubility than the compounds of P, Ca or Mg. Additionally, K ions as well as other monovalent cations do not form complex bonding with organic matter. The smallest content of Mg in the central zone of the lake can be attributed to the development of phytoplankton biomass, which uses Mg as a building block in the chlorophyll structure.

The study indicates horizontal variability in the total content of the analysed elements in the bottom sediments (Table 1). The lowest content

Table 1

Content of the tested metals in bottom sediments sampled in different zones of Starzyc Lake

Element	Zone of lake	Content of the elements in sediment (mg g ⁻¹ d.m.)					
		inflow		centre		outflow	
		mean	SD	mean	SD	mean	SD
P	2005	2.73	0.67	1.43	0.42	1.03	0.15
	2006	3.43	0.15	1.7	0.26	1.17	0.15
	2009	1.9	0.1	1.43	0.21	0.97	0.06
	all the years	2.69 ^a	0.31	1.52 ^b	0.30	1.06 ^b	0.12
Ca	2005	97.9	0.71	86.6	1.22	24.7	1.71
	2006	78.1	0.92	78.4	0.33	58.1	1.62
	2009	64.2	2.01	26.4	0.31	25.7	0.42
	all the years	80.07 ^a	1.21	63.80 ^a	0.62	36.17 ^b	1.25
Mg	2005	4.17	0.01	2.60	0.01	2.40	0.03
	2006	4.10	0.01	2.50	0.02	2.47	0.02
	2009	1.37	0.02	1.20	0.01	0.70	0.01
	all the years	3.21 ^a	0.01	2.10 ^b	0.01	1.86 ^c	0.02
K	2005	4.53	0.04	2.83	0.05	0.63	0.03
	2006	3.53	0.01	2.07	0.05	0.43	0.02
	2009	1.33	0.02	1.13	0.01	0.40	0.01
	all the years	3.13 ^a	0.02	2.01 ^b	0.04	0.49 ^c	0.02

Table 2

Average content of elements in examined layers of bottom sediments, 0-10, 10-20 and 20-30 cm thick

Layer of sediment (cm)	Content of the elements in sediment (mg g ⁻¹ d.m.)							
	P		Ca		K		Mg	
	mean	SD	mean	SD	mean	SD	mean	SD
0-10	1.99 ^a	0.95	58.9 ^a	29.9	1.87 ^a	1.42	2.33 ^a	0.79
10-20	1.71 ^a	0.76	65.1 ^a	38.2	1.84 ^a	1.29	2.37 ^a	0.67
20-30	1.57 ^a	0.82	56.0 ^a	22.3	1.92 ^a	1.31	2.47 ^a	0.70

was found in sediments taken from the effluent zone to the Krapiel River, and the highest content was determined in sediments sampled from the watercourse influent zone. The biggest differences between the levels of the analysed macroelements in bottom sediments were determined for Ca and K. The content of these metals in bottom sediments taken from the influent zone from drainage ditches was six times higher than the content found in samples obtained from the effluent zone.

During the five-year long period of pulverizing aeration of Starzyc Lake, there was a significant decrease in the TP content, on average by 43%, in

bottom sediments taken from the influent zone (Figure 2). No significant differences were determined in the mean TP content in bottom sediments taken from the other parts of the lake. The content of all the analysed metals in bottom sediments of the lake decreased gradually during the analysed period (Table 1). The content of Ca was most stable and decreased on average by 21% in the analysed period. As for Mg and K, the differences were comparable: 64% and 56%, respectively. The P-PO_4 concentration in near-bottom water layers decreased significantly (Figure 2): the mean content in 2005 was $0.19 \text{ mg P dm}^{-3}$, and fell down to $0.11 \text{ mg P dm}^{-3}$ in 2009. The concentration was also characterized by horizontal variability: in the influent zone it

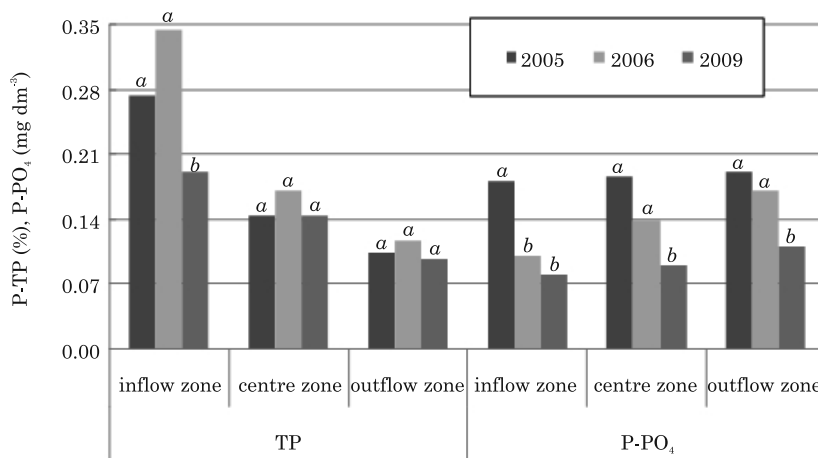


Fig. 2. Changes of the content of TP in the bottom sediments surface layer and P-PO_4 in benthic water during the research period

was on $0.12 \text{ mg P dm}^{-3}$ and in the effluent zone it reached $0.16 \text{ mg P dm}^{-3}$ on average. The results indicate that the bottom sediments were the source of P-PO_4 for the lake during the analysed period. This process is dependent on many other factors and in flow-through lakes the prolonged retention time in the central basin favours mineralisation of organic matter in bottom sediments (GAJEWSKI, CHRÓST 1995).

A significant positive linear correlation between the Ca content and TP in bottom sediments (Figure 3) and a decrease of the P-PO_4 concentration in near-bottom water layers together with an increase in pH (Figure 4) indicate that in the central zone and in the zone of effluent waters phosphorus was binding with Ca. Two significant functions of linear regression were calculated for the relationship between pH and the P-PO_4 concentration in the near-bottom water layers. The first function includes the results obtained prior to the use of PIX coagulant, while the second includes the results obtained in 2009, after the chemical reclamation began. The decrease in pH and the P-PO_4 concentration in the lake's near-bottom water layers observed during the analysed period indicates that the phosphorus content in the near-bottom layers of water could have been affected by the chemical binding of phospho-

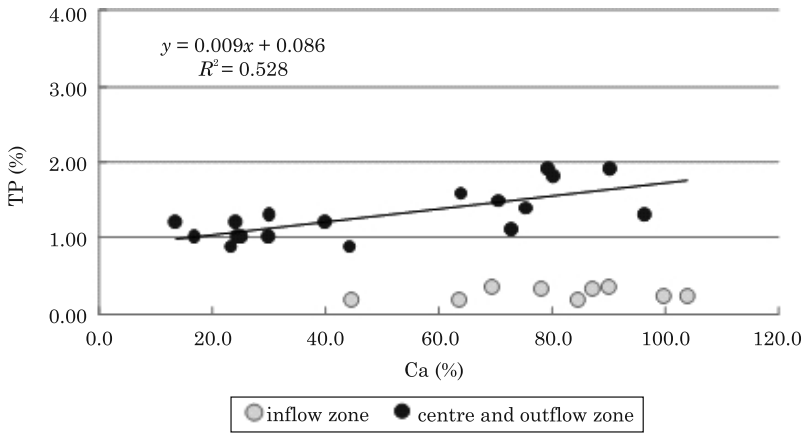


Fig. 3. Relation between the content of TP and Ca in the bottom sediments

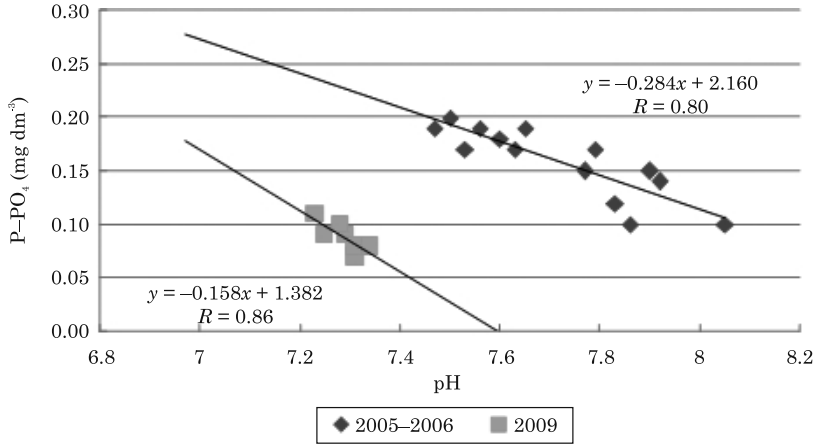


Fig. 4. Relation between pH and P-PO₄ concentration in benthic water

rus which occurred in 2008 and 2009 in response to the use of PIX 113 coagulant, which includes strong acidic electrolytes: 43% Fe₂(SO₄)₃ and 1% H₂SO₄.

Monitoring studies conducted on Starzyc Lake in 2010 by the Regional Inspectorate of Environmental Protection in Szczecin show small efficiency of reclamation with the use of a pulverizing aerator. After the completion of a seven-year long period of aeration, the ecological state of the lake was classified as poor. Such classification was due to the high content of phytoplankton, deoxygenation and high total phosphorus in the near-bottom layers of water. The fact that the reclamation conducted with the above method has failed can be explained by inadequate protective actions pursued in and around Starzyc Lake. Reclamation measures will not improve water quality unless it is possible to eliminate the excessive inflow of biogenic compounds to the lake (Lossow 1998).

SUMMARY

During the five-year long period of pulverizing aeration of Starzyc Lake, no significant differences in the annual content of total phosphorus in bottom sediments were found. The only exception was the sediment sampled from the influent zone, where a decrease in total phosphorus by an average 43% was observed. The results show a gradual decrease of the content calcium, potassium and magnesium (by 21, 56 and 64% respectively), as well as a decrease in phosphate phosphorus in the near-bottom layers of water (on average 0.19 mg P dm⁻³ in 2005, and 0.11 mg P dm⁻³ in 2009). The horizontal variability in the content of the analysed elements was found in bottom sediments collected from various zones of the lake. The lowest content was found in sediment material collected from the zone outflow water to the Krapiel River, and the highest content was recorded in the watercourse inflow zone. It has been found that bottom sediments constituted an indigenous source of phosphorus during the reclamation processes conducted on Starzyc Lake.

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THE IMPACT OF NITROGEN FERTILIZATION AND *RHIZOBIUM* INOCULATION ON THE YIELD AND QUALITY OF *TRIGONELLA FOENUM-GRAECUM* L.

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Abstract

Fenugreek is one of the oldest known medicinal plants that have been used in traditional medicine in many parts of the world. The aim of this study was to determine the effects of nitrogen fertilization and *Rhizobium meliloti* inoculation on the yield, yield components and seed quality of fenugreek. The experiment was performed during two growing seasons. Chlorophyll content was determined with the use of the SPAD-502 chlorophyll meter. After harvest, biometric measurements were performed and the chemical composition of vegetative organs and seeds was determined in fenugreek plants. At 43 DAS, the chlorophyll content of control plant leaves was determined as 46 SPAD, and it decreased with plant growth. In treatments fertilized with nitrogen, the chlorophyll content of leaves increased from 48.5 (N_{0.5}) - 56.1 (N₁) SPAD (43 DAS) to 58.2 (N_{0.5}) - 60.6 (N₁) SPAD (58 DAS). Seed inoculation with *Rhizobium meliloti* was more highly correlated with seed quality than plant habitus and yield components. Inoculation decreased (by 11.5%) the crude fat content of fenugreek seeds, and increased phosphorus, calcium and sodium concentrations. Nitrogen fertilization significantly influenced morphological parameters and yield components, and it contributed to a significant increase in the total protein content of seeds.

Key words: Fenugreek, morphological parameters, nitrogen, quality, yield.

WPLYW NAWOŻENIA AZOTEM I INOKULACJI *RHIZOBIUM* NA PLON I JAKOŚĆ *TRIGONELLA FOENUM-GRAECUM* L.

Abstrakt

Kozieradka pospolita jest jedną z najstarszych roślin leczniczych, była wykorzystywana w tradycyjnej medycynie w wielu częściach świata. Celem badań było określenie wpływu nawożenia

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nia azotem i inokulacji *Rhizobium meliloti* na plonowanie i zawartość składników w nasionach kozieradki. Eksperyment przeprowadzono w dwóch sezonach wegetacyjnych. Zawartość chlorofilu określono za pomocą chlorofilomierza SPAD-502. Po zbiorze wykonano pomiary biometryczne i oznaczono skład chemiczny nasion i wegetatywnych części roślin. W 43. dniu po siewie (DAS) zawartość chlorofilu w liściach roślin kontrolnych wynosiła 46 SPAD i zmniejszała się wraz ze wzrostem roślin. Na obiektach nawożonych azotem zawartość chlorofilu w liściach zwiększała się z 48,5 ($N_{0.5}$) - 56,1 (N_1) SPAD (43 DAS) do 58,2 ($N_{0.5}$) - 60,6 (N_1) SPAD (58 DAS). Inokulacja materiału siewnego *Rhizobium meliloti* w większym stopniu wpływała na jakość nasion niż na pokrój roślin i komponenty plonu, zmniejszyła (do 11,5%) zawartość tłuszczu surowego i zwiększyła zawartość fosforu, wapnia i sodu w nasionach kozieradki. Nawożenie azotem istotnie różnicowało parametry morfologiczne roślin i komponenty plonu, co skutkowało istotnym wzrostem zawartości białka w nasionach.

Słowa kluczowe: kozieradka pospolita, cechy morfologiczne, azot, jakość, plon.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual herb of the family *Fabaceae*, which is commonly encountered in Southwest Asia and the Mediterranean region. Fenugreek is cultivated across Western Europe and China for its aromatic seeds, and it is grown for fodder in selected parts of Europe and Northern Africa (KINJI, RHADI 2012, KOŁODZIEJ, ZEJDAN 2000, MANISHA, ANGOORABALA 2013). This herbaceous plant is widely used for medicinal purposes, and its seeds are rich in protein, macronutrients, micronutrients, steroids, saponins, mineral salts and vitamins (MADAR, STARK 2002). AMIN et al. (2005) demonstrated that fenugreek extracts effectively prevent and inhibit the development of breast cancer.

Rhizobium meliloti are nitrogen-fixing bacteria that lower the plants' demand for nitrogen fertilization and thus contribute to environmental protection (ABDELGANI et al. 1999). Nitrogen-fixing bacteria supply soil with nitrogen and other nutrients. Soil-dwelling microorganisms form a symbiotic relationship with fenugreek plants (PROVOROV, TIKHONOVICH 2003). The emergence of new production systems contributes to the popularity of fenugreek. The climatic, soil and nutritional requirements of fenugreek plants have been researched by numerous authors (KIRSHNAYYA, MANJARI 1995, KINJI, RHADI 2012). In fenugreek, nitrogen fertilization contributes to intensified growth, deferred maturation, leaf health, stem development and luxuriant foliage with the desired dark-green color. Efforts are being made to improve the yield and qualitative composition of fenugreek to make it amenable to large-scale production. The aim of this study was to investigate the effects of nitrogen fertilization and *Rhizobium meliloti* inoculation on the yield, yield components and seed quality of fenugreek.

MATERIAL AND METHODS

A pot experiment in a completely randomized design with six replicates was performed in a greenhouse of the University of Warmia and Mazury in Olsztyn, Poland (latitude 53° 46'N, longitude 20° 25'E), during two growing seasons Fenugreek plants were grown in modified Kick-Braukmann pots filled with 10 kg of light soil developed from heavy loamy sand. The soil was slightly acidic ($\text{pH}_{\text{KCl}} = 5.5$) and abundant in available nutrients (P – 107, K – 124 and Mg – 19 mg kg⁻¹ of soil). Greenhouse temperature ranged from 11.0°C to 22.8°C in the first season of the study and from 8.2°C to 32.3°C in the second season. Relative humidity in the greenhouse ranged from 40% to 95%. The following treatments were applied: inoculation – (1) non-inoculated (control), (2) inoculation with *Rhizobium meliloti*; nitrogen fertilization (urea) rates: N₀ (control), N_{0.5} and N_{1.0} g pot⁻¹. All plants were fertilized with magnesium (MgSO₄ 7H₂O) at 0.3 g per pot, phosphorus (KH₂PO₄) at 0.5 g per pot, and potassium (KH₂PO₄+K₂SO₄) at 2.5 g per pot. Regular pest control treatments were applied throughout the experiment.

The chlorophyll content of leaves was measured at 7-day intervals with the use of the SPAD-502 chlorophyll meter (Minolta, Japan). The samples were collected at 43 DAS (Days After Sowing), flowering, 51 DAS (40% of pods with final length), 58 DAS (80% of pods with final length), at the beginning of seed ripening and filling the pod cavity (65 DAS).

Harvesting took place at the full maturation stage. Every plant (12) from each pot was sampled, and the following parameters were determined: plant height, weight of aerial parts in individual plants, number of pods per plant, thousand seed weight, seed weight per plant. Plant material was mineralized in concentrated sulfuric acid (VI) with the use of hydrogen dioxide as an oxidant. Total nitrogen content was determined calorimetrically with hypochlorite. A conversion factor of 6.25 was applied to determine crude protein content by Soxhlet extraction. Phosphorus content was determined by the vanadium-molybdenum method, potassium, calcium and sodium levels were determined by flame spectrophotometry (AES), and magnesium concentrations– by atomic absorption spectrometry (AAS).

The results were statistically analyzed in the Statistica v9® application. The statistical significance of the factors was evaluated by Anova for a completely randomized design. The treatments were compared by Tukey's HSD at $P < 0.01$. All results were expressed as mean values for the two-season experimental period.

RESULTS AND DISCUSSION

Seed inoculation had an insignificant influence on the chlorophyll content of fenugreek leaves (data not shown). Nitrogen fertilization contributed to significant differences in chlorophyll concentrations in the leaves of the analyzed species (Figure 1). At 43 DAS, chlorophyll concentrations were determined at 56.1 and 48.5 SPAD for fertilizer rates of 0.5 and 1.0 g N per pot, respectively. In control plants (N_0), chlorophyll content was significantly

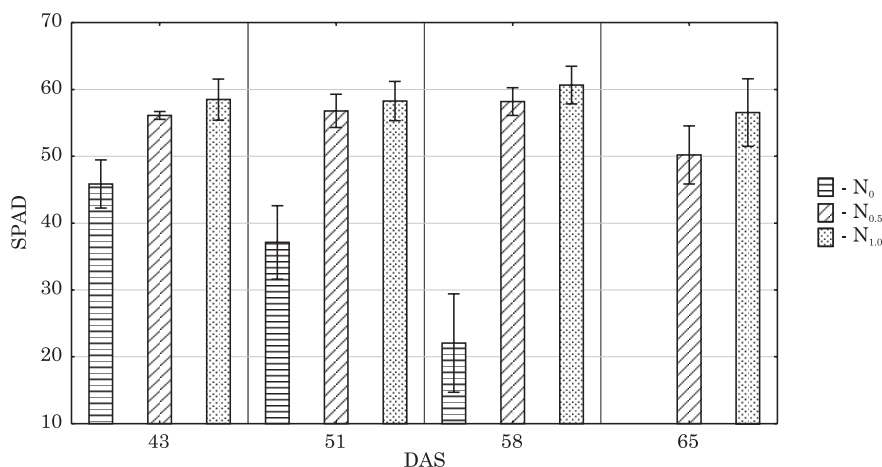


Fig. 1. Leaf chlorophyll content, subject to the applied nitrogen rate (mean values and confidence intervals, $P < 0.01$ HSD = 6.55)

lower at 46 SPAD. On successive measurement days, chlorophyll concentrations continued to decrease in control plants, but remained fairly constant in leaves fertilized with nitrogen, because nitrogen is a structural element of chlorophyll and protein molecules, and thereby affects formation of chloroplasts and accumulation of chlorophyll in them (DAUGHTRY et al. 2000). At 58 DAS, control leaves contained significantly less chlorophyll (approximately 22 SPAD) than the leaves fertilized with nitrogen ($N_{0.5}$ – 58.2 SPAD and $N_{1.0}$ – 60.6 SPAD). In control treatments, premature withering (65 DAS) significantly influenced plant habitus, the dry matter content of the analyzed vegetative organs and yield components (Figure 1, Table 1). N level also affected chlorophyll content, N concentration at anthesis, protein, and oil yield of Safflower *Carthamus tinctorius* L. (DORDAS, SIOULAS 2008). BOJOVIC, STOJANOVIC (2005), FRITSCHI, RAY (2007) and HOULES et al. (2007) demonstrated a positive correlation between N fertilization rates and leaf chlorophyll content in many plant species.

Seed inoculation and increasing nitrogen rates led to significant differences in selected morphological parameters, the weight of selected plant organs and yield components (Table 1). Inoculated plants were 9.5% shorter

Table 1

Selected morphometric parameters and yield components of fenugreek plants

Specification	Plant height (cm)	Number of		Weight of (g)			Number of seeds	Thou- sand seed weight (g)	Seed weight (g)	
		bran- ches	Pods	stems	leaves	peri- carps				
Non-inoculated										
N _{0.0}	25.90	0.692	1.94	0.140	0.157	0.112	16.04	7.70	0.123	
N _{0.5}	48.95	1.163	1.97	0.933	0.502	0.105	11.50	14.39	0.170	
N _{1.0}	41.77	1.567	2.36	0.857	0.550	0.128	16.36	12.95	0.211	
Mean	38.87	1.141	2.09	0.643	0.403	0.115	14.63	11.68	0.155	
Inoculated										
N _{0.0}	26.20	0.250	1.95	0.143	0.168	0.127	13.34	7.55	0.101	
N _{0.5}	42.57	1.443	2.30	0.740	0.513	0.137	15.39	12.53	0.193	
N _{1.0}	36.87	1.553	1.75	0.803	0.547	0.140	13.51	14.50	0.163	
Mean	35.21	1.082	2.00	0.562	0.409	0.134	14.08	11.53	0.172	
Mean for dose										
N _{0.0}	26.05	0.471	1.95	0.142	0.163	0.119	14.69	7.62	0.112	
N _{0.5}	45.76	1.303	2.13	0.837	0.508	0.121	13.45	13.46	0.182	
N _{1.0}	39.32	1.560	2.05	0.830	0.548	0.134	14.93	13.72	0.187	
HSD <i>P</i> <0.01 for:										
N rate	1.39	0.352	0.38	0.052	0.059	0.012	2.18	0.48	0.029	
Inoculated	1.13			0.043				0.68		
Interaction	1.96			0.074						

than control, which contributed to lower stem weight. Inoculation significantly increased pericarps weight. The highest number of pods (2.36) and seeds (16.36) per plant was reported in non-inoculated plants fertilized with 1 g N per pot. In plants inoculated with *Rhizobium meliloti* and treated with 0.5 g N per pot, the number of pods and seeds per plant was determined at 2.30 and 15.39, respectively (Table 1). The application of 0.5 and 1.0 g N per pot significantly influenced morphological parameters (plant height, number of branches, stem weight) and yield components (thousand seed weight, seed weight). The tallest plants (45.76 cm) were noted in treatments fertilized with 0.5 g N per pot. The number of branches increased significantly with a rise in nitrogen application rates. Stem branching was not observed in control plants (N₀) – Table 1. The application of 0.5 g N per pot increased leaf weight 3-fold and stem weight nearly 6-fold in comparison with unfertilized plants. A similar relationship was observed in a field experiment (BANAFAR et al. 1995) where the application of 60 N kg ha⁻¹ and 40 P kg ha⁻¹ significantly increased the weight of fenugreek leaves. In the study of (DARAMOLA et al. 2013) increase in stem dry matter of other legumes crop (cowpea) accumulation was observed as N rates increased. Besides it, the content of macronu-

trients in green forage of red clover dependent on from phase development (ZUK-GOLASZEWSKA et al. 2010). Nitrogen fertilization contributed to a significant increase in thousand seed weight and seed weight (by 76.6% and 60%, respectively) compared with the control one. In the study of DETROJA et al. (1996), the application of 30 kg nitrogen per ha significantly influenced thousand seed weight (14.32 g) in comparison with unfertilized fenugreek plants (11.38 g). SHARMA (2000) reported the highest number of branches (4.4) and pods per plant (64), and the highest seed yield per plot (725 g) and per ha (18.2 g) in treatments fertilized with 60 kg per ha in comparison with control. In a study by DATTA et al. (2005), the yield of fenugreek seeds also increased significantly at fertilization rates of 0, 15, 25 and 45 kg N ha⁻¹. Seed yield increased to 1.07 t ha⁻¹ in treatments fertilized with 45 kg N ha⁻¹ in comparison with control (0.66 t ha⁻¹). CHAUDHARY (1999) demonstrated that the application of 40 kg nitrogen per ha and 40 kg phosphorus per ha contributed to an increase in the thousand seed weight (12.11 g) of fenugreek plants relative to control (11.76 g). In the other research application of organic manures and *Rhizobium* inoculation enhanced the seed yield (NAIMUDDIN et al. 2014). In this study, an increase in nitrogen fertilization rates (N_{1.0}) had no significant effect on the mentioned parameters (Table 1).

The nitrogen content of vegetative organs in fenugreek plants was correlated with both seed inoculation and fertilization rate (Table 2). The highest nitrogen concentrations were reported in the stems and leaves of non-inoculated plants fertilized with 1.0 g N per pot (10.26 and 27.22 g kg⁻¹ d.m., respectively), which contributed to a higher nitrogen content of pods (10.14 g kg⁻¹ d.m.). The vegetative organs of inoculated plants contained less potassium and calcium, but more sodium and magnesium (Table 2). Seed inoculation with *Rhizobium meliloti* increased the nitrogen content of pods by 41.3%. The highest nitrogen concentrations (14.24 g kg⁻¹ d.m.) were determined in the pods of plants that were inoculated and fertilized with a high nitrogen rate.

Seed inoculation with *Rhizobium meliloti* significantly lowered (by 11.5%) crude fat content and increased phosphorus, calcium and sodium concentrations (by 6.1%, 28.2% and over 130%, respectively) in fenugreek seeds (Table 3). In other study reported by HEMAVATHY and PRABHAKAR, (1989) seeds of fenugreek contained 7.5% crude fat. Potassium and magnesium levels remained fairly constant regardless of inoculation and nitrogen fertilization. The application of nitrogen contributed to a significant increase in the total protein content of fenugreek seeds (Table 3). The highest total protein content (333.9 g kg⁻¹ d.m.) was reported in the seeds of inoculated plants supplied with the highest nitrogen rate. In the research GENDY (2013) *Rhizobium* inoculation increased nitrogen, carbohydrates, potassium and protein percentages of fenugreek.

Table 3

Total protein, crude fat and macronutrient concentrations in fenugreek seeds

Specifica- tion	Total protein	Crude fat	P	K	Na	Ca	Mg
(g kg ⁻¹ d.m.)							
Non-inoculated							
N _{0.0}	241.5	57.10	7.65	23.20	0.442	0.96	2.74
N _{0.5}	311.5	56.30	7.73	16.07	0.580	1.12	2.32
N _{1.0}	315.1	53.10	8.03	14.21	0.866	1.42	2.25
Mean	289.4	55.50	7.80	17.83	0.629	1.17	2.44
Inoculated							
N _{0.0}	222.1	49.30	8.23	22.68	1.842	1.41	2.45
N _{0.5}	295.9	50.60	8.08	15.63	1.448	1.42	2.13
N _{1.0}	333.9	47.30	8.55	14.18	1.111	1.68	2.15
Mean	283.9	49.07	8.28	17.50	1.467	1.50	2.24
Mean for N rate							
N _{0.0}	231.8	53.20	7.94	22.94	1.14	1.19	2.60
N _{0.5}	303.7	53.45	7.90	15.85	1.01	1.27	2.23
N _{1.0}	324.5	50.20	8.29	14.20	0.99	1.55	2.20
HSD $P=0.01$ for:							
N rate	13.40						
Inoculated		3.59	0.34		0.25	0.23	
Interaction	18.95				0.43		

CONCLUSIONS

1. Nitrogen fertilization significantly affected the chlorophyll content of fenugreek leaves. In the leaves of plants fertilized with nitrogen, chlorophyll concentrations were determined in the range of 48.5 - 56.1 SPAD (43 DAS) to 58.2 - 60.6 SPAD (58 DAS), subject to the applied nitrogen rate. At 43 DAS, the chlorophyll content of control plant leaves was determined at 46 SPAD, and it decreased along plant growth.

2. Nitrogen fertilization had a greater influence on the weight of vegetative organs and the seed yield of fenugreek than seed inoculation. Fertilization improved morphological parameters (plant height, number of branches, stem weight), yield components (thousand seed weight, seed weight), and total protein content.

3. Seed inoculation with *Rhizobium meliloti* had a greater effect on seed quality than on plant habitus or yield components. Inoculation significantly decreased the crude fat content of fenugreek seeds, but it increased phosphorus, calcium and sodium concentrations.

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CONTENT OF Fe, Mn, Cu AND Zn IN LIGHT SOIL AFTER INTRODUCING ANIONS IN THE FORM OF CALCIUM SALTS AND AFTER IRRIGATION

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Abstract

Mineral fertilisation pertains to the supply of cations and anions affecting the chemism of soil environment, which leads to changes in the solubility of chemical compounds, including Fe, Mn, Cu and Zn. The purpose of this research was to determine the effects of anions CO_3^{2-} , SO_4^{2-} , NO_3^- applied in the form of calcium salts and of irrigation on the solubility of Fe, Cu, Mn and Zn compounds in light soil. In order to attain our objective, a soil column (PO no 54427) was used for laboratory examinations of irrigated soil. A two-factor laboratory experiment was set up with the method of complete randomisation in three replications, where the first factor was an anion coupled with calcium cations: I – 0, II – CaCO_3 , III – CaSO_4 , IV – $\text{Ca}(\text{NO}_3)_2$; and the second one was the soil depth: a – 0-10, b – 10-20, c – 20-30 cm. Soil material was collected from the humus accumulation horizon of brown-rusty soil (0-10 cm). It was characterised by very acid reaction and the amount of $\phi < 0.02$ mm fraction lower than 13%. The calcium salt dose was determined on the basis of the soil's hydrolytic acidity and its grain-size distribution. Soil was placed in a soil column and irrigated. After the water sprinkling, the soil material was analysed for the content of chemical elements soluble in H_2O , HCl at the concentration 0.1 mol dm^{-3} and concentrated acids $\text{HNO}_3 + \text{HClO}_4$. Calcium incorporated into the soil as well as the soil irrigation affected the soil reaction depending on the applied accompanying anion. The highest concentration of Mn forms measured in concentrated acids was found after applying CaCO_3 , and the significantly lowest one was determined in response to CaSO_4 . The content of the Cu forms soluble in concentrated acids was the largest in the soil supplied with CaSO_4 . Calcium salts applied in the study modified differently the soil resources of Mn and Zn forms measured in 0.1 mol dm^{-3} HCl and the forms of Fe, Cu and Zn soluble in water.

Key words: sprinkling, anions, solubility, light soil.

ZAWARTOŚĆ Fe, Mn, Cu i Zn W GLEBIE LEKKIEJ PO WPROWADZENIU ANIONÓW W POSTACI SOLI WAPNIA I NAWADNIANEJ

Abstrakt

Nawożenie mineralne gleby polega na dostarczeniu kationów oraz anionów wpływających na chemizm środowiska glebowego, co prowadzi do zmiany stopnia rozpuszczalności związków chemicznych, w tym także Fe, Mn, Cu i Zn. Celem badań było określenie wpływu anionów CO_3^{2-} , SO_4^{2-} , NO_3^- zastosowanych w formie soli wapnia i nawadniania na rozpuszczalność związków Fe, Cu, Mn i Zn w glebie lekkiej. Do realizacji celu badań wykorzystano urządzenie kolumnowe do laboratoryjnych badań gleby z nawadnianiem (PO nr 54427). Doświadczenie laboratoryjne 2-czynnikowe założono metodą kompletnej randomizacji w 3 powtórzeniach. Pierwszym czynnikiem był anion towarzyszący kationowi wapnia: I – 0, II – CaCO_3 , III – CaSO_4 , IV – $\text{Ca}(\text{NO}_3)_2$; natomiast drugim miąższość gleby: a – 0-10, b – 10-20, c – 20-30 cm. Materiał glebowy użyty w doświadczeniu pobrano z poziomu akumulacji próchnicy gleby brunatnej rdzawej (0-10 cm). Odnaczał się on bardzo kwaśnym odczynem i zawartością frakcji o $\phi < 0.02$ mm w ilości nie przekraczającej 13%. Dawki soli wapnia wyznaczono na podstawie wartości kwasowości hydrolitycznej z uwzględnieniem składu granulometrycznego gleby. Glebę umieszczono w urządzeniu kolumnowym i nawadniano. W materiale glebowym po nawadnianiu oznaczono zawartość form pierwiastków chemicznych rozpuszczalnych w H_2O , w HCl o stężeniu 0.1 mol dm^{-3} oraz w stężonych kwasach $\text{HNO}_3 + \text{HClO}_4$. Wapń dodany do gleby i nawadnianie miały wpływ na odczyn gleby w zależności od anionu towarzyszącego. Największe stężenie Mn ogółem stwierdzono po zastosowaniu CaCO_3 , a istotnie najniższą wartość – po dodatku CaSO_4 . Zawartość formy ogólnej Cu była największa w glebie, w której zastosowano CaSO_4 . Zastosowane w doświadczeniu sole wapnia odmiennie kształtowały zasobność gleby lekkiej w formy Mn i Zn oznaczone w 0.1 mol dm^{-3} HCl, a także w formy Fe, Cu, Zn rozpuszczalne w wodzie.

Słowa kluczowe: deszczowanie, aniony, rozpuszczalność, gleba lekka.

INTRODUCTION

Mineral fertilisation pertains to the supply of cations and anions affecting the chemism of soil environment, which leads to changes in the degree of solubility of chemical elements, including Fe, Mn, Cu and Zn. Water solubility of the above elements is negatively correlated with the increase in soil pH, whereas a growing amount of organic carbon has been observed to result in an increase in the concentration of Fe, Mn, Cu and Zn in the soil solution (ŁABĘTOWICZ, RUTKOWSKA 2002, RUTKOWSKA et al. 2004, JAKUBUŚ 2007). In the opinion of MARTÍNEZ and MOTTO (2000), H^+ ion activity affects the solubility of Cu and Zn. At pH = 6.2 for Zn and 5.5 for Cu, the soil content of these metals remains on a natural level, whereas below these pH values their solubility is increasing. Higher pH values and lower soil acidity are obtained by supplying the soil with calcium carbonate (PATRA, MOHANTY 1994, BADORA, FILIPEK 1999, KOWALENKO, IHNAT 2010), magnesium carbonate or bicarbonate of calcium and magnesium (BADORA, FILIPEK 1999).

BADORA and FILIPEK (1999) found higher pH in 0.01 CaCl_2 as affected by $\text{Ca}(\text{NO}_3)_2$ supplied with manure. In turn, pH values were reduced after fertilising light soil with $(\text{NH}_4)_2\text{SO}_4$ and KCl or $(\text{NH}_4)_2\text{SO}_4$ and K_2SO_4 or $(\text{NH}_4)_2\text{SO}_4$

with KCl, in comparison with manure fertilisation and CaCO_3 . According to BŁAZIAK (1998), MgSO_4 did not cause a change in pH, but significantly increased the values of soil exchangeable and hydrolytic acidity.

Agricultural practices lead to changes in the concentration, availability and mobility of anions in arable land. JAKUBUŚ (2007) is of the opinion that the content of Fe, Mn, Cu and Zn soluble in DTPA solution depends on the share of grain crops in a rotation, the dose of nitrogen fertiliser and sprinkler irrigation.

Having been incorporated into soil, cation and anion forms of nitrogen fertiliser differentiate the yield and chemical composition of field crops. Nutrient uptake by plants depends on the kind of anion coupled with cation introduced into the soil (WARCHOŁOWA, MROCZKOWSKI 1982, MICHAŁEK 2000). Small differences in copper desorption from very acid grey-brown podsollic soil developed from slightly loamy sand and silt loam by Mg^{2+} and NH_4^+ cations with accompanying Cl^- anion were found by PASZKO et al. (2000) and PASZKO (2000). In their opinion, anions introduced into soil with fertilisers may have a greater influence on the Cu depletion from the soil.

The purpose of this research was to determine the effects of anions CO_3^{2-} , SO_4^{2-} , NO_3^- coupled with calcium cations on the solubility of Fe, Cu, Mn and Zn in light soil after sprinkling.

MATERIAL AND METHODS

In order to attain our objective, a soil column (PO no 54427) for irrigated soil examinations was used. Two-factor laboratory experiment was set up by the method of complete randomisation, in three replications, where the first factor was an anion coupled with calcium cations: I – 0, II – CaCO_3 , III – CaSO_4 , IV – $\text{Ca}(\text{NO}_3)_2$; and the second one was the soil depth: a – 0-10, b – 10-20, c – 20-30 cm.

Soil material for the experiment was collected from the humus accumulation horizon of brown-rusty soil (0-10 cm), in a field of Experimental Agricultural Station in Lipnik. It was characterised by very acid reaction and 13% share of $\phi < 0.02$ mm fraction. The calcium salt dose was determined on the basis of the soil's hydrolytic acidity and grain-size distribution. The data on the chemical properties of the soil material used in our laboratory experiment before sprinkling, and the quantities of applied calcium salts are presented in WOJCIESZCZUK and WOJCIESZCZUK (2009).

Soil material, placed in a soil column for laboratory investigations of irrigated soil, was sprinkled 8 times with deionised water. The water dose was the same for all fertiliser variants. After sprinkling, 36 soil samples were taken from columns for analysis. In total, 7.4 litres of water were used per one column during one irrigation cycle. Under the humid climatic conditions,

this amount of water would soak through a soil column in more than 12 years (LEVESQUE, HANNA 1966).

After irrigation, the soil material was analysed to determine the content of chemical elements Fe, Mn, Cu and Zn soluble in H_2O , in HCl at the concentration 0.1 mol dm^{-3} and in concentrated acids $HNO_3 + HClO_4$. Physicochemical analyses were carried out by the methods described by OSTROWSKA et al. (1991). The concentration of metals was measured spectrophotometrically by the ASA methods.

The results obtained in laboratory investigations were verified statistically by two-factorial analysis of variance (Anova) with a software package Statistica 9 software at $\alpha=0.05$. The significance of differences was estimated using the Newman - Keuls test.

RESULTS AND DISCUSSION

In the light, irrigated soil with $CaCO_3$ amendment, pH measured in $1 \text{ mol KCl dm}^{-3}$ was higher than in the unfertilised treatment with the supplement of $CaSO_4$ or $Ca(NO_3)_2$ (Table 1). WOJCIESZCZUK and WOJCIESZCZUK (2009) give pH values in $1 \text{ mol KCl dm}^{-3}$ for light, non-irrigated soil after the application of $CaCO_3$ within the range 6.34 - 6.45, whereas in unfertilised soil and in the soil with $CaSO_4$ and $Ca(NO_3)_2$, the values of pH_{KCl} ranged from 3.57 to 3.67. According to KOWALENKO and IHNAT (2010), fertilisation of acid soil with $CaCO_3$ increases its pH in water and this effect is enhanced with growing amounts of the applied fertiliser. With time, pH was decreasing in successive years of experiments. PATRA and MOHANTY (1994) stated that $CaCO_3$ introduced into the soil flooded with deionised water, under rice cover, caused an increase of pH measured *in situ* from 5.2 to pH 7.0-7.2. These researchers noticed that in the inundated soil without fertilisers, pH (6.0) was also higher prior to the studies. In our investigations, soil irrigation widened the range of pH_{KCl} from 3.53 to 3.88 in unfertilised objects with the amendment of $CaSO_4$ and $Ca(NO_3)_2$ – Table1. As a result of sprinkling and calcium carbonate application, the values of pH_{KCl} decreased by 1.48-1.69 units in comparison with the non-irrigated soil (WOJCIESZCZUK, WOJCIESZCZUK 2009). MOTO-

Table 1

Soil pH values measured in $1 \text{ mol KCl dm}^{-3}$ after the application of calcium salts and irrigation

Property	Soil depth (cm)	Form of calcium salt				Mean
		I – control	II – $CaCO_3$	III – $CaSO_4$	IV – $Ca(NO_3)_2$	
pH_{KCl}	0-10	3.53	4.97	3.60	3.57	4.42
	10-20	3.73	4.76	3.73	3.80	4.27
	20-30	3.74	4.65	3.80	3.88	4.20
Mean		3.68	4.82	3.72	3.77	

WICKA-TERELAK and DUDKA (1991) also found that supplementing the soil developed from sand with elemental sulphur caused acidification of humus (0-20 cm), from pH_{KCl} 4.2 to pH_{KCl} 3.7 in bare soil at the 0-10 cm depth and from pH_{KCl} 4.0 to pH_{KCl} 3.8 at the 11-20 cm depth. Due to the leaching of chemical elements in the 21-40 cm layer of bare soil, the authors recorded higher pH_{KCl} , since pH_{KCl} was in the range of 5.0-4.3 in the 21-30 cm depth, but higher pH_{KCl} values were found at the depth of 31-40 cm (pH_{KCl} 5.3-5.8).

We obtained a similar distribution of pH_{KCl} values in the unfertilised variants with the supplement of CaSO_4 and $\text{Ca}(\text{NO}_3)_2$; in the soil supplied with CaCO_3 , the highest pH_{KCl} was found in the 0-10 cm layer, was decreasing with depth. WOJCIESZCZUK et al. (2004) report that the hydrolytic acidity of light soil after sprinkling was decreasing with depth. In our studies, increasing pH_{KCl} values in deeper soil layers were observed in unfertilised soil with the amendment of CaSO_4 and $\text{Ca}(\text{NO}_3)_2$; in turn, in the soil enriched with CaCO_3 , the highest pH_{KCl} was in the 0-10 cm layer, again decreasing with depth.

In the conducted laboratory experiment, sprinkling affected the solubility of various forms of chemical compounds and their mobility in soil. Compared to non-irrigated soils (WOJCIESZCZUK, WOJCIESZCZUK 2009), sprinkling reduced the content of Fe and Cu compounds soluble in concentrated acids, whereas their content in 0.1 mol dm^{-3} HCl increased. It was only the sprinkling treatment of the soil fertilised with CaCO_3 that reduced the amount of Fe and Cu compounds soluble in H_2O .

The mean content of the Mn forms soluble in 0.1 mol dm^{-3} HCl significantly depended on the depth from which the soil material was taken, but this was not observed in the case of the Mn forms soluble in concentrated acids and in water (Tables 2 and 3). Soil material from the 0-10 cm layer was characterised by a lower mean content of Mn measured in 0.1 mol dm^{-3} HCl than that one from the 10-30 cm depth (Table 4). In the topsoil supplied with NO_3^- , a considerably lower amount of Mn forms soluble in 0.1 mol dm^{-3} HCl was detected than in unfertilised soil from the 20-30 cm layer.

Solubility of Mn compounds determined in concentrated acids varied and depended on both the kind of an anion and the depth. A markedly higher amount of total Mn was found in the 0-10 cm layer in the soil with CaCO_3 in comparison with the content determined in all layers of the soil with CaSO_4 and $\text{Ca}(\text{NO}_3)_2$ – Table 2. At the depth of 10-30 cm, substantially higher amounts of Mn forms soluble in concentrated acids were found under the influence of carbonate anion rather than sulphate.

In contrast to non-irrigated soil (WOJCIESZCZUK, WOJCIESZCZUK 2009), sprinkling lowered the mean content of Mn compounds soluble in concentrated acids from 123.4-126.5 mg kg^{-1} to 102.4-116.7 mg kg^{-1} . The only exception was the variant with calcium carbonate, where the mean value was higher by 14.5 mg kg^{-1} after water application. Water treatment of the soil contain-

Table 2

Content of Fe, Mn, Cu and Zn forms soluble in concentrated acids $\text{HNO}_3 + \text{HClO}_4$ in soil after irrigation depending on applied form of calcium salt (mg kg^{-1})

Chemical element	Soil layer (cm)	Form of calcium salt				Mean
		I – control	II – CaCO_3	III – CaSO_4	IV – $\text{Ca}(\text{NO}_3)_2$	
Fe	0-10	4939	5291	5803	5573	5402
	10-20	5096	5407	5373	5442	5330
	20-30	5056	5399	5219	5356	5258
Mean		5030	5366	5465	5457	
Mn	0-10	119.2 ^{abc}	139.3 ^c	105.7 ^{ab}	106.0 ^{ab}	117.6
	10-20	116.6 ^{abc}	136.7 ^{bc}	101.4 ^a	107.9 ^{ab}	115.7
	20-30	114.1 ^{abc}	135.6 ^{bc}	100.2 ^a	106.0 ^{ab}	114.0
Mean		116.7 ^b	137.2 ^c	102.4 ^a	106.6 ^{ab}	
Cu	0-10	5.10 ^{ab}	4.45 ^a	5.15 ^{ab}	4.92 ^{ab}	4.90
	10-20	4.70 ^{ab}	4.92 ^{ab}	5.24 ^{ab}	5.10 ^{ab}	4.98
	20-30	4.24 ^a	5.21 ^{ab}	5.97 ^b	5.64 ^{ab}	5.26
Mean		4.67 ^a	4.86 ^a	5.45 ^b	5.21 ^{ab}	
Zn	0-10	19.88	20.17	21.95	20.51	20.63
	10-20	19.61	21.36	21.86	20.66	20.87
	20-30	18.09	21.70	29.28	21.59	22.67
Mean		19.19	21.08	24.36	20.92	

Key: ^{a, b, c} – homogenous groups were obtained using the Newman - Keuls test at $\alpha=0.05$.

ing calcium carbonate and nitrate forms increased the mean content of Mn in $0.1 \text{ mol dm}^{-3} \text{ HCl}$ by 4.6 and 3.1 mg kg^{-1} , respectively, in relation to the values presented by WOJCIESZCZUK and WOJCIESZCZUK (2009). Irrespective of the applied anion, the mean content of water soluble Mn was lower after sprinkling than in unfertilised soil. The concentration of Mn compounds soluble in water ranged from 0.328 to 0.860 mg kg^{-1} .

Supplying the soil with CaCO_3 lowers the solubility of manganese compounds (BADORA 2001, RUTKOWSKA et al. 2009, WOJCIESZCZUK, WOJCIESZCZUK 2009, KOWALENKO, IHNAT 2010). On the other hand, amending the soil with nitrate or ammonium form of nitrogen increases the content of Mn^{2+} in the soil solution. JAKUBUŚ (2007) recorded an increase in the amount of bioavailable Mn as a result of nitrogen fertiliser. This author also stated that there was more active Mn (extracted with DTPA) in the soil without sprinkling than in irrigated soil. In BŁAZIAK'S opinion (1998), a significantly higher content of active Mn (determined according to Schachtschabel) was found in excessively moist soil (moisture 33% volume), and the amendment of calcium and magnesium oxide brought about an over two-fold decrease in its content. GONDEK (2009) claims that liming and watering heavy soil resulted in a lower concentration of mobile forms of Cu, Mn and Zn (soluble in NH_4NO_3 at the

Table 3

Content of Fe, Mn, Cu and Zn forms soluble in H₂O in soil after irrigation depending on the calcium salt applied (mg kg⁻¹)

Chemical element	Soil layer (cm)	Form of calcium salt				Mean
		I – control	II – CaCO ₃	III – CaSO ₄	IV – Ca(NO ₃) ₂	
Fe	0-10	3.51	2.83	4.84	6.58	4.44
	10-20	3.74	5.03	10.32	10.26	7.34
	20-30	6.36	5.63	7.31	10.18	7.37
Mean		4.54 ^a	4.49 ^a	7.49 ^{ab}	9.01 ^b	
Mn	0-10	0.527	0.297	0.833	0.893	0.637
	10-20	0.930	0.370	0.480	0.857	0.659
	20-30	1.123	0.317	0.810	0.783	0.758
Mean		0.860	0.328	0.708	0.844	
Cu	0-10	0.300 ^{abc}	0.197 ^a	0.267 ^{ab}	0.327 ^{abc}	0.272 ^a
	10-20	0.297 ^{abc}	0.270 ^{ab}	0.307 ^{abc}	0.417 ^{cb}	0.322 ^a
	20-30	0.340 ^{abc}	0.380 ^{cb}	0.450 ^c	0.553 ^d	0.431 ^b
Mean		0.312 ^a	0.282 ^a	0.341 ^a	0.432 ^b	
Zn	0-10	0.150	0.093	0.183	0.160	0.147
	10-20	0.130	0.120	0.173	0.203	0.157
	20-30	0.187	0.130	0.163	0.227	0.177
Mean		0.155 ^{ab}	0.114 ^a	0.173 ^b	0.197 ^b	

Key: ^{a, b, c} – homogenous groups were obtained using Newman - Keuls test at $\alpha=0.05$.

concentration 1 mol dm⁻³) than the one found in soil without liming. Soil irrigation and the NPK [NH₄NO₃, Ca(H₂PO₄) H₂O, KCl] and NPKS [(NH₄NO₃, Ca(H₂PO₄) H₂O, KCl (NH₄)₂SO₄] fertilisation applied by GONDEK (2009) increased the content of mobile Cu and Mn in the treatments without liming; in the case of Zn, the differences were significant in comparison with the unfertilised object.

In this research, the mean content of the forms of Cu soluble in concentrated acids was significantly higher in unfertilised soil with CaSO₄ than in unfertilised soil supplied with CaCO₃, whereas there was no significant difference in the soil with the amendment of Ca(NO₃)₂ – Table 2. After sprinkling, a significantly higher amount (5.97 mg kg⁻¹) of copper compounds soluble in concentrated acids was detected at the 20-30 cm depth after CaSO₄ application than in the same layer of unfertilised soil (4.24 mg kg⁻¹) and the 0-10 cm layer (4.45 mg kg⁻¹) of the soil supplemented with CaCO₃ (Table 2).

Anions coupled with calcium cations had no significant effect on the mean content of copper in 0.1 mol dm⁻³ HCl (Table 3). Considerable differences were observed in individual layers. In the topsoil, a markedly lower content of the forms of Cu soluble in 0.1 mol dm⁻³ HCl was found as a result of

Table 4

Content of Fe, Mn, Cu and Zn forms soluble in HCl at the concentration of 0.1 mol dm⁻³ in soil after irrigation depending on the calcium salt applied (mg kg⁻¹)

Chemical element	Soil layer (cm)	Form of calcium salt				Mean
		I – control	II – CaCO ₃	III – CaSO ₄	IV – Ca(NO ₃) ₂	
Fe	0-10	275.8	273.9	290.9	281.1	280.4
	10-20	357.3	271.6	319.9	338.6	321.8
	20-30	368.2	272.9	348.8	372.9	340.7
Mean		333.8	272.8	319.9	330.9	
Mn	0-10	53.3 ^{ab}	57.7 ^{ab}	45.6 ^{ab}	40.5 ^a	49.3 ^a
	10-20	70.8 ^{ab}	54.6 ^{ab}	49.0 ^{ab}	58.5 ^{ab}	58.2 ^b
	20-30	74.8 ^b	56.7 ^{ab}	48.4 ^{ab}	61.8 ^{ab}	60.5 ^b
Mean		66.3 ^b	56.4 ^{ab}	47.7 ^a	53.6 ^a	
Cu	0-10	2.25 ^{ab}	2.32 ^{ab}	2.17 ^{ab}	1.87 ^a	2.15 ^a
	10-20	2.36 ^{ab}	2.08 ^{ab}	2.04 ^{ab}	1.99 ^{ab}	2.12 ^a
	20-30	2.52 ^{ab}	2.69 ^b	2.61 ^{ab}	2.68 ^b	2.62 ^b
Mean		2.38	2.36	2.27	2.18	
Zn	0-10	6.49 ^{ab}	5.76 ^{ab}	4.06 ^a	4.60 ^a	5.22 ^{ab}
	10-20	4.51 ^a	3.81 ^a	4.73 ^a	3.45 ^a	4.13 ^a
	20-30	6.80 ^{ab}	9.65 ^b	3.82 ^a	4.01 ^a	6.07 ^b
Mean		5.93 ^{ab}	6.41 ^b	4.20 ^a	4.02 ^a	

Key: ^{a,b,c} – homogenous groups were obtained using the Newman - Keuls test at $\alpha=0.05$.

nitrate anion and water in comparison with the amount obtained in the soil material from the 20-30 cm layer in the same column and the soil with CaCO₃.

Our studies showed that irrigation and Ca(NO₃)₂ application resulted in higher mean values of the forms of Cu soluble in water in relation to the other fertiliser variants. The mean value of the content of Cu compounds soluble in water was substantially higher in the 20-30 cm layer (Table 3). The amount of this form of copper in particular layers of irrigated soil was affected by the anion coupled with calcium cation. The highest content of Cu compounds soluble in water was determined in the 20-30 cm layer in the soil with NO₃⁻, whereas the lowest content of this copper form was determined in the topsoil with CO₃²⁻. Soil fertilisation differentiated the content of copper compounds soluble in water within the distinguished layers. No significant differences were found only in unfertilised soil.

HOCH et al. (2011) think that the fertilisation of light soil with ammonium nitrate and potassium salt had a greater effect on the content of Cu in HCl at the concentration 1 mol dm⁻³, whereas calcium amendment resulted in reducing the content of this form of copper. RUTKOWSKA et al. (2009)

demonstrate that an increase in Cu content in the soil solution was caused by ammonium nitrate, which lowered pH values. According to GONDEK (2009), irrigation of heavy soil and its fertilisation with NPK [NH_4NO_3 , $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, KCl] and NPKS [$(\text{NH}_4\text{NO}_3, \text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}, \text{KCl}, (\text{NH}_4)_2\text{SO}_4)$] increased the content of the Cu mobile form (soluble in NH_4NO_3 at the concentration 1 mol dm^{-3}), whereas liming decreased its amount. This study shows that the content of Fe and Cu forms soluble in water was significantly lower in irrigated soil with CaCO_3 supplement than in soil with the amendment of $\text{Ca}(\text{NO}_3)_2$. In the opinion of WOJCIESZCZUK and WOJCIESZCZUK (2009), the content of the forms of Cu soluble in water was significantly smaller in the control object, although it did not vary significantly in the objects with CaCO_3 , CaSO_4 and $\text{Ca}(\text{NO}_3)_2$.

The mean content of Zn in concentrated acids ranged from 19.19 to 24.36 mg kg^{-1} , but the differences were statistically insignificant (Table 2). Calcium carbonate and sprinkling raised the mean concentration of Zn compounds soluble in $0.1 \text{ mol dm}^{-3} \text{ HCl}$. After water treatment of light soil supplemented with SO_4^{2-} and NO_3^- , there were significantly more Zn compounds soluble in water compared with the soil supplied with CO_3^{2-} (Table 3). The situation was reverse in the case of Zn determined in $0.1 \text{ mol dm}^{-3} \text{ HCl}$, where its amount was higher in the soil with CO_3^{2-} than after the application of SO_4^{2-} , or NO_3^- (Table 4). JAKUBUŚ (2007) states that the quantity of bioavailable Zn increased after nitrogen fertilisation and irrigation. BŁAZIAK (1998) indicates that magnesium sulphate and soil moisture growing up to 23.5% and 33.0% volume significantly reduced the content of the Zn forms soluble in HCl at the concentration 0.1 mol dm^{-3} . GONDEK (2009) recorded an increase in the content of the mobile Zn form after fertilising heavy soil with NPK and NPKS, and its decrease after liming. According to RUTKOWSKA et al. (2009), reduced the amount of Zn in the soil solution by supplying the soil with CaCO_3 . Also, KOWALENKO and IHNAT (2010) demonstrate that carbonate anions lowered the amount of available Zn (extracted from soil by the Mehlich-3 method) in relation to the variant without CaCO_3 . The results presented by WOJCIESZCZUK and WOJCIESZCZUK (2009) show that CaCO_3 , CaSO_4 and $\text{Ca}(\text{NO}_3)_2$ contributed to a decline in the content of Zn determined in $0.1 \text{ mol dm}^{-3} \text{ HCl}$ in non-irrigated soil.

The mean content of the Zn forms soluble in $0.1 \text{ mol dm}^{-3} \text{ HCl}$ depended on the layer from which the soil material for analysis was separated after the completion of water treatment, but it did not significantly affect the amount of zinc compounds soluble in concentrated acids and water. The mean content of exchangeable zinc was considerably higher at the depth of 10-20 cm (Table 4). The concentration of zinc in $0.1 \text{ mol dm}^{-3} \text{ HCl}$ in particular layers was affected by anions introduced into the soil. The highest value (9.65 mg kg^{-1}) was obtained at 20-30 cm after the application of CO_3^{2-} , at the same time being significantly different from the lowest value determined in the same column at 10-20 cm (3.81 mg kg^{-1}). The values for Zn in

0.1 mol dm⁻³ HCl in all layers of the soil where SO₄²⁻ and NO₃⁻ were applied and from the 10-20 cm layer of unfertilised soil were considerably lower than those from the soil with CO₃²⁻ supplement at the depth of 20-30 cm.

There were no significant differences in the content of particular forms of iron depending on the depth. In our laboratory experiment, only the content of iron compounds soluble in water was significantly dependent on the form of calcium salt applied. A significantly higher amount of Fe compounds soluble in water was recorded in the soil with Ca(NO₃)₂ in comparison with its content in the soil without fertilisation and after the application of CaCO₃ (Table 3). AMMARI and MENGEL (2006) found that the soil content of Fe bound to organic complexes in aqueous extract ranged from 40-91% and its concentration was significantly correlated ($r=0.77$) with hydrogen ion activity. WOJCIESZCZUK and WOJCIESZCZUK (2009) found that in non-irrigated soil the content of Fe compounds soluble in water after the application of CaSO₄ and Ca(NO₃)₂ was 0.11 and 0.09 mg kg⁻¹, respectively. In our studies, sprinkling the light soil supplied with SO₄²⁻ and NO₃⁻ increased the solubility of Fe compounds in water up to 7.49 and 9.01 mg kg⁻¹ (Table 3).

In the light soil subjected to sprinkling, the content of Fe in 0.1 mol dm⁻³ HCl ranged from 272.8 to 333.8 mg kg⁻¹ (Table 3). Comparing these results with the amount of Fe compounds soluble in 0.1 mol dm⁻³ HCl in non-irrigated soil (WOJCIESZCZUK, WOJCIESZCZUK 2009), it was noticed that water treatment increased solubility of these compounds by 104.6-183.1 mg kg⁻¹. A smaller increase in irrigated soil versus non-irrigated one was observed after the application of CO₃²⁻, and the highest one occurred after using NO₃⁻. JAKUBUŚ (2007) stated that nitrogen fertilisation resulted in the growing amount of bioavailable Fe in soil, and its content was increasing with higher doses of nitrogen fertiliser. The content of Fe in the soil solution rises under the influence of ammonium nitrate, whereas CaCO₃ reduces the content of iron (RUTKOWSKA et al. 2009). The decrease in the amount of available iron (extracted from the soil by the Mehlich-3 method) affected by CO₃²⁻ was proven by KOWALENKO and IHNAT (2010).

CONCLUSIONS

1. Calcium amendment and irrigation affected soil reaction depending on the accompanying anion applied.
2. CO₃²⁻, SO₄²⁻, NO₃⁻ anions introduced into light soil coupled with calcium cation and irrigation influenced differently the solubility of compounds of Fe, Mn, Cu and Zn in the soil.
3. The calcium salts and irrigation applied differentiated the mean content of Fe, Mn, Cu and Zn depending on the layer from which the soil material was collected. Statistically significant differences were found for the

amount of Mn, Cu, Zn compounds soluble in 0.1 mol dm^{-3} HCl and the form of Cu soluble in H_2O .

4. In the case of the content of Mn and Cu compounds soluble in concentrated acids, and amount of Mn, Cu, Zn compounds soluble in 0.1 mol dm^{-3} HCl as well as the form of Cu soluble in water, there was an interaction between the activity of anions coupled with calcium cations and the depth from which the soil material was separated after irrigation.

5. Mineral fertilisation and irrigation should be adjusted to soil properties and plant nutrient requirements so as to reduce the loss of chemical elements in the plant root system. The results show that chemical element leaching is affected not only by cations but also by the anions introduced.

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EFFECT OF DIVERSIFIED FERTILIZATION WITH NITROGEN, SULPHUR AND BORON ON FATTY ACIDS PROFILE IN OIL FLAX SEEDS*

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Abstract

Following strict standards imposed on raw materials for food production, the most important aim in plant cultivation is to obtain high quality yields with desirable chemical composition. It can be achieved, for example, through fertilization, which modifies the content and share of organic components (protein, fat) in crops. This study was conducted in 2009, 2010, 2011, and dealt with the effect of nitrogen, sulphur and boron fertilization on the content and chemical composition of fatty acids in light- and dark-coloured seeds of oil flax. The content of saturated acids, with the following number of carbon atoms C8, C10, C12, C16, C18, C22, and unsaturated acids with carbon atoms C16:1, C18:2 and C18:3, was especially modified by the weather conditions. In 2009, with the lowest mean values of precipitation and air temperatures during the plant growing season, the highest content of oleic acid (19.94%) and MUFA (20.1%) was determined. In turn, the highest air temperature and mean precipitation values in 2011 favoured a high content of linolenic acid (64.3%) and PUFA (78.5%)

Regardless of the year of flax cultivation, the dark-seed cultivar had a higher share of oleic acid $C_{18:1}$, $n-9$ in the sum of fatty acids (by about 6%), and a lower share of linolenic acid $C_{18:3}$, $n-3$ (by about 10%).

The cultivar with light-coloured seeds accumulated higher amounts of capric, palmitoleic, behenic acids, as well as 6.7% more of palmitic acid and 5.3% more of linolenic acid than the dark-seeded cultivar.

The three-year experiment demonstrated that the introduction of a nitrogen dose of 40 kg ha⁻¹ was advantageous only to the accumulation of palmitic and stearic acids, while the simultaneous application of 15 kg B and 70 kg S ha⁻¹ resulted in a decreased lignoceric acid content. The tested fertilization did not affect significantly the content of MUFA, PUFA and saturated fatty acids (SFA).

No statistically significant differences were determined in the total content of saturated fatty acids in flax oil of the examined cultivars. The dark-seeded cultivar presented a higher value of mono-unsaturated fatty acids, by 19.3%, while the light-seeded cultivar Oliwin contained 4.4% more of essential unsaturated fatty acids.

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The average content of saturated fatty acids (SFA) in flax oil reached the level of 8.24%. The percentage of Essential Fatty Acids (EFA = PUFA – polyunsaturated Fatty Acids) was about 74.9%, while the percentage of MUFA (Monounsaturated Fatty Acids) was nearly 17%.

In the experiment, the $C_{18:2}$ ($n-6$) to $C_{18:3}$ ($n-3$) ratio for the light-seeded cultivar was 0.24:1, compared to 0.32:1 for the dark -seeds variety. The ratio of saturated to unsaturated fatty acids reached 1:8.97 for the examined cultivars, being higher in the light-seeded cultivar.

Key words: flax oil, nitrogen, sulphur, boron, fatty acids, MUFA, PUFA, SFA.

WPLYW ZRÓŻNICOWANEGO NAWOŻENIA AZOTEM, SIARKĄ I BOREM NA PROFIL KWASÓW TŁUSZCZOWYCH W NASIONACH LNU OLEISTEGO

Abstrakt

Ze względu na określone wymagania stawiane surowcom do produkcji żywności, istotnym zadaniem w uprawie roślin jest uzyskanie plonów o wysokiej jakości i pożądanym składzie chemicznym. Jednym ze sposobów jest nawożenie, które modyfikuje ilość i wzajemne proporcje związków organicznych (białka, tłuszczu) w badanych odmianach.

W latach 2009, 2010, 2011 badano wpływ nawożenia azotem, siarką i borem na zawartość i skład kwasów tłuszczowych w jasnych i ciemnych odmianach lnu oleistego. Zawartość kwasów nasyconych, o liczbie atomów węgla C8, C10, C12, C16, C18, C22 oraz nienasyconych C16:1, C18 o 1, 2 i 3 wiązaniach była w szczególności modyfikowana przebiegiem warunków pogodowych. W 2009 r. o najniższym poziomie opadów i średniej temperaturze powietrza w okresie wegetacyjnym roślin odnotowano najwyższą zawartość kwasu oleinowego (19,94%) i jednonienasyconych kwasów tłuszczowych (MUFA – Monounsaturated Fatty Acids) – 20,1%, a najwyższe temperatury powietrza i średnia ilość opadów atmosferycznych w 2011 r. sprzyjały wysokiej zawartości kwasu linolenowego (64,3%) i wielonienasyconych kwasów tłuszczowych (PUFA - Polyunsaturated Fatty Acids) – 78,5%.

Bez względu na rok uprawy wyższy udział kwasu oleinowego $C_{18:1}$, $n-9$ w sumie kwasów tłuszczowych stwierdzono u odmian o ciemnych nasionach (o ok. 6%), a jednocześnie niższy udział kwasu linolenowego $C_{18:3}$, $n-3$ (o ok. 10%).

Odmiana o jasnych nasionach kumulowała więcej kwasów kaprynowego, palmitoleinowego, behenowego oraz o 6,7% kwasu palmitynowego i o 5,3% więcej kwasu linolenowego, w porównaniu z odmianą o nasionach ciemnych.

W 3-letnim doświadczeniu wykazano, że zastosowanie dawki azotu w ilości 40 kg ha⁻¹ okazało się korzystne w gromadzeniu jedynie kwasów palmitynowego i stearynowego, natomiast jednoczesne zastosowanie 15 kg B ha⁻¹ i 70 kg S ha⁻¹ spowodowało zmniejszenie zawartości kwasu lignocerynowego. Zastosowane nawożenie nie modyfikowało w istotny sposób zawartość MUFA, PUFA i nasyconych kwasów tłuszczowych (SFA – Saturated Fatty Acids).

Nie stwierdzono istotnych różnic w sumarycznej zawartości nasyconych kwasów tłuszczowych w oleju lnianym badanych odmian. Odmiana o ciemnych nasionach (Szafir) zawierała więcej jednonienasyconych kwasów tłuszczowych (o 19,3%), a odmiana o jasnych nasionach (Oliwin) – więcej niezbędnych nienasyconych kwasów tłuszczowych (o 4,4%).

Średnia zawartość nasyconych kwasów tłuszczowych (SFA) w oleju lnianym wynosiła 8,24%, NNKT- niezbędnych nienasyconych kwasów tłuszczowych (EFA = PUFA) stwierdzono ok. 74,9%, natomiast jednonienasyconych kwasów tłuszczowych (MUFA) ok. 17%.

Wykazano, że stosunek $C_{18:2}$ ($n-6$) do $C_{18:3}$ ($n-3$) wynosił u odmiany o jasnych nasionach 0,24:1, u odmiany ciemnonasiennej – 0,32:1, natomiast stosunek kwasów nasyconych do nienasyconych u badanych odmian kształtował się na poziomie 1:8,97 i był wyższy u odmiany jasnonasiennej.

Słowa kluczowe: olej lniany, azot, siarka, bor, kwasy tłuszczowe, NNKT, MUFA, SFA.

INTRODUCTION

Flax is a plant with a wide range of applications, including the chemical, cosmetics and food industries. The area cropped with oil flax is predicted to increase, owing to the increased demand for plant oils, the climate in Poland, suitable for oil flax cultivation, as well as the advantageous dietary properties of flax seeds (ZAJĄC *et al.* 2010). As oil flax seeds are used in bakery, consumption of bread introduces healthy fatty acids into the human organism. Flax oil contains unsaturated fatty acids (SFA), undesirable nutrients in the human diet, as well as unsaturated fatty acids (PUFA, MUFA), prone to oxidation (VERSCHUREN, ZEVENBERGEN 1990). Oxidation depreciates the nutritive value of fatty acids, first of all by decreasing their content of EFA (PUFA) and the content of soluble vitamins in fats (ŻBIKOWSKA, RUTKOWSKA 2008). Oxidizability of fats depends on their chemical composition. Therefore, determination of the composition of fatty acids, apart from the evaluation of their quality, can be useful as far as the applicability of fatty acids is concerned (ŻBIKOWSKA, RUTKOWSKA 2008).

Therefore the type of fat does significantly affect not only on physical and sensoric properties of products, but also on their health – promoting properties (ŻBIKOWSKA, RUTKOWSKA 2008). Thus, the important task in agricultural production is not only to obtain high yields, but also to provide the highest quality of raw material. The yield size, the content of fat and its quality is modified by fertilization (ZAJĄC *et al.* 2010).

Nitrogen is regarded as the most important yield – forming macroelement (CZUBA *et al.* 1999), yet too high doses of this element lead to the decrease in fat content in seeds (ZAJĄC 2005).

In the case of oil plants, it is crucial to provide them with sufficient amount of sulphur, which stimulates nitrogen absorption by plants and increases the quantity of FAT in seeds (KOZŁOWSKA-STRAWKA, KACZOR 2003).

In modern cultivation of flax a significant role is also played by boron fertilization (DIEPENBROCK, PORKSEN 1992, ANTONIEWICZ, ZAJĄC 2005).

The deficit of boron becomes a serious problem on acid soils, where soil erosion brings about high lose of this chemical element resulting from washing it out. Boron is the basic microelement of all higher plants and a structural component (BOLANOS *et al.* 2004).

Additionally, B is one of the nutrients responsible for changes in the concentration and metabolism in vascular plants (BLEVINS, LUKASZEWSKI 1998).

In Poland, the research on comparison of productivity of bright – and dark – coloured seeds cultivars was conducted. The mentioned cultivars were assessed on the basis of their field – forming value, oil content and chemical composition of fatty acids (ZAJĄC *et al.* 2010). PIOTROWSKA, FUROWICZ 1999) proved that the light – coloured cultivars accumulated higher amount of FAT in seeds than the dark – seed ones.

In the working hypothesis it is assumed that application of diverse nitrogen fertilization, at simultaneous introduction of fertilization with boron and sulfur in the cultivars of dark and light seeds, will result in beneficial effect on the increase in linolenic and linoleic acid content in linseed oil, while their ration will achieve desirable values.

The aim of field and laboratory experiments was determination of the effect of nitrogen, sulphur and boron fertilization on fatty acids profile in oil flax cultivars of bright – coloured and dark – coloured seeds.

MATERIAL AND METHODS

In the years 2009-2011, in Experimental Station belonging to Department of Plant Cultivation in Pawłowice (eastern longitude 17°12' and northern latitude 51°31') field experiments, using the method of sub blocks in correlated "split – plot" system, in four replications and with four variable factors were carried out.

The first factor to be examined were flax cultivars Oliwin – bright – coloured seeds and Szafr – dark – coloured seeds. The second factor was nitrogen fertilization in the following doses: N_1 – 0 kg ha⁻¹, N_2 – 20 kg ha⁻¹, N_3 – 40 kg ha⁻¹, N_4 – 60 kg ha⁻¹. The third examined factor was combined fertilization with sulphur and boron S_1+B_1 , S_2+B_2 , S_3+B_3 , where: S_1 – 30, S_2 – 50, S_3 – 70 kg S ha⁻¹ and B_1 – 5, B_2 – 10 and B_3 – 15 kg B ha⁻¹. In pre – sowing fertilization of oil flax, triple superphosphate in the amount of 40 kg P ha⁻¹, 60% potassium salt in the quantity of 70 kg K ha⁻¹, and 46% urea was applied. Ammonium nitrate was introduced in the form of top – dressing fertilization. Sulphur and boron fertilization was introduced in the stages of herringbone and budding

Fatty acids profile was determined using the method of gas chromatography with flame – ion detection FID and with the use of column ZB-WAX (30 m × 0.25 mm i.d., 0.25 µm film thickness). The analysis was done in the following conditions: the carrier gas was helium flowing at the speed of 20 m s⁻¹. The temperature in injection cell – 250°C, detector temperature – 270°C, column work temperature programme – 50°C for 1 min, temperature rise to 180°C at the speed of 15°C min⁻¹, temperature rise to 230°C at the speed of 7°C min⁻¹, temperature rise to 400°C at the speed of 30°C min⁻¹, temperature 400°C for 10 min. Total time of analyses equaled 33 min. Quantitative interpretation of chromatograms was done through comparison of retention times of methyl esters of fatty acids of the examined sample with retention times of methyl esters standards for fatty acids, using Supelco Component FAME Mix C8 - C24. The obtained results were subjected to statistical analysis according to Statistica 8.0 PL (StatSoft Polska) Programme.

The soil in Pawłowice is classified as autogenic, brown soil, originating

from light loam on medium loam, assigned to agriculturally usable good wheat complex of soil class III b. The soil richness in mineral components and its pH were determined at the Department of Plant Nutrition, Wrocław University of Environmental and Life Sciences. In the years of investigation, phosphorus content in the soil ranged from a medium to a very high level, potassium content was between a low and a very high range, magnesium – high to very high content, while pH range was slightly acidic (Table1)

The course of weather conditions in the years of investigation-distribution of temperature and rainfall for Pawłowice were shown in Tables 2 and 3.

Table1

The content of P, K, Mg (mg 100 g⁻¹) in the soil and pH (in 1 n KCl)

Years	pH	Mg	P	K
2009	5.74 _a	8.08 _b	6.93 _b	11.3 _b
2010	6.22 _a	15.0 _a	6.05 _b	17.3 _a
2011	5.87 _a	7.53 _b	8.88 _a	20.8 _a

Table 2

Climate characteristics according to SCHMUCK (1960)

Specification	Value
Average temperature of the vegetative period	14.5°C
Number of days of the temperature above 0°C	300
Number of days of the temperature above 15°C	95
Average number of ground frost - days more than	91
Annual sum of rainfall (mm)	500-600
Field works can start in	the second decade of April

The year 2009 characterized the lowest summary precipitation in growing period, ranging, approximately, 440 mm and the lowest mean value of monthly temperatures. In 2010 the highest summary precipitation amounting 540 mm at the lowest sum of mean monthly value of air temperatures equal 93°C was recorded. The hottest year in the course of three – year experiment proved to be 2011.

RESULTS AND DISCUSSION

Unique composition of flax oil was the subject of research for a number of authors (ZAJĄC et al. 2010). The content of particular fatty acids in flax oil, a considerable parameter from the nutrition point of view underwent

Table 3

Weather conditions in the years 2009-2011 (according to meteorological station in Wrocław-Swojec) for Pawłowice

Years	Average monthly day temperatures (°C)											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2009	-2.3	0.2	4.6	12.0	14.2	15.8	19.5	19.3	15.4	7.9	6.8	-0.4
2010	-5.9	-1.1	4.2	9.3	12.7	17.9	21.4	18.9	12.5	7.0	6.5	-5.2
2011	0.62	-1.62	4.39	11.91	14.81	19.1	18.19	19.27	15.49	9.34	3.81	3.89
Mean values of the years (1979-2008)	-0.5	0.1	3.4	8.7	14.2	17.1	18.9	18.4	13.8	9.3	3.9	0.8
Years	monthly sums of rainfall (mm)											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2009	34.6	46.8	48.3	30.9	67.6	141.7	134.2	53.5	12	76	32.5	51.9
2010	40.6	11	44.9	45.4	140.7	32.9	78.6	109.1	134.1	5.7	66.4	63.2
2011	35.6	10.5	45.2	27	49.4	95.7	170.9	78.9	30.4	42.6	0	48.7
Mean values of the years (1979-2008)	29.2	26.4	34.6	35.4	53.4	67.1	85.9	69.6	47.5	35.7	41.1	36.0

numerous analyses as far as human nutrition was concerned (VERSCHUREN, ZEVENBERGEN 1990, GAMBUŚ et al. 2004, JUTTELSTAD 2004, ŻBIKOWSKA, RUTKOWSKA 2008) and animal (BAROWICZ et al. 1997, BAROWICZ 2000, BAROWICZ, BREJA 2000, PIETRAS et al. 2000, SAWOSZ et al. 2000, NIEMIEC et al. 2001, BAROWICZ et al. 2002, CIEŚLAK et al. 2003, JELIŃSKA et al. 2003, BOROWIEC et al. 2004, MICEK et al. 2004, BRZÓSKA 2005, BHATTACHARYA et al. 2006, BIAŁEK et al. 2009). The mentioned investigation also involved nutrition factors taking into account the division into brown - and yellow - seed cultivars (BAROWICZ et al. 1997, SAEIDI, ROWLAND 1999, BARTECZKO et al. 2001, BOROWIEC et al. 2001a, BIAŁEK et al. 2009). The composition and content of flax seeds fat result from the effect of the environmental factor (ALBRECHTSEN, DYBING 1973, HOCKING et al. 1987, DIEPENBROCK, PORKSEN 1992, DIEPENBROCK et al. 1995, PIOTROWSKA, FUROWICZ 1998, CASA et al. 1999, GRANT et al. 1999, AUFHAMMER et al. 2000, ZAJĄC et al. 2001, 2002). In 2009, characterized by the lowest mean values of precipitation and air temperatures in growing period, the highest value of oleic acid (19.94%) and MUFA, ranging 20.1% was recorded, while the highest air temperature values and mean precipitation values were recorded in 2011, which favored high content of linolenic acid (64.3%) and PUFA (78.5%) – Tables 4 and 5. In the experiment by ZAJĄC et al. (2001), no statistically

Table 4
Fatty acids content in oil flax seeds (means for the factors 2009-2011)

Specification	Caprylic acid	Capric acid	Lauric acid	Myristic acid	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Ara-chidic acid	Behenic acid	Lignoceric acid
Years													
2009	0.324a	0.036a	0.013b	0.084	6.63a	0.091b	0.131b	19.94a	12.50c	59.19b	0.074a	0.152a	0.760a
2010	0.009b	0.026	0.032a	0.077	6.06a	0.118a	2.052a	16.93b	17.77a	56.79b	0.038b	0.057b	0.039b
2011	0.005b	0.034a	0.043a	0.120	4.41b	0.035b	2.300a	13.21c	14.24b	64.30a	0.037b	0.154a	0.031b
Cultivar													
Oliwin	0.089	0.031a	0.031	0.099	6.24a	0.098a	1.331b	14.86b	14.84	61.68a	0.046	0.162a	0.290
Szafir	0.136	0.028b	0.028	0.082	5.82b	0.064b	1.658a	18.53a	14.83	58.40b	0.053	0.080b	0.263
Fertilization N (kg ha ⁻¹)													
N1	0.099	0.033	0.039	0.088	5.93	0.103a	1.368a	16.00	14.89	60.92	0.055	0.126	0.308
N2	0.064	0.028	0.029	0.099	6.20	0.053b	1.171b	16.94	14.81	60.12	0.045	0.128	0.268
N3	0.119	0.033	0.023	0.081	5.93	0.085a	1.864a	16.38	14.86	60.04	0.041	0.129	0.327
N4	0.168	0.033	0.027	0.095	6.06	0.084a	1.575a	17.45	14.80	59.30	0.056	0.102	0.202
Fertilization S + B (kg ha ⁻¹)													
S1+B1	0.134	0.034	0.028	0.075	6.19	0.080	1.322	16.49	15.29	59.82	0.049	0.128	0.317a
S2+B2	0.109	0.032	0.032	0.085	6.08	0.078	1.589	16.65	14.44	60.34	0.042	0.130	0.325a
S3+B3	0.094	0.030	0.029	0.112	5.82	0.085	1.573	16.95	14.78	60.13	0.058	0.105	0.187b

Table 5

Sum of SFA, MUFA and PUFA in oil flax seeds
(means for the factors 2009-2011)

Specification	SFA	MUFA	PUFA
Years			
2009	8.200	20.10a	71.70c
2010	8.385	17.06b	74.56b
2011	8.121	13.34c	78.54a
Cultivar			
Oliwin	8.334	15.04b	76.62a
Szafr	8.137	18.63a	73.24b
Fertilization N (kg ha ⁻¹)			
N1	8.042	16.16	75.81
N2	8.037	17.04	74.92
N3	8.546	16.56	74.90
N4	8.317	17.58	74.10
Fertilization S + B (kg ha ⁻¹)			
S1B1	8.278	16.62	75.11
S2B2	8.426	17.80	74.78
S3B3	8.003	17.09	74.91

significant differences in fatty acids content between subsequent years of flax cultivation were reported.

The composition of fatty acids is strictly connected, among others, with a genetic factor (TURNER 1991, DRIBNENKI, GREEN 1995, FROMENT et al. 1998, SANKARI 2000, BOROWIEC et al. 2001, ZAJĄC et al. 2001, GAMBUSI et al. 2003, DIEDERICHSEN, RANEY 2006, ZAJĄC et al. 2012). In the experiment by the author, Oliwin cultivar accumulated higher amounts of capric, palmitoleic, behenic acids, as well as more by 6.7% of palmitic acid and by 5.3% of linolenic acid. Similar results were obtained by BOROWIEC et al. (2001) and FROMENT et al. (1998), while ZAJĄC et al. (2001) did not report statistically significant differences between the examined cultivars, regarding the content of fatty acids. The only exception was the content of oleic acid (differences determined between cultivars) and of palmitic acid (differences determined between the years of cultivation). The dark – seed cultivar, in the experiment by ZAJĄC et al. (2001), characterized higher content of oleic acid (25.0%) than the light – seed cultivar (19.0%). The content of oleic acid, in the experiment by the author, was lower than the values obtained by FROMENT et al. (1998) and by ZAJĄC et al. (2001).

Statistically significant differences in summary content of saturated fatty acids in flax oil of the examined cultivars were not determined. Yet those

differences were proved for the content of MUFA and PUFA, which amounted, average, 16.8% and 74.9% respectively. The dark – seeds cultivar, Szafir, featured higher value of mono – unsaturated fatty acids, by 19.3%, while the light – seeds cultivar, Oliwin- proved to contain higher content of indispensable unsaturated fatty acids by 4.4%.

The fertilization of plants with sulphur (KRZYWY et al. 2001), boron (SHORROKS 1997, BLEVINS, LUKASZEWSKI 1998, BOLANOS et al. 2004, HEIDARABADIA et al. 2011) or nitrogen (AUFHAMMER et al. 2000, ZAJĄC, KULIG 2001, ZAJĄC et al. 2001) had a diverse impact on the quality and content of fat, although the analysis of fatty acids composition, resulting from simultaneous application of sulphur, boron and nitrogen fertilization, was not taken into account as an object of research, probably because of high cost of such analyses. In three – year – lasting experiment it was possible to prove that introduction of nitrogen dose 40 kg ha⁻¹ was advantageous only in the case of accumulation of palmitic and stearic acids, while combined application of 15 kg B ha⁻¹ and 70 kg S ha⁻¹ resulted in decreased value of lignoceric acid content. The introduction of fertilization did not significantly affect the content of MUFA, PUFA and saturated fatty acids (SFA).

In the experiment by ZAJĄC et al. (2001), regardless the year of flax cultivation, the dark – seed cultivar characterized higher share of oleic acid C_{18:1}, *n*-9 in the sum of fatty acids (by about 6%), at lower share of linolenic acid C_{18:3}, *n*-3 (by about 10%). The yellow – seed cultivar featured more beneficial relation between the quantity of saturated and unsaturated fatty acids, as well as C_{18:2} (*n*-6) and C_{18:3} (*n*-3) ratio. In the experiment by the author, C_{18:2} (*n*-6) to C_{18:3} (*n*-3) ratio was for Oliwin cultivar – ranged 0.24:1 and 0.32:1 – for Szafir cultivar. The ratio of saturated to unsaturated fatty acids amounted 1:8.97 for the examined cultivars and it was higher for Oliwin cultivar.

CONCLUSIONS

1. High content of stearic acid occurred in the hottest year, 2010, while the wet year, 2009, favored accumulation of palmitic and linoleic acid.

2. Bright – colour seeds cultivar of oil flax in comparison to dark – colour seeds, characterized higher content of C10:0, C16:0, C16:1, C18:3 i C22:0 by 19%, while the content of stearic and oleic acid were of lower values.

3. Nitrogen fertilization, with the dose amounting 40 and 60 kg ha⁻¹, did advantageously effect on the content of palmitic and stearic acid. High doses of sulphur and boron brought about the decrease in lignoceric acid in the fat made from oil flax.

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CHEMICAL COMPOSITION OF PEA (*PISUM SATIVUM* L.) SEEDS DEPENDING ON TILLAGE SYSTEMS

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Abstract

Tillage systems determine conditions for plant growth and development, which undoubtedly influences the crop quality. The present study evaluated the mineral composition and content of phytate phosphorus, protein and starch in pea seeds under the conditions of conventional tillage (shallow ploughing and harrowing after harvest of the previous crop, ploughing in the autumn), reduced tillage (only cultivator after harvest of the previous crop), and herbicide tillage (only Roundup 360 SL after harvest of the previous crop). The study demonstrated a higher content of total ash, phosphorus (P) and potassium (K) in pea seeds from the conventional and reduced tillage systems as well as a higher content of calcium (Ca) in seeds from the reduced tillage compared to the herbicide system. The conventional tillage also increased the content of iron (Fe) compared to the reduced and herbicide systems. In turn, the content of magnesium (Mg) and zinc (Zn) did not depend on the tillage system or the study year, whereas the content of copper (Cu) was significantly higher in the reduced tillage compared to the conventional and herbicide systems. The content of phytate-P in pea seeds was affected only by the study year. The total protein content of pea seeds was similar in all the tillage systems and study years, whereas the starch content was higher in the conventional than in the reduced and herbicide tillage, and was also differentiated by the study year.

Key words: legumes, mineral elements, phytate-phosphorus, soil tillage.

SKŁAD CHEMICZNY NASION GROCHU SIEWNEGO (*PISUM SATIVUM* L.) W ZALEŻNOŚCI OD SYSTEMU UPRAWY ROLI

Abstrakt

Uprawa roli kształtuje warunki wzrostu i rozwoju roślin, co niewątpliwie wpływa na jakość plonu. W badaniach oceniano skład mineralny, zawartość fosforu fitynowego oraz białka i skrobi w nasionach grochu siewnego w warunkach konwencjonalnej uprawy roli, uproszczonej i herbicydowej. Wykazano, że więcej popiołu ogółem, fosforu (P) i potasu (K) zawierały nasiona grochu z uprawy konwencjonalnej i uproszczonej niż herbicydowej, natomiast więcej wapnia (Ca) zawierały nasiona z uprawy uproszczonej niż herbicydowej. Konwencjonalna uprawa roli wpłynęła również na zwiększenie zawartości żelaza (Fe), w stosunku do uprawy uproszczonej i herbicydowej. Z kolei zawartość magnezu (Mg) oraz cynku (Zn) kształtowała się niezależnie od systemu uprawy roli i lat badań, zaś miedzi (Cu) była istotnie większa w uprawie uproszczonej niż konwencjonalnej i herbicydowej. Na zawartość P-fitynowego w nasionach wpływały tylko lata badań. Zawartość białka ogółem w nasionach grochu była podobna we wszystkich systemach uprawy roli i latach badań, zaś zawartość skrobi była większa w uprawie konwencjonalnej niż uproszczonej i herbicydowej. Cechę tę istotnie różnicowały również lata badań.

Słowa kluczowe: strączkowe, skład mineralny, fosfor fitynowy, uprawa roli.

INTRODUCTION

The objective of a tillage system is to assure optimal conditions for the growth and yielding of plants (MORRIS et al. 2010). However, solutions applied in crop cultivation practice are not always optimal because their effectiveness is influenced by the interaction of many natural and economic factors of a farm (GRUBER et al. 2012). Investigations by LOCKE et al. (2002) and WOŹNIAK (2012) demonstrated that the no-till system was increasing crop infestation with weeds, which may contribute to yield reduction (HEMMAT, ESKANDARI 2004), and deterioration of crop quality (WOŹNIAK 2013b). In studies conducted by HEMMAT and ESKANDARI (2004), the yield of chickpea sown in drylands in a no-till system was significantly higher than in the ploughing, reduced and minimal systems. Also GUY and COX (2002) achieved higher yields of pea seeds in the no-till than in the conventional system. In turn, in moderately humid soil, the yield of pea was higher in the conventional system than in the reduced and herbicide ones (WOŹNIAK 2013a). The tillage system and fertilization also influence the chemical composition of crop (WOŹNIAK, MAKARSKI 2012). In the research by KRASKA (2011), the conservative tillage system increased the content of phosphorus and copper in grain of spring wheat, as compared to the ploughing system. In turn, as WOŹNIAK and MAKARSKI (2012) reported, the ploughless tillage system increased the content of ash, zinc and copper in wheat grain, while the ploughing system increased the content of potassium, magnesium and manganese. WOŹNIAK (2013b) demonstrated that tillage systems affected the content of protein and ash in grain of durum wheat. In this respect, poorer quality grain was obtained in herbicide tillage rather than in the conventional and reduced systems.

Legume seeds are rich in mineral elements, but also in phytates (inositol hexaphosphate). These compounds have anti-nutrient properties because they decrease the availability of major minerals, particularly of iron, zinc and calcium (TAVAJJOH et al. 2011). They form complexes with iron and zinc, which in some cases may cause deficiency of these elements in human diet (SANDBERG 2002). They are the main cause of phosphorus accumulation in plant tissues (KUMAR et al. 2010). They are synthesized during seeds maturation and constitute from 60 to 90% of total phosphorus (LOEVUS 2002). On the other hand, phytates display some beneficial effects as they reduce the risk of ischaemic heart disease, atherosclerosis and diabetes development as well as show antioxidative properties (KUMAR et al. 2010).

The aim of the present study was to evaluate the effect of tillage systems on the content of macro- and microelements, phytate phosphorus as well as protein and starch in pea seeds.

MATERIAL AND METHODS

A controlled field experiment was conducted in 2009-2011 at the experimental station in Uhrusk, of the University of Life Sciences in Lublin. The experiment was established on Rendzic Phaeozem soil (IUSS Working Group WRB, 2006) with the particle-size distribution and texture of sandy loam. The content of available forms of phosphorus in the soil reached 214 mg P kg⁻¹, whereas the potassium content was 237 mg K kg⁻¹; the soil had a slightly alkaline pH value (pH_{KCl}=7.2). The content of total N in the soil was 1.03 g kg⁻¹, and that of organic C equalled 7.60 g kg⁻¹. The experiment was carried out in the system of randomized blocks, in 3 replications, on plots with the area of 24 m² each. The test plant was the Bohun cultivar pea, cultivated from seeds in three tillage systems: (1) conventional (CT), (2) reduced (RT), and (3) herbicide (HT).

The conventional tillage included shallow ploughing and harrowing after harvest of the previous crop (spring wheat) and ploughing in the autumn. The reduced tillage included only field cultivation after harvest of the previous crop, and herbicide tillage consisted of a treatment with Roundup 360 SL (a.s. glyphosate, 4 L ha⁻¹) after harvest of the previous crop. In the springtime, a cultivation set composed of a cultivator, a string roller and a harrow was used on all the plots.

In each year, pea seeds were sown in the first decade of April, in quantities of 100 seeds per m² in row spacing of 20 cm. Fertilization before sowing included 20 kg N ha⁻¹, 17.5 kg P ha⁻¹ and 66.5 kg K ha⁻¹. Before sowing, the seeds were dressed with Zaprawa Nasienna T (a.i. carbendazim 20% and thiuram 45%). Karate Zeon 050 CS insecticide (a.i. lambda-cyhalothrin) was applied in a dose of 0.1 L ha⁻¹ for pest control, whereas Afalon Dyspersyjny

450 SC (a.i. linuron) in a dose of 1.5 L ha⁻¹ directly after sowing and Fusilade Forte 150 EC (a.i. fluazyfop-P-butyl) in a dose of 1 L ha⁻¹ after sprouting of monocotyledonous weeds were used for weed control.

The content of mineral components in pea seeds was established after dry mineralization of the samples at a temperature of 600°C. The resultant ash was dissolved in 5 mL of 6 M HCl, then filled up to the volume of 50 mL with redistilled water. Measurements were carried out with the method of Atomic Absorption Spectrometry with acetylene-air flame excitation in a UNICAM 939 apparatus. Phytate-phosphorus was extracted from ground samples with 5% TCA for 60 min. Then, the extract was centrifuged for 10 min at the speed of 3000 rpm. Phytate-P present in the supernatant was determined with the spectrophotometric method ($\lambda=500$ nm) using Wade reagent – 0.3 g FeCl₃ · 6H₂O + 3.0 g sulfosalicylic acid in 1 L (LATTA, SKIN 1980, DRAGIČEVIĆ et al. 2011). The content of nitrogen in pea seeds was determined with the Kjeldahl method and converted into total protein (N x 6.25). The starch content was assayed by shaking the seed samples with a TRIS buffer (pH=9.2) until complete solubilization of protein. The remaining precipitate was hot-dissolved in water. The starch content was determined spectrophotometrically ($\lambda=660$ nm) in the form of a complex with iodine.

The results achieved were developed statistically with the analysis of variance method (Anova) in Statistical PL software, whereas significant differences between mean values were evaluated with the Tukey's HSD test, $P<0.05$.

RESULTS

The evaluation of variance components (F -Value) demonstrated that the ash content of pea seeds was affected by the study year more than by the tillage system (Table 1). Pea seeds harvested from plots cultivated in the conventional system were characterized by a significantly higher content of total ash than seeds from plots with the herbicide tillage (Table 2). Values of these traits were also differentiated by the study year. A significantly higher ash content was determined in seeds from 2010 than in those from 2009 and 2011. Pea seeds originating from the conventional and reduced

Table 1
 F -Value for mineral composition of pea seeds, $P<0.05$

Effects	DF	Ash	P	Phytate-P	K	Mg	Ca	Fe	Zn	Cu
		F -Value								
*TS	2	3.92	10.50	0.96	86.41	1.04	3.76	42.47	2.18	5.21
**Y	2	13.11	4.36	7.10	0.41	2.50	6.29	1.21	0.92	0.54
TS x Y	4	0.61	1.05	0.71	1.11	1.06	0.72	0.23	0.02	0.56

* TS – tillage systems, ** Y – years

Table 2

Content of ash, macroelements and phytate-P in pea seeds

Tillage systems (TS)	Years (Y)			Mean
	2009	2010	2011	
Total ash (%)				
CT	2.90	3.12	2.81	2.94
RT	2.81	3.03	2.80	2.88
HT	2.78	2.90	2.76	2.81
Mean	2.83	3.02	2.79	-
HSD _{0.05} for TS – 0.12 ; Y – 0.12				
P (g kg ⁻¹ d.m.)				
CT	3.41	3.62	3.90	3.64
RT	3.61	3.63	3.66	3.63
HT	3.17	3.14	3.45	3.26
Mean	3.40	3.47	3.67	-
HSD _{0.05} for TS – 0.25; Y – 0.25				
Phytate-P (g kg ⁻¹ d.m.)				
CT	1.73	1.72	1.85	1.77
RT	1.72	1.72	1.79	1.74
HT	1.77	1.70	1.94	1.80
Mean	1.74	1.71	1.86	-
HSD _{0.05} for TS – ns ; Y – 0.11				
K (g kg ⁻¹ d.m.)				
CT	10.26	10.16	10.56	10.33
RT	10.04	10.04	10.37	10.15
HT	8.50	8.43	8.17	8.37
Mean	9.60	9.54	9.70	-
HSD _{0.05} for TS – 0.42 ; Y – ns				
Mg (g kg ⁻¹ d.m.)				
CT	1.02	1.06	0.97	1.02
RT	1.02	0.94	0.93	0.97
HT	1.03	1.01	0.67	0.90
Mean	1.02	1.00	0.86	-
HSD _{0.05} for TS – ns; Y – ns				
Ca (g kg ⁻¹ d.m.)				
CT	0.67	0.64	0.52	0.61
RT	0.71	0.64	0.62	0.66
HT	0.59	0.62	0.48	0.56
Mean	0.66	0.63	0.54	-
HSD _{0.05} for TS – 0.09; Y – 0.09				

Explanations: CT – conventional tillage, RT – reduced tillage, HT – herbicide tillage, HSD_{0.05} – honestly significant difference, ns – not significant, $P < 0.05$

systems contained from 11.3 to 11.7% more phosphorus (P) than seeds from the herbicide tillage. A higher content of phosphorus was noted in the crop of 2011 than of 2009. The evaluation of variance components indicated that the content of this element in pea seeds was influenced by the tillage system more than by the study year. Also, the content of phytate-P was significantly higher in 2011 than in 2009 and 2010, although it was unaffected by the tillage systems. The content of potassium (K) in pea seeds depended only on the tillage system and was 21.3 to 23.4% higher in the conventional and reduced systems compared to the herbicide system. In turn, the content of magnesium (Mg) in seeds was not influenced by the tillage system or by the year. In contrast, the content of calcium (Ca) in pea seeds was observed to depend on both the tillage system and the year. A significantly higher Ca content was determined in seeds harvested from plots with the reduced tillage than in the herbicide system. A higher content of this element was found in pea seeds from 2009 than in those from 2011. The *F*-Value indicated that the calcium content of pea seeds was influenced more by the year than by the tillage system.

The content of iron (Fe) in pea seeds was found to depend only on the tillage system (Table 3). Its higher value (from 20.1 to 26.0%) was determined in seeds harvested from the conventional rather than from the reduced

Table 3

Content of microelements in pea seeds

Tillage systems (TS)	Years (Y)			Mean
	2009	2010	2011	
Fe (mg kg ⁻¹ d.m.)				
CT	78.43	78.37	77.82	78.21
RT	66.18	65.74	63.48	65.13
HT	64.01	63.03	59.17	62.07
Mean	69.54	69.05	66.82	-
HSD _{0.05} for TS – 4.75; Y – ns				
Zn (mg kg ⁻¹ d.m.)				
CT	34.04	33.63	35.78	34.48
RT	38.32	36.96	39.67	38.31
HT	36.13	35.90	38.33	36.79
Mean	36.16	35.50	37.93	-
HSD _{0.05} for TS – ns; Y – ns				
Cu (mg kg ⁻¹ d.m.)				
CT	6.80	6.63	6.88	6.77
RT	8.38	7.72	7.29	7.80
HT	6.87	6.83	6.85	6.85
Mean	7.35	7.06	7.01	-
HSD _{0.05} for TS – 0.90; Y – ns				

Explanations see Table 2

Table 4

F-Value for protein and starch content in pea seeds, $P < 0.05$

Effects	DF	Total protein	Starch
		<i>F</i> -Value	
TS*	2	1.52	15.44
Y	2	0.87	24.39
TS x Y	4	2.56	1.75

* TS – tillage systems, Y – years

Table 5

Content of total protein and starch in pea seeds

Tillage systems (TS)	Years (Y)			Mean
	2009	2010	2011	
Total protein (g kg ⁻¹ d.m.)				
CT	212.8	206.4	199.0	206.1
RT	204.5	218.4	212.5	211.8
HT	209.0	207.6	207.8	208.4
Mean	209.0	210.8	206.5	-
HSD _{0.05} for TS – ns; Y – ns				
Starch (g kg ⁻¹ d.m.)				
CT	431.2	406.3	348.6	395.3
RT	386.0	330.5	249.8	322.1
HT	383.0	327.8	316.9	342.6
Mean	400.0	354.9	316.9	-
HSD _{0.05} for TS – 34.7; Y – 34.7				

Explanations see Table 2

and herbicides tillage. In turn, the content of zinc (Zn) was unaffected by the tillage system or the study year, whereas the content of copper (Cu) was significantly higher in the reduced tillage than in the conventional and herbicide systems.

The evaluation of variance components (*F*-Value) demonstrated that the starch content of pea seeds was influenced more demonstrably by the study year than by the tillage system (Table 4). The content of protein in pea seeds was similar in all the tillage systems and study years (Table 5). In turn, the starch content was significantly influenced by both the tillage systems and the years. In the conventional tillage, it was higher than in reduced tillage (by 22.7%) and herbicide system (by 15.4%). Its higher value was also noted in the crop from 2009 than in crops from 2010 (by 12.7%) and 2011 (by 26.2%).

DISCUSSION

As demonstrated in previous investigations (KRASKA 2011, WOŹNIAK, MAKARSKI 2012, WOŹNIAK 2013b), a tillage system may influence the quality and chemical composition of the crop. In our study, the pea seeds harvested from plots with the conventional tillage contained more ash than those from the herbicide system. This may have resulted from less favorable conditions of pea growth in the herbicide system than in the conventional tillage, which in consequence led to a worse supply of plants with nutrients. Some earlier research by WOŹNIAK (2013a) showed that pea seeds harvested from plots with a herbicide system were smaller and their weight was significantly lower compared to seeds from the conventional tillage. It was probably due to the competition of pea plants with weeds, because the herbicide tillage was accompanied by a significantly more severe weed infestation compared to the conventional system (WOŹNIAK 2012). In a study by WANG et al. (2010), the content of ash in pea seeds depended on a study year, plot location and cultivar. In the cited work, the ash content ranged from 2.57 to 2.79%, being slightly lower than in our experiment (2.76 to 3.12%). In the conventional and reduced tillage, the pea seeds were characterized by a higher content of the macroelements: phosphorus (P), potassium (K) and calcium (Ca), and of microelements: iron (Fe) – compared to the herbicide tillage, and copper (Cu) – in the reduced tillage versus the conventional and herbicide systems. According to AMARAKOON et al. (2012), the content of microelements in different genotypes of pea cultivated in North Dakota, USA, was variable and ranged from 46 to 54 mg kg⁻¹ for iron (Fe), from 39 to 63 mg kg⁻¹ for zinc (Zn), and 1350 mg kg⁻¹ for magnesium (Mg). In our experiment and in the study by SANDBERG (2002), the content of Zn, Mg and Ca in pea seeds was the same, whereas the content of Fe was higher. According to AMARAKOON et al. (2012), pea is a good source of Fe, Zn and Mg, but a poor source of Ca. In the research by KRASKA (2011), the conservative tillage was observed to increase the content of P and Cu in wheat grain compared to the ploughing system. The latter was increasing the content of K, Mg, Mn and S in the grain, compared to the conservative tillage variants. WOŹNIAK and MAKARSKI (2012) reported that the ploughless tillage increased the content of Zn and Cu in wheat grain, compared to the ploughing tillage. It could be due to the fact that the absorbability of microelements in soils well permeable to air is lower than in soils less permeable to air. In turn, under the ploughing system, the wheat grain was characterized by a higher content of K and Mg. Higher levels of the elements in grain may be explained by their higher availability to plants, because they are more easily translocated into deeper layers of soil (particularly in well-aerated soil) in the ploughing system. This observation was also confirmed in our study on pea, where the content of phosphorus (P) in pea seeds was higher in the conventional and reduced tillage than in the herbicide tillage, whilst the content of phytate phosphorus was at a similar

level in all the tillage systems. According to LOEWUS (2002), over 60% of phosphorus in pea seeds may occur in the form of phytate-P. In our experiment, phytate-P constituted from 47 to 56% of the total phosphorus accumulated in seeds. As reported by SANDBERG (2002), the content of phytate-P in pea seeds ranges from 0.6 to 3.30 g kg⁻¹. In our study, the content of phytate-P also fell within this range and spanned from 1.74 to 1.80 g kg⁻¹ depending on a tillage system.

The research conducted by WANG et al. (2010) demonstrated significant differences in the content of protein, starch, crude fiber, fat, ash, and phytates in pea seeds depending on a cultivation system and study site. In our experiment, the content of total protein in pea seeds was at a similar level in all tillage systems, whereas the content of starch was higher in seeds harvested from plots with the conventional tillage than in those from the reduced and herbicide systems.

In conclusion, a higher content of total ash, phosphorus (P) and potassium (K) was determined in pea seeds originating from the conventional and reduced systems than from the herbicide tillage, whereas a higher content of calcium (Ca) was assayed in seeds from the reduced than from the herbicide tillage. A higher content of iron (Fe) was found in pea seeds harvested from plots with the conventional tillage than from the reduced and herbicide systems, whereas a higher content of copper (Cu) was noted in seeds from the reduced than from the conventional and herbicide systems. The content of phytate phosphorus was differentiated only by the study years, whereas the content of magnesium (Mg) and zinc (Zn) was not affected by the tillage systems or the study years. The content of total protein in pea seeds was similar in all the tillage systems and all study years, whereas the starch content was higher in seeds from the conventional tillage than from the reduced and herbicide systems.

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EFFECT OF ORGANIC AND NITROGEN FERTILIZATION ON SELECTED COMPONENTS IN POTATO TUBERS GROWN IN A SIMPLIFIED CROP ROTATION

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Abstract

Fertilization is one of the factors of agro-technical practice that determines potato yield volume and quality. This research involved the early table potato cultivar Bila grown in the first year of the tenth (2006) and the eleventh rotation cycle (2009) in a long-term, static field experiment (set up in 1979). A three-year simplified crop rotation included potato – winter rye 1 – winter rye 2. The experimental factors covered: I organic fertilization (without FYM and with FYM at the amount of 30 t ha⁻¹), II nitrogen doses 0, 60, 120, 180 kg N ha⁻¹ under potato and 0, 40, 80, 120 kg N ha⁻¹ under rye. The aim of the present research was to evaluate the yielding and content of selected chemical components of the tubers of cv. Bila potato grown in a simplified system depending on different organic and mineral fertilization regimes, implemented for many years. The results show a significantly positive effect of FYM and nitrogen fertilization on the tuber yield volume and on the content of total protein and magnesium, as well as a negative effect on the content of nitrates(V). However, FYM fertilization increased but nitrogen fertilization decreased the content of ascorbic acid. The highest nutritive value of tubers was achieved after the application of 60 kg N ha⁻¹. However, a dose higher than 120 kg N ha⁻¹ applied with FYM increased the content of nitrates(V) above the toxicity threshold (200 mg NO₃⁻ kg⁻¹ of the fresh product).

Key words: potato, protein, magnesium, vitamin C, nitrates(V).

WPLYW NAWOŻENIA ORGANICZNEGO I AZOTU NA WYBRANE SKŁADNIKI BULW ZIEMNIAKA UPRAWIANEGO W UPROSZCZONYM ZMIANOWANIU

Abstrakt

Nawożenie jest jednym z czynników agrotechniki decydujących o wielkości i jakości plonu ziemniaka. Badania nad wczesną, konsumpcyjną odmianą ziemniaka Bila, pochodzącą z pierwszego roku 10. (2006 r.) i 11. rotacji (2009 r.) wieloletniego polowego doświadczenia statycznego (od 1979 r.), prowadzono w 3-letnim uproszczonym zmianowaniu: ziemniak – żyto ozime 1 – żyto ozime 2. Czynnikiem doświadczenia były: I – nawożenie organiczne (bez obornika ozime i z obornikiem w ilości 30 t ha⁻¹), II – dawki azotu 0, 60, 120, 180 kg N ha⁻¹ pod uprawę ziemniaka i 0, 40, 80, 120 kg N ha⁻¹ pod uprawę żyta.

Celem badań była ocena plonowania oraz zawartości wybranych składników chemicznych w bulwach ziemniaka odmiany Bila uprawianego w systemie uproszczonym, w zależności od wieloletniego zróżnicowanego nawożenia organicznego i mineralnego.

Wykazano istotnie pozytywne działanie obornika i nawożenia azotem na wielkość plonu bulw oraz zawartość białka ogólnego i magnezu, a negatywne na zawartość azotanów(V). Natomiast zawartość kwasu askorbinowego istotnie wzrastała pod wpływem nawożenia obornikiem, a malała pod wpływem azotu.

Najwyższą wartość odżywczą miały bulwy nawożone dawką 60 kg N ha⁻¹, natomiast przekroczenie dawki 120 kg N ha⁻¹ na tle obornika powodowało wzrost zawartości azotanów(V) powyżej progu toksyczności (200 mg NO₃⁻ kg⁻¹ świeżego produktu).

Słowa kluczowe: ziemniak, białko, magnez, witamina C, azotany(V).

INTRODUCTION

Potatoes grown for the food processing industry most often come from traditional plantations, where intensive mineral fertilization and intensive plant protection technologies are employed (RYTEL 2010). In Poland, potatoes have many uses (ROGOZIŃSKA et al. 2005, JABŁOŃSKI 2006, POBEREŻNY, WSZELACZYŃSKA 2011). Fertilization, especially with mineral nitrogen, is one of the agro-technical factors determining potato yield volume (BLECHARCZYK, MAŁECKA 2000, CIEĆKO et al. 2000, ROGOZIŃSKA et al. 2005, JABŁOŃSKI 2006, 2009). Unsurprisingly, high nitrogen doses have an unfavourable effect on the tuber quality (PESHIN, SINGH 1999, LESZCZYŃSKI 2000, BELANGER et al. 2002, PROŚBA-BIAŁCZYK 2004, ROGOZIŃSKA et al. 2005, JABŁOŃSKI 2006). Organic fertilisers can alleviate the effects of unbalanced mineral fertilization, especially in light soil. With the above in mind, this research was undertaken in order to define whether and to what extent varied nitrogen fertilization applied for many years with or without FYM determined the yield volume, the content of total protein and magnesium, vitamin C and nitrates(V) in the tubers of the table potato cultivar Bila grown in a simplified crop rotation.

The aim was to evaluate the yielding and selected chemical components of the tubers of cv. Bila potato grown in a simplified system, depending on different, long-term organic and mineral fertilization regimes.

MATERIAL AND METHODS

The research involved an early cultivar of table potato called Bila cultivar, grown in the first year of the tenth (2006) and the eleventh rotation (2009) of a long-term, static field experiment. The experiment was set up in 1979 at the Agricultural Experimental Station at Wierzychucinek (the Kujawy and Pomorze Province, today Experimental Station of the Faculty of Agriculture and Biotechnology, the University of Technology and Life Sciences in Bydgoszcz) on Luvisol representing the good wheat complex. The experiment was carried out in a three-year simplified crop rotation cycle: potato – winter rye (1) – winter rye (2). Once winter rye (2) was harvested, straw was left to be ploughed in. The experiment followed the design:

- | | |
|-------------------|--------------------------------|
| 1. PKN_0 | 5. $\text{PKN}_0 + \text{FYM}$ |
| 2. PKN_1 | 6. $\text{PKN}_1 + \text{FYM}$ |
| 3. PKN_2 | 7. $\text{PKN}_2 + \text{FYM}$ |
| 4. PKN_3 | 8. $\text{PKN}_3 + \text{FYM}$ |

Plant	Fertilization levels (kg ha^{-1})					
	P	K	N_0	N_1	N_2	N_3
Potato	35	100	0	60	120	180
Winter rye (1)	35	66	0	40	80	120
Winter rye (2)	35	83	0	40	80	120

The fertilisers were applied as triple superphosphate (46%), potassium salt (50%) as well as ammonium nitrate (34%). The nitrogen doses were applied without and with FYM in the amount of 30 t ha^{-1} . The potato experiment was set up in a split-plot design with four replications, where factor I comprised FYM fertilization, and factor II included nitrogen doses. Potato tubers were planted in the third decade of April.

The total tuber yield was determined and the potato samples were analysed for the following:

- total nitrogen (with the Kiejdahl method). Tuber samples (dry weight) for N_{tot} assessment were mineralized in a mixture of H_2SO_4 and H_2O_2 in a Digest Automat K-438, auto-Sampler K371, and then analyzed in an Autokjedahl Unit K-370 apparatus (GOZDECKA, GĘSIŃSKI 2009);
- magnesium (with atomic absorption spectrophotometry AAS in the dry weight), the same mineralization procedure as for the N_{tot} determination was followed. The magnesium content was measured with a VARIAN AA240FS Fast Sequential Atomic Absorption Spectrometer system USA (OSTROWSKA et al. 1991);
- vitamin C levels were measured spectrophotometrically (absorbance was measured at 520 nm with a Shimadzu UV-1800, UV Spectrophoto-

meter system, Japan) by a method in which 2,6-dichlorophenolindophenol dye is reduced by ascorbic acid. The details are described elsewhere (EGOAVILLE et al. 1988);

- nitrates(V) were determined with the use of an ion-selective Elmetron make (KUNSCH et al. 1981) using a multi-purpose computer device: CX-721.

The results of the 3-year research were statistically verified, applying analysis of variance for two-factor experiments. The data were analysed using Sigma Stat software (SPSS, Chicago, USA). The main effects were tested by Anova, and pairwise multiple comparisons were made using the Tukey's Test at the 0.05 level. Coefficients of correlation (r) and regression (y) were calculated to present the relationships between the traits.

The soil analyses made prior to and after 28 years of the experiment show that the fertilization applied and the absence of liming changed the classification of the soil reaction from slightly acid (pH 5.6) to acid (pH 4.0) in the case of the treatments with FYM and very acid (pH 3.7) in respect of the treatments without FYM. These results have been presented in an earlier report (JANOWIAK et al. 2010).

RESULTS AND DISCUSSION

The tuber yield of the early potato cultivar Bila was on average 30.2 t ha^{-1} , ranging from 24.6 t ha^{-1} to 37.4 t ha^{-1} (Table 1). The lowest yield was recorded for the control, without fertilization, and the highest one – for the treatment with FYM and NPK with nitrogen in the dose of 120 kg N ha^{-1} . The above data are concordant with the results by BLECHARCZYK et al. (2008), who completed long-term research on potato grown in a simplified crop rotation system and in monoculture; in both systems, the highest tuber yield was reported after FYM fertilization combined with NPK (29.4 t ha^{-1}). In several reports (BLECHARCZYK, MAŁECKA 2000, MERCIK, STĘPIEŃ 2006), combined orga-

Table 1

Total yield of potato tubers (t ha^{-1})

Natural fertilization	Nitrogen dose (kg ha^{-1})				Mean
	0	60	120	180	
Without manure	24.6	27.6	26.2	25.5	25.9
Manure	33.0	34.1	37.4	34.0	34.6
Mean	28.8	30.8	31.8	29.8	30.2

LSD $\alpha=0.05$:

- natural fertilization (I) – 2.39
- nitrogen fertilization (II) – 2.43
- interaction (II/I) – n.s.

nic and mineral (NPK) fertilization increased the total tuber yield by about 10%, as compared with the yield recorded for the mineral fertilization only. The literature shows that FYM fertilization enhances the physicochemical soil properties and increases the level of plant yielding (BLECHARCZYK, MAŁECKA 2000, JANKOWSKA-HUFLEJT 2006, MERCIK, STĘPIEŃ 2006, BLECHARCZYK et al. 2008). The statistical analysis of the current results demonstrated that FYM applied during potato growth significantly determined the tuber yield. After the FYM application, a 33.6% tuber yield increase was noted relative to the treatment without FYM. Contrary results were reported by BLECHARCZYK et al. 2008, where the FYM application, irrespective of the farming system (simplified crop rotation, monoculture), decreased the tuber yield by 14.6%. Significantly smaller yield was found for potatoes cultivated in the organic system (on manure) in comparison to the conventional one. The three-year average decrease was 35.9% (HAMOUZ et al. 2005), which corroborates the 30% decrease reported by PROŚBA-BIAŁCZYK (2004) .

According to CIEĆKO et al. (2000), JABŁOŃSKI (2006, 2009), nitrogen fertilization is the factor that best enhances the yield volume. In the present research, mineral fertilization with nitrogen, with or without FYM, significantly increased the tuber yield up to the dose of 120 kg N ha⁻¹. The application of the highest nitrogen dose (180 kg N ha⁻¹), on the other hand, decreased the above trait (Table 1). BLECHARCZYK et al. (2008), applying only mineral fertilization (NPK) with nitrogen at the level of 90 kg N ha⁻¹, recorded a 22.8% decrease in the yield relative to the comprehensive fertilization treatment (FYM+NPK). JABŁOŃSKI (2004), investigating varied fertilization, recorded the highest potato tuber yields for 150 kg N ha⁻¹. In another report, the same author (JABŁOŃSKI 2006) showed that the higher the level of nitrogen fertilization, the higher the potato yield, up to the dose of 135 kg N ha⁻¹. In the present research, the application of the highest nitrogen dose carried out for many years, with and without FYM, considerably decreased the soil pH value from pH=5.6 to pH=4.0 and pH=3.7, respectively, which inhibited the adequate plant growth and development. Such a result was also reported in the 29th potato rotation (JANOWIAK et al. 2010). JANKOWSKA-HUFLEJT (2006), testing the effect of long-term potato fertilization with FYM combined with mineral fertilization in various combinations, thus verifying a clearly acidifying effect of nitrogen. A decrease in soil pH was recorded especially for the treatments with mineral fertilization containing nitrogen. The present research and the reports by the above authors demonstrate that FYM slightly alleviated those effects and, according to JABŁOŃSKI (2004), setting an optimal nitrogen dose should account for both the soil richness in nutrients and the soil reaction.

Protein is considered one of the basic nutrients of potato tuber and it shows a high biological value corresponding to animal protein. In the fresh weight of tubers, protein constitutes 1.7-2.3%, of which 35-68% is the true protein, also referred to as 'pure' protein (MITRUS et al. 2003, PEKSA 2003, ZARZECKA, GUGAŁA 2006, RYTEL 2010). In the present research, the content

of total protein in potato tubers, on average, ranged from 85 to 117 g kg⁻¹ in the dry weight (the experiment mean of 101 g kg⁻¹), being significantly determined by nitrogen fertilization as well as natural fertilization (Table 2). The significantly highest protein content (33.0% higher than the control) was determined in potato tubers from the treatment where nitrogen was applied in the dose of 180 kg N ha⁻¹ with FYM. MITRUS *et al.* (2003) showed that the protein content in the tubers of potato cultivars (Irga and Ekra) increased

Table 2

Total protein content in potato tubers (g kg⁻¹ d.m.)

Natural fertilization	Nitrogen dose (kg ha ⁻¹)				Mean
	0	60	120	180	
Without manure	85	103	101	108	99
Manure	88	95	111	117	103
Mean	87	99	106	112	101

LSD $\alpha=0.05$:

natural fertilization (I) – 3.1
 nitrogen fertilization (II) – 4.3
 interaction (II/I) – n.s.

significantly under the influence of nitrogen fertilization. The increase in the total protein content after the application of the higher tested fertilizer dose (90 kg N ha) was 10.76% for cv. Irga cultivar and 11.08% for cv. Ekra, albeit to the detriment of the biological value.

ZARZECKA, GUGAŁA (2006) and GUGAŁA *et al.* (2008), who investigated the effect of traditional and simplified farming systems on the content of total protein in cv. Wiking, recorded a higher protein content in the traditional farming system: 99.5 g kg⁻¹ of dry weight versus 97.9 g kg⁻¹ in the simplified system. On the other hand, KLIKOCA (2002), who performed an experiment on the traditional and simplified systems, recorded a growing tendency for the content of that nutrient in both systems, although she did not prove it to be significant. KRASKA (2002), DZIENIA *et al.* (2004), LACHMAN *et al.* 2005 as well as GUGAŁA *et al.* (2008), however, showed that the cultivation method did not have a significant effect on changes in the content of total protein in potato tubers.

Potato tubers contain more than 1% of mineral compounds (LESZCZYŃSKI 2000, RYTEL 2010). In the present research, the magnesium content ranged from 0.77 g kg⁻¹ to 0.91 g kg⁻¹, with an average of 0.84 g kg⁻¹ in the dry weight (Table 3). The applied nitrogen fertilization significantly affected the magnesium content in potato tubers. The highest mean Mg content was recorded in the tubers fertilised with the dose of 60 kg N ha⁻¹. Higher nitrogen doses gradually decreased the content of that nutrient in tubers. Similar tendencies were also observed for the treatments with FYM. The results were submitted to the linear regression analysis. The relationships for which the coefficient

Table 3

Magnesium content in potato tubers (g kg^{-1} d.m.)

Natural fertilization	Nitrogen dose (kg ha^{-1})				Mean
	0	60	120	180	
Without manure	0.85	0.91	0.80	0.77	0.83
Manure	0.85	0.89	0.83	0.82	0.85
Mean	0.85	0.90	0.81	0.79	0.84

LSD $\alpha=0.05$:

natural fertilization (I) – 0.019

nitrogen fertilization (II) – 0.036

interaction (II/I) – n.s.

of determination was higher than 35 are given in Figures 1-3. There was a significant negative coefficient of correlation between the nitrogen dose and the magnesium content in tubers ($r=-0.60$) – Figure 1. According to CIEĆKO et al. (2000), different levels of mineral fertilization (NPK) did not significantly modify the Mg content in potato tubers, although its highest concentration was reported, similarly to the present research, after the nitrogen dose of 60 kg N ha^{-1} . RUDZIŃSKA-MĘKAL, MIKOS-BIELAK (2001) claim the opposite. According to these authors, the magnesium content in potato tubers mostly depends on the duration of the plant growing season, hence the earliness of a cultivar plays a role. The cited authors, who investigated 5 potato cultivars submitted to FYM application and fertilised with the nitrogen dose of 100 kg

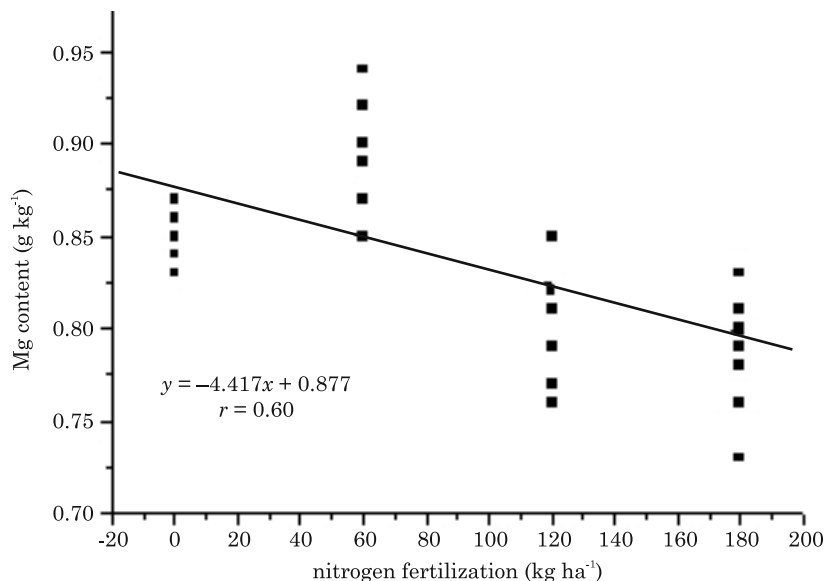


Fig. 1 Relationship between nitrogen fertilization and magnesium content in potato tubers

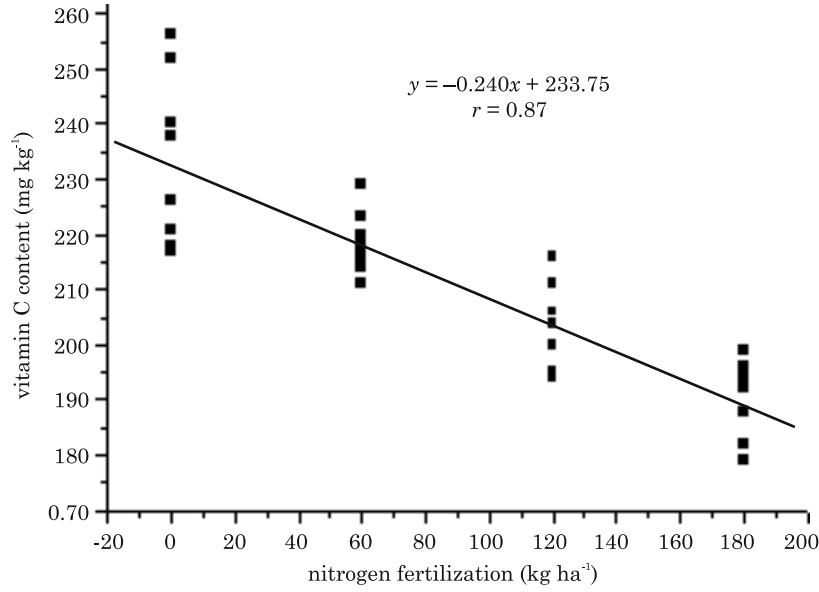


Fig. 2 Relationship between nitrogen fertilization and vitamin C in potato tubers

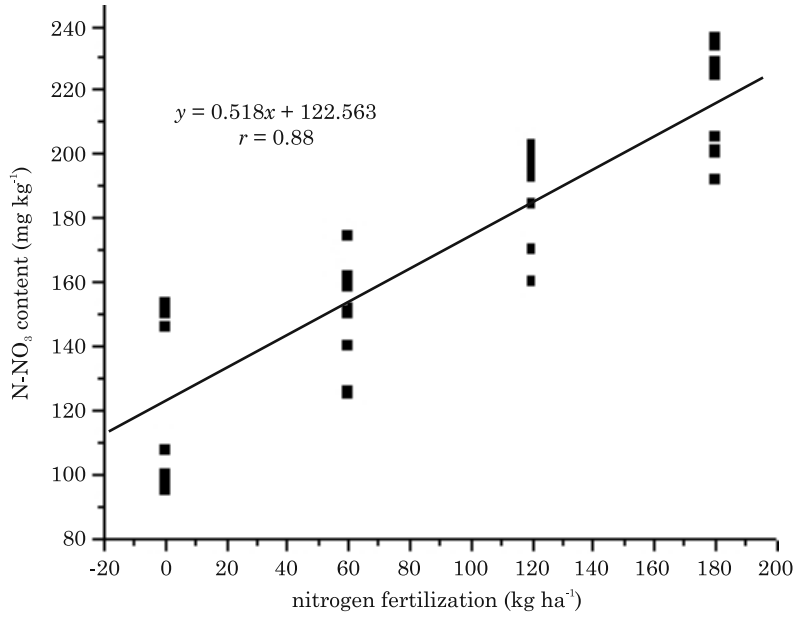


Fig. 3 Relationship between nitrogen fertilization and nitrates(V) content in potato tubers

N ha⁻¹, found the lowest content of magnesium nutrient in the early cultivars Drop and Perkoz (0.09 to 0.10%), a higher Mg content in the medium late cultivars Irga and Grot (0.11%), and the highest one in the late cultivar Elba (0.12%).

The human magnesium requirements are mostly satisfied through consumption of plant products (LESZCZYŃSKI 2000, WSZELACZYŃSKA 2001, WSZELACZYŃSKA, POBEREŻNY 2011). With its high share in the diet of an average Pole, potato is perceived as an essential Mg source (RUDZIŃSKA-MEKAL, MIKOS-BIELAK 2001, WSZELACZYŃSKA 2001, RYTEL 2010). Drawing on the results, it was calculated that the consumption of 200 g of potato with the highest magnesium content, that is after the application of 60 kg N ha⁻¹, introduces 38 mg of that nutrient into the body (180 mg day⁻¹ of magnesium in the dry weight – Table 3), which covers 13% of the adult's daily requirement, the finding which coincides with the earlier results (LESZCZYŃSKI 2000, WSZELACZYŃSKA 2001).

Potato is also an essential source of vitamin C. Its content is about 200 mg kg⁻¹ of fresh tuber weight, although it varies in a wide range (from 30-300 mg) depending on the cultivar and the year of cultivation (HAMOUZ et al. 1999, KRASKA 2002, BROWN 2005, ZARZYŃSKA, GOLISZEWSKI 2005, ZARZECKA et al. 2007, HAMOUZ et al. 2009), which was demonstrated by the present results, since the content of ascorbic acid in the fresh weight of potato tubers ranged from 1867 to 247 mg kg⁻¹ (Table 4). ZARZECKA et al. (2007) recorded an average content of ascorbic acid in cv. Wiking potato tubers to be 221.0 mg kg⁻¹ of the fresh weight. In that research, a simplified potato growing method did not differentiate the vitamin C content significantly, which coincides with the results reported by HAMOUZ et al. (1999), KRASKA (2002), ZARZYŃSKA, GOLISZEWSKI (2005), HAMOUZ et al. (2009), who observed that the growing method did not change the content of ascorbic acid. Different results were recorded by SAWICKA, KUŚ (2002), who found that the growing methods (integrated, organic) differentiated the vitamin C content. In the present research, the increasing nitrogen doses steadily decreased the vitamin C content by 6.4%, 12.2% and 17.5% (Table 4) as compared to the treatment

Table 4

Ascorbic acid content in potato tubers (g kg⁻¹ f. m.)

Natural fertilization	Nitrogen dose (kg ha ⁻¹)				Mean
	0	60	120	180	
Without manure	221	216	201	187	206
Manure	247	221	209	199	219
Mean	234	219	205	193	212

LSD $\alpha=0.05$:

natural fertilization (I) – 6.1
 nitrogen fertilization (II) – 13.1
 interaction (II/I) – n.s.

without fertilization. This tendency coincides with the calculated negative coefficient of correlation ($r=-0.87$) between the nitrogen dose and the vitamin C content (Figure 2). HAMOUZ et al (2009) proved the negative effect of an increased level of nitrogen fertilization equal 180 kg N ha on the AA content. According to these authors, the AA content decreased by an average 12.4% in the experimental years in comparison with the control (100 kg N ha, 44 kg P ha, 108 kg K ha, 30 kg Mg ha). However, LIN et al. (2004) discovered that nitrogen fertilization had very small impact on the AA content; only high doses of nitrogen that lead to the yield depression significantly reduced the AA content. Reversely, the effect of organic fertilization on the content of ascorbic acid is less unambiguous. In the present research, organic fertilization significantly increased the vitamin C content – by more than 6%. WSZELACZYŃSKA et al. (2007), investigating the effect of organic fertilization on the vitamin C content in cv. Bila potato tubers, recorded its slight and insignificant increase. Among the cultivars they investigated, the highest vitamin C content was recorded in the early cultivars Bila and Bard. ZARZECKA (2006), having reviewed the results of research carried out by many authors for over 10 years, with the focus on the effect of organic fertilization on the tuber quality, showed that there was no obvious effect of organic fertilization on the content of vitamin C in potato tubers. Those results confirm the hypothesis that FYM alleviates the effects of the long-term application of high mineral nitrogen doses, since the coefficient of correlation ($r=-0.87$) points to a significantly negative relationship between nitrogen fertilization and the content of vitamin C (Figure 2).

For the consumer's health, it is very important that potato tubers contain the lowest content of harmful compounds, including nitrates(V). In the investigated potato flesh, the content of nitrates(V) ranged from 100 to 231 mg kg⁻¹ of fresh weight (Table 5). The lowest content of nitrates(V) was recorded in potato tubers from the control (with neither FYM nor nitrogen).

The fertilization applied in the present research significantly determined the content of nitrates(V) in tubers. The FYM fertilization as well as higher

Table 5

Nitrates(V) content in potato tubers (mg kg⁻¹ f. m.)

Natural fertilization	Nitrogen dose (kg ha ⁻¹)				Mean
	0	60	120	180	
Without manure	100	135	177	200	153
Manure	150	161	199	231	185
Mean	125	148	188	215	169

LSD $\alpha=0.05$:

natural fertilization (I) – 13.1

nitrogen fertilization (II) – 14.4

interaction (II/I) – n.s.

nitrogen doses significantly increased the content of nitrates(V) in potato: by 21.3, 18.6, 50.3 and 72.2%, respectively, as compared with the control. The relationship between nitrogen fertilization and the content of nitrates(V) is supported by a significant positive value of the coefficient of correlation ($r=0.88$) – Figure 3. According to AMR, HADIDI (2001), DZIENIA et al. (2004), HAMOUZ et al. (2005), LACHMAN et al. (2005), ZARZECKA, GUGAŁA (2006) as well as IERNA (2009), the strongest effect on the content of nitrates(V) is produced by nitrogen fertilization, the cultivar and weather conditions, whereas the cultivation method does not change the content of that nutrient in tubers. The Ministry of Health, in the Regulation of January 13, 2003 (*Regulation ...*), defined the maximum level of pollution with nitrates(V) in potato tubers at $200 \text{ mg NO}_3^- \text{ kg}^{-1}$ of the fresh product (*Regulation ...* 2003). Considering the range of toxicity, and thus the toxic effect of nitrates(V), in the context of the fertilization of table potato, the nitrogen dose of 120 kg N ha^{-1} should not be exceeded.

CONCLUSIONS

1. The fertilization with FYM and nitrogen of potatoes grown in a simplified crop rotation significantly affected the tuber yield volume. The highest total tuber yield was recorded after the use of nitrogen at the dose of 120 kg N ha^{-1} with FYM applied.

2. After the FYM treatment, the content of magnesium and vitamin C increased significantly, although after the application of increasing nitrogen rates (120 and 180 kg N ha^{-1}) there was a decrease in the content of those nutrients, as compared with their content determined after the nitrogen dose of 60 kg N ha^{-1} .

3. The content of total protein and nitrates(V) in potato tubers increased due to FYM within the whole range of the applied nitrogen doses. Interestingly, exceeding the dose of 120 kg N ha^{-1} applied with FYM increased the content of nitrates(V) above the threshold of toxicity.

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REVIEW PAPERS

LIHIUM AND THE APPLICATION OF ITS COMPOUNDS IN DIFFERENT FIELDS OF MEDICINE

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Abstract

Although lithium has not been classified as an essential element for humans, it can influence numerous metabolic processes and exert diverse effects in the human body, both positive and negative ones. Its actions enable the use of lithium compounds in therapy of different illnesses. It is mostly used for cure of psychiatric disorders: as a mood stabilizer and for intensifying the action of antidepressants. However, its compounds have been also tried in other fields of medicine: as an adjuvant in patients with thyroid diseases undergoing radioiodine therapy, in dermatology, in cure of neurodegenerative and ophthalmic illnesses as well as in tumour therapy. Lithium displays beneficial action only within a determined range of its serum concentration and an overrun of the safe threshold can cause side effects. Due to this fact, the Li serum level as well as many other factors, e.g.: body weight, creatinine, renal and thyroid functions should be monitored during the whole therapy. Many studies were undertaken to clarify the mechanism by which lithium affects organisms but the results still remain unsatisfactory. Nevertheless, some interesting aspects of lithium action have been revealed, including its effect on the enzymatic activity, neurodegenerative processes, apoptosis, formation of cytokines as well as neurotransmission and oxidant balance.

Key words: lithium, psychiatric disorders, neurodegenerative and ophthalmic illnesses, thyroid diseases, dermatology, side effects and precautions in lithium therapy, mechanism of lithium action.

LIT I ZASTOSOWANIE ZWIĄZKÓW TEGO PIERWIASTKA W RÓŻNYCH DZIEDZINACH MEDYCYNY

Abstrakt

Obecność litu w organizmie może wywierać różnorodny wpływ zarówno korzystny, jak i negatywny na liczne procesy metaboliczne, jednakże nie został on jeszcze zaliczony do pierwiastków niezbędnych dla człowieka. Działanie litu umożliwiło zastosowanie jego związków w terapii różnych chorób. Jest on głównie stosowany w przypadkach zaburzeń psychicznych jako stabilizator nastroju oraz środek potencjalizujący działanie leków przeciwdepresyjnych. Przeprowadzono również próby zastosowania jego związków w innych dziedzinach medycyny: do wspomagania terapii radiojodem stosowanej w chorobach tarczycy, w dermatologii, w leczeniu schorzeń neurodegeneracyjnych i chorób oczu oraz w leczeniu nowotworów. Lit wywiera działanie terapeutyczne jedynie wtedy, gdy jego stężenie w surowicy mieści się w określonym zakresie, a przekroczenie dopuszczalnej wartości może spowodować działania uboczne. Z tego powodu stężenie litu w surowicy, podobnie jak wiele innych czynników, takich jak: masa ciała, poziom kreatyniny oraz funkcje tarczycy i nerek, musi być monitorowane podczas całej terapii. W celu wyjaśnienia mechanizmu oddziaływania litu na organizm przeprowadzono wiele badań, ale rezultaty są wciąż niezadowalające, niemniej jednak odkryto liczne, interesujące aspekty działania litu, m.in. wpływ na aktywność enzymatyczną, procesy neurodegeneracyjne i neurotransmisyjne, apoptozę, produkcję cytokin oraz na procesy pro- i antyoksydacyjne.

Słowa kluczowe: lit, schorzenia psychiatryczne, choroby neurodegeneracyjne, choroby oczu, schorzenia tarczycy, dermatologia, efekty uboczne i środki ostrożności w terapii litem, mechanizm działania litu.

Lithium belongs to the first group in the periodic table of elements. Chemists are not very much concerned with lithium compounds as it is not widespread in the Earth's crust and its properties can be predicted from its place in the periodic table (ARAL, VECCHIO-SADUS 2008). In contrast, Li compounds are widely applied in medicine and the physiological aspects of the effect of lithium on organisms have been studied for the last sixty years. Research has revealed that lithium is necessary for the correct functions of animal organisms (ZARSE et al. 2011). As for the humans, it has not been classified as an essential element (ARAL, VECCHIO-SADUS 2008). However, a cohort study performed by Japanese scientists showed an inverse correlation between the Li concentration in drinking water and the mortality in 18 Japanese municipalities (ZARSE et al. 2011).

As lithium salts occur in mineral waters (ARAL, VECCHIO-SADUS 2008), this element had been applied in medicine long before it was discovered. In ancient times, people suffering from nervous disorders were recommended to drink mineral waters (GORGOTAS, GERSHON 1981). In the 1940s, the beneficial effect of lithium in manic states was found and since then lithium in the form of its carbonate (Li_2CO_3) has been used for therapy of affective disorders (PIETRUCZUK, WITKOWSKI 2008). This drug is regarded as the first choice one in therapy of affective disorders (ZHONG, LEE 2007, WOŹNIAK 2008, RYBAKOWSKI 2010) and one third of patients subjected to Li therapy display total remission of recurrences without any adjuvant (RYBAKOWSKI, SUWALSKA

2010). Among mood-stabilizers, lithium is best documented considering prevention of suicidal behaviours (KLIWICKI, RYBAKOWSKI 2009). Furthermore, lithium can be used as an adjuvant to augment the pharmacological effect of antidepressants drugs (HANSON et al. 2011). Interestingly, bipolar disorders belong to hereditary illnesses (MÖLLER 2003) and a clinical course of disease also seems to be inherited (DUFFY et al. 2002). Considering these facts, the usefulness of genetic research for predicting the effectiveness of therapy and predisposition to psychiatric disorders has been studied (SZCZEPANKIEWICZ et al. 2009, REMLINGER-MOLENDA, RYBAKOWSKI 2010).

Lithium is absorbed from the gastrointestinal tract and excreted mainly in urine (COLLINS et al. 2010). After oral administration, it reaches the maximum serum concentration in about 30 minutes and the plateau occurs after 14 - 24 hours (ARAL, VECCHIO-SADUS 2008). As kidneys are practically the only way of lithium excretion, this element cannot be used in patients with renal insufficiency (STRZELECKI 2006). Markers of renal disturbances such as proteinuria, increased N-acetyl- β -glucoaminidase activity in urine and enhanced Cu excretion, were suggested to be useful indicators of nephrotoxicity of lithium (CHMIELNICKA, NASIADEK 2003).

Lithium therapy can show high efficacy but it is necessary to take appropriate precautions. The reason is that lithium displays beneficial action only within a strictly determined range of its serum concentration (MIŠAK 2005, NG et al. 2006). If the safe threshold is overrun, side effects can occur (CHIU et al. 2007). Another problem is the lack of correlation between the applied Li dose and its serum level (NG et al. 2006). Moreover, scientists from India found that the plasma lithium level underwent seasonal variations (MEDHI et al. 2008). The same observations were reported by Dutch researchers, who also stated that the Li level was influenced by the ambient temperature, although these effects were therapeutically irrelevant (WILTING et al. 2007). Lithium therapy is usually long-term, therefore the serum or plasma Li concentration must be monitored during the whole administration period (WILKOWSKA et al. 2006, MEDHI et al. 2008). It is recommended to be within the range of 0.6-1.2 mmol L⁻¹ (CHIU et al. 2007). In India, the effective maintenance plasma level in manic-depressive patients was established to range from 0.35 to 1.0 mmol L⁻¹ (MEDHI et al. 2008). Similar values were reported by British authors (COLLINS et al. 2010). Besides, considering side effects of lithium, it is recommended to monitor creatinine, thyrotrophin and thyroid hormones in plasma, particularly in women, as well as renal functions (MIŠAK 2005). STRZELECKI (2006) reported that at the onset of a therapy the lithium concentration should be controlled twice a week and parathormone and antithyroperoxidase antibodies ought to be measured before Li administration. In 2009, according the UK guidelines regarding Li therapy, renal and thyroid functions tests before administration and every 6 months during the Li treatment are advised; it is also recommended to measure the serum Li every 3 months (COLLINS et al. 2010). The symptoms of adverse effects of lithium could be disturbances of the central nervous, cardiovascular

and alimentary systems (NG et al. 2006, CHIU et al. 2007), renal disorders (HWANG et al. 2010), hyperparathyroidism (STRZELECKI 2006), disturbances of carbohydrate and lipid metabolism (OLSZEWSKA, RYBAKOWSKI 2007, VIJAIMOHAN et al. 2010) as well as alterations of the thyroid volume, enhancement of the thyroid stimulating hormone level and depression of the secretion of thyroid hormones (OZSOY et al. 2010). Animal studies also revealed that subcutaneous administration of lithium resulted in depleted thymus mass (LEVINE, SALTZMAN 2006). The teratogenic effects of lithium were also described (NGUYEN et al. 2009) although a more recent article stated that the teratogenic risk resulting from Li therapy should be softened (GENTILE 2011). However, a lithium treatment was found to raise the risk of cardiovascular malformation in pregnancy and perinatal complications (GALBALLY et al. 2010), thus it is still recommended that lithium ought to be avoided during gestation (HOWLAND 2009). Several methods for lithium dosage prediction have been described. These *a priori* calculations are based on different data, including: the patient's age, gender, body weight, blood urea level, creatinine and lithium clearance, and concurrent administration of tricyclic antidepressants. However, the use of these methods is connected with the risk of under- or overdosing, hence the claim that nothing can replace monitoring of the Li serum level (CHIU et al. 2007).

Lithium may exert beneficial effects not only in cases of psychiatric disorders. Studies revealed that lithium could show action against the herpes virus (REMLINGER-MOLENDA, RYBAKOWSKI 2010). Lithium succinate and gluconate were suggested to be effective as topical drugs in seborrhoeic dermatitis. The local application was found to decrease the risk of side effects and might be used even in renal insufficiency, although not in the third trimester of pregnancy (SPARSA, BONNETBLANC 2004).

Owing to its ability to inhibit secretion of thyroid hormones, lithium has been studied as a possible adjuvant in patients with thyroid diseases subjected to radioiodine therapy (BAL et al. 2002, LIU et al. 2006, BOGAZZI et al. 2010, OSZUKOWSKA et al. 2010). Lithium was found to inhibit hyperthyroidism resulting from radioiodine treatment (VANNUCCHI et al. 2005). It was emphasized that Li enhanced radioiodine retention in the thyroid without increasing its uptake. Thus, a risk of side effects was lower because the Li treatment was shorter and the serum Li concentrations lower than used in psychiatric diseases (VANNUCCHI et al. 2005, BOGAZZI et al. 2010).

Numerous studies showed possible applications of lithium to cure neurodegenerative illnesses, although it was still recommended that the proper precautions should be taken to avoid side effects (ZHONG, LEE 2007). These studies were based on the evidence of a broad range of effects of lithium on processes involved into the pathogenesis of neurodegenerative disorders e.g. Alzheimer's disease (NAKASHIMA et al. 2005, KESSING et al. 2008). Lithium inhibits the activity of glycogen synthase kinase (GSK) (BIELECKA, OBUCHOWICZ 2008, CAMINS et al. 2009), whose isoform GSK3 β stimulates τ protein phos-

phorylation (LEROY et al. 2010). Inhibition of GSK-3 β activity may also decrease β -catenin degradation (BIELECKA, OBUCHOWICZ 2008, CHIU, CHUANG 2010). Lithium may inhibit β -amyloid formation (NAKASHIMA et al. 2005) although contradictory observations were also described (FEYT et al. 2005). Lithium treatment, both *in vivo* and *in vitro*, was found to cause significant increase in the neuroprotective protein Bcl-2 level (CAMINS et al. 2009). Animal studies revealed that lithium administration significantly enhanced anti-apoptotic protein Bcl-2 and decreased pro-apoptotic Bax in testes of cadmium-exposed rats (AL AZEMI et al. 2010). Another study performed on aluminium-treated rats revealed that lithium administration could display a neuroprotective action by improving the histoarchitecture of the cerebrum and cerebellum, which confirmed the potential use of Li against neurotoxicity (BHALLA, DHAWAN 2009). All the evidence of lithium being able to alleviate neurodegenerative processes encourage research on its administration in cases of disorders of the nervous system such as Alzheimer's, Parkinson's and Huntington's diseases as well as ethanol-induced neurotoxicity (LUO 2010). Other perspectives of Li application were connected with its possible efficacy in therapy of Canavan's disease (JANSON et al. 2005, ASSADI et al. 2010). The results of animal studies revealed that lithium used in the same doses as those applied in cases of bipolar disorders may be useful for prevention and cure of neonatal brain injury (LI et al. 2010). Continued lithium treatment was believed to reduce the rate of dementia (KESSING et al. 2008) although observations contradicting this conclusion were also reported (DUNN et al. 2005). Nonetheless, lithium was considered as an adjuvant in cases of dementia resulting from HIV infection (DOU et al. 2005).

Another possible application of lithium in cases of some ophthalmic diseases is connected with its action on the nervous system. Studies revealed that lithium could sustain the survival and regeneration of retinal ganglion cells. Bcl-2 protein was believed to be involved into the mechanism of this effect. The authors suggested that lithium therapy could be beneficial in cure of glaucoma, neuritis of optic nerve and degeneration of retinal ganglion cells (HUANG et al. 2003).

Studies concerning the possibility of Li application in cases of tumour have been performed on mice. Inhibition of the tumour growth by lithium carbonate has been shown. Moreover, additional positive effects were observed as white blood cells were unaffected, superoxide dismutase activity was enhanced and lipid peroxidation was inhibited (ZHANG et al. 1998). Moreover, a recent animal study pointed to a possible application of lithium for protection against side effects of the anti-neoplastic agent doxorubicin (RAHIMI BALAEI et al. 2010). On the contrary, JOHNSON et al. (2001) reported that lithium gamolenate should not to be recommended in cases of the pancreatic cancer.

Although lithium compounds have been used in medicine for more than sixty years, different aspects of their influence on organisms are still being

studied. Numerous new “targets” of Li action have been displayed, but the mechanism of lithium action remains unclear (AGHDAM, BARGER 2007, BIELECKA, OBUCHOWICZ 2008, CHIU, CHUANG 2010, ZARSE et al. 2011).

The inositol depletion hypothesis was one of the theories regarding the mechanism of action of lithium cations on organisms. Lithium inhibits the activity of inositol monophosphatase, which leads to a decrease in the brain myo-inositol level and subsequently to the downregulation of the phosphatidylinositol cycle (AGAM et al. 2002). It was regarded to be the explanation of the positive Li action in psychiatric diseases (BRANDISH et al. 2005). Some studies have undermined this hypothesis (BERRY et al. 2004), although the inositol theory is still taken into account (CHIU, CHUANG 2010).

Observations regarding the proinflammatory cytokines interleukins IL-1 β and IL-6, which play an important role in the immune system's functions and also affect the brain and neuroendocrine system, can contribute to the clarification of the mechanism of Li action. In LPS-stimulated monocytes of non-lithium-treated patients suffering from bipolar disorder, production ratio of these interleukins was found to be changed, whereas lithium treatment resulted in restoration of the aberrant ratio (KNIJFF et al. 2007).

Studies on the influence of lithium on the organism also revealed its effect on enzymatic activity (GOULD, MANJI 2005, MARTINS et al. 2008). Lithium can affect the activity of arachidonic acid cascade enzymes (RAPOPORT, BOSETTI 2002) as well as nitrogen oxide synthase (WEGENER et al. 2004) and the mitochondrial respiratory chain enzymes (MAURER et al. 2009).

Lithium treatment influences the neuronal transmission in a complex way (PIETRUCZUK, WITKOWSKI 2008, PERMODA-OSIP, RYBAKOWSKI 2009). It was found to affect the serotonin neurotransmission by modifying the serotonin release and turnover. This fact was supposed to be connected with the ability of lithium to intensify the action of antidepressants (BIRKENHÄGER et al. 2007, SCHEUCH et al. 2010).

Animal studies revealed that lithium can also affect levels of selected amino acids, causing their decrease in the brain (O'DONNELL et al. 2003) and kidneys (HWANG et al. 2010).

Interactions between lithium and bioelements seem to be another aspect of Li action in organism. Lithium was shown to increase significantly the serum calcium and phosphorus in rats, which seemed to result from bone demineralization and renal impairment (SHARMA, IQBAL 2005). Dietary lithium was found to prevent aluminium storage in the cerebrum and cerebellum of rats subjected to Al-exposure (BHALLA, DHAWAN 2009). Increase in the intracellular sodium level was suggested to be involved into the pathogenesis of bipolar disorder, while *in vitro* research showed that lithium decreased the ouabain-induced enhancement of the intracellular Na in human glial cells. The authors believed that their research results could contribute to a better understanding of the action mechanism of lithium (HUANG et al. 2007).

Lithium treatment was also found to influence pro- and antioxidant pro-

cesses, which are believed to be involved in the pathogenesis of numerous illnesses. The results are inconsistent, as some studies have revealed that Li administration can affect the activity of antioxidant enzymes (BHALLA, DHAWAN 2009, AL-AZEMI et al. 2010), whereas others observed no influence of lithium on the activity of superoxide dismutase (ENGİN et al. 2005). In the cerebrum and cerebellum of rats exposed to aluminium, lithium treatment resulted in a significant depression of the lipid peroxidation, level of reactive oxygen species as well as activities of antioxidant enzymes such as superoxide dismutase and catalase (BHALLA, DHAWAN 2009). The serum activity of the superoxide dismutase isoform Zn-Cu SOD was increased in cadmium-exposed rats receiving lithium orally, whereas animals with no cadmium Li treatment showed no significant effect (AL-AZEMI et al. 2010). Some authors also stated that Li can inhibit lipid peroxidation processes (SHAO et al 2005, BHALLA, DHAWAN 2009, AL-AZEMI et al. 2010). Contrary to that, an animal study carried out on Li-treated mice displayed an enhanced lipid peroxidation level in the liver (NCIRI et al. 2009). Moreover, the lithium salt of cysteine was found to produce an antioxidant effect (OVSEPIAN et al. 2010).

Lithium may affect the metabolism of carbohydrates (WIERNSPERGER, RAPIN 2010), as it was reported to show an insulin-like effect (DE ALMEIDA SOUZA et al. 2010). The hormonal balance, e.g. availability of thyroxine (CONSTANTINO et al. 2005) as well as levels of luteinizing hormone, follicle stimulating hormone, prolactin and testosterone (AL-AZEMI et al. 2010) were also reported to be subjected to the influence of lithium.

All the findings described above show that lithium is an element of great potential and that future investigations may bring to light surprising and entirely unexpected facts which will contribute to the elucidation of lithium action and allow new medical applications.

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MAGNESIUM IN MEDICINE AND TREATMENT

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Abstract

Magnesium (Mg^{2+}) plays a critical role in numerous metabolic function in our body. Normal levels of both intra- and extra cellular magnesium are necessary for correct cellular processes. The specific clinical conditions in which Mg^{2+} deficiency has been implicated to play a pathophysiological role include depression, ischaemic heart disease, arrhythmias, preeclampsia, asthma, intradialytic hypotension or critical illness. Correct administration of magnesium can eliminate perioperative pain and muscle spasms, keep blood flowing smoothly, and prevent platelet stickiness. Although magnesium sulphate is now widely used to prevent or control eclamptic convulsions, there is no consensus on the optimal dosage and concentration of magnesium sulphate for the management of eclamptic seizures. Owing to its bronchodilating and anti-inflammatory effects, Mg^{2+} is an encouraging adjuvant therapy for paediatric patients, although its use is often limited to patients who do not respond to conventional treatment. Studies have also suggested that magnesium might be useful as an agent to control the rigidity and spasms of severe tetanus or for the enhancement of neuromuscular blockade during anaesthesia, but its actual efficacy as an anticonvulsant or an adjuvant to analgesics and anaesthetics to induce and maintain anaesthesia remains unclear. Moreover, a negative correlation has been shown between intradialytic changes of the serum magnesium and hypotensive episodes during dialysis sessions. Low Mg^{2+} haemodialysis solution resulted in both hypomagnesaemia and hypocalcaemia. Mg^{2+} compounds are now becoming recognized as safe, effective and cost-efficient alternatives to other phosphorus binders, with a significant added benefit of substantially reducing the risk and impact of cardiovascular diseases.

It seems that magnesium is underused in clinical conditions, considering its therapeutic capacity. On the other hand, numerous studies have found conflicting results, questioning the implicit efficacy of magnesium in several clinical conditions.

Key words: magnesium, eclampsia, asthma, tetanus, intradialytic hypotension, anesthetic.

Abstrakt

Magnez (Mg^{2+}) odgrywa kluczową rolę w wielu procesach metabolicznych w organizmie ludzkim. Odpowiednie stężenie magnezu, zarówno wewnątrz- jak i zewnątrzkomórkowego, zapewnia prawidłowe funkcjonowanie komórki. Niedobór Mg^{2+} może odgrywać rolę w patofizjologii depresji, choroby niedokrwiennej serca, zaburzeń rytmu serca, drgawek rzucawkowych, astmy, hipotonii śróddializacyjnej czy stanach krytycznych. Stosowanie odpowiednich dawek magnezu może zmniejszać ból okołoperacyjny, łagodzić skurcze mięśni, warunkować zachowanie płynności krwi czy zmniejszać ryzyko zakrzepów lub zatorów. Pomimo że siarczan magnezu jest powszechnie stosowany w celu zapobiegania lub kontroli drgawek rzucawkowych, brak jest jednomyślności co do optymalnej dawki czy stężenia roztworu siarczanu magnezu. Rozszerzające oskrzela oraz przeciwzapalne właściwości Mg^{2+} zachęcają do wykorzystania preparatów magnezowych w celu uzupełnienia terapii w leczeniu astmy, szczególnie dziecięcej, jednak jego użycie często ogranicza się do pacjentów, którzy nie reagują na leczenie konwencjonalne. Badania sugerują również, że terapia magnezowa może być pomocna w opanowaniu skurczu oraz sztywności mięśni u chorych na tężec lub może wzmacniać blokadę nerwowo-mięśniową w trakcie znieczulenia. Jednak skuteczność Mg^{2+} jako rzeczywistego czynnika przeciwdrgawkowego lub czynnika uzupełniającego leki przeciwbólowe czy anestetyk pozostaje niejasna.

Liczne badania wykazały ujemną korelację między śróddializacyjnymi zmianami stężenia magnezu w surowicy pacjentów a częstością występowania hipotensji podczas dializy. Stosowanie płynu dializacyjnego o niskim stężeniu Mg^{2+} powoduje nie tylko hipomagnezemię, lecz również hipokalcemię.

Związki magnezowe stają się obecnie uznawane za bezpieczne, skuteczne i opłacalne w porównaniu z innymi związkami wiążącymi fosforany. Na ich korzyść przemawia dodatkowo fakt, że Mg^{2+} zmniejsza ryzyko lub łagodzi skutki chorób układu krążenia. Biorąc pod uwagę terapeutyczne możliwości, jakie ma magnez, wydaje się, że jest on niewystarczająco wykorzystywany w warunkach klinicznych. Jednakże liczne badania wykazują sprzeczne wyniki, kwestionując oczekiwaną skuteczność magnezu.

Słowa kluczowe: magnez, rzucawka, astma, tężec, hipotonia śróddializacyjna, anestetyk.

INTRODUCTION

Magnesium (Mg^{2+}) is the second most abundant intracellular divalent cation and is a cofactor for hundreds of metabolic reactions in the body, such as glycolysis, Krebs cycle, β -oxidation, activation of amino acids, synthesis and breakdown of DNA, neurotransmission or ion transport across cell membranes (PASTERNAK et al. 2010). Mg^{2+} also causes vasodilation by direct action as well as indirectly by sympathetic blockade and inhibition of catecholamine release. Magnesium dilates both the epicardial and resistance coronary arteries. It balances cholesterol and is essential for endocrine stability and function. Most importantly, magnesium prevents calcification of the cardiac tissues. Magnesium has been directly implicated in hypokalaemia, hypocalcaemia, tetany and dysrhythmia. Moreover, it regulates enzymes controlling intracellular calcium, thereby affecting smooth muscle vasoconstriction, which is important to the pathophysiology of several critical illnesses (TONG,

RUDE 2005). Because of these vital roles, the magnesium level may be affected by stressors to the body, such as in certain disease states. Supplementation with magnesium may have therapeutic effects in these situations (GUERRERA et al. 2009). Correct administration of magnesium can reduce perioperative pain (SEYHAN et al. 2006) and muscle spasms (ELSHARKAWY et al. 2006, BICHARA, GOLDMAN 2009), keep blood flowing smoothly and prevent platelet stickiness.

The specific clinical conditions in which magnesium deficiency has been implicated to play a pathophysiological role include ischaemic heart disease, arrhythmias (HERROEDER et al. 2011), pre-eclampsia (EUSER, CIPOLLA 2009, PALMER, NEWBY 2009), asthma (CHEUK et al. 2005), intradialytic hypotension (ELSHARKAWY et al. 2006, PAKFETRAT et al. 2010) or critical illness (TANG, RUDE 2005); magnesium deficiency is a common yet underdiagnosed problem in an intensive care unit (ICU). The problem is that the extracellular level of the ion may not reflect its intracellular deficiency, because only 1% of the total body Mg^{2+} is in the extracellular fluid. Moreover, one cannot clearly define a patient's response to magnesium treatment based on his/her magnesium serum level.

ECLAMPSIA

Magnesium sulphate is recommended as the first-line medication for prophylaxis and treatment of eclampsia (PALMER, NEWBY 2009). Though the specific mechanisms of action remain unclear, the effect of magnesium sulphate in the prevention of eclampsia seems to be multi-factorial, including its action as a vasodilator, with effects produced either peripherally or through the cerebral circulation, and an agent protecting the blood-brain barrier (BBB) to decrease cerebral oedema formation; and acting as a central anticonvulsant by inhibiting N-methyl-D-aspartate receptors (EUSER, CIPOLLA 2009). The most commonly used administration methods are the so-called Pritchard's and Zuspan's regimens. In the Pritchard's regimen, a loading bolus dose of 4 g of $MgSO_4$ is given slowly intravenously over 5-10 minutes and this is followed by 10 g given intramuscularly (5 g in each buttock). Subsequently, 5 g is given intramuscularly into alternate buttocks every 4 h. According to the Zuspan's regimens, a loading dose of 4 g is given intravenously over a period of 10 minutes with a maintenance dose of 1-2 g every hour by controlled infusion pump (TUKUR 2009). The above doses should be respected due to the risk of overdosing side effects: muscle atonia, respiratory failure leading to death. The absence of a knee jerk may indicate magnesium overdosing. It must be remembered that doses exceeding 30 mg may result in the CNS depression, respiratory problems and reduced Apgar scores in neonates. A daily dose of $MgSO_4$ should *not exceed* 20-24 g. Patients with the renal failure after a loading dose are administered i.v. magnesium sulphate at a dose of 1.0 g h^{-1} under the control of serum magnesium concentration. It has been noticed that magnesium sulphate is superior to phenytoin and diaze-

pam in preventing the recurrence of seizures and maternal death in patients with eclampsia (NALEWCZYŃSKA et al. 2008). Magpie and co-authors' trial showed that the reduction in the risk of eclampsia following prophylaxis with magnesium sulphate was associated with a 16% decrease in the risk of death or serious morbidity potentially related to pre-eclampsia 2-3 years later (MAGPIE et al. 2007). In 2006 and then in 2008, the maximum recommended concentration of magnesium sulphate for i.v. infusion was set at 20% in the British Columbia Perinatal Health Program and later, by the Society of Obstetricians and Gynaecologists of Canada. Because the 20% concentration of MgSO_4 was much higher than the 2% to 8% concentrations used previously there were concerns about a potential increase in the incidence of phlebitis. So Palmer and Newby undertook a retrospective chart audit for patients who had received magnesium sulphate (2% to 8% or 20% solution) for eclampsia prophylaxis, concluding the lack of any evidence of documented cases of phlebitis as well as fewer side effects when 20% magnesium sulphate was used (PALMER, NEWBY 2009).

Nowadays, researchers aim to investigate whether low dose MgSO_4 regimens might assure less toxicity and an improved neonatal outcome without significant adverse effects in control of eclamptic convulsions. SETH et al. demonstrated that both maternal and fetal outcomes were comparable in either low dose (4 g i.v. loading dose continued with 2 g i.m. every 3 h), single loading dose (4 g i.v. and 5 g i.m. only) or the standard Pritchard's regimen of magnesium sulphate; a low dose as well as a single dose were associated with significantly lower toxicity related to magnesium sulphate (SETH et al. 2010). Similarly, EKELE et al. showed that limiting the dosage of magnesium sulphate to 14 grams loading dose (4 g intravenous and 10 g intramuscular) was effective as an anticonvulsant in 92.6% of eclampsia patients and only 7% of the patients needed the continuation of maintenance doses (EKELE et al. 2009). Chowdhury et al. also proved that a much lower maintenance dose of 0.6 g h^{-1} , considerably less than $1\text{--}2 \text{ g h}^{-1}$ advocated by Zuspan, was as effective in Asian women as the Pritchard's i.m. regimen and could prevent the recurrence of convulsions. Thus, researchers found that lower dosage of MgSO_4 may increase the safety margin without compromising effectiveness and, moreover, one might prefer to avoid the possibility of painful injections in the i.m. regimen. On the other hand, they guessed that since the study in a western population showed that an ideal serum magnesium level was obtained with either the Pritchard's i.m. regimen or the i.v. regimen with a maintenance dose of $\geq 2 \text{ g h}^{-1}$, the difference in the response between western and Asian women was only because the former had a higher body weight than the latter (CHOWDHURY et al. 2009).

Although magnesium sulphate is now widely used to prevent or control eclamptic convulsions, there is no consensus on the optimal dosage and concentration of magnesium sulfate for the management of eclamptic seizures.

ASTHMA

In the smooth muscle of bronchi, magnesium decreases the intracellular calcium level by blocking its entry through non-voltage-operated channels and its release from the endoplasmic reticulum. Mg^{2+} acting as Ca^{2+} antagonist may directly inhibit actin-myosin interactions, which results in muscle cell relaxation. The magnesium ion also reduces muscle fibre excitability by inhibiting the release of acetylcholine from cholinergic nerve-endings. Additionally, magnesium stimulates nitric oxide and prostacyclin synthesis, which might depress asthma severity; as well as this, it also stabilizes T cells and inhibits mast cell degranulation, alleviating inflammation (BICHARA, GOLDMAN 2009). Magnesium deficiency in asthmatic patients may potentiate the calcium intracellular flow into the smooth muscle cells, thereby leading to increased myosin phosphorylation and thus to the enhanced contractility of muscles. Since a decrease in magnesium concentration was noted during adrenaline or salbutamol infusion, which are used in acute asthma, it is suggested that the magnesium flow between extracellular and intracellular compartments may be under the influence of β -adrenergic receptors. During a stress reaction, i.e. acute asthma exacerbation, magnesium escapes from cells while calcium enters them. Hypomagnesaemia increases acetylcholine cell depolarization, causing an increase in the excitability of bronchial smooth muscles and their contraction. Additionally, the shortage of Mg enhances the muscle's reactivity to catecholamines and reduces the relaxant action of prostaglandins (KOWAL et al. 2007).

Magnesium sulphate is not recommended for *routine use* in asthma exacerbations. However, in some cases of severe asthma attacks, a single dose of i.v. $MgSO_4$ is appropriate and recommended by the Global Initiative for Asthma (GINA). It applies to adults having the baseline forced expiratory volume in 1 s (FEV1) 25-30% of the predicted value; adults who do not respond to conventional treatment; children whose FEV1 remains less than 60% of the predicted value after an hour of treatment. Magnesium is administered intravenously at a dose of 2 g in a single 20-minute infusion. Additional monitoring is unnecessary and no side effects of such treatment are indicated (SILVERMAN et al. 2002).

Several clinical trials regarding the use of magnesium in adults and children with asthma were summarized by ROWE et al. (2000), CHEUK et al. (2005), BLITZ et al. (2005) as well as MOHAMMED and GOODACRE (2007). ROWE et al. and CHEUK et al. examined the effect of i.v. magnesium sulphate while BLITZ et al. studied its inhaled form in the treatment of asthma exacerbations, whereas MOHAMMED and GOODACRE's meta-analysis included both intravenous and nebulised magnesium sulphate usage in patients presenting with acute asthma.

Rowe and co-authors' thorough review on the effect of i.v. $MgSO_4$ versus placebo used in patients with acute asthma demonstrated that hospital admission was not statistically reduced owing to the use of $MgSO_4$ and patients

receiving magnesium sulphate displayed non-significant improvement in the peak expiratory flow rates (PEFR) together with clinically unimportant changes in vital signs and lack of side effects (ROWE et al. 2000). The meta-analysis written in 2005 by CHEUK et al., which compared i.v. magnesium sulphate to placebo in treating paediatric patients with moderate to severe asthmatic attacks, with co-therapies of inhaled β_2 agonists and systemic steroids, showed that MgSO_4 was effective in preventing hospitalisation and led to significantly improved outcomes of short-term pulmonary function tests and clinical symptom scores (CHEUK et al. 2005). A meta-analysis of six trials made by BLITZ et al. investigating usage of inhaled MgSO_4 combined with a β_2 -agonist for an acute asthma exacerbation confirmed beneficial effects with respect to improved pulmonary function, although there was no evidence of its positive impact on hospital admission rates (BLITZ et al. 2005). Contrary, in the study of AGGARWAL et al., who compared nebulized (thrice at intervals of 20 min) magnesium sulphate (500 mg) with salbutamol (0.5 mg) or salbutamol alone in the treatment of acute asthma, there was no significant rise in PEFR at any point in patients nebulized MgSO_4 . The researchers concluded there was no therapeutic advantage in adding MgSO_4 to salbutamol nebulisation in the treatment of patients with acute, severe or life threatening asthma (AGGARWAL et al. 2006). MOHAMMED and GOODACRE completed a meta-analysis of randomised and quasi-randomised trials of intravenous or nebulised MgSO_4 in acute asthma, which showed that the i.v. treatment was associated with weak evidence of any effect on the respiratory function in adults, no significant effect on hospital admission, and a significant effect on respiratory function and hospital admission in children. With respect to the nebulised treatment, it was associated with weak evidence of its effect on respiratory function and on hospital admission in adults or else no significant effect on the respiratory function in children (MOHAMMED, GOODACRE 2007).

Thus, intravenous magnesium sulphate seems to be beneficial in the treatment of moderate to severe asthma in children. Owing to its bronchodilating and anti-inflammatory effects, magnesium is an encouraging adjuvant therapy for paediatric patients, but its use is often limited to patients who do not respond to conventional treatment. A cross-sectional online survey of two national paediatric emergency physician associations in Canada and the United States conducted using a modified Dillman's technique showed that although 88% of participants report knowing that Mg^{2+} is effective, only 14 of 199 (7%) and 142 of 199 (71%) give it to prevent hospitalizations and to prevent admissions to an intensive care unit (ICU), respectively (SCHUH et al. 2010).

TETANUS

Magnesium is a presynaptic neuromuscular blocker with properties of a vasodilator, catecholamine release blocking agent and an anticonvulsant, all of which are desirable for the control of spasticity and autonomic dysfunction

in tetanus (KARANIKOLAS et al. 2010). Thwaites et al. reported the results of a randomised placebo controlled trial of magnesium sulphate infusions for the treatment of severe tetanus, which showed that magnesium did not reduce the need for mechanical ventilation in adults with severe tetanus but it reduced the requirement for other drugs to control muscle spasms and cardiovascular instability (THWAITES et al. 2006). In the later study, Thwaites et al. confirmed that the patients who received Mg^{2+} had significantly less autonomic dysfunction and required less cardiovascular stabilizing drugs; additionally, the researchers noticed that patients had lower urinary concentrations of adrenaline. Therefore, they suggested that adrenaline was important in the pathophysiology of severe tetanus, and magnesium controlled the autonomic dysfunction by decreasing the adrenaline release, which affected its urinary excretion (THWAITES et al. 2008). KARANIKOLAS et al. observed that even a high-dose (total Mg dose 758 g) prolonged (26 days) intravenous Mg therapy, if carefully titrated and monitored, could be continued for a long time without obvious side effects or major organ toxicity. They reported that the i.v. Mg^{2+} therapy resulted in an excellent muscle contraction control by patients within hours, without the need for additional administration of muscle relaxants. However, because of the persistence of painful muscle rigidity during their daily wake-up tests, i.v. Mg^{2+} infusions were continued for a long period (26 days) in two of three patients (KARANIKOLAS et al. 2010). In contrast, Mathew et al. reported that the intravenous magnesium therapy might not be useful as a sole agent to control the rigidity and spasms in severe tetanus, therefore additional sedative adjuncts were required to control the disease (MATHEW et al. 2010).

Since magnesium therapy can cause serious adverse effects, including muscle weakness, paralysis and hypotension, further research is needed before recommending Mg^{2+} as the first-line therapy for tetanus (KARANIKOLAS et al. 2010).

DIALYSIS

Intradialytic changes in the serum Mg^{2+} affect systemic hemodynamics, especially when haemodialysis is performed with a low calcium dialysate. Since Mg^{2+} exerts direct modulatory action on cardiac excitability and vascular smooth muscle contraction and relaxation (ELSHARKAWY et al. 2006), hypomagnesaemia in haemodialysis patients *can result in impaired myocardial contractility*, thereby favouring hypotension. Moreover, in haemodialytic (HD) patients, reduced Mg^{2+} level may lead to increased atherosclerosis susceptibility, because it has shown that both serum and intracellular Mg had a significant and independent negative association with the carotid intima-media thickness, which is a predictor of future vascular events and a marker of early atherosclerosis (NAVARRO-GONZÁLEZ et al. 2009). Additionally, Mg-based phosphate binders have always been used with excellent results (DE FRANCISCO et al. 2010).

KYRIAZIS et al. investigated the way dialysate Mg^{2+} concentrations could affect blood pressure during hemodialysis. The researchers identified a dialysis solution containing $0.25 \text{ mmol l}^{-1} \text{ Mg}^{2+}$ and $1.25 \text{ mmol l}^{-1} \text{ Ca}^{2+}$ as a significant risk factor for developing intradialytic hypotension (IDH), and showed that increasing the Mg^{2+} level to 0.75 mmol l^{-1} could prevent frequently seen IDH. Moreover, the low-Mg haemodialysis solution resulted in both hypomagnesaemia and hypocalcaemia (decreasing serum magnesium and calcium levels by 35% and 8%, respectively), which was associated with an impairment of myocardial contractility not compensated by total peripheral resistance (KYRIAZIS et al. 2004). These results coincide with the studies by PAKFETRAT et al. or ELSHARKAWY et al., linking intradialytic changes in the serum Mg^{2+} with hypotension episodes during a dialysis session (ELSHARKAWY et al. 2006, PAKFETRAT et al. 2010). Turgut et al. observed magnesium supplementation (magnesium citrate orally at a dosage of 610 mg every other day for 2 months) was negatively associated with the common carotid intima-media thickness (IMT), so it may play an important protective role in the development and/or acceleration of arterial atherosclerosis in patients with chronic renal sufficiency (TURGUT et al. 2008). This has confirmed some earlier research by TZANAKIS et al., which had shown that both increased serum and intracellular Mg^{2+} levels significantly improve the carotid IMT in HD patients (TZANAKIS et al. 2004).

In patients with chronic kidney disease (CKD) requiring haemodialysis, renal elimination of phosphate is impaired, resulting in hyperphosphataemia. Although certain magnesium salts have high phosphate-binding capacity, their use in the treatment of hyperphosphataemia is very limited, largely due to the fear of the occurrence of hypomagnesaemia.

TZANAKIS et al. carried out a study in hemodialytic patients to evaluate the efficacy and safety of MgCO_3 as a phosphate-binder when given with a concurrent low dialysate magnesium solution. The study showed that MgCO_3 administered in the mean daily dose of 6.21 tablets (range 3-9) containing 441 mg of elemental magnesium (range 213-639 mg) might be an effective and inexpensive way to control serum phosphate levels in HD patients, and the administration of MgCO_3 in combination with a low dialysate Mg^{2+} concentration (0.48 mmol l^{-1}) prevented the risk of severe hypermagnesaemia (TZANAKIS et al. 2008). Similarly, MCINTYRE et al. demonstrated that fermagate, a calcium-free iron and magnesium hydroxycarbonate binder, administered (1 g or 2 g) three times daily just before meals for 21 days was associated with statistical reductions in the mean serum phosphate from 2.16 mmol l^{-1} to 1.71 and 1.47 mmol l^{-1} , respectively, in HP patients. Adverse event (AE) incidence in the 1 g dose of fermagate was statistically comparable to the placebo group but the 2 g arm was associated with a statistically higher number of patients reporting AEs than the 1 g arm, particularly gastrointestinal AEs, as well as a higher number of discontinuations, thus complicating the interpretation of the efficacy this dose. Thus, the study proved

that the efficacy and tolerability of ferrogate were dose-dependent and a daily oral 1g dose of the medicament might be promising in the treatment of hyperphosphatemia in patients who are receiving haemodialysis (McINTYRE et al. 2009). Another study investigated the effect of calcium acetate/magnesium carbonate (CaMg) on serum phosphorus levels compared with sevelamer hydrochloride (HCl). Patients received calcium acetate 435 mg containing 110 mg elemental calcium combined with magnesium carbonate 235 mg containing 60 mg elemental magnesium (OsvaRen®) or sevelamer-HCl 800 mg (Renagel®) for 24 weeks. A starting dose of the drugs was at least four tablets per day. Thereafter, the dose was increased by one to three tablets per day to achieve an adequate phosphate level in serum. The researchers showed CaMg was non-inferior to the comparator at controlling serum phosphorus levels and it had a good tolerability profile, so it might represent an effective treatment of hyperphosphataemia (DE FRANCISCO et al. 2010).

Although the use of dialysis fluid containing $0.25\text{--}0.4\text{ mmol l}^{-1}\text{ Mg}^{2+}$ usually keeps magnesaemia within normal limits, magnesium compounds are not widely used in HD patients because nephrologists have an inordinate fear of hypermagnesaemia and the belief that Mg^{2+} administration is frequently accompanied by gastrointestinal disorders. However, currently magnesium compounds are becoming recognised as safe, effective and cost-efficient alternatives to other phosphorus binders with the significant added benefit of substantially reducing the risk and impact of cardiovascular diseases.

ANAESTHESIA

Because Mg^{2+} ions block the open N-methyl-D-aspartate (NMDA) channel in a voltage-dependent manner, they may contribute to the anesthetic, amnesic as well as anticonvulsant properties of propofol. Moreover, Mg^{2+} competes with calcium ions at the neuromuscular junction and enhances the effect of neuromuscular blockers by decreasing presynaptic acetylcholine release. It also has an inhibitor effect on the postsynaptic potential, thereby reducing the excitability of the muscular fiber membrane.

A study by CIZMECI and OZKOSE (2007) showed that magnesium sulphate can be used safely as an adjuvant to total i.v. anesthesia for day case surgeries, with the effect from potentialization of neuromuscular blockade. The patients received $15\%\text{ MgSO}_4$ 50 mg kg^{-1} in 100 ml of saline before induction of anesthesia, followed by $8\text{ mg kg}^{-1}\text{ h}^{-1}$ infusion of magnesium sulphate until the end of surgery. The researchers noticed that propofol requirements were significantly lower in patients received MgSO_4 than in those who received only saline, and the haemodynamic variables were similar in the two groups. Moreover, the neuromuscular potency of vecuronium was greater and the verbal numeric scale values for pain were found to be significantly lower in the Mg^{2+} group compared to the control one (CIZMECI, OZKOSE 2007). The serum magnesium level was in the normal range at the induction of anesthesia in the both groups, but its *postoperative concentration* was significantly

lower in the saline than in the MgSO_4 group. ALTAN et al. confirmed that the amount of propofol used in the maintenance of anesthesia was lower in the presence of magnesium sulphate (30 mg kg^{-1} as a bolus before induction and $10 \text{ mg kg}^{-1} \text{ h}^{-1}$ by infusion; moreover, they noticed that anesthetic induction was more rapid in patients who received MgSO_4 (ALTAN et al. 2005). SEYHAN et al. demonstrated that magnesium (40 mg kg^{-1} bolus followed by $10 \text{ mg kg}^{-1} \text{ h}^{-1}$) infusion caused significant reductions in intraoperative propofol, atracurium as well as postoperative morphine consumption, although increasing the magnesium dosage further did not offer any advantages, but induced haemodynamic consequences (SEYHAN et al. 2006). RAY et al. agreed that the perioperative use of magnesium sulphate (30 mg kg^{-1} as a bolus before induction and $10 \text{ mg kg}^{-1} \text{ h}^{-1}$ by infusion) was able to attenuate the haemodynamic response to tracheal intubation. On the other hand, they noticed Mg^{2+} may cause bradycardia and hypotension as well as result in a delay in recovery. Thus, the researchers concluded that MgSO_4 needed careful management to be used as an adjuvant agent to general anaesthetics (RAY et al. 2010).

GHATAK et al. indicated positive effects of magnesium as an adjuvant to epidural bupivacaine (19 ml of epidural bupivacaine 0.5% along with 50 mg magnesium), Mg^{2+} reduced the time of onset and establishment of the epidural block up to T6 level without any side effects (GHATAK et al. 2010). SHOEIBI et al. found that an intrathecal injection of 0.5 ml magnesium sulphate 10% with 1.5 ml lidocaine 5% for spinal anesthesia resulted in a significant enhancement of the duration of analgesia after spinal anesthesia and surgery. It is possible that the analgesic effect of magnesium occurred at the supra-spinal level and might be related to its systemic absorption (SHOEIBI et al. 2007). Ko et al. confirmed an inverse relation between cumulative postoperative analgesic consumption and the cerebrospinal fluid (CSF) magnesium concentration. On the other hand, researchers showed that perioperative i.v. administration of MgSO_4 (50 mg kg^{-1} intravenous magnesium sulfate as a bolus dose followed by a continuous infusion of $15 \text{ mg kg}^{-1} \text{ h}^{-1}$ for 6 h) did not increase the CSF magnesium concentration and had no effects on postoperative pain. Thus, they suggested that perioperative intravenous magnesium infusion may not be useful for preventing postoperative pain (Ko et al. 2001). Because magnesium prolongs muscle relaxation, continuous monitoring of neuromuscular function during surgery is required, and muscle relaxants should be applied accordingly.

Although the above studies suggest a clinically relevant effect of magnesium, its actual efficacy as an adjuvant to analgesics and anaesthetics to induce and maintain anaesthesia remains unclear (HERROEDER et al. 2011). Clinicians are therefore very often hesitant to use magnesium in perioperative treatment. This issue requires evaluation in large clinical trials.

SUMMARY

In conclusion, it seems that magnesium is used insufficiently in comparison to its therapeutic capacity. Primary limitations associated with the use of Mg^{2+} in treatment are due to the fear of overdose. The problem is to determine the best magnesium dosage regime: dose, i.v. or i.m. administration. However, experiments have shown that administration of 2 g MgSO_4 (16 mEq) in a bolus enhanced the magnesium serum concentration from 1.8 to 3.1 mEq l^{-1} (0.9-1.55 mmol l^{-1}) 30 minutes after the infusion and that administration of 2-4 g MgSO_4 (16-32 mEq) to patients with normal renal function over 30-60 minutes has a low probability of side effects. Fast infusions (1-2 min) of 2 g MgSO_4 (16 mEq) also caused minimal side effects, but they required patient monitoring (SILVERMAN et al. 2002). On the other hand, numerous studies have found conflicting results, questioning the implicit efficacy of magnesium in several clinical conditions. In short, future studies are required to know how magnesium contributes to pathological processes, whether Mg^{2+} administration serves the purpose of merely correcting an underlying deficiency state or how to use a specific pharmacologic effect of magnesium as well as to clearly determine under what circumstances should it be used therapeutically and what doses should be recommended.

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