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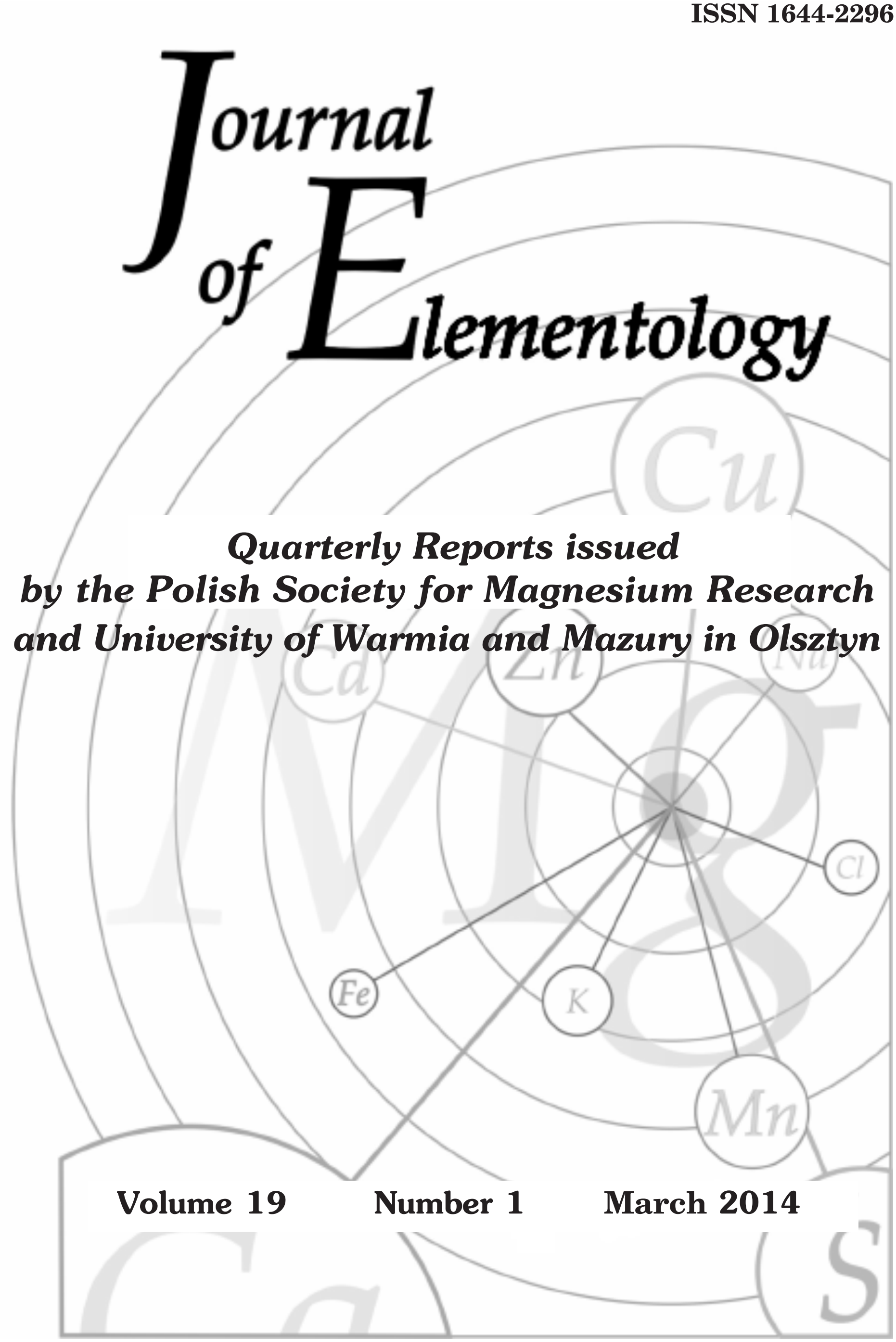

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Editorial Office

University of Warmia and Mazury in Olsztyn
Chair of Agricultural Chemistry and Environment Protection
Oczapowskiego Street 8, 10-719 Olsztyn, Poland
phone:+48 89 5233231
jelementol@uwm.edu.pl

English Language Proofreading
Jolanta Idźkowska

Statistical Reviewer
Janusz Gołaszewski

Webmaster
Sławomir Krzebietke

www.uwm.edu.pl/jelementol

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ORIGINAL PAPERS

THE PROFILE DISTRIBUTION OF ZINC IN ARABLE ALLUVIAL SOILS WITH NATURALLY ELEVATED CONTENT OF CALCIUM CARBONATE

Agata Bartkowiak[1], Jacek Długosz[1], Ryszard Zamorski[2]

[1]Chair of Soil Science and Soil Protection[1]
[2]Department of Biochemistry
University of Technology and Life Sciences in Bydgoszcz

Abstract

The objective of the study was to analyze profile spatiation of total and DTPA-extractable zinc forms in alluvial arable soils. Six soil profiles located in the Unisław Basin were analyzed. The soils were classified as alluvial black earths. The alluvial material of the examined soils was overlying calcareous sinter situated 100 cm deep into the ground. Soil samples were analyzed for the grain size composition, reaction, Corg and the $CaCO_3$ content. The total zinc concentration was assessed after mineralization in HF+ $HClO_4$ mixture according to Crock and Severson (1980), while zinc forms extractable with DTPA were assayed with ASA as described by Lindsay and Norvell (1978). The soils did not show symptoms of zinc pollution. Its total, Zn concentrations ranged between 6.25 and 18.78 mg kg^{-1}, while an average content was 11.16 mg kg^{-1}. The analyzed soil samples contained zinc forms extractable with DTPA in amounts fluctuating between 0.32 and 2.6 mg kg^{-1}. The highest concentrations of total and available zinc were noted in the surface horizons of the soils and in the horizons enriched with organic carbon.

Keywords: zinc in soil, alluvial soils, total and DTPA-extractable forms.

PROFILOWE ROZMIESZCZENIE CYNKU W ALUWIALNYCH GLEBACH UPRAWNYCH O NATURALNIE PODWYŻSZONEJ ZAWARTOŚCI WĘGLANU WAPNIA

Abstrakt

Celem pracy była analiza profilowego rozmieszczenia cynku w formie całkowitej i ekstrahowanej DTPA w glebach aluwialnych użytkowanych rolniczo.

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dr inż. Agata Bartkowiak, Chair of Soil Science and Soil Protection, University of Technology and Life Sciences, Bernardyńska 6, 85-029 Bydgoszcz, Poland, e-mail: bartkowiak@utp.edu.pl

Przedmiotem badań było 6 profili glebowych zlokalizowanych na terenie Basenu Unisławskiego. Gleby te sklasyfikowano jako mady czarnoziemne. Materiał aluwialny badanych gleb zalegał na martwicy wapiennej, która występowała na głębokości ok. 100 cm. Z podstawowych właściwości w analizowanych próbkach glebowych oznaczono: uziarnienie, odczyn, zawartość C- org. oraz $CaCO_3$. Całkowitą zawartość Zn oznaczono po mineralizacji w mieszaninie kwasów HF+ $HClO_4$ metodą Crocka i Seversona (1980), natomiast formę ekstrahowaną DTPA metodą Lindsaya i Norvella (1978), z zastosowaniem metody ASA. Badane gleby nie wykazywały zanieczyszczenia cynkiem, którego całkowita zawartość wynosiła 6.25–18.78 mg kg^{-1}. Zawartość cynku ekstrahowanego DTPA wahała się od 0.32 do 2.6 mg kg^{-1}. Największe ilości cynku całkowitego i przyswajalnego odnotowano w poziomach powierzchniowych analizowanych gleb oraz w poziomach wzbogaconych w węgiel organiczny.

Słowa kluczowe: cynk w glebie, gleby aluwialne, formy całkowite i ekstrahowane DTPA .

INTRODUCTION

Zinc is a widespread element in nature. It is found in compounds incidentally released to the environment, but it is also an ingredient of mineral fertilizers, pesticides and waste substances applied in agricultural practice. Because of the physiological functions it plays in live organisms, zinc is considered an essential element in nutrition of plants and animals (Allowey 2004). However, any concentration higher than allowable can impair the biological activity of soil. Zinc can be toxic to plants due to zinc compounds accumulating in their vegetative and generative parts, causing adverse effects on the yield volume and quality of crops (Roszyk et al. 1988). Secondary to this, a threat to higher organisms appears. Similarly to other trace elements, the mobility of zinc in soil and its ability to form bonds depends on the soil sorption capacity, i.e. soil physical and chemical properties (Dube et al. 2001). High zinc mobility in soil creates a risk of its excessive uptake by plants (Kabata-Pendias, Pendias 2001, Terelak , Tujaka 2003). The degree of tolerance of plants towards zinc depends mainly on soil factors, such as reaction, fractional composition and organic matter content, as well as other conditions, e.g. zinc form and plant species (Baran 2009, 2011, Spiak et al. 2000).

The objective of the study was to investigate the profile spatiation of total zinc and its forms extractable with DTPA in profiles of alluvial arable soils with a naturally elevated content of calcium carbonate.

MATERIAL AND METHODS

The subject of this research consisted of six soil profiles located in the Unislaw Basin, and sampled in 2009. Those soils are classified as Mollic Limnic Fluvisol (Calcaric) – profiles 1 and 2, Limnic Epigleyic Phaeozem

– profile 4 and Epigleyic Limnic Fluvisol (Calcaric) – profile 6 (IUSS WRB 2006). The surface (Ap) and subsurface soil horizons (Aa) were composed of mineral alluvial depoists, in which various mineral and organic limnic materials (Lc) were embedded. Soil samples were taken from morphologically distinct layers. They were air-dried and passed through a 2 mm mesh. The fractions with grains finer than 2 mm were separated for the fractional composition according to Jackson (Gonet and Cieśla 1988). The objective was to remove humus, carbonates and free iron oxides. Afterwards, the samples were analyzed for the content of specific fractions from 2 mm to 0.05 mm, using the sieving method, while the share of particles less than 0.05 mm large was determined with the pipette method (Soil Survey Investigations 1996).

Beside the above analysis of soil fractions, the samples were checked for active acidity in H_2O and exchangeable acidity in 1M KCl. Potentiometric assays were performed with a CPC-551 pHmeter (Lityński et al. 1976), concentrations of organic carbon were assessed with the use of a Dutch SKALAR TOC, and the $CaCO_3$ content was determined with the volumetric method according to Schleiber (Lityński et al. 1976). The total zinc concentrations were assayed after mineralization of samples in a mixture of HF and $HClO_4$, as described by Crock and Severson (1980), while the Zn forms easily available and extractable with DTPA were determined according to Lindsay and Norvell (1978). The zinc total content and mobile forms were assayed with atom absorption spectroscopy on a Philips PU 9100X spectrometer. The reliability of the results was attested against SV-M certified material. Additionally, control samples were run, which were analyzed in the same way as the investigated samples. All the determinations were made in triplicates. The results are presented as arithmetic means. Possible correlations between the results were checked with the aid of Statistica software. A correlation matrix of the analyzed factors was based on the Pearson's correlation coefficients using $p< 0.05$ to indicate the 95% probability levels.

RESULTS AND DISCUSSION

Basic physicochemical properties of the soil samples are presented in Table 1. As evidenced by the data, the soils had neutral or alkaline reaction. Thus, pH values expressed in pH/H_2O ranged between 7.30 and 7.87, while those determined in 1M KCl varied from 6.88 to 7.48. The measured values were influenced by high amounts of $CaCO_3$, which made up as much as 76.1% of the material in the limnic horizons. High concentrations of calcium carbonate were shown in the ploughable humus horizons, which contained on average 22.0% of this compound. The above accumulation of carbonates could have been caused by the capillary rise of groundwater saturated with calcium ions to the topsoil. Another possible reason was frequent application

Table 1

Some physicochemical properties of the soils

Profile no	Horizon	pH		Corg	$CaCO_3$	Fraction < 0.002 mm
		H_2O	1M KCl	(g kg^{-1})	(%)	
I	Ap	7.67	7.03	55.8	16.2	48.3
	Ld	7.76	7.12	77.3	29.8	70.6
	Lc	7.51	6.88	195.2	36.7	71.4
	Lm1	7.86	7.24	30.6	59.5	70.6
	Lm2	7.86	7.39	9.5	9.8	61.2
II	Ap	7.59	7.13	63.9	21.8	63.9
	Ah	7.68	7.15	65.9	27.2	60.2
	Ld	7.64	7.17	63.9	40.2	81.5
	Lm1	7.59	7.2	24.4	68.4	47.6
	Lm2	7.63	7.14	59.8	46.0	78.3
	Lc	7.64	7.16	112.9	56.5	44.1
	Lm3	7.65	7.2	14.7	51.6	35.5
III	Ap	7.84	7.41	60.9	24.4	40.5
	Ah1	7.57	7.27	65.7	25.6	38.7
	Ah2	7.26	6.96	63.1	5.3	11.4
	Cr	7.61	7.09	65.8	2.4	6.8
	Ld1	7.68	7.14	7.9	8.5	35.0
	Ld2	7.75	7.30	9.7	39.8	29.9
IV	Ap	7.60	7.30	59.4	26.5	51.0
	Ah	7.68	7.27	19.7	6.7	18.3
	Cr	7.87	7.26	4.9	4.5	32.9
	Cr	7.79	7.16	5.3	0.6	31.9
	Ld	7.86	7.41	1.4	12.1	42.7
V	Ap	7.60	7.33	80.3	23.6	60.0
	Ah	7.53	7.26	78.0	24.9	64.5
	Ld	7.53	7.21	65.0	39.1	57.2
	Lc	7.67	7.4	46.5	48.7	66.0
	Lm	7.57	7.48	14.4	76.1	61.9
VI	Ap	7.38	7.20	50.2	15.9	66.5
	Ah	7.37	7.30	5.1	69.3	29.3
	Ld1	7.37	7.3	29.0	35.2	651
	Lm	7.42	7.25	2.3	70.1	58.1
	Ld2	7.44	7.30	4.1	20.5	23.0
	Ld3	7.46	7.31	2.8	40.6	19.4
	Ld4	7.46	7.35	2.2	37.5	16.2

of agronomic measures, especially ploughing. The amount of organic carbon ranged from 1.4 g kg^{-1} to 195.2 g kg^{-1}. Different concentrations of organic carbon were probably caused by the location of the sampling site in the profile, which can be considered typical for multilayer systems of alluvial soils. Accumulation of organic matter is most strongly determined by the type and kind of soils, although the differentiation of this parameter is also attributable to different land use (PRANAGAL 2004, SKŁODOWSKI 1994).

Our analyses of the fractional composition of the samples revealed that the content of clay particles (∅ < 0,002 mm) ranged from 6.8 to 81.5%. According to the WRB (2006), the analyzed samples were rich in clay (c) and silty clay (ipł). High concentrations of clay, such as found in the examined soils, confirm that stagnant waters create conditions suitable for sedimentation (MYŚLIŃSKA 2001). In turn, a high share of sands could indicate an intensive activity of running water and/or streams from melting continental glaciers. Such runoff could wash off finer fractions from surface clay horizons.

The soils analyzed herein did not manifest symptoms of zinc pollution, with the Zn total concentration between 6.25 and 18.78 mg kg^{-1}, and the average content 11.16 mg kg^{-1} (Table 2). Similar values were determined by JAWORSKA and DĄBKOWSKA-NASKRĘT (2012) in agricultural luvisols. According to TERELAK et al. (2000), an average zinc concentration in soils is 23.3 mg kg^{-1} in the Province of Kujawy and Pomorze, and 32.4 mg kg^{-1} in whole Poland. The highest concentrations of zinc were found in the humus horizons enriched with organic matter. KABATA-PENDIAS and PENDIAS (2001) confirmed that organic matter has a distinct ability to bind zinc. Our statistical analysis of the results revealed a relationship between the content of Zn ions and organic matter ($r = 0.408$ $p < 0.05$) – Figure 1. The presence of zinc in soils is mainly connected with its concentration in the parent rock and their abundance in iron and clay minerals (CZARNOWSKA 1984, 1996). Alluvial soils are those of the highest changeability of zinc concentrations, both spatial and the profile one. As for alluvial soils in the Lower Vistula Valley, their content of zinc is mostly dependent on the site (distance from the main stream) and fractional composition. KOBIERSKI and PIOTROWSKA (2010) reported that alluvial soils of the Unisław Basin located 50, 200, 600 and 900 m away from the Vistula riverbed and containing from 7 to 31% of clay fraction had between 30.1 and 228.5 mg kg^{-1} of total zinc. Flood sedimentation of fine material brought by the river caused a significant reduction of impurities transported to the estuary. This is why periodically flooded lowlands are reservoirs of collected suspensions and their heavy metals (TAYLOR 1996). Moreover, specific genesis of soils developed from alluvial sediments (sedimentation processes) affects the profile differentiation of those metals (DĄBKOWSKA-NASKRĘT 1994, DĄBKOWSKA-NASKRĘT, KĘDZIA 1996, ORZECHOWSKI, SMÓLCZYŃSKI 2010).

The region of the Unisław Basin has long-lasting agricultural tradition. The soils in this area present high production potential and are intensively cultivated, which changes their properties considerably. Fertilizers and plant protection preparations can raise the levels of heavy metals in such soils.

Table 2

Total content and DTPA-extractable forms of zinc in the soil samples

Profile no	Horizon	Total content	Extractable (DTPA)	DTPA / Total content
		(mg kg^{-1})		(%)
I	Ap	14.48	2.3	15.88
	Ld	9.76	0.66	6.72
	Lc	12.25	0.98	8.00
	Lm1	11.23	0.68	6.06
	Lm2	8.68	0.58	6.68
II	Ap	15.13	2.29	15.14
	Ah	15.83	1.68	10.61
	Ld	9.68	0.85	8.78
	Lm1	10.13	0.72	7.10
	Lm2	10.30	0.67	6.50
	Lc	11.38	1.03	9.06
	Lm3	12.00	0.64	5.33
III	Ap	18.58	1.92	10.33
	Ah1	18.78	2.40	12.78
	Ah2	11.78	1.41	11.97
	Cr	6.25	0.70	11.20
	Ld1	9.75	0.51	5.23
	Ld2	7.98	0.48	6.02
IV	Ap	12.63	1.88	14.89
	Ah	7.33	0.58	7.91
	Cr	9.15	0.50	5.46
	Cr	7.43	0.61	8.21
	Ld	7.85	0.45	5.73
V	Ap	14.23	2.60	18.27
	Ah	11.75	2.36	20.09
	Ld	11.03	1.52	13.78
	Lc	11.88	0.96	8.08
	Lm	12.05	0.32	2.56
VI	Ap	16.68	1.80	10.79
	Ah	9.90	0.54	5.45
	Ld1	9.95	0.52	5.23
	Lm	6.65	0.52	7.82
	Ld2	9.93	0.56	5.64
	Ld3	12.98	0.36	2.77
	Ld4	6.53	0.44	6.73

The determined profile spatiation of zinc in the examined soils showed some accumulation of Zn ions in the surface horizons, which probably reflects the anthropogenic changes in that region. The surface horizons contained the highest amounts of total zinc, which were decreasing down the profile.

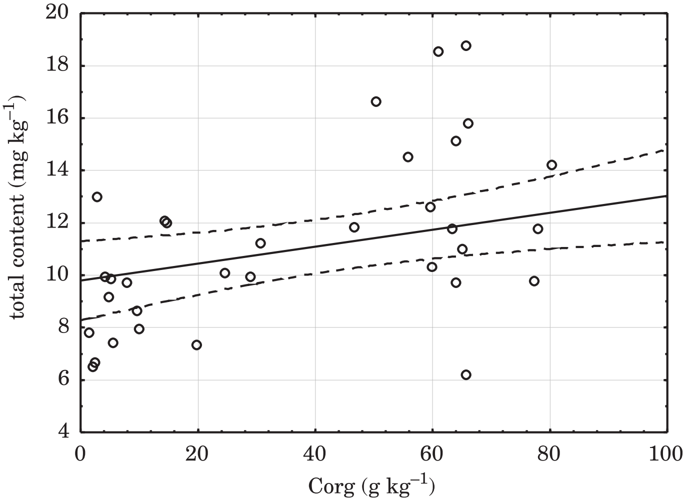

Fig.1 Correlation between the content of total zinc and organic carbon

A similar observation was made by Dąbkowska-Naskręt (1994) in her study on arable alluvial soils.

Extraction with diethynene triaminopentaacetic acid (DTPA) was used in order to evaluate the mobility of zinc forms available for plants. Those compounds first accumulate in the humus horizons of many types of soils (Niemyska-Łukaszuk, Ciarkowska 1999). Zinc is perceived as a mobile element, whose availability and mobility are influenced by a number of factors, such as the content of organic matter, concentration of iron compounds, pH and fractional composition of the soil (Rogóż, Grudnik 2004). The soil reaction is seen as one of the most important factors affecting zinc availability (Kwiatkowska-Malina, Maciejewska 2011). The analyzed soils samples contained from 0.32 to 2.6 mg kg^{-1} of zinc extractable with DTPA, while the corresponding average for all the samples was 1.05 mg kg^{-1}. The share of available forms in the total content of zinc was 2.56%–20.09% (Table 2). The highest amounts of available zinc were found in the surface horizons of the soils and in the horizons rich in organic carbon. A positive and significant correlation was noted between the zinc forms extractable with DTPA and concentrations of organic carbon in the analyzed soil samples ($r = 0.505$, $p<0.05$) – Figure 2. A change in soil reaction considerably influenced the concentration and mobility of Zn ions. Higher soil reaction was accompanied by a decrease in the zinc content because of its more intensive binding by iron and aluminum oxides and/or precipitation to less soluble forms. Alkaline reaction of the samples could have decreased concentrations of available zinc, down to the lower threshold of its content in soils with the particle-size distribution typical for clays (Kabata-Pendias, Pendias 2001).

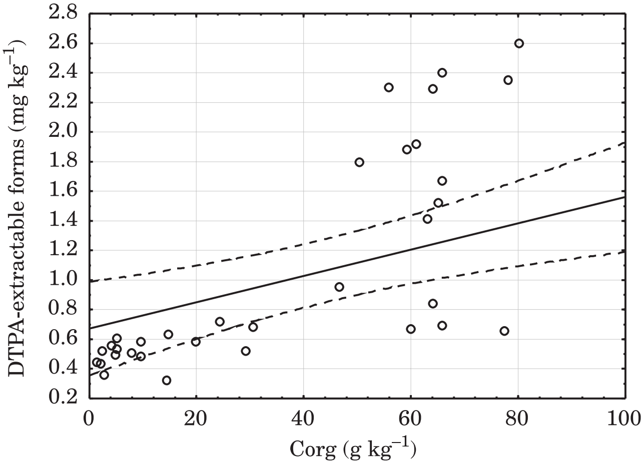

Fig. 2 Correlation between DTPA-extractable forms of zinc and organic carbon

CONCLUSIONS

1. The analyzed soils did not show symptoms of zinc pollution. Its total concentration ranged from 6.25 to 18.78 mg kg^{-1}.

2. The highest concentrations of total and available zinc were noted in the surface horizons of the soils and in the horizons enriched with organic carbon.

3. The profile distribution of zinc in the soils reflected the accumulation of this ion in the surface horizons, which can be a result of anthropogenic changes in that region.

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PHYSICOCHEMICAL PROPERTIES OF SURFACE SOIL LAYER AFTER THE FLOOD IN THE MIDDLE VISTULA RIVER VALLEY

Wiesław Bednarek[1], Sławomir Dresler[2], Przemysław Tkaczyk[3], Agnieszka Hanaka[2]

[1]Chair of Agricultural and Environmental Chemistry
University of Life Sciences
[2]Department of Plant Physiology
Maria Curie-Skłodowska University, Lublin, Poland
[3]Regional Chemical-Agricultural Station Lublin, Poland

Abstract

Environmental studies conducted after the 2010 flood in the middle Vistula River Valley focused on assessment of the physicochemical properties of soil (0-20 cm) sampled from horticultural plantations (2493 samples). Soil pH_{KCl} as well as the content of available P, K and Mg forms were determined. Selected samples (48) were analysed for the content of heavy metals (Cu, Zn, Cd, Pb, Cr, Ni, As, Hg), mineral N, $S\text{-}SO_4$, salinity and dry mass. The tested soil pH ranged from acid to neutral; the phosphorus content was in the average range, while the content of available potassium was 161.0 mg K kg^{-1} and that of available magnesium exhibited the value of 160.5 mg Mg kg^{-1}. The mean value of pH_{KCl} and available P were not elevated in the flooded soils versus the same soils before the flood, while the available K and Mg were higher. The content of mineral nitrogen and sulphate sulphur as well as the salinity level were only slightly dependent on the soil agronomic category and soil pH. The content of heavy metals in the soil was higher than before the flood, but did not exceed natural values. The analyses did not demonstrate any significant deterioration of the physicochemical parameters of soils after the 2010 flood, which could negatively affect the quality and yield of cultivated fruit trees and shrubs.

Keywords: flood, soil physicochemical properties, soil agronomic category, heavy metals, macroelements.

WŁAŚCIWOŚCI FIZYKOCHEMICZNE POWIERZCHNIOWEJ WARSTWY GLEBY PO POWODZI W DOLINIE ŚRODKOWEJ WISŁY

Abstrakt

W badaniach środowiskowych przeprowadzonych po powodzi, która wystąpiła w 2010 r. w Dolinie Środkowej Wisły, oceniano właściwości fizykochemiczne gleb (0-20 cm) pobranych

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prof. dr hab. Wiesław Bednarek, Chair of Agricultural and Environmental Chemistry, University of Life Sciences, Akademicka 15, 20-033 Lublin, Poland, e-mail: wieslaw.bednarek@up.lublin.pl

spod plantacji sadowniczych (2493 próbki). Oznaczono w nich pH_{KCl} oraz zawartość przyswajalnych form P, K i Mg. W wybranych 48 próbkach oznaczono dodatkowo zawartość metali ciężkich (Cu, Zn, Cd, Pb, Cr, Ni, As, Hg), N-mineralny, $S\text{-}SO_4$, zasolenie i suchą masę. Odczyn badanych gleb mieścił się w zakresie od kwaśnego do obojętnego, zasobność fosforu przyswajalnego była przede wszystkim średnia, zawartość potasu przyswajalnego wynosiła 161.0 mg K kg^{-1}, magnezu przyswajalnego 160.5 mg Mg kg^{-1}. W glebach po powodzi, w porównaniu z glebami sprzed tego zdarzenia, nie stwierdzono podwyższonego pH_{KCl} i zawartości P-przyswajalnego, ale odnotowano wyższą zawartość K i Mg przyswajalnego. Zawartość azotu mineralnego, siarki siarczanowej i zasolenia w niewielkim stopniu zależała od kategorii agronomicznej i odczynu gleby. Zawartość metali ciężkich w glebie była wyższa niż przed powodzią, ale nie przekroczyła zawartości naturalnych. Wykazano, że po powodzi z 2010 r. nie stwierdzono znaczącego pogorszenia właściwości fizykochemicznych gleb, które mogłyby negatywnie oddziaływać na jakość i plonowanie uprawianych roślin, w tym drzew i krzewów owocowych.

Słowa kluczowe: powódź, właściwości fizykochemiczne gleby, kategoria agronomiczna gleby, metale ciężkie, makroelementy.

INTRODUCTION

Floods occur in various parts of the world, including Poland, causing substantial economic losses and contributing to changes in various environmental elements, e.g. in soil (EULENSTEIN et al. 1998, KUCHARZEWSKI, NOWAK 2000, NAGEL et al. 2003, ŠERÁ et al. 2008).

Heavy rainfalls on May 14-18 and June 1-2, 2010 in the upper Vistula River basin resulted in two flood waves, which caused substantial damage in the agricultural environment of the Lublin Province (BEDNAREK et al. 2011). The first wave in the Lublin Province flooded 23 villages and approximately 90% of the Wilków Commune. Admittedly, May 2010 was extremely wet across all the country. The highest level of monthly precipitation was reported in Bielsko-Biała (511.5 mm; 509% of the station's norm) and in Kraków (302.4 mm; 130.8% of the long-term norm) (*Bulletin* ... 2010*a*). In June that year, the highest monthly precipitation level was recorded in Nowy Sącz (193.9 mm; 186,1% of the norm) and Tarnów (179.4 mm; 172.5% of the norm). In Warsaw, the monthly rainfall sum was 86.8 mm (121.9% of the long-term norm) (*Bulletin* ... 2010*b*). The flood caused considerable loss in agriculture and horticulture (EULENSTEIN et al. 1998, KUCHARZEWSKI, NOWAK 2000). The largest damage to horticultural plantations was reported from the Sandomierz Valley and Wilków in the Lublin Region. In communes situated in the Vistula valley near Sandomierz, orchards and blueberry plantations were flooded up to the height of 1.5 to 4 m twice or thrice in several weeks. In Wilków Commune, floodwater covered 1400 ha of horticultural plantations for several weeks. Apple orchards, which constitute 70% of horticultural plantations, prevailed in all the flooded regions (ZIELIŃSKI 1997).

The aim of the study was to assess physicochemical properties of soils sampled from horticultural areas in the middle Vistula River valley in the Lublin Region after the 2010 flood.

MATERIAL AND METHODS

In July and August 2010, soil (0-20 cm) was sampled from orchards flooded in May and June that year. Alluvia deposited during the flooding were sampled together with the soil. In total, 2493 samples were taken, most from the communes of Wilków (1365) and Łaziska (951). 121 samples were taken in and around Józefów nad Wisłą, 32 in Janowiec, 20 in Puławy and 4 in Opole Lubelskie. About 47% of the sampled soils belonged to the agronomic category composed of medium-heavy soils (containing 20-35% of particles with diameter <0.02 mm), 40% were heavy soils (>35% of particles with diameter <0.02 mm), 10% – light soils (10-20% of particles with diameter <0.02 mm), 2% – very light soils (up to 10% of particles with diameter <0.02 mm), and 1% represented organic soils. The number of the samples depended on the surface area that had been flooded and presence of orchards in a given region. Some basic soil properties were determined, i.e. the agronomic category, pH_{KCl}, available forms of phosphorus, potassium, and magnesium; also, the K:Mg ratio was calculated for the sake of rational soil fertilisation with these chemical elements (Sadowski et al. 1990). In order to assess more thoroughly the effect of flooding on soil properties, some samples were additionally analysed for dry mass, mineral nitrogen, $S\text{-}SO_4$, salinity as well as the levels of copper, zinc, cadmium, lead, chromium, nickel, arsenic and mercury. These additional chemical analyses were performed on soil samples from orchards located in the following villages: Wilków – 3, Las Dębowy – 4, Braciejowice – 4, Dobre – 1, Zastów Karczmiska – 2, Niedźwiada Duża – 1, Zagłoba – 9, Kąty – 5, Zakrzów – 9, Kępa Solecka – 6, Machów – 1, Kępa Chotecka – 1, Kol. Wilków – 1, and Zastów Polanowski – 1. In total, additional analyses were performed on 48 soil samples, which had been taken according to the criterion of an additional environmental risk caused by flooding, e.g. stores of mineral and natural fertilizers or pesticides. The chemical analyses were carried out in an accredited laboratory of the Regional Chemical-Agricultural Station in Lublin. The following were determined: soil agronomic category, pH in 1 mol KCl dm^{-3}, phosphorus and potassium with the Egner-Riehm method (DL), magnesium with the ASA method after extraction from soil with 0.0125 mol $CaCl_2$ dm^{-3}, mineral nitrogen in its nitrate and ammonium forms determined colorimetrically (Dresler et al. 2011), sulphate sulphur with the nephelometric method, salinity, dry mass and trace elements with the ASA method after mineralization in a mixture of concentrated HCl and HNO_3 (3:1) acids (*A catalogue of methods* ... 2011). The results were analysed statistically, employing one-way non-orthogonal analysis of variance, with the Tukey's confidence semi-intervals ($p = 0.05$). Moreover, correlations between some soil properties were calculated. Since pH_{KCl} showed a nearly normal distribution, arithmetic means of this index were calculated (Gruba et al. 2010). For statistical calculations and graphic representation of the correlations between the agronomic soil

Table 1

Soil pH, content of available macroelements and the K:Mg ratio in soil (0-20 cm)

Region of soil sampling	_n_	pH_{KCl}	P	K	Mg	K:Mg	Agronomic category
			(mg kg^{-1})				
Józefów	121	6.6ns**	71.7a*	177.2_ab_	78.8_e_	3.13_c_	2.25_d_
Janowiec	32	6.7ns	63.2_ab_	135.1_ab_	173.8_abcd_	0.87_ab_	2.88_c_
Łaziska	951	5.6ns	57.5_b_	183.6_b_	171.0_cd_	1.21_b_	3.22_a_
Opole Lub.	4	5.4ns	50.0_ab_	179.8_ab_	216.3_ab_	0.85_ab_	3.75_ab_
Puławy	20	5.7ns	47.6_ab_	125.5_ab_	157.4_ab_	0.99_ab_	3.10_abc_
Wilków	1365	6.0ns	66.0_a_	164.8_a_	165.4_ac_	1.07_a_	3.40_b_
Total	2493	6.0ns	59.3	161.0	160.5	1.35	3.1

_n_ – sample number, * – homogeneous groups, ** – not significant

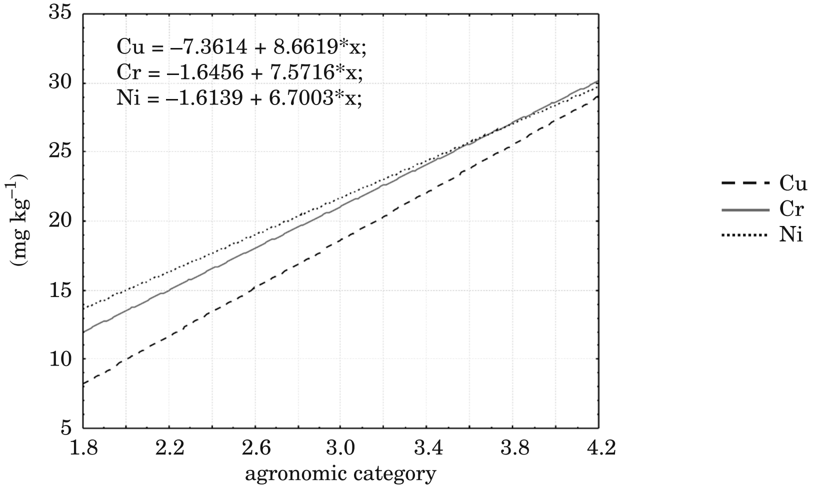

Fig. 1. Correlation between the content of Cu, Cr and Ni in soil versus the agronomic category

category and soil content of Cu, Cr and Ni, very light soils (very coarse textured) were assigned value 1, light – 2, medium – 3 and heavy ones – 4 (Table 1, Figure 1).

RESULTS AND DISCUSSION

The soil pH in the studied area was varied: from acidic (pH_{KCl} 5.5), slightly acidic (pH_{KCl} 5.6-6.0) to neutral (pH_{KCl} 6.6-6.7) – Table 1. Organic and very light soils had the highest acidity with the pH value of 5.1 and 5.2, respectively. The pH value in light, medium, and heavy soils was slightly

Table 2

Soil pH, content of available macroelements and the K:Mg ratio in soil (0-20 cm) in relation to the agronomic category

Agronomic category	n	pH_{KCl}	P	K	Mg	K:Mg
			(mg kg^{-1})			
Heavy	994	5.9ns**	54.2*b**	161.4*a*	190.3*d*	0.88*a*
Light	255	5.9ns	71.1*a*	166.2*ab*	92.3*b*	2.25*c*
Medium	1173	5.9ns	68.7*a*	184.5*b*	160.4*c*	1.22*b*
Very light	45	5.2ns	51.2*ab*	107.2*c*	48.4*a*	2.64*d*
Organic soil	26	5.1ns	262.4*c*	161.0*abc*	338.4*e*	0.53*a*
Total	2493	5.6ns	64.9	171.7	165.2	1.50

n – sample number, * – homogeneous groups, ** – not significant

acidic, i.e. 5.9 (Table 2). After the flood in the environs of Wrocław, the average increase in soil acidity was from pH 5.6 to pH 5.1 (KUCHARZEWSKI, NOWAK 2000). On the German side of the Odra River, the pH declined on average from 6.2 to 5.9 (EULENSTEIN et al. 1998). A possible cause of the pH decrease may have been the anaerobic fermentation processes induced by soil waterlogging (EULENSTEIN et al. 1998). In turn, investigations conducted in flooded areas in the Czech Republic revealed more neutral values in most samples than those found before the flood (ŠERÁ et al. 2008); interestingly, the pH changes did not exert unfavourable effects on the riparian ecosystem. In 2007, an assessment of floodplain soils in the middle Elbe River area showed that their pH ranged between 4.3 and 7.9 (ZIMMER et al. 2011). In a study on flooded rice soils by FAGERIA et al. (2011), it was found that the pH of acidic soils increased and that of alkaline soils declined. Soil sampled from a Mexican mangrove forest analysed in 2009 was characterized by a high pH (CERÓN-BRETÓN et al. 2011). Areas that had been flooded more frequently and intensively contained more sulphur. Investigations carried out in three locations in a floodplain of the mouth of the Dovey River in Wales, the UK, showed that a 2.3- to 3.5-fold increase in precipitation was accompanied by an increase in soil salinity and pH. Depending on the location, the salinity gradient was significant, reaching a 13-fold difference. A similar gradient was found for soil pH (DAUSSE et al. 2012). One should take into consideration the fact that soil pH may control mobility of heavy metals, which grows with a declining pH (NAGEL et al. 2003). Furthermore, the investigations presented in the aforementioned paper provide evidence that the content of easily soluble heavy metals in calcareous soils is highly dependent on pH.

In the majority of soils, the content of available phosphorus was on a moderate level relative to the limit values, being high in only one location (Table 1). It was significantly higher in the soils from Józefów and Wilków, than in the soil from Łaziska, where it was significantly lower than elsewhere. The significance of the differences may have been related to the amount of phosphorus fertilizers applied and the number of samples taken (Table 1).

In very light and heavy soils, the content of this element was moderate, in light and medium soils – high, and in organic soils – very high (Table 2). In organic soils, its content was significantly higher than in mineral soils, and in light and medium soils, it was higher than in heavy soils.

The content of available potassium in the soils was fairly varied and ranged from 125.5 to 183.6 mg K kg^{-1} (Table 1). Its content was high in very light, light and medium soils, and moderate in heavy soils (Table 2). The content of available magnesium in the soils sampled from the particular regions was even more diverse. The lowest amount of this element was detected in the soils sampled from Józefów (78.8), and the highest appeared in the soils taken from Opole Lubelskie (216.3 mg Mg kg^{-1}). The content of this element in very light soils was high, and very high in light, medium and heavy soils. The broadest K:Mg ratio was found in the soils from Józefów nad Wisłą (3.13). In the soils sampled from the other locations it was close to 1. The narrowest ratio was characteristic for organic soils (0.53), and the broadest one – for very light soils (2.64). The magnitude of these ratios should be regarded as proper, never exceeding the threshold of 3.5 (SADOWSKI et al. 1990). EULENSTEIN et al. (1998) conducted investigations in flooded areas near the Odra River and, in most cases, demonstrated a slight decline in the available P and Mg content as well as an increased level of K in soil. The authors claimed that the increased K content may have been due to the flooding of a fertilizer storage and transfer of solved fertilizers into flood water. The changes in the P, Mg and K content were within the ranges of variation caused by fertilization. Importantly, the flood did not change significantly the nutrient content in the soil.

The analyses performed before the flood (2007-2009, $n = 511$) showed the following means of the soil parameters: pH_{KCl} – 6.0 (5.6-6.5); the content of available phosphorus – 61.6 (49.5-76.5) mg P kg^{-1}, potassium – 137.2 (94.5-210.7) mg K kg^{-1} and magnesium – 85.8 (35.4-120.7) mg Mg kg^{-1}. These results did not differ significantly from the pH_{KCl} values and available phosphorus content after the flood, whereas the levels of available potassium and magnesium, in particular, were higher. Potassium and magnesium may have been relatively easily washed out of soil, or else these elements may have been imported into the studied area with the flood wave.

GĄSIOR et al. (2003) found that long-term persistence of flood waters resulted in a several-fold increase of concentration of the basic element forms, i.e. calcium to 3.7 g kg^{-1}, magnesium to 5.4 g kg^{-1} and potassium to 9.5 g kg^{-1}. CHODAK and PERLAK (1999) found that the sorption complex in soils covered with stagnant flood water for a long time was enriched primarily with Ca^{2+} cations and, to a lesser extent, with Na^{+}, Mg^{2+} and K^{+}, which lead to a slight increase in their sorption capacity and saturation of the sorption complex with alkaline cations. The properties of soils analysed by these authors after the flood showed no significant changes compared with these parameters before the flood. In another study, CHODAK et al. (1999) found a high content

Table 3

Some soil properties (0-20 cm) in relation to the agronomic category

Agronomic category	pH_{KCl}	N_{min}	d.m.	$S\text{-}SO_4$	Salinity
		(mg kg^{-1})	(%)	(mg kg^{-1})	(mg NaCl dm^{-3})
Light	7.02ns*	20.6ns	88.8ns	1.67ns	0.24ns
Medium	6.29ns	32.1ns	89.6ns	1.72ns	0.27ns
Heavy	6.42ns	31.8ns	87.6ns	2.18ns	0.28ns

* – not significant

Table 4

Some soil properties (0-20 cm) in relation to soil pH

Soil pH	N_{min}	d.m.	$S\text{-}SO_4$	Salinity
	(mg N kg^{-1})	(%)	(mg kg^{-1})	(mg NaCl dm^{-3})
Acidic	41.2ns**	92.3*a**	1.22*a*	0.28ns
Slightly acidic	28.8ns	89.5*a*	1.80*ab*	0.30ns
Neutral	34.6ns	85.9*b*	2.39*b*	0.28ns
Alkaline	18.0ns	90.7*a*	1.68*ab*	0.25ns

* – homogeneous groups, ** – not significant

of macroelements and trace elements, particularly Zn and Cr, in alluvia transported by floodwaters. They also determined a neutral or alkaline pH in allotment garden soils. An experiment carried out by LIPIŃSKI and BEDNAREK (1999) in areas flooded in 1997 showed a considerable proportion of soils with a medium, high and very high levels of soluble forms of P, K and Mg, which were distinctly different from the mean values in the same regions. Similarly, FAGERIA et al. (2011) reported an increased content and availability of P and Mg accompanied by a decrease in the concentration and availability of S, Zn, and Cu in lowland rice soils. In another study conducted by BEDNAREK et al. (2011) after the 2010 flood in the middle Vistula River valley, it was found that the pH_{KCl} and the content of available phosphorus, potassium and magnesium in the layer 0-20 cm of arable land and grassland did not basically differ from the values reported before the flood. The soil agronomic category was significantly positively correlated with pH (r_{xy} = 0.465) and the available magnesium content (r_{xy} = 0.564), and negatively correlated with available phosphorus (r_{xy} = -0.730) and the K:Mg ratio (r_{xy} = -0.342). the soil pH was significantly positively correlated with the content of available phosphorus (r_{xy} = 0.315), available potassium (0.195), available magnesium (0.120) and the K:Mg ratio (r_{xy} = 0.140).

The other soil properties were assessed after the additional chemical analyses of 48 samples had been performed. No significant differences in the dry mass, mineral nitrogen, sulphate sulphur content and salinity were found between light, medium and heavy soils (Table 3). No major differences in the soil properties were found in relation to the pH, either (Table 4). In the flooded soils, the mineral N (N_{min}) content was significantly lowered, which was most probably associated with the denitrification process that occurred in the prevalent anaerobic conditions during the flood (EULENSTEIN et al. 1998). The total N content in flooded areas fluctuated, decreasing in most soils (ŠERÁ et al. 2008). In turn, the high content of heavy metals indicates intensive sulphate oxidation and may be the cause rather than the result of a specific pH (NAGEL et al. 2003).

The mean content of copper in the soil sampled in 14 villages was 22.8 mg Cu kg^{-1} d.m., being the lowest in Kępa Solecka (12.4) and the highest in Zastów Polanowski (52.9 mg Cu kg^{-1}). The mean content of zinc in the soils of the studied regions was 69.3 mg Zn kg^{-1}, being the lowest in the soils of Kępa Solecka (47.9 mg), and the highest in Zastów Karczmiska and Niedźwiada Duża (97.4 mg Zn kg^{-1}). A 10 sites, the content of cadmium was 0.27 mg Cd kg^{-1}; the highest level was found in the soil from Braciejowice (0.32 mg Cd kg^{-1}), and the mean content was 0.28 mg Cd kg^{-1}. The mean content of lead in the sampled soils was 12.1 mg Pb kg^{-1}; again, the lowest in Kępa Solecka (7.73 mg Pb kg^{-1}) but the highest in Zastów Karczmiska and Niedźwiada Duża (18.6 mg Pb kg^{-1}). The mean content of chromium was 24.7 mg Cr kg^{-1}, with the lowest level found in Kępa Solecka (13.4), and the highest one in Zastów Karczmiska (55.8 mg Cr kg^{-1}). The mean content of nickel in the analyzed soils was 24.9 mg Ni kg^{-1}, with the lowest result in Kępa Solecka (17.5), and the highest one in Zastów Karczmiska (35.4 mg Ni kg^{-1}). In turn, the mean content of arsenic in the soil samples was 4.09 mg As kg^{-1}, being the lowest levels in Dobre (2.64) and the highest in Zastów Karczmiska and Niedźwiada Duża (7.26 mg As kg^{-1}). The mean content of mercury in the soil samples was 0.046 mg Hg kg^{-1}, with the lowest one in Kępa Solecka (0.026) and the highest – in Niedźwiada Duża (0.069 mg Hg kg^{-1}).

Compared with the investigations carried out in this region by LIPIŃSKI and BEDNAREK (1999) after the 1997 flood, the present study has revealed an increased content of copper, zinc, chromium and nickel in the soil, while the levels of cadmium and lead were similar as before the flood. Notably, even if the levels of Cu, Zn and Ni were higher, they did not exceed the upper limits of natural amounts of these elements in soils (KABATA-PENDIAS et al. 1993, *Regulation* ... 2002). Similarly, CHODAK and PERLAK (1999) found elevated levels of zinc, copper and lead in the alluvial sediments after the 1997 flood. In turn, GĄSIOR et al. (2003) claimed that a flood did not induce a rise in the basic forms of Co, Ni and Cd, irrespective of the soil management practices. Long-term flooding resulted in the elevation of copper, zinc, chromium and manganese levels, although not to the extent that would be detrimental to crop plants. In another study carried out in the village Czernin (Mielec Community), GĄSIOR and PAŚKO (2007) found increased levels of soluble forms of some heavy metals (Cu, Zn, Pb, Cd Ni) following a 30-day long flooding. Simultaneously, they reported that such amounts in flooded soils did not pose a threat to agricultural production. In turn, a comparative study conducted in the Wrocław region before the 1997 flood afterwards, in

Table 5

Content of trace elements in soil (0-20 cm) in relation to the agronomic category

Agronomic category	Cu	Zn	Cd	Pb	Cr	Ni	As	Hg
	(mg kg^{-1})							
Light	11.8*a**	48.6*ab*	0.27ns**	7.36*a*	10.5*a*	13.5*a*	2.28ns	0.023*a*
Medium	18.3*a*	59.9*a*	0.29ns	10.9*a*	21.6*a*	22.0*b*	4.10ns	0.040*a*
Heavy	27.4*b*	78.9*b*	0.28ns	13.5*b*	28.4*b*	28.3*c*	4.21ns	0.054*b*

* – homogeneous groups, ** – not significant

1998, showed that the content of available Mn, Zn, Cu and Fe in the topsoil was considerably changed depending on the soil agronomic category (KUCHARZEWSKI, NOWAK 2000). The content of the two former elements decreased by an average 24%, and the two latter one declined by 13%. There was a general dependence between a higher soil firmness and smaller differences between the content of microelements before and after a flood. However, the content of the analysed microelements was in the typical range of concentrations for arable soils.

The content of cadmium and arsenic in the soil did not depend significantly on the agronomic category, whereas the levels of copper, lead, chromium, nickel and mercury were significantly higher in heavy than in medium and light soils (Table 5). Furthermore, the content of copper, zinc, nickel and arsenic was not significantly correlated with the soil pH, whereas the level of cadmium was significantly higher in alkaline than in the other types of soils. In flooded areas on the German side of the Odra River, the content of 3 elements, i.e. Cd, Cu, and Zn, exceeded the accepted German standards (EULENSTEIN et al. 1998). Additionally, the study revealed an increase in the Sr and Al content accompanied by a decrease in the Mn level in the post-flood areas, compared with regularly flooded land. In an area situated 146 m from the Rhein River, which is flooded on average for 47 days per year, at the mean soil pH 7.8, the biggest differences in the results were obtained for Cd (6.4-fold), Pb (5.1-fold), and Cu (5-fold), while the smallest differences were found for Ni (2.7-fold) and Hg (3-fold) (SCHIPPER et al. 2011). Moreover, a comparison of results from the three studied areas demonstrated that two factors, i.e. distance from the river (146 m versus 262 or 269 m), as well as flooding period (47 days per year as compared with 12), elevated the mean content of all the metals tested, i.e. As, Cd, Cr, Cu, Hg, Pb and Zn, with the exception of Ni (SCHIPPER et al. 2011). Areas in the middle part of the Elbe River that were flooded for a longer time and exposed to more intensive floods exhibited a statistically higher concentration of heavy metals such as Cd, Cu, Hg, Pb and Zn, compared with areas that were less frequently and less intensively flooded (ZIMMER et al. 2011). Furthermore, the concentration of Cd, Cu, Hg, and Zn in the 0-10 cm layer was significantly higher than in the 10-20 cm layer. Presence of lead, chromium and mercury in soils in relation to the soil pH varied most considerably

Table 6

Content of trace elements in soil (0-20 cm) in relation to the soil pH

Soil pH	Cu	Zn	Cd	Pb	Cr	Ni	As	Hg
	(mg kg^{-1})							
Acidic	20.1ns**	68.6ns	0.28a*	12.8ab	30.0a	23.5ns	4.92ns	0.051ab
Slightly acidic	23.1ns	78.1ns	0.27a	13.0b	28.0a	26.9ns	4.40ns	0.052b
Neutral	23.2ns	63.0ns	0.27a	10.8a	20.8b	23.6ns	3.76ns	0.041a
Alkaline	23.1ns	67.5ns	0.35b	13.2ab	23.6ab	25.5ns	3.48ns	0.047ab

* – homogeneous groups, ** – not significant

Table 7

Correlation between the trace element content and some soil properties (correlation coefficients, $n = 48, p \leq 0.05$)

Variable	Cu	Zn	Cd	Pb	Cr	Ni	As	Hg
Agronomic category	0.542	0.426	*	0.505	0.451	0.611	*	0.530
pH_{KCl}	*	*	0.309	*	–0.375	*	–0.356	*
$P_{available}$	0.485	*	*	0.303	*	*	0.295	0.292
$K_{available}$	0.418	*	*	*	*	*	*	*
$Mg_{available}$	0.494	0.367	*	0.367	*	0.594	*	0.299
N_{min}	0.487	*	*	0.294	*	0.326	0.293	0.345
d.m.	*	*	*	*	*	*	*	*
$S\text{-}SO_4$	*	*	*	*	*	*	*	*
Salinity	0.434	*	*	*	*	0.306	0.288	0.359

* – non-significant correlation

(Table 6). The biggest differences were found in the mean content of Cd, Cr and Cu between the 3 studied locations, ranging between 2.3- and 3.2-fold values (SCHIPPER et al. 2011).

Comparative analyses of the heavy metal content in post-flood areas on the Polish and German sides of the Odra River demonstrated comparable values within an acceptable range, posing no risk to agricultural production, with the exception of Cd, Cu and Zn (EULENSTEIN et al. 1998). In turn, the investigations carried out by ŠERÁ et al. (2008) showed elevated levels of all analysed heavy metals, i.e. Zn, Pb, Cr, Ni and Cd, with the exception of Hg, in most soil samples. The highest rise in the heavy metal content was reported from the riparian areas in Morava, which was probably caused by contamination imported from the upper part of the river basin. According to the cited authors, an increased content of heavy metals may threaten riparian ecosystems, especially when flood-induced metal mobility in soil profiles in stimulated.

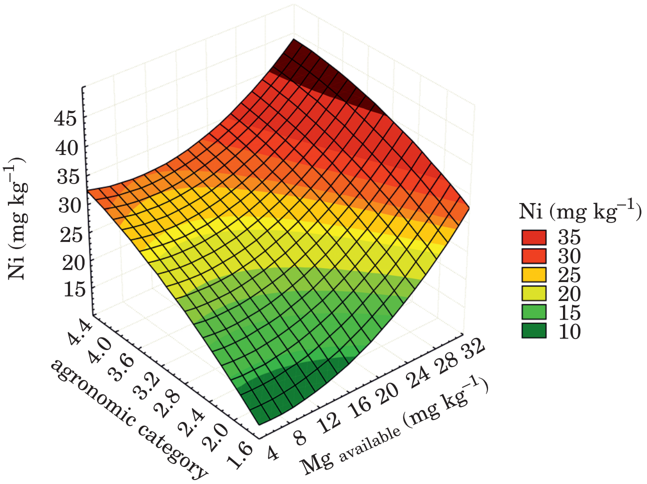

Fig. 2. Correlation between the content of Ni in soil, the agronomic category and available Mg content in soil

In the current research, it was found that the content of copper and mercury in the tested soils were most strongly correlated with the examined properties (agronomic category, pH, the content of available phosphorus, potassium and magnesium, the content of mineral nitrogen, dry mass, sulphate sulphur and salinity). The content of cadmium was the least dependent on these properties. An effect of the agronomic category and the content of available Mg on the Ni content in soil was demonstrated (Table 7, Figs 1, 2). Similarly, in their study of the eastern side of the middle Vistula River valley, Gąsior and Paśko (2007) found that the content of soluble forms of elements in flooded soils was correlated with the proportion of particles with diameter <0.02 mm, pH (Ca, Mg, Fe, Mn, Cu, Pb, Cd, Co and Ni), and humus content (Fe, Pb and Cd).

CONCLUSIONS

After the flood which struck the middle Vistula River valley in 2010, the pH of soils form horticultural plantations was acidic, slightly acidic and neutral, while the content of available phosphorus was primarily moderate. The content of this element was moderate in very light and heavy soils and high in light and medium soils.

The content of available potassium in the soils ranged from 125.5 to 183.6 mg K kg^{-1}. The potassium content was high in very light, light and medium soils, and moderate in heavy soils. The K:Mg ratio was most often close to 1.

The content of available magnesium in orchard soils ranged from 78.8 to 216.3 mg Mg kg^{-1}. The content of this element was high in very light soils, and very high in light, medium and heavy soils.

Compared with soils before the flood, the values of pH_{KCl} and the available phosphorus content were not elevated, unlike the levels of available potassium and magnesium, which had risen.

The content of dry mass, N_{min}, S-SO_4 and salinity was not significantly correlated with the soil agronomical category and pH. The content of trace elements (Cu, Zn, Cd, Pb, Cr, Ni, As, Hg) in the soil remained on a level equal the geochemical background but was slightly higher than before the flood.

The investigations indicated no considerable deterioration of the physicochemical soil properties after the 2010 flood, which could affect negatively yield volumes and quality of cultivated plants, including fruit trees and shrubs.

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COPPER AND OTHER TRACE ELEMENTS IN SEDIMENTS OF LAKES NEAR KONIN (POLAND)*

Izabela Bojakowska, Joanna Krasuska

Państwowy Instytut Geologiczny-Państwowy Instytut Badawczy

Abstract

High concentrations heavy metals accumulate in sediments of some lakes, possibly excerting adverse effects on lake-dwelling organisms. Sediment samples were collected from the surface layer of the profundal zone in 14 lakes located near Konin, in the southern part of the Gniezno Lake District, in order to determine the content of Ag, As, Ba, Cd, Co, Cr, Cu, Hg, Ni, Pb, Sr, V, Zn, Ca, Fe, Mg, Mn, P, S and TOC. Significant differences in the content of trace elements in sediments of the lakes were observed. A very high copper content was found in sediment of the lakes included in the cooling system of the Konin-Pątnów power plant, esepecially in comparison with its content in sediments of the lakes outside that system. The lake sediment samples from lakes within the power plant cooling system are also characterized by higher levels of barium, mercury, manganese, strontium and zinc, but lower concentrations of lead, sulfur and organic matter. In the sediments of lakes within the power plant cooling system, the geometric means of the content of elements were as follows: 385 mg kg^{-1} Cu, 309 mg kg^{-1} Ba, 20 mg kg^{-1} Pb, 613 mg kg^{-1} Sr, 129 mg kg^{-1} Zn and 0.152 mg kg^{-1} Hg; and in sediments of the lakes whose water was not used by the power plant cooling system: 14 mg kg^{-1} Cu, 93 mg kg^{-1} Ba, 33 mg kg^{-1} Pb, 193 mg kg^{-1} Sr and 63 mg kg^{-1} Zn. The copper pollution of sediments of the lakes Licheńskie, Gosławskie, Pątnowskie, Wąsowsko-Mikorzyńskie and Ślesińskie is most likely created by passing the lake water through the Konin-Pątnów power plant cooling system. Copper brought to the lakes in water discharged from the cooling system probably originates from corroded heat exchangers made of copper or copper alloys.

Keywords: lake sediments, trace elements, corrosion, power plant cooling water.

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prof. dr hab. Izabela Bojakowska, Państwowy Instytut Geologiczny-Państwowy Instytut Badawczy, e-mail: izabela.bojakowska@pig.gov.pl

* This paper contains results of monitoring carried out under the National Environmental Monitoring System (Państwowy Monitoring Środowiska – PMŚ) to control current levels of potentially harmful elements and persistent organic pollutants in sediments of rivers and lakes, as well as their changes over time.

MIEDŹ I INNE PIERWIASTKI ŚLADOWE W OSADACH JEZIOR W REJONIE KONINA (POLSKA)

Abstrakt

Wysokie stężenia metali ciężkich akumulowanych w osadach niektórych jezior mogą szkodliwe oddziaływać na organizmy bytujące w tych jeziorach. Ze strefy profundalnej 14 jezior położonych w rejonie Konina, w południowej części Pojezierza Gnieźnieńskiego, pobrano próbki 5 cm powierzchniowej warstwy osadów. W próbkach określono zawartość Ag, As, Ba, Cd, Co, Cr, Cu, Hg, Ni, Pb, Sr, V i Zn oraz Ca, Fe, Mg, Mn, P, S i TOC. Zaobserwowano duże zróżnicowanie w zawartości pierwiastków śladowych w osadach jeziornych. W osadach jezior, których wody wykorzystywane są w systemie chłodzącym elektrowni Konin-Pątnów, stwierdzono bardzo wysoką zawartość miedzi w porównaniu z jej zawartością w osadach jezior, których wody nie są wykorzystywane przez elektrownię. W osadach jezior, których wody włączono w system chłodzący, zawierają również więcej baru, rtęci, manganu, strontu i cynku, a jednocześnie mniej ołowiu, siarki i materii organicznej. W osadach jezior antropogenicznie zmienionych średnie geometryczne zawartości wynosiły: 385 mg kg^{-1} Cu, 309 mg kg^{-1} Ba, 20 mg kg^{-1} Pb, 613 mg kg^{-1} Sr, 129 mg kg^{-1} Zn i 0,152 mg kg^{-1} Hg, a w osadach jezior antropogenicznie niezmienionych: 14 mg kg^{-1} Cu, 93 mg kg^{-1} Ba, 33 mg kg^{-1} Pb, 193 mg kg^{-1} Sr, 63 mg kg^{-1} Zn i 0,084 mg kg^{-1} Hg. Na zanieczyszczenie miedzią osadów jezior Licheńskiego, Gosławskiego, Pątnowskiego, Wąsowsko-Mikorzyńskiego oraz Ślesińskiego ma najprawdopodobniej wpływ wykorzystywanie wody jezior w systemie chłodzącym elektrowni Konin-Pątnów. Miedź wnoszona do jezior przez wody wykorzystane w obiegu chłodzącym elektrowni najprawdopodobniej pochodzi z korozji wymienników ciepła wykonanych z miedzi lub jej stopów.

Słowa kluczowe: osady jeziorne, pierwiastki śladowe, korozja, elektrowniane wody chłodnicze

INTRODUCTION

Under natural conditions, lake benthic sediments build up by accumulation of material derived from erosion and weathering of rocks in the catchment area (including grains of quartz, feldspars, carbonate minerals and clay minerals) and through on-site sedimentation (remains of dead plants and animals as well as substances that precipitate from lake water, such as carbonates, phosphates, sulfides, hydrous iron and aluminum hydroxides). Concentrations of trace elements in sediments mainly depend on properties of geological formations occurring in the catchment area of a lake. The content of elements in lake sediments is also affected by morphological properties, such as the size of the catchment area, the surface area and depth of a given lake and the shape of the lake basin. In industrial, urbanized and agricultural areas, lake sediments are contaminated by heavy metals contained in wastewater which is sometimes discharged to surface waters. An increase in the concentration of heavy metals in lake sediments is often caused by their deposition from the atmosphere as well as from rain and snowmelt runoff from urbanized and agricultural areas (Birch et al. 2001, Lindstrom, 2001, Mecray et al. 2001, Rocher et al. 2004, Reiss et al. 2004, Wildi et al. 2004). Heavy metals and other hazardous substances found in lake sediments may accumulate in the trophic chain up to a level which is toxic to organisms, especially predators. Such elements and compounds may also be harmful to humans (Šmejkalová et al. 2003, Vink 2009). Moreover, sediments with high amounts of hazdrous constituents are a potential source of environmental contamination.

Copper is an essential element in many organisms because of its involvement in the carbohydrate metabolism and in functions of several enzymes. However, excessive concentrations of copper can be toxic. Besides, copper is one of the most toxic metals in an aquatic environment (Beaumont et al. 2000, Dethloff et al. 2001), with algae being particularly sensitive to this metal. Regarding fish, high copper concentrations cause gill damage and disturbances in the transport and excretion of sodium and potassium chlorides as well as Na+/K+-ATPase inhibition (Cerqueira, Fernandes 2002, Grosell et al. 2002, 2004, Takasusuki et al. 2004, De Boeck et al. 2007, Sorour, Harbey 2012). Copper, known to man for over 6000 years, is widely used in different industries, e.g. its alloys can be found in many tools, brake discs, heat exchangers, jewellery, electrical systems; copper is also a component of algaecides, fungicides and molluscicides. It is released to the environment from many sources, including coal combustion, copper ore processing, copper processing in metallurgy, transport, agriculture (microfertilizer, pesticides, feed additive, control of algae and pathogens in fish breeding ponds). Due to the harmful effects of copper to aquatic organisms, its permissible level in sediments has been set at 149 mg kg^{-1} (MacDonald et al. 2000. Alkalinity, hardness and pH of water strongly influence the speciation of copper in sediments and consequently its availability to aquatic organisms (Carvalho, Fernandes 2006). In the hypergene zone, copper is mobile under aerobic conditions, especially in an acidic environment. However, it is readily captured by sediment components, mainly organic matter and hydrated iron oxides.

MATERIAL AND METHODS

The Gniezno Lake District is located in the eastern part of the Great Poland Lake District, between the Warta River valley and Gopło Lake. Its relief was shaped by the Vistulian Glaciation. Among the lakes, N-S and NE-SW-oriented ribbon lakes are dominant. All Pleistocene and Holocene deposits (tills and ice-dammed lake sediments, clays, sands, muds, peats and gyttjas) in this area are characterized by a low content of potentially harmful metals and metalloids. Sediment samples were collected from the 5-cm thick surface layer of the profundal zone in 14 lakes (Brdowskie, Budzisławskie, Godowskie, Gosławskie, Licheńskie, Lubstowskie Mąkolno, Pątnowskie, Powidzkie, Powidzkie Małe, Suszewskie, Ślesińskie, Wąsosko-Mikorzyńskie and Wilczyńskie) located in the south of the Gniezno Lake District near Konin (Figure 1, Table 1). Each sediment sample was averaged from two or three independent samplings taken from a single deeper place of the lake.

Fig. 1. Geographic location of sampled lakes

Table 1

The characteristics of sampled lakes

No	Lake	Area (ha)	Maximum depth (m)	Average depth (m)	Volume (thousand m^3)
Lakes outside in the power plant cooling system					
1	Brdowskie	194.7	4.9	2.5	4 796.2
2	Budzisławskie	140.8	35.2	10.8	15 240.8
3	Głodowskie	93.9	8.2	4.5	2 600.3
4	Lubstowskie	85.3	5.9	2.5	2 142.7
5	Mąkolno	82.5	6.7	3.2	2 636.8
6	Powidzkie	1 035.9	45.4	12.7	131 279.2
7	Powidzkie Małe	52	7.5	3.5	1 820
8	Suszewskie	81.7	21.8	6.5	5 325.7
9	Wilczyńskie	173.8	23.2	7.3	12 615.4
Lakes within the power plant cooling system					
10	Gosławskie	454.5	5.3	3.9	13 485.3
11	Licheńskie	147.6	12.6	4.5	67 12.3
12	Pątnowskie	282.6	5.5	2.6	7 255.4
13	Ślesińskie	152.3	24.5	7.6	11 5500
14	Wąsosko- -Mikorzyńskie	251.8	36.5	11.5	29 050.1

The sediment samples were dried at room temperature and sieved through 0.2-mm mesh nylon sieves to obtain a grain fraction finer than 0.2 mm, which was used for analytical studies. The sediment fraction with grains less than 0.2 mm faithfully reflects the concentration of trace elements in rocks of the catchment area of a lake or river, and therefore this grain fraction is most commonly used in geochemical mapping and monitoring studies. Concentrations of copper as well as Ag, As, Ba, Cd, Co, Cr, Cu, Ni, Pb, Sr, Ti, V, Zn and elements included in the phases whose compounds can retain pollutants in aquatic sediments (Ca, Mg, Fe, Mn, P and S) were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) from solutions obtained after digestion in *aqua regia*. The detection limits for the elements were as follows: Ag and Cd – 0.5 mg kg^{-1}, Co, Cr, Cu, Mn, Ni, Sr and Zn – 1 mg kg^{-1}, As – 3 mg kg^{-1}, Pb – 2 mg kg^{-1}, P and S – 0.005%, Ca, Fe, Mg – 0.01%. The mercury concentration was determined on a solid sample using the absorption spectrometry method with pre-concentration on a gold amalgamator trap with the detection limit of 0.005 mg kg^{-1}. Total organic carbon (TOC) was determined by coulometric titration of a solid sample, after removal of carbonate carbon by hydrochloric acid, with the determination limit of 0.01%.

The results were subjected to statistical analysis. Statistical parameters such as a mean, geometric mean, median, minimum, maximum, standard deviation and correlation matrix were determined using the Statistica software. While computing averages, geometric means, medians, standard deviation and correlation matrix, whenever the determined content of an element was below the detection limit set for a given analytical method, half of the detection limit value was taken for calculations.

RESULTS AND DISCUSSION

Concentrations of some trace elements varied over a wide range of concentrations: for copper – 9-674 mg kg^{-1}, barium – 57-409 mg kg^{-1}, mercury – 0.058-0.366 mg kg^{-1}, nickel – 5-25 mg kg^{-1}, lead – 16-53 mg kg^{-1}, strontium – 53-758 mg kg^{-1} and for zinc – 30-184 mg kg^{-1} (Table 2). It has been found that the sediments from the lakes Gosławskie, Licheńskie, Pątnowskie, Ślesińskie and Wąsosko-Mikorzyńskie are characterized by much higher levels of copper, barium, mercury, manganese, strontium and zinc, and lower concentrations of lead, sulfur and organic matter than sediments from the other lakes, i.e. Brdowskie, Budzisławskie, Godowskie, Lubstowskie, Mąkolno, Powidzkie, Powidzkie Małe, Suszewskie and Wilczyńskie. The average concentrations of copper, nickel, lead, mercury and zinc in the sediments of the latter group of lakes are comparable with concentrations of these elements in sediments of lakes from other regions (Table 3) and are similar to the geochemical background values. They are distinguished only by a higher content of barium and strontium. The sediments from the lakes involved in the Konin-Pątnów power plant cooling system have a distinctly high concentration of copper: the geometric mean is 385 mg kg^{-1}, which is 35-fold higher than a mean copper concentration in lake sediments. In Poland, the average Cu concentration in sediments is 7 mg kg^{-1}. The average content of barium

Table 2

Statistical parameters of elements in the lake sediments

Element	Lakes within the power plant cooling system						Lakes outside the power plant cooling system					
	a	*b*	*c*	*d*	*e*	*f*	*a*	*b*	*c*	*d*	*e*	*f*
Arsenic (mg kg^{-1})	3	<3	3	<3	4	<3	6	4	6	<3	11	4
Barium (mg kg^{-1})	317	309	329	217	409	80	100	93	92	57	196	44
Chromium (mg kg^{-1})	9	8	8	7	11	2	8	7	6	4	16	4
Zinc (mg kg^{-1})	133	129	133	88	184	35	71	63	67	30	144	37
Cadmium (mg kg^{-1})	<0.5	<0.5	<0.5	<0.5	<0.5	0.0	0.8	0.7	0.7	0.5	2.0	0.6
Cobalt (mg kg^{-1})	2	2	2	1	3	1	2	2	1	1	4	1
Copper (mg kg^{-1})	415	385	412	203	679	174	14	14	13	9	24	5
Nickel (mg kg^{-1})	21	20	19	17	25	4	8	8	6	5	13	3
Lead (mg kg^{-1})	20	20	21	16	26	4	38	33	36	12	69	19
Mercury (mg kg^{-1})	0.179	0.152	0.149	0.060	0.366	0.114	0.091	0.084	0.089	0.037	0.160	0.037
Strontium (mg kg^{-1})	618	613	618	526	758	91	252	193	195	53	717	211
Vanadium (mg kg^{-1})	10	10	10	7	15	3	12	11	9	6	17	4
Phosphorus (%)	0.091	0.089	0.091	0.070	0.114	0.019	0.078	0.075	0.069	0.049	0.120	0.024
Magnesium(%)	0.41	0.41	0.41	0.37	0.43	0.03	0.30	0.29	0.27	0.22	0.45	0.07
Manganese (mg kg^{-1})	1017	935	934	587	1874	505	501	451	500	166	874	224
Sulphur (%)	0.650	0.640	0.613	0.520	0.814	0.126	0.980	0.927	0.956	0.440	1.580	0.328
Calcium (%)	22.51	22.46	21.37	21.19	24.95	1.71	17.50	15.96	19.74	4.76	24.32	6.47
Carbon org. (%)	5.37	5.32	4.99	4.68	6.81	0.85	8.53	8.22	7.29	5.43	13.82	2.57
Iron (%)	0.88	0.86	0.85	0.60	1.13	0.21	0.78	0.54	0.85	0.09	1.38	0.52

a – mean, *b* – geometric mean, *c* – median, *d* – minimum, *e* – maximum, *f* – standard deviation

in the sediments of the above lakes was three times as high as in whole Poland and the average content of nickel, mercury and zinc was double the Polish average.

The results showed that the concentrations of arsenic, cadmium, cobalt, chromium and vanadium in the sediments were low and characterized by a relatively low variability. They varied within the following intervals: for arsenic <3-11 mg kg^{-1}, cadmium – <0.5-1.3 mg kg^{-1}, cobalt – 1-4 mg kg^{-1}, chromium – 4-16 mg kg^{-1} and vanadium – 6-15 mg kg^{-1} (Table 2). These values are similar to the ones measured in sediments of lakes in the drainage basins of the Ełk and Augustów Lake District, the Włodawa and Łęczna Lake Districts and in the Suwałki Lake District (Table 3).

The copper concentration in sediments of lakes connected to the Konin-Pątnów power plant cooling system shows a significant correlation ($r > 0.9$, p=0.05, n=5) with the chromium, mercury and zinc concentrations. And its content in sediments of the lakes outside

Table 3

Geometric means of the content of trace elements in lake sediments of different regions (mg kg^{-1})

Element	Ełk and Augustów Lake District	Suwałki Lake District*	Łęczyca and Włodawa Lake District**	Konin Lake District	
				lakes outside the power plant cooling system	lakes within the power plant cooling system
Arsnic	6	7	7	4	<2
Barium	94	94	70	93	309
Cadmium	0.6	0.7	1.3	0.7	<0.5
Chromium	10	10	8	7	8
Cobalt	2	3	3	2	2
Copper	13	11	11	14	385
Lead	26	28	39	33	20
Mercury	0.098	0.083	0.146	0.084	0.152
Nickel	7	8	8	8	20
Strontium	83	66	183	193	613
Vanadium	15	15	11	11	10
Zinc	78	74	101	63	129

* BOJAKOWSKA, GLIWICZ (2009*a*)

** BOJAKOWSKA, GLIWICZ (2009*b*)

the power plant cooling system is correlated ($r \approx 0.6$-0.7, $p = 0.05$, $n = 9$) with the concentrations of arsenic, cobalt, nickel and chromium.

The water from the lakes Licheńskie, Gosławskie, Pątnowskie, Wąsowsko-Mikorzyńskie and Ślesińskie has been drawn to the Konin-Pątnów power plant cooling system from over 50 years. Consequently, the lakes have been loaded with copper from corroded heat exchangers made of copper or its alloys. This undermines the long-held opinion that cooling water from power plants is chemically pure and its discharge to surface waters causes only thermal pollution of the environment. The past and present use of copper sulfate as a compound limiting the occurrence of algal blooms, e.g. in fish ponds and power plant cooling ponds, can be an additional source of copper in lakes.

The problem of environmental pollution with copper due to the discharge of cooling water from power plants is rarely discussed. Most studies focus on biological aspects of the impact of discharging heated cooling water on the aqueous environment, like disturbances in the species composition of flora and fauna. The scope of research is only occasionally expanded onto chemical investigations of organisms in water habitats, and the risk of chemical pollution with copper is very rarely considered. A negative impact of copper on snails and copper bioaccumulation in the tissues of riverine snails were found in receiver cooling water the Clinch River, Virginia USA (REED-JUDKINS et al. 1997). Concentrations of copper in plankton due to the cooling system of a nuclear power plants in Beloyarsk were discussed by GUSEVA and CHABOTINA (2000). The accumulation of copper and other heavy metals in the tissues of *Vallisneria spiralis* (L.), *Sinanodonta woodiana* (Lea), and *Dreissena polymorpha* (Pall.) from lakes around Konin in Poland was demonstrated by KRÓLAK et al. (2007).

The current study on lakes sediments in the region of Konin has revealed their high copper concentrations (a geometric mean of 385 mg kg^{-1}), which may cause harmful effects of copper on aquatic organisms (the boundary limit is >150 mg kg^{-1}). The analyzed lake sediments are also characterized by very high levels of calcium, organic matter and iron components, which can significantly affect the bioavailability of copper. However, it is necessary to perform further tests, such as determinations of the copper content in fish tissues and histological examinations of fish gills, because these lakes are used for recreational and angling purposes on a large scale.

CONCLUSIONS

1. The sediments of the lakes Licheńskie, Gosławskie, Pątnowskie, Wąsowsko-Mikorzyńskie and Ślesińskie, whose waters are drawn into the Konin-Pątnów power plant cooling system, are characterized by higher concentrations of copper, barium, mercury, manganese, strontium and zinc, and lower levels of lead, sulfur and organic matter than uncontaminated lakes.
2. The geometric mean content of copper in the sediments of lakes included in the cooling system is 385 mg kg^{-1} and is 35-fold higher than its average content in lake sediments from other regions.
3. The content of copper, barium, mercury, lead, strontium and zinc in the analyzed lake sediments, except lakes within the power plant cooling system, was comparable to the content in lake sediments of other lake districts.
4. The content of arsenic, cadmium, cobalt, chromium and vanadium in the analyzed sediments was low and similar to the content of these elements in lake sediments of other lake districts.
5. Copper contamination of sediments of the lakes involved in the cooling system is probably due to corrosion of heat exchangers made of copper or its alloys.

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EFFECT OF DIFFERENT FORMS OF SELENIUM ON TRACE ELEMENTS IN THE BLOOD SERUM AND LIVER TISSUE OF LAMBS

Agnieszka Chałabis-Mazurek, Grażyna Wałkuska

Department of Preclinical Veterinary Sciences
University of Life Sciences in Lublin

Abstract

The objective of the present research was to evaluate the dynamics of changes in the mineral content in blood serum and liver of lambs under dietary selenium supplementation. The study was conducted on 48 lambs assigned to three equal groups. The experiment started when the lambs were 5 weeks old, proceeded for 8 weeks and consisted of two stages. During the first, 4-week stage, the lambs were divided into three groups: group I – clinical control group, II – which received 0.2 mg Se/day in the form of enriched yeast, and III – which was fed 0.2 mg Se/day in the form of sodium selenite (POCH, Poland). During the second, 4-week stage, the lambs did not receive any supplements. Blood samples were taken after 1, 2, 3 and 4 weeks of the experiment. Liver samples were collected from slaughtered animals after 2, 4, 6 and 8 weeks since the onset of the experiment. Selenium supplementation significantly increased the Se concentration in the blood serum and liver of lambs. Selenium compounds significantly contributed to changes in the analyzed minerals, i.e. manganese, zinc, copper and iron. The results of this experiment suggest that selenium supplementation might lead to a decrease in levels other health-promoting elements such as Zn, Cu and Fe, and an increase in the Mn levels in the liver. Differences in the influence of organic and mineral forms of selenium on the Mn, Zn, and Cu content in the blood serum and liver were noticed. A significant decrease in the copper and iron content may induce symptoms of the deficiency of these trace elements in animals.

Keywords: manganese, zinc, copper, iron, selenium, lambs.

WPŁYW RÓŻNYCH FORM SELENU NA ZAWARTOŚĆ METALI ŚLADOWYCH W SUROWICY KRWI I WĄTROBIE JAGNIĄT

Abstrakt

Celem badań była ocena dynamiki zmian zawartości składników mineralnych w surowicy i w wątrobie jagniąt po suplementacji diety selenem. Badanie przeprowadzono na 48 jagnię-

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dr Agnieszka Chałabis-Mazurek, Department of Preclinical Veterinary Sciences, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland, e-mail: agnieszka.mazurek@up.lublin.pl

tach w wieku 5 tygodni. Doświadczenie trwało 8 tygodni i zostało podzielone na dwie części. W pierwszych 4 tygodniach jagnięta podzielono na trzy równe grupy: Grupę I stanowiły jagnięta kontrolne, grupę II – jagnięta otrzymujące drożdże wzbogacone w selen w dawce 0,2 mg/dzień/zwierzę, grupę III – jagnięta, którym podawano selen w tej samej dawce co w grupie II w formie seleninu sodu. W drugiej części doświadczenia jagnięta nie otrzymywały suplementacji. Próbki krwi pobierane były po 1, 2, 3, 4 tygodniach eksperymentu, zaś wątroby po uboju po 2, 4, 6, 8 tygodniach od rozpoczęcia doświadczenia.

Zastosowanie doustnej suplementacji selenem w sposób istotny przyczyniło się do wzrostu koncentracji tego pierwiastka w surowicy krwi i wątrobie jagniąt, ale jednocześnie spowodowało znaczne zmiany w koncentracji innych składników mineralnych, tj. manganu, cynku, miedzi i żelaza. Wyniki tego doświadczenia wskazują, że podawanie selenu może prowadzić do obniżenia poziomu ważnych dla zdrowia pierwiastków Cu, Zn i Fe oraz wzrostu koncentracji Mn w wątrobie. Stwierdzono różnice w oddziaływaniu organicznych i mineralnych form selenu na zawartość Mn, Zn, Cu w surowicy krwi i wątrobie jagniąt.

Znaczny spadek zawartości miedzi i żelaza stwierdzony po zastosowaniu suplementacji selenem może sugerować ryzyko wystąpienia u jagniąt objawów związanych z niedoborem tych pierwiastków.

Słowa kluczowe: mangan, cynk, miedź, żelazo, selen, jagnięta.

INTRODUCTION

Maintenance of good health of animals, their adequate growth and performance depend on appropriate concentrations of trace elements and their correct balance in tissues and organs. The required animal supply with micro- and macro-elements is governed by the animal species, physiological status, age and production type (HYLLAND et al. 2009).

One of the mineral elements critical for the optimal functioning of organisms is selenium (Se), an essential trace element known to demonstrate complex biological activities, for example participating in the reproductive process (VAN NIEKERK et al. 1996, HEMINGWAY 2003, MAKHOUL et al. 2004, LEKATZ et al. 2010 *a*, *b*,) or being present in enzymatic proteins (BIK 2003). Analysis of an animal's demand for should account for the fact that Se biological availability depends not only on sources of feedstuffs or the form of Se, but also on interactions with other dietary elements. Some dietary ingredients compete in the absorption process or form compounds insoluble in the rumen or intestines, which leads to excretion of elements unavailable to an animal organism (GRELA, SEMBRATOWICZ 1997). Moreover, selenium bioavailability and retention in ruminants is also influenced by diet composition, for example absorption of Se is better in sheep fed a concentrate-based diet than in ones receiving forages (KOENIG et al. 1997). This and other experiments have revealed that the selenium form is of key importance in its retention in an animal organism. Nutrition of ruminants, especially sheep and goats, is based on homegrown forages, therefore a local soil selenium deficit results in a low selenium content in plant feeds, and consequently a low level of this trace element in farm animals (HARTIKAINEN 2005). There are a number of methods to prevent selenium deficiency in both humans and animals (Szarek et al. 1997), of which oral supplementation is the preferred one. Most commonly, functional food products are enriched with selenium, analogously to adding this element to pharmaceutical preparations for humans and for breeding animals. Another solution is dietary supplementation of feeds. However, it is vital for selenium supplementation of animals to be preceded by reliable recognition of the deficiency status and to be accompanied by monitoring the availability to supplemented animals with elements antagonistic to selenium (manganese, copper, iron, zinc).

The objective of the present research was to assess the dynamics of changes in mineral content in the blood serum and liver of lambs under the dietary supplementation with selenium.

MATERIAL AND METHODS

The study included 48 lambs, young rams of the synthetic meat-prolific line BCP, with the mean body mass of 4-5 kg and aged 5 weeks. During the investigations, the lambs were raised and kept with mothers. They received extra feed such as meadow hay and crushed oats. The experimental animals were assigned into 3 equal groups; two groups were supplied with oral selenium supplement from 5 weeks of age. Group I comprised the experimental lambs without any dietary additives. The animals from group II received 0.2 mg Se/day/animal – Se enriched yeasts (Sel-plex, Alltech, Serbia and Montenegro). Group III consisted of the lambs given the same dose of selenium but in the form of sodium selenite (POCH, Poland). The preparations were administered daily for 4 weeks during the morning feeding. Throughout the mineral supplementation period, the lambs had blood samples collected every seven days to monitor the trace element concentration in their organisms. Blood was drawn from the external jugular vein to separator tubes and centrifuged at 3000/rpm to obtain serum.

After 2 and 4 weeks of mineral supplementation and 2 and 4 weeks after the supplementation had been stopped, some of the animals were slaughtered to obtain liver samples. The level of elements in blood serum and in the liver was determined with atomic absorption spectrometry using atomic absorption spectrometers: with electrothermal induction and deuter Zeeman-effect background correction, SpektrAA 220Z (Varian, Australia) and with the flame atomization system Avanta PM (GBC Scientific Equipment, Australia). Samples of liver were immediately deep-frozen, freeze-dried (lyophilized) and homogenized before analysis. For particular determinations, three parallel samples of the liver, about 0.5 g each, were taken for analysis. Samples were digested under a higher-pressure level in a microwave stove Multiwave 3000 (Anton Paar, Austria), using concentrated nitric acid and hydrochloric acid (3:1 v/v) for each sample. Afterwards, the samples were quantitatively transferred into 50 ml plastic flasks and measured with the AAS method. All results have been recalculated to wet mass. The water content was the basic difference between the weight of samples before and after lyophilization. Simultaneously, the reagent blank and certified reference material NIST 1570a (bovine muscle powder) were mineralized. The blood serum selenium content was established after GFAAS atomic absorption spectrometry modified by NEVE and MOLLE (1986). Each blood serum sample was diluted in a 1:3 ratio with matrix modifier: 0.5 g dm^{-3} Cu acetate (II); 1.0 g dm^{-3} nitrate Mg (II); 0,15% TritonX-100 in 2% HNO_3. At the same time, determinations of the reagent blank and reference material Seronorm (Nycomed and Co., Norway) were performed.

The results were analyzed statistically with two-factor analysis of variance, which included the following experimental factors in the mathematical model: the feeding group, sample collection time. The statistical computations were made using a Statistica statistical software package. Significance of differences between the groups was estimated by the Duncan's test.

RESULTS AND DISSCUSION

The research results obtained during the first stage of the experiment displayed marginal, low values of the blood serum selenium concentrations in the lambs. The four-week administration of selenium in either form manifested itself as changes in levels of this element in both blood serum and the liver of the animals. The peak serum response indicating the highest Se concentration increase was recorded in the group of lambs with a dietary supplement of selenium yeasts in the form of Sel-plex preparation. After the mineral supplementation was discontinued, the blood serum selenium content remained on a similar level, but decreased significantly in the liver. However, it continued to be significantly higher than in the control group (CHAŁABIS-MAZUREK, WAŁKUSKA 2008*a b*). The above research results served as the basis for discussing the determinations made during the second research stage.

The results on the manganese concentration in the blood serum during the supplementation period are summarized in Table 1. The manganese content in blood of the control group lambs remained fairly constant throughout the experiment and ranged between 0.052 up to 0.063 mg dm^{-3}. A statistically significant difference in the elemental concentration was noted only between the first and the last week of the research. After the first week of selenium supplementation, the Mn content in the experimental groups was about twice as high as in the control. Importantly, the same week was distinguished by the highest selenium increase in the examined lambs. In

Table 1

Blood serum manganese content (mg dm^{-3}) in lambs fed different forms of supplementary selenium (n =16; x ± s)

Group	Week				Differences between weeks
	1	2	3	4	
Control	0.052 0.009	0.054 0.011	0.053 0.014	0.063^{a} 0.014	^{x}1 - 4
Sel-plex	0.027 0.007	0.038 0.007	0.045 0.016	0.048^{b} 0.016	
Sodium selenite	0.021 0.004	0.024 0.004	0.027 0.004	0.026c 0.010	^{x}1 - 4

Key: statistically significant differences between groups marked with different letters:
$a, b, c - p \le 0.05$; $A, B, C - p \le 0.01$
^{x} $p \le 0.05$; ^{xx} – $p \le 0.01$ – statistically significant differences between weeks

Table 2

Blood serum zinc content (mg dm^{-3}) in lambs fed different forms of supplementary selenium (n =16; x ± s)

Group	Week				Differences between weeks
	1	2	3	4	
Control	2.220^{A} 0.461	2.963 0.366	2.015 0.302	2.103 0.182	
Sel-plex	1.457^{B} 0.264	1.630 0.107	2.003 0.174	2.545 0.502	^{xx}4 – 1,2 ^{x}1 – 2 ^{x}3 – 1,4
Sodium selenite	2.087^{A} 0.441	1.985 0.287	2.105 0.176	2.370 0.412	

Key: cf. Table 1.

the subsequent weeks, a substantial growth of the blood serum manganese concentration was observed in the group of lambs administered Sel-plex preparation against the first week. On the other hand, in the group of lambs fed an additional inorganic selenium form, the manganese level remained unchanged.

After four weeks of dietary mineral supplementation, statistically significant differences in the manganese concentration between the groups were observed. In the control group, this trace element level reached 0.063 mg dm^{-3} and in the lamb group supplemented with selenium yeasts it averaged 0.048 mg dm^{-3}, while in the group with dietary sodium selenite, the manganese concentration was approximately 2.5-fold lower than in the control.

The results on the zinc level in the blood serum of lambs are presented in Table 2. After one-week selenium supplementation, a significantly lower content of the element (1.457 mg dm^{-3}) was found in the group of lambs given the organic form of selenium, compared to the control group and the group administered sodium selenite (2.220 and 2.087 mg dm^{-3}, respectively).

The second week was marked with a significant rise in the zinc concentration in the Sel-plex supplied group against the values obtained in the first week. The tendency continued during the subsequent weeks, unlike in the group with the mineral form of selenium, where no significant changes in the zinc content were reported.

Oral selenium supplementation contributed to a significant decline in the copper concentration in the blood serum of lambs with the dietary supplements (Table 3). The mean content of this trace mineral in blood serum of lambs from the control was found within 0.665 mg dm^{-3} and 0.751 mg dm^{-3} and did show any significant changes in any week. In the first experimental week, group II given the organic form of selenium had a copper level lower by 44% than the control, while in group III, it was lower by 22%. Similar relationships were found in the following weeks.

The data in Table 4 show the influence of selenium supplementation on the iron concentration in the blood serum. A significant decrease in this element versus the control was observed in both groups fed additional selenium. The blood serum iron content in the animals from the control group ranged from 5.183 mg dm^{-3} up to 6.265 mg dm^{-3}, while in the groups with dietary Se supplementation they were between 3.478 mg dm^{-3} and 3.935 mg dm^{-3} (group II) and from 2.970 mg dm^{-3} up to 4.555 mg dm^{-3} (group III).

Table 3

Blood serum copper content (mg dm^{-3}) in lambs fed different forms of supplementary selenium (n =16; x ± s)

Group	Week				Differences between weeks
	1	2	3	4	
Control	0.751^{A} 0.183	0.665^{A} 0.045	0.711^{A} 0.200	0.698^{a} 0.039	
Sel-plex	0.424^{B} 0.052	0.499^{B} 0.102	0.526^{B} 0.102	0.546^{b} 0.028	
Sodium selenite	0.583^{A} 0.121	0.565^{B} 0.166	0.560^{B} 0.043	0.559^{b} 0.308	

Key: cf. Table 1.

Table 4

Blood serum iron content (mg dm^{-3}) in lambs fed different forms of supplementary selenium (n =16; x ± s)

Group	Week				Differences between weeks
	1	2	3	4	
Control	6.093^{A} 0.592	5.183^{A} 0.539	5.763^{A} 0.138	6.265^{A} 0.530	^{x}1 – 2,3 ^{x}4 – 2,3
Sel-plex	3.478^{B} 0.403	3.547^{B} 0.416	3.935^{B} 0.797	3.660^{B} 0.848	
Sodium selenite	4.490^{C} 0.542	3.163^{B} 0.403	2.970^{B} 0.437	4.555^{B} 0.891	^{xx}1 – 2,3 ^{xx}3 – 4

Key: cf. Table 1.

Many authors claim that changes in the mineral content of the liver are the most reliable indicator of the trace mineral status of an animal (GEHRKE, LACHOWSKI 1999). Table 5 presents the Mn, Zn, Cu and Fe content in the

Table 5

Mineral content (mg kg^{-1} wet mass) in lamb liver during and after dietary supplementation with different selenium forms, (n =16; x ± s)

Trace element	Research week	Group			Differences between groups
		control (1)	sel-plex (2)	sodium selenite (3)	
Mn	2	2.063 0.029	3.432^{A} 0.183	2.470^{A} 0.156	^{xx}1 - 2,3 ^{xx}2 - 3
	4	2.123 0.035	3.318^{AC} 0.120	3.396^{B} 0.166	^{xx}1 - 2,3
	6	2.134 0.098	2.274^{B} 0.130	3.285^{B} 0.096	^{xx}1 - 3 ^{xx}2 - 3
	8	2.239 0.086	3.233^{BC} 0.114	2.368^{A} 0.093	^{xx}1 - 2 ^{xx}2 - 3
Zn	2	27.25 0.731	22.34^{A} 1.448	21.59^{A} 0.847	^{xx}1 - 2,3
	4	26.71 1.637	20.93^{A} 0.941	22.72^{A} 0.487	^{xx}1 - 2,3
	6	27.88 1.046	25.63^{B} 2.479	28.57^{B} 0.591	^{xx}1 - 2
	8	25.25 1.539	24.45^{B} 1.312	27.79^{B} 0.574	^{xx}3 - 1,2
Cu	2	49.37 4.789	26.28^{a} 1.366	31.78^{a} 2.265	^{xx}3 - 1,2
	4	48.52 3.111	19.73^{b} 1.004	27.68^{b} 1.491	^{xx}3 - 1,2
	6	48.42 3.215	15.49^{c} 1.041	18.77^{c} 0.501	^{xx}3 - 1,2
	8	46.82 0.459	9.212^{d} 0.699	12.31^{d} 0.440	^{xx}3 - 1,2
Fe	2	73.54 7.657	46.99^{A} 2.960	45.88^{A} 1.600	^{xx}1 - 2,3
	4	63.91 6.393	36.48^{B} 2.376	37.19^{B} 2.322	^{xx}1 - 2,3
	6	65.82 2.731	29.79^{C} 0.994	33.26^{B} 1.419	^{xx}1 - 2,3
	8	66.14 2.826	29.83^{C} 1.127	33.06^{B} 1.738	^{xx}1 - 2,3

Key: cf. Table 1.

lamb liver during and after supplementation with the mineral and organic form of selenium. Application of a dietary selenium supplement produced a significant rise of the hepatic manganese content in lambs receiving selenium preparations as compared to the control. The tendency held true in both 2 and 4 week of trace mineral supplementation. After the selenium supplementation was discontinued, in 6 research week, a significant decrease appeared in the manganese concentration in the liver of lambs from group II against the values recorded during the trace mineral supplementation and the Mn content in group III. However, the determined value proved to be significantly higher than the control.

The zinc content in the control group of lambs in the successive weeks remained on a similar level: from 27.25 in 2 week up to 25.25 mg kg^{-1} of wet mass in the final week. During the trace element supplementation, significantly less zinc was determined in the groups receiving selenium preparations compared to the control. No significant differences were reported in the Zn level in the experimental groups. In 6 and 8 experimental week, a significant rise was observed in the zinc content in the treatment groups compared to the selenium supplementation period.

In both periods i.e., of additional trace mineral supply and after its termination, the copper and iron concentrations declined significantly in both groups of lambs fed supplementary selenium preparations. This tendency was observed throughout all the research. After two supplementation weeks, the Cu and Fe levels were 47% and 36% lower, respectively, in group II and 36% and 38% in group III than in the control. Afterwards, despite the discontinuation selenium supplementation, the content of both minerals persistently declined, down to the following values in the last experimental week: Cu 9.212 and Fe 29.830 mg kg^{-1} in group II, Cu 12.308 and Fe 33.056 mg kg^{-1} in group III. The biggest decrease in the copper concentration was reported in the lambs whose diet was enriched with selenium yeasts. In the case of iron, no effect of a selenium form on the liver mineral content in lambs was determined.

The above results imply potential interaction between selenium and the analyzed trace minerals. However, consideration should be given to the fact that a number of other factors may have additionally affected the course of each mineral element concentration in both the blood serum and liver (SPEARS 2003). The antagonistic effect between selenium and other elements such as cadmium (Cd), lead (Pb) or mercury (Hg) has been described by EL-SHARAKY et al. (2007), ABDOLLAHI (2001) and SU et al. (2008). The investigations of other authors have confirmed the zinc-selenium and copper-selenium antagonism (JENSEN 1975, HAUSE, WELCH 1989). Iron (Fe) and manganese (Mn) have also been shown to be selenium antagonists (MARTIN et al. 1989).

The present research shows that dietary supplementation with different selenium forms affected the content of Mn, Zn, Cu and Fe in the blood serum and liver of lambs. Manganese is found in virtually all tissues and organs of animal organism, with its highest concentration recorded in the pancreas, liver, bone tissue and hair and hair cover. Most animal organisms show high tolerance to this mineral element. Manganese deficiency is more often associated with its disturbed availability than a low Mn level in feedstuffs (GEHRKE 1997b). In animals, the Mn shortage causes animal growth inhibition, disordered urea synthesis and mineral metabolism in bones as well as inferior reproduction performance. The present research has shown a lower Mn content in the blood serum throughout the supplementation period in both experimental groups against the control. A contrary tendency was observed in the liver. Selenium supplementation has elevated the Mn concentration in both lamb groups with administered dietary selenium compounds. Selenium supplementation of lambs seems to be beneficial with respect to the Mn function as an oxidant contained in superoxide dismutase, whose activity is conditioned by the presence of selenium and manganese. Beside copper and iron, magnesium proves to be an essential trace element for hemoglobin synthesis.

Zinc belongs to the minerals whose shortage is more strongly related to its reduced assimilation in the digestive track than to its low content in a diet (KLEBANIUK, GRELA 2008). The effects of zinc deficiency may strike many bodily functions, including delayed and inhibited growth, impaired reproduction, immune system suppression and biochemical changes in blood. Cases of dermatitis and abnormal glossy-white hair appearance are also reported (WHITE et al. 1994, DANEK 2002). Throughout the experiment, the blood serum zinc content fluctuated, showing a rising tendency after the 4-week supplementation treatment. However, statistically significant differences were noted in the lamb group given organic selenium supplementation. A probable explanation could be the differentiated effeminacy of inorganic and organic selenium compounds included in ruminant diet (TAYLOR 2005). The zinc concentration in the liver appeared different. The initial decline during the supplementation period was followed by a significant growth in the successive weeks, as compared to the control, especially in the group of lambs whose diet included the inorganic form of selenium. Similar results were obtained by CHMIELNICKA et al. (1988). This behavior of zinc in the liver may be governed, inter alia, by the influence of metallothionein MT, a low molecular mass protein likely to participate in the uptake, transport and regulation of zinc in biological systems; it is formed through the synthesis stimulated in mucous cells by competitive zinc and copper atoms (ECK, PALLAUF 1999, OH et al. 1981). Metallothionein also acts as a zinc and copper store in the cell and has been documented to be a potent scavenger of free radicals (PARK et al. 1985).

Copper proves to be an essential component of different proteins and metalloenzymes. This trace element contributes to iron metabolism, hemoglobin synthesis and erythrocyte production. The most pronounced changes were noted in the Cu and Fe concentration in liver. The observed behavior of the mineral elements indicates a strong antagonistic interaction in their deposit and metabolism in the liver. The current results agree with those reported by other authors (BIK, BEDNAREK 1997), who noted a significant decrease in the copper level in sheep's blood serum after a subcutaneous injection of sodium selenite aqueous solution.

A low concentration of copper (the liver being its main storage organ) is likely to induce in future some clinical signs associated with this mineral deficit. Ruminants, sheep and goats predominantly, prove to be very susceptible to copper deficiency; also, wooly breeds and dairy animals are more sensitive than meat ones. Common symptoms of copper shortage include anemia or anemization, depending on the severity and duration of the deficit. Hypochromic anemia reported in animals and associated with copper shortage constitutes a major challenge in a differential diagnosis, to distinguish from an iron deficit-induced disease (SUTTLE et al. 1987). Prolonged copper shortage leads to lesions and deformations in bone tissue as well changes in the appearance and structure of animal hairs and wool (GEHRKE 1997a). The vital role in copper regulation is attributed to the plasma ceruloplasmin concentration (RANSOM-STERN et al. 2007). The present research did not include an assessment of the activity of this enzyme, although the blood serum copper content appeared to be significantly lower compared to the control.

The experiment performed by SOUTH et al. (2000) on mice showed that selenium deficiency played a role in iron accumulation in the liver and in cholesterol plasma; it affected the triglyceride level as well. Iron is essential to virtually all living organisms, it is a key component of cytochromes, an important electron carrier and plays a critical role in cellular respiration. This trace element is found deficient mainly in juvenile animals fed monotonous diets or milk alone, with no iron supplementation for long periods. As a consequence, an insufficient iron supply to animals causes the depletion of the body iron reserves in the liver, kidneys, spleen and bone marrow, leading to anemia. Besides, a reduced iron concentration in plasma is observed, alongside an enhanced iron-binding capacity by serum proteins (UNDERWOOD, SUTTLE 1999).

The influence of Se supplementation on reproductive efficiency can be varied (HEMINGWAY 2003). Apart from the main source of Se, such as organic and inorganic supplements, we should also consider a period of supplementation before mating and check the vitamin E status of animals.

Se supplementation between 15 and 35 days after mating and during the first month of pregnancy is not recommended because it reduces the embryonic survival rate. On the other hand, Se supplementation from 21 days before gestation until near term in ewes (days 135 of gestation) is suggested (LEKATZ 2010b).

Ample evidence shows that Se supplemented in the maternal diet may be of vital importance for the development of ruminant offspring; also in humans, a diet supplemented with Se can contribute to decreasing mortality and morbidity of preterm infants (MAKHOUL et al. 2004).

CONCLUSIONS

Selenium supplementation significantly increased the Se concentration in the blood serum and liver of lambs. Selenium compounds significantly contributed to changes of the minerals: manganese, zinc, copper and iron, in the blood serum and liver. Selenium supplementation distinctly depressed the Cu and Fe content in the blood serum and liver in both selenium-supplemented lamb groups. The highest copper concentration decrease was reported in the lambs whose diet was enriched with selenium yeasts. In the case of iron, no effect of the selenium form on the hepatic mineral content in lambs was determined. At the same time, selenium compounds had a synergistic effect on the Mn content in liver, especially in the group of lambs whose diet included inorganic selenium. The Zn concentration in the liver responded differently. The initial decline during the supplementation period was followed by a significant growth of the Zn content in the liver of lambs whose diet contained sodium selenite.

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ENVIRONMENTAL CONDITIONS CAUSING SELENIUM DEFICIENCY IN SHEEP

Agnieszka Chałabis-Mazurek, Grażyna Wałkuska

Department of Toxicology and Environmental Protection
University of Life Sciences in Lublin

Abstract

The research was carried out on the Experimental Farm in Uhrusk, which belongs to the University of Life Sciences in Lublin. Two-year experiment were conducted in order to assess the influence of environmental conditions on the selenium content in sheep. The investigations included meadows grazed by sheep, from which samples of soils and meadow herbage were obtained for analysis of selenium content. The animal material consisted of 64 lambs and their mothers, whose blood serum was taken for determinations of the selenium content in their organism. The research results showed that the analyzed soils and meadow grasses were characterized by low selenium content. Also, the selenium content in fodder fed to the sheep and lambs was below the animals' standard demand for this element. The low selenium level found in the sheep's blood serum distinctly proved a deficiency of this element in their organisms. The results show that environmental conditions have a significant influence on the supply of selenium to adult sheep and their offspring. Selenium deficiency in particular trophic chain links can present a serious threat to the health of animals fed with homegrown fodders.

Keywords: selenium, sheep, blood serum, milk, plants, soil.

UWARUNKOWANIA ŚRODOWISKOWE NIEDOBORU SELENU U OWIEC

Abstrakt

Eksperyment przeprowadzono w Gospodarstwie Doświadczalnym w Uhrusku należącym do Uniwersytetu Przyrodniczego w Lublinie. W cyklu dwuletnim oceniano wpływ warunków środowiskowych na zawartość selenu w organizmie owiec. Z łąk przeznaczonych do wypasu owiec pozyskano próbki gleby i roślin łąkowych, a następnie poddano analizie na zawartość selenu. Materiał zwierzęcy stanowiły 64 jagnięta i ich matki, od których pobrano krew w celu pozyskania surowicy do oznaczania zawartości selenu. W badanych glebach i florze łąkowej wykazano niską zawartość selenu. Również zawartość selenu w paszy dla owiec i jagniąt była

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dr Agnieszka Chałabis-Mazurek, Department of Toxicology and Environmental Protection, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland, e-mail: agnieszka.mazurek@up.lublin.pl

poniżej zapotrzebowania zwierząt na ten pierwiastek. Niska koncentracja selenu w surowicy krwi badanych zwierząt wyraźnie wskazuje na niedobór tego mikroelementu w ich organizmach. Z badań wynika, że uwarunkowania środowiskowe mają istotny wpływ na podaż selenu dla dorosłych owiec i ich potomstwa. Niedobór selenu w poszczególnych ogniwach łańcucha pokarmowego może stanowić poważne zagrożenie dla zdrowia zwierząt karmionych paszami produkowanymi lokalnie.

Słowa kluczowe: selen, owca, surowica krwi, mleko, rośliny, gleba.

INTRODUCTION

Selenium is a crucial trace element, responsible for the proper functioning, growth, and development of a living organism. It is also characterized by a very narrow margin between the lowest acceptable level of intake and the levels causing toxicity (Zhang et al. 2001). It plays a complex biological function, mainly related to its presence in enzymatic proteins. Selenium is a constituent of 35 enzymes and selenoproteins (Chen et al. 1999). Selenium has four natural oxidation states – 2 (selenides), 0 (elemental), +4 (selenites) and +6 (selenates) (Barcelaux 1999).

An insufficient supply of this element results in an inferior productivity of animals. Several metabolic diseases have been linked to selenium deficiency, including white muscle disease (WMD) in calves and lambs (Smart et al. 1981), hepatosis in pigs, exudative diathesis and pancreatic degeneration in poultry.

Ensuring that animals are in good health requires a good biological value of fodder, which in turn depends on the mineral composition of soils. Elemental selenium and precipitated metal selenides are not bioavailable for plant uptake. It is only the water soluble forms that plants can readily take up. Beside plants, some microbial populations can reduce selenium to volatile chemical forms (Combs 2001, Chasteen, Bentley 2003). High soil selenium not available to plants is due to the acid soil reaction causing a lower level of water soluble and therefore phytobioavailable selenium. Plants absorb soil selenium most effectively when it is in the form of selenite or selenate and synthesized selenomethionine (Se Met) (Rayman 2004).Hence, causes of deficiency or relative excess of a given mineral should be sought in soil, the first link in the trophic chain composed of soil – plant – animal (Hartikainen 2005). Numerous authors have proven empirically that large parts of Poland, including the Lublin District, lie on selenium-deficient soils (Piotrowska 1984, Biernacka, Małuszyński 2006, Patorczyk-Pytlik, Kulczycki 2009).

Locally produced fodder is mainly used in the nutrition of lambs, which may suffer from mineral deficits due to some local deficiency of elements in soil and, consequently, in plants (Patorczyk-Pytlik 2009, Bombik et al. 2010). Animals fed on fodder with less than 0.1 mg kg^{-1} selenium may develop symptoms of diseases related to selenium deficiency.

The aim of the present work has been to determine selenium levels in organisms of adult sheep and their offspring.

MATERIAL AND METHODS

The investigations were carried out in two replicates completed at the turn of May and June in two consecutive calendar years (2005 and 2006). They covered meadows on two very different soil habitats: mineral lessive soil (alternate meadow) and peat-organic soil (permanent meadow). Both meadows lie in an area periodically flooded by the Bug River. They are characterized by a poor variety of plant communities. Soil samples were taken from the surface layer (0-20 cm deep) scattered at 12 randomly selected sites representative of the whole meadow area. Laboratory assays were made on averaged samples of friably dry soil. Soluble selenium forms in soils were determined in a solution obtained after six-hour extraction of 3 g of soil by 20 cm^3 0.05 M EDTA, whereas total selenium content was checked in a solution achieved after mineralization of 1 g of soil with a 3:1 mixture of oxygenating acids HNO_3 and HCl.

Meadow plants were sampled from 1 m^2 squares located at the same 12 sites as the soil samples. Plant samples were digested in a microwave stove Multiwave 3000 (Anton Paar, Austria). A similar resolution procedure was applied before determination of the selenium content in fodder and milk. The determination of selenium in the extract and mineralizates was carried out in an atomic absorption spectrometer SpektrAA 220Z (Varian, Australia) with electro-thermal induction and deuter Zeeman-effect background correction.

The experiment was repeated twice in two consecutive years (2005 and 2006). The evaluation of sheep's selenium status was based on analyses of this element in blood serum. The investigation involved 64 five-week-old ram lambs of the synthetic prolific-meaty line BCP and their dams. During the experiment, the lambs were nursed on their dams' milk and the adult sheep were fed on fodder produced on the experimental soils. In order to obtain serum, blood was drawn from the *vena jugularis* to heparinized test tubes (Medlab Poland) and centrifuged at 3000 rpm. Selenium in the blood serum of lambs and their dams was determined by the GFAAS method according to the modified protocol by NEVE and MOLLE (1986). Simultaneously, determinations were conducted on a reagent trial and reference material Seronorm (Nycomed and Co., Norway). The criteria of analytical reliability were as follows: LOD – 8 µg dm^{-3}, LOQ – 16 µg dm^{-3}.

The results were statistically processed by analysis of variance run in the statistical program Statistica (Data Analysis Software System Version 6).

RESULTS AND DISCUSSION

The tested soils differed significantly both in the total and soluble selenium content (Table 1). The highest determined total selenium concentration in organic soil was almost 10-fold higher than the total content of this element in mineral soil. The small amount of selenium detected in mineral soil (< 0.5 mg kg^{-1}) is considered insufficient (Ramirez-Bribiesca et al. 2001). The concentration of easily soluble selenium forms, which are the ones mainly absorbed by plants, constituted a small proportion of this element in the tested soils: about 10% in samples of mineral soil and about 6% in organic soil. Similar results were obtained by other authors (Hamada, Hattori 1989). The low availability of selenium from organic soils may result from the fact that this type of soil contains a prevalent share of stable compounds with iron oxides, hydroxides and organic matter, which makes selenium less than fully available to plants despite its high total content.

Presence of stable selenium compounds is also proven by the reaction of the analyzed soils. In the two consecutive years, the soils had similar values of pH (7.4 pH_{H20} and 6.6_{KCl} in mineral soil and 7.3 and 6.3, respectively, in organic soil), which are characteristic of neutral soils, with selenins which are difficult to be absorbed by plants (Johnson 1991, Wang, Chen 2003).

The low concentration of selenium bioavailable forms in soil significantly determined the concentration of this element in meadow flora, below the

Table 1

pH reaction; total and soluble selenium contents in two different soil systems

Year	Parameters		Soil habitat	
			I	II
2005	pH	H_2O	7.4	7.3
		KCl	6.6	6.3
	total Se ± SD (mg kg^{-1})		0.075^{a} 0.006	0.726^{b} 0.014
	soluble Se ± SD (mg kg^{-1})		0.007^{a} 0.001	0.042^{b} 0.01
2006		H_2O	7.2	7.3
		KCl	6.5	6.3
	total Se ± SD (mg kg^{-1})		0.065^{a} 0.004	0.722^{b} 0.013
	soluble Se ± SD (mg kg^{-1})		0.007^{a} 0.003	0.038^{b} 0.040

I – mineral soil – habitat of ‘alternate meadow’

II – organic soil – habitat of ‘permanent meadow’

Statistically significant differences between selenium content in soils from two soil habitats were designated with different letters: *a*,*b* – $p \leq 0.01$.

Table 2

Selenium content in grasses of two different soil habitats

Year	Se (mg kg^{-1} d.m.) ± SD in habitat	
	I	II
2005	0.063^{a} 0.006	0.043^{b} 0.003
2006	0.061^{a} 0.005	0.043^{b} 0.005

I – mineral soil – habitat of 'alternate meadow'
II – organic soil – habitat of 'permanent meadow'
Statistically significant differences between selenium contents in grasses from different soil habitats were designated with different letters, *a-b* at $P \leq 0.01$.

level covering the sheep's daily demand for this micronutrient (0.1 mg kg^{-1} d.m. of fodder).

Significant differences were observed in the selenium content in meadow herbage from the two soil systems (Table 2). In grasses grown on mineral soils the concentration of selenium was even 1.5-fold higher than in grasses grown on organic soils. The results can support the dependence between concentrations of particular selenium forms in different soils and Se bioavailability. Similar observations were reported by other authors (Munier-Lamy et al. 2007).

Additionally, fodder used in winter (oat and meadow hay) was analyzed. The tests showed that the mean selenium concentration in oat was 0.053 and 0.043 mg kg^{-1} d.m. in the first and second year, respectively, while meadow hay contained 0.071 and 0.068 mg Se kg^{-1} d.m. in the two years. The research results reported by other authors led to the conclusion that the sheep's demand for selenium is covered by fodder with about 0.1 mg Se kg^{-1} d.m. content, and lower values should be considered as insufficient, possibly leading to diseases caused by selenium deficiency (Cloete et al. 1999, Beytut, Karatas 2002). The dietary requirement of Se by both ruminants and non-ruminants is 0.1 mg kg^{-1} d.m. in a diet, while the maximum tolerable level of Se has been increased from 2 (NRC 1980) to 5 mg kg^{-1} d.m. (NRC 2005). In diagnosed Se deficiencies, supplementation with inorganic Se sources is used, for example: sodium selenite, sodium selenate, as well as with an organic form of Se, e.g. selenized yeast (Pappas et al. 2008). Selenium may be administered to livestock as *ad libitum* Se mineral supplement, fertilization, injection, oral drenching, distribution in water or ruminal pellets (Szarek et al. 1997).

Table 3 presents selenium concentrations in blood serum and milk of adult sheep and in blood serum of lambs. The selenium level in blood serum is one of the diagnostic clues used for an assessment of the degree of Se sup-

Table 3

Selenium content in sheep blood serum (µg dm^{-3}) and sheep milk (mg dm^{-3}) as well as in lamb blood serum (µg dm^{-3}) (n = 32; $\bar{x}$ ± SD)

Parameters	Sheep blood serum		Sheep milk		Lamb blood serum	
	2005	2006	2005	2006	2005	2006
Mean	29.33	29.21	0.018	0.017	24.57	22.56
SD	3.79	5.01	0.002	0.003	2.735	1.90
Min	25.42	20.32	0.015	0.012	20.12	18.32
Max	39.89	44.42	0.022	0.022	30.36	27.32

ply to an organism. The mean selenium concentrations in the blood serum of adult sheep reached similar values in the consecutive years: 29.33 in 2005 and 29.21 µg dm^{-3} in 2006. Lower values were obtained when analyzing selenium concentrations in blood serum of sheep offspring (24.57 and 22.56 µg dm^{-3}, respectively). It should be noted that the concentrations values reported in the present work are in the range of 6-30 µg dm^{-3}, which Puls (1994) considers as indicative of selenium deficiency in sheep's blood serum. Selenium concentrations in blood serum above the deficiency threshold were found only in about 16% of adult sheep and about 6% of lambs in the first year of research and in 31% of dams in the second year of research. All the lambs tested in 2006 were selenium-deficient.

Significantly higher selenium levels in sheep's blood serum were obtained by other authors, who at the same time proved a large effect of the year of research and selenium level in fodder on this element in sheep's blood serum (Lipecka et al. 2003).

Determination of low selenium concentrations in lamb blood serum can encourage search for ways to supplement this element in lambs. Selenium supplementation could reduce the risk of subclinical symptoms of alimentary muscle dystrophy in lambs whose selenium concentration in blood fell below 26 µg dm^{-3} (Sobiech, Kuleta 2002). Juxtaposing the above results with ours seems to suggest that the lambs tested herein may be at risk of alimentary muscle dystrophy.

The low selenium concentrations determined in lambs' blood serum probably resulted from Se low levels in their mothers' milk (0.018 in 2005 and 0.017 mg dm^{-3} in 2006). The research showed the mean selenium levels in milk below the critical value of 0.02 mg dm^{-3} suggested by Underwood, Suttle (1999). Other authors detected similarly low selenium concentrations in milk (Brzóska et al. 2000, Valle et al. 2003, Khan et al. 2006). Selenium deficiency found in sheep's milk during lactation can be caused by a relatively low content of this element in fodder (Ursini et al. 1999).

Our observations are also supported by the high correlation coefficients between selenium concentrations in blood serum and milk of mother sheep and in blood serum of lambs, presented in Table 4.

Table 4

Correlations between selenium concentrations in blood serum and milk of sheep and blood serum of lambs

Selenium level	Milk of mother sheep	Blood serum of lambs
Iin blood serum of mother sheep	0.94*	0.77*
In milk of mother sheep	-	0.72*

* statistically significant differences ($p \leq 0.01$)

Statistically, all the correlations turned out to be significant at $p \leq 0.01$. It is possible, then, that selenium level in blood serum and milk of sheep may be a good indicator in the assessment of Se supply to sheep. The correlations discovered herein are confirmed by other authors (Kaup 1998, Rock et al. 2001, Davis et al. 2006, Karimi-Poor et al. 2011).

Moreover, the dependencies described above can help to design supplementation of sheep's diets with preparations containing selenium in order to prevent deficiencies of this element in their offspring. This concept is supported by research of Lacetera et al. (1999), Muniz-Naveiro et al. (2006), Abdel-Ghany et al. (2008), who have proven that selenium supplementation of sheep's and cows' diets during lactation results in a large increase of this element concentration in collostrum and milk, which protects newly-born lambs and calves from the risk of diseases related to selenium deficiency. Selenium in the form of seleno-amino acids, selenomethionine and selenocysteine are absorbed through the active amino acid transport mechanism and are more bioavailable than selenite or selenate (Vandeland et al. 1994). In monogastric animals, the relative selenium absorption is greater than in ruminants (Bopp et al. 1982) and organic forms of selenine are more easily absorbed, thus being a better source of Se in tissues and blood (Pehrson et al. 1999). The intestinal active absorption of selenium concerns selenomethionine and selenocysteine, and varies between 90-95 % for selenite and less than 10% less for selenate (Lee et al. 1996). In ruminants, the relative absorption ranges between 29-50% for selenate (Suttle, Jones 1989). The lower absorption in ruminants is due to microbial reduction of selenium form in the rumen to selenides and elemental selenium, which are not bioavailable (Peter et al. 1982). Some rumen microbes reduce selenium more efficiently, while others effectively incorporate it into selenium containing amino-acids. The incorporation proteins as well as systemic absorption can be competitively inhibited by natural methionine and cysteine (Serra et al. 1996).

Recent research shows that positive effects of Se supplementation on productivity and breeding in animals depend on many factors, such as environmental, nutritional and management factors (source of Se, time and length of the treatment, presence of interfering elements, diet feeding pattern) (Palmieri, Szarek 2011).

CONCLUSION

The research proves that environmental conditions have a significant influence on the supply of selenium to adult sheep and their offspring. Selenium deficiency in particular trophic chain links can pose a serious threat to the health of animals fed homegrown feeds.

Administration of selenium to animals for therapeutic or prophylactic reasons should be preceded by a thorough analysis of its content in organs and tissues. Moreover, the kind of compound, dose and duration of the supplementation should be well considered. Another crucial factor is the determination of the Se content in fodders fed to animals.

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EFFECT OF SALT STRESS CAUSED BY DEICING ON THE CONTENT OF MICROELEMENTS IN LEAVES OF LINDEN*

Wojciech Dmuchowski[1,2], Aneta H. Baczewska[1],
Dariusz Gozdowski[2], Beata Rutkowska[2], Wiesław Szulc[2],
Irena Suwara[2], Paulina Brągoszewska[2,3]

[1]Botanical Garden-Center for Conservation of Biological Diversity
Polish Academy of Sciences, Warsaw
[2] Warsaw University of Life Sciences – SGGW, Warsaw
[3]Institute of Environmental Protection – National Research Institute, Warsaw

Abstract

Application of large amounts of NaCl to control slippery roads in winter leads to soil salinity and consequently to ionic imbalances, changes in pH, changes in physicochemical properties of the soil and the death of roadside trees. The aim of this study was to evaluate the effect of salt stress on the content of microelements in the leaves of roadside trees and on the health trees. The subject of research were trees of the Crimean linden (*Tilia* 'Euchlora') growing in the median strip of one of the main streets in Warsaw. The roadside trees contained much higher amounts of Cl and Na than trees in a park (control). There was a significant correlation between the Cl and Na content in leaves of the trees and their health state. As the content of these elements increased, the health condition of leaves clearly deteriorated. There was no significant effect of soil salinity on the micronutrient content in leaves. The content of Cu, Fe, Zn and Mn in linden tree leaves were on levels considered normal, with values not indicative of any deficiency or toxicity. The presence of Fe and Zn in leaves had no significant effect on the health of leaves of the trees. A statistically significant negative relationship was found between the index of leaf damage and their content of Cu and Mn. This means that a higher degree of leaf damage corresponded to a lower content of Cu and Mn. Based on regression analysis, it was estimated an increase in the Cl content in soil solution by approximately 1000 mg dm^{-3} caused an average 0.2% increase in the Cl content in leaves.

Keywords: deicing, urban trees, salt stress, microelements, linden, soil pollution.

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dr hab. Wojciech Dmuchowski, Botanical Garden-Center for Conservation of Biological Diversity, Polish Academy of Sciences, 2 Prawdziwka St., 02-973 Warsaw, Poland, e-mail: w.dmuchowski@obpan.pl

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WPŁYW STRESU SOLNEGO SPOWODOWANEGO ZASOLENIEM GLEBY NA ZAWARTOŚĆ MIKROELEMENTÓW W LIŚCIACH LIP

Abstrakt

Stosowanie dużych ilości NaCl do zwalczania śliskości dróg w okresie zimowym prowadzi do zasolenia gleb, a w konsekwencji do zaburzeń równowagi jonowej, zmian pH oraz zmian właściwości fizykochemicznych gleby i zamierania drzew przyulicznych. Celem badań była ocena wpływu stresu solnego na zawartość mikroelementów w liściach drzew ulicznych i stan zdrowotny drzew. Przedmiotem badań były drzewa lipy krymskiej (*Tilia* Euchlora) rosnące w pasie międzyjezdniowym głównej ulicy w Warszawie. Drzewa uliczne zawierały znacznie więcej Cl i Na niż drzewa z parku (kontrola). Stwierdzono istotne zależności między zawartością Cl i Na w liściach drzew a ich stanem zdrowotnym. Wraz ze wzrostem zawartości tych pierwiastków wyraźnie pogarszał się stan zdrowotny liści. Nie stwierdzono istotnego wpływu zasolenia gleby na zawartość mikroelementów w liściach. Zawartość Cu, Fe, Zn i Mn w liściach lip była na poziomie uznanym za „normalny”, nie stwierdzono wartości wskazujących na niedobór lub poziom toksyczny. Zawartość Fe oraz Zn w liściach nie miała istotnego wpływu na stan zdrowotny liści drzew. Stwierdzono natomiast ujemne statystycznie istotne zależności między indeksem uszkodzenia liści a zawartością w nich Cu i Mn. Oznacza to, że wraz ze wzrostem stopnia uszkodzenia liści, zmniejszała się zawartość Cu i Mn. Na podstawie analizy regresji oszacowano w przybliżeniu, że zwiększenie zawartości Cl w roztworze glebowym o 1000 mg dm^{-3} powoduje przeciętne zwiększenie zawartości Cl w liściach o 0,2%.

Słowa kluczowe: stres solny, drzewa miejskie, mikroelementy, zanieczyszczenie gleby, lipy.

INTRODUCTION

Land degradation by salts is a major threat to sustainable crop production in many arid and semi-arid regions of the world (MAVI et al. 2012). In the countries of Northern and Central Europe and in the United States and Canada, we have to deal with soil salinity caused by widespread de-icing of winter streets and sidewalks with salt. NaCl is most commonly used because its inexpensive and effective at de-icing; calcium and magnesium chlorides and calcium and magnesium acetals are applied in smaller amounts (AKBAR et al. 2006, BERKHEIMER, HANSON 2006). According to the United States Geological Survey data, in the U.S., between 9.5 and 20.9 million tons of NaCl were applied on roads annually in 1993-2005 (SANDER et al. 2007). In Sweden, 200,000-300,000 tons of NaCl are used on roads every winter (THUNQVIST 2004). The dosage of salt per kilometer of a road is 40-80 tons per year (AKBAR et al. 2006). For example, in Toronto (Canada) the annual budget for de-icing is $ 50 million (GUTHRIE 2006). But the hidden costs of de-icing, like corrosion and the environmental damage, are in general much greater than the direct cost of road salting (FAY, SHI 2012).

Of the de-icing salt applied on a road, 20-63% ends up being transported by air and deposited on the ground 2 to 40 m away from the road. At least ninety percent of the total deposition occurs within 20 m (BLOMQVIST, JOHANSSON 1999, LAX, PETERSON 2009). MCBEAN and AL-NASSRI (1987) concluded that 90% of the deposited salt was found within 13 m.

It is estimated that over 50% of salt applied on the road permeates into surface water and groundwater. Salt concentration in fresh water bodies may increase up to 30 times compared with unpolluted waters (Ramakrishna, Thunqvist 2004, Viraraghavan 2005, Green et al. 2008, Nowotny et al. 2008).

NaCl applied on roads contributes to soil pollution in the roadside zone, causing a number of adverse changes which lead to soil degradation. The following are examples of adverse changes in soil: alteration in soil structure, decreased soil permeability and aeration, accompanied by increased overland flow, surface runoff and erosion. More intensive erosion results in nutrients and heavy metals being transported from the roadside to surface water. Another possible consequence is the reduction in hydraulic conductivity as pores become blocked by the release of fine particulates and soil colloids, promoting the leaching of base cations and consequently modifying the soil exchange pools. This change affects soil microflora and microfauna communities (Amrhein, Strong 1990, Fritzsche 1992, Defourny 2000, Bergstedt 2001, Norrström, Bergstedt 2001, Bryson, Barker 2002, Green et al. 2008, Nelson et al. 2009, Mavi et al. 2012).

Salt affects trees by inducing water stress, nutrient imbalance and ion toxicity, causing biochemical, anatomical and morphological changes, also making them more susceptible to disease, as a result of which it interferes with their growth and development, and may even cause their death, which is not an uncommon consequence of salt stress to roadside trees (Czerniawska-Kusza et al. 2004, Franklin, Zwiazek 2004, Dmuchowski et al. 2007, 2011*a*,*b*, Oleksyn et al. 2007, Polanco et al. 2008, Baczewska et al. 2011*a*, Gałuszka et al. 2011, Tahkokorpi et al. 2012).

The aim of this research was to assess the impact of salt stress on the health of roadside trees, and especially on changes in the content of microelements in leaves. The subject of research was the Crimean linden (*Tilia* Euchlora), which is often planted along streets in Warsaw.

MATERIAL AND METHODS

The study focused on the median strip of Żwirki and Wigury Avenue. This is one of the main streets of Warsaw, hence it carries a heavy flow of traffic. The control area was a park located about 150 meters away near the avenue and separated from it by dense hedges of trees and bushes. The top soil on both surfaces was of the same type, described as salty soil. Both areas were populated by Crimean linden (*Tilia* Euchlora) trees about 85 years old.

The soil samples (18) were collected from both surfaces around the selected trees (18). The trees were divided into six categories based on the health status of their leaves. The evaluation of the health of leaves was conducted with a modified method of Duda et. al. (1994). This classification consists of six health categories (the leaf damage index), where 0 meant a healthy tree and 5 is a seriously damaged one (damage of up to 75% of the leaf surface area). The observations of the health were conducted in mid-September 2010. Three trees were selected from each health status category and the control surfaces. Soil samples were taken from the soil under each tree at three locations 1 m away from the trunk. They were collected at three depths (0-20, 20-40, 40-60 cm) with a soil drill. The samples were dried to the air humidity level, ground and passed through a 2 mm mesh sieve.

Air-dry soil samples weighing 100 g were saturated with redistilled water to 150% of full water capacity, and then incubated at room temperature for 66 hours to achieve the ionic equilibrium between the solid and the liquid phases. Soil solutions were obtained from the prepared soil samples by vacuum suction (Wolt, Graveel 1986, Łabętowicz 1995) with a single oil vacuum pump (Dynavac OP4). The solutions were filtered through blotting paper filter into polyethylene bottles, then frozen and stored for chemical analysis.

The following determinations, with three replications, were run on the soil solutions:

- the total content of Zn, Cu, Fe, Mn, Na by atomic emission spectrometry method (ICP-AES, apparatus IRYS Advantage ThermoElementar). The apparatus was calibrated against patterns prepared from Single Element Standards for ICP Solution by UltraScientific. In order to check the calibration curve, the solutions were used to attest the instrument and calibration (QC) at concentrations of 0.1 ppm and 1 ppm before the samples were studied, at every 20 samples against the Combined Quality Control Standard from Ultra Scientific company;
- pH in the fresh soil solutions – with the potentiometric method;
- chlorides – with the flow colorimetric method (a Skalar apparatus).

For the calculations, means from the three sampling sites were used, separately for each tree. The analytic results were obtained in an accredited laboratory, and the accuracy of determinations was ensured by a control system based on the criteria contained in the standard PN-EN ISO/IEC/17025.

The leaf samples used for chemical determination were collected separately from each tree during the last week of July 2011. The leaves were collected from the outer belt of the tree crown – around its fill perimeter at a height of about 4 m. The leaf material was placed in linen bags and dried at 70°C. The dried materials were ground to a powder in a stainless steel impact mill (Fritsch 14702) and stored in tightly sealed plastic containers until the time of analysis. The leaves were washed for one minute in distilled water before they were dried and ground.

For the determination of metals, powdered samples were ashed in a muffle oven (Naberthern L40/11/P320) using the following time/temperature protocol: 120°C – 2h, 200°C – 1h, 300°C – 1h, and 450°C – 5h. The ashes were digested in 30% HCl (Merck suprapure) and filtered through a filter paper (Allen et al. 1974). The analyses were performed by flame AAS (Perkin Elmer 1100A), connected to deuterium background correction, hollow cathode lamps and acetylene burner (Roberts 1991). Three replicate subsamples of each sample were processed. Three blanks were run with each batch of samples; thus, each sample was blank corrected.

Chloride was determined by the potentiometric titration method using an ion-selective electrode and an Orion Star Plus ion meter (La Croix et al. 1970).

For quality control (QC), the elemental content in the plant samples was determined using certified reference materials from the NIST- USA - Apple leaves nr 1515. The results were in good agreement with the certified values. The recovery range was from 90 to 96%.

The means were compared using analysis of variance and the Tukey's procedure of multiple comparisons. Relationships were evaluated using correlations and simple linear regression. The statistical analyses were performed in Statistica 10 (StatSoft) software, with the significance level at 0.05 probability level (Sokal, Rohlf 1995).

RESULTS AND DISCUSSION

The study included Crimean linden trees, characterized by high sensitivity to street side urban conditions. Just 38% of the trees growing in 1973 along Warsaw's main streets survived until 2008 (Dmuchowski et al. 2011*b*). In Poland, the main cause of roadside trees dying is elevated soil salinity caused by winter deicing of streets and sidewalks (Chmielewski et al. 1999, Brogowski et al. 2000, Czerniawska-Kusza et al. 2004, Bach, Pawłowska 2006, Oleksyn et al. 2007, Kochanowska, Kusza 2010, Wrochna et al. 2010, Baczewska et al. 2011a, Dmuchowski et al. 2011a, Gałuszka et al. 2011).

Table 1 shows the results of analyses of soil from three depth levels, including the content of chlorine, metals and soil pH. The pH of surface soil along the street ranged at 7.36-7.52, depending on the depth, and was statistically different from the soil in the park, which was 6.72-7.02. The fact that salinity raises alkalinity of soil has been described in many publications. Gałuszka et al. (2011) determined pH 7.8-8.0 in soil near streets with heavy traffic in Kielce (Poland). In forest soil near a road where deicing was used, Černohlávková et al. (2008) detected pH 7.6 at a distance of 1 m from the road, 5.5 at 10 m, and just 3.8 in the control soil. Similar results were obtained by Green et al. (2008), with 6.7-8.1 pH at 3 m from the main road, and in the control soil 3.8. Hofman et al. (2012) studied soil in protected areas near a main road heavily salted in winter (6.6-13 t km^{-1} year^{-1})

Table 1

pH and the average content of particular elements in the three depths of the soil solution

Element	Depth (cm)	Park (control)	Street
pH	0 - 20	7.02*a*	7.52*b*
	20 - 40	6.72*a*	7.36*b*
	40 - 60	6.93*a*	7.38*b*
Cl (mg dm^{-3})	0 - 20	1470*a*	3599*b*
	20 - 40	1304*a*	2561*b*
	40 - 60	1186*a*	2526*b*
Na (mg dm^{-3})	0 - 20	430*a*	2392*b*
	20 - 40	418*a*	1540*b*
	40 - 60	477*a*	1517*b*
Cu (mg dm^{-3})	0 - 20	1.6*a*	1.9*a*
	20 - 40	1.3*a*	1.9*b*
	40 - 60	1.1*a*	1.6*b*
Fe (mg dm^{-3})	0 - 20	5.3*a*	6.7*a*
	20 - 40	4.0*a*	8.3*a*
	40 - 60	2.8*a*	5.7*a*
Mn (mg dm^{-3})	0 - 20	4.5*a*	12.8*b*
	20 - 40	3.7*a*	7.1*a*
	40 - 60	2.4*a*	5.7*b*
Zn (mg dm^{-3})	0 - 20	1.6*a*	2.3*b*
	20 - 40	1.4*a*	2.4*b*
	40 - 60	1.5*a*	2.1*b*

The average values are assigned letters indicating differences between the park and street. The same letter (*a*) stands for a non-significant difference between the means. Different letters (*a* and *b*) stand statistically significantly different means.

where the pH decreased from 7.4-7.8 m to 4.0-4.1in the transect 1-15 m. Similar results were reported by Norrström and Jacks (1998) and Norrström and Bergstedt (2001), Bryson and Barker (2002), Czerniawska-Kusza et al. (2004), Nelson et al. (2009), Kochanowska and Kusza (2010).

Street soils contained more of the analyzed elements than the park soils, and the differences were not statistically significant only in the case of Fe, which could have been due to the very high divergence of results. Particularly large differences occurred for Cl, which – depending on the level - was detected in the concentrations equal 1186-1470 mg dm^{-3} in the park, and 2526-3599 mg dm^{-3} near the street, and for Na, which appeared in the levels of 418-477 mg dm^{-3} and 1517-2392 mg dm^{-3}, respectively.

Leaves from trees growing along the street contained much more Cl and Na than those from the park (control) – Table 2, Figure 1. Leaves from the control trees contained an average of 0.36% Cl and 78.3 mg kg^{-1} Na, and those from the street trees had 0.73-1.92% Cl, and 174-2766 mg kg^{-1} Na. Significant correlations were found between the content of these elements in the leaves and their state of health (Figure 1). With an increase in the content of these elements, the health condition of tree leaves clearly deteriorated. It is accepted that the normal level of Cl in linden leaves should be less than 0.3%, with 0.6-0.8% considered as a threshold value beyond which clear leaf damage appears (SHORTLE, RICH 1970, PRACZ 1978, PAULEIT 1988, DMUCHOWSKI, BADUREK, 2004, MUNCK et al. 2010) gives 0.37% as the value of chlorine concentration at which the first faint signs of leaf damage appear. This level was exceeded in leaves of all the street trees studied.

The Na content also significantly affected the health status of leaves. Relatively healthy trees with an index of leaf damage at 0-1 contained 168-174 mg Na kg^{-1}, while badly damaged ones, indexed at 4-5, had 1338-2766 mg Na kg^{-1}. It is not easy to interpret the results of the sodium content in leaves because the literature does not provide us with any information about toxicity limits. There is only one report, by MUNCK et al. (2010), in which > 650 mg kg^{-1} is said to be the limit value beyond which tree leaves contain distinctly fewer ions. Sodium is characterized by high lability both in soil and plants, and its excess causes mainly ionic imbalance, rather than a simple toxic effect (ALAOUI-SOSSE 1998, DMUCHOWSKI et al. 2011*a*).

The content of Cu, Fe, Zn and Mn in the linden leaves was on levels considered normal, with values not indicative of any deficiency or toxicity. A normal level for Cu has been determined at 2-20 mg kg^{-1} and toxic levels are above 30 mg kg^{-1} (HEWITT, SMITH 1974, KABATA-PENDIAS, PENDIAS 2001). MADEJON et al. (2006) determined as the normal Mn content in leaves of *Quercus* at 17 mg kg^{-1}, while KABATA-PENDIAS and PENDIAS (2001) identified 10-25 mg kg^{-1} as sufficient for most plants. SMITD (1988) concluded that a normal level of Zn in tree leaves was 25-80 mg kg^{-1}, but KABATA-PENDIAS and PENDIAS (2001) claimed it was 10-100 mg kg^{-1}, although all these researchers stated that values above 100 mg kg^{-1} were toxic. LINZON et al. (1976) found 250 mg kg^{-1} as a limiting toxic level of Zn for deciduous trees. No values were found in the literature for deficient and toxic levels of iron in leaves of trees.

Table 2

The average content of elements in linden leaves, depending on the leaf damage index

Element	Leaf damage index						
	control	0	1	2	3	4	5
Cl (%)	0.36*a*	0.73*ab*	0.78*ab*	1.25*bc*	1.64*cd*	1.77*cd*	1.92*d*
Na (mg kg^{-1})	78*a*	174*a*	168*a*	198*a*	353*a*	1338*ab*	2766*b*
Cu (mg kg^{-1})	10.61*ab*	14.8*b*	13.9*b*	12.4*ab*	10.4*ab*	10.5*ab*	9.4*a*
Zn (mg kg^{-1})	29.1*a*	27.3*a*	29.6*a*	26.8*a*	24.3*a*	26.6*a*	24.4*a*
Fe (mg kg^{-1})	163*a*	311*a*	308*a*	286*a*	251*a*	292*a*	256*a*
Mn (mg kg^{-1})	49.6*a*	92.6*a*	74.9*a*	66.5*a*	47.7*a*	48.3*a*	42.9*a*

The means are assigned letters indicating homogeneous groups. If the letter is the same, the difference between the means is not statistically significant. If the letters are different, then the two means differ statistically significantly.

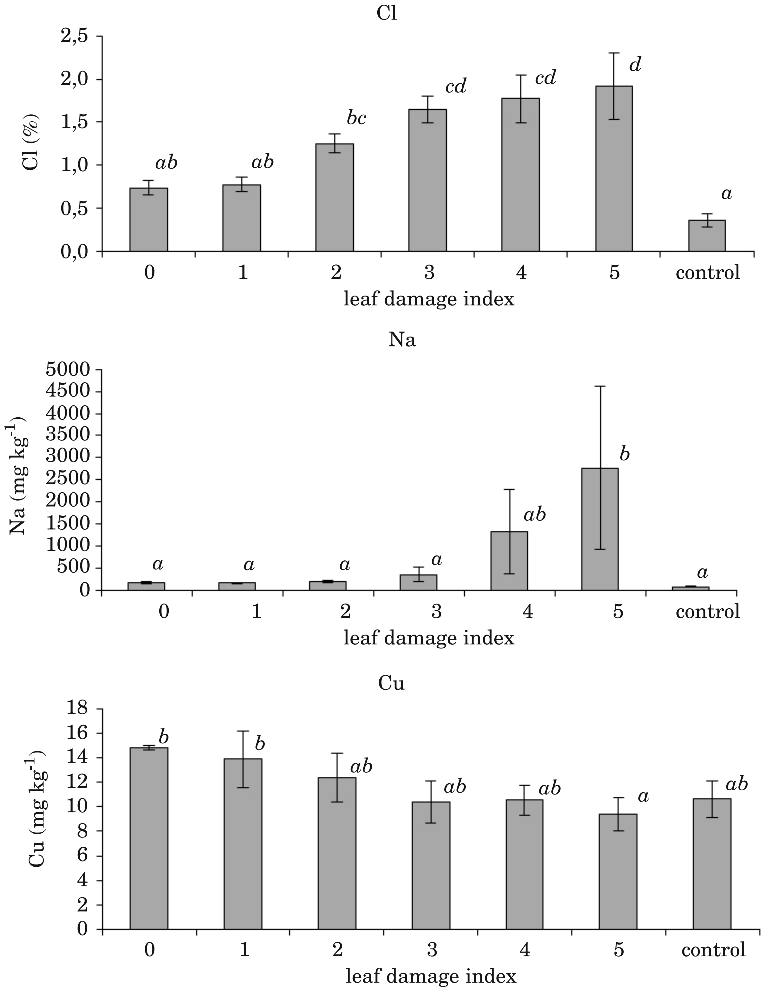

Fig. 1. The average content of Cl, Na and Cu in the leaves, depending on the leaf damage index
Error bars present values of standard deviations (SD) and letters indicate homogenous groups of means (if there is even one common letter, then the difference between the means is not statistically significant)

Iron and zinc in leaves had no significant effect on the health of linden leaves (Table 3). A statistically significant negative relationship was found between the leaf damage index and the content of Cu and Mn. Thus, a higher degree of leaf damage or a worse health status were correlated with the Cu and Mn content in leaves. In all the examined trees, the Cu and Mn content did not appear deficient, hence the above dependence may have been caused by an ionic imbalance due to the high content of Cl and Na in leaves. Baczewska et al. (2011*a*) and Dmuchowski et al. (2011a) demonstrated

Table 3

Correlation coefficients between pH and the content of elements in the soil solution versus their leaf content and the leaf damage index

Element	Depth (cm)	Leaf damage index	Cl	Na	Cu	Zn	Fe	Mn
		-	0.91**	0.69**	0.81**	-0.30	-0.34	-0.49*
pH	0-20	0.22	0.26	0.32*	-0.26	0.00	-0.16	-0.08
	20-40	0.17	0.20	0.21	-0.20	-0.34*	-0.14	-0.47**
	40-60	0.05	0.07	-0.10	-0.08	-0.22	-0.04	-0.31*
Cl	0-20	0.40**	0.44**	0.46**	-0.21	-0.09	0.03	-0.20
	20-40	0.38**	0.43**	0.47**	-0.27*	-0.32*	-0.11	-0.30*
	40-60	0.21	0.25	0.53**	-0.14	-0.25	-0.08	-0.31*
Na	0-20	0.42**	0.44**	0.60**	-0.29*	-0.27	-0.04	-0.24
	20-40	0.36**	0.42**	0.61**	-0.23	-0.26	-0.03	-0.27
	40-60	0.44**	0.43**	0.51**	-0.26	-0.11	-0.02	-0.22
Cu	0-20	0.24	0.23	0.34*	-0.08	0.01	0.06	-0.24
	20-40	-0.03	-0.08	0.04	0.02	0.01	0.11	0.11
	40-60	0.05	0.10	-0.12	-0.18	0.19	-0.14	-0.06
Fe	0-20	0.06	0.19	0.06	-0.12	-0.18	-0.05	-0.13
	20-40	-0.20	-0.26	-0.16	0.23	0.18	0.12	0.35*
	40-60	-0.19	-0.05	-0.14	0.13	0.16	0.05	0.21
Mn	0-20	-0.21	-0.12	0.01	0.28*	0.04	0.23	0.16
	20-40	-0.10	-0.19	-0.12	0.14	0.27*	0.09	0.42**
	40-60	-0.14	-0.11	0.02	0.18	0.23	0.05	0.21
Zn	0-20	0.08	0.15	0.10	0.05	-0.01	0.15	0.00
	20-40	0.15	0.06	0.11	-0.06	0.14	0.11	0.01
	40-60	0.01	0.01	0.08	0.04	0.17	0.09	0.02

* Statistically significant correlations at the level of $P<0.05$
** Statistically significant correlations at the level of $P<0.01$

that the ionic imbalance caused by soil salinity resulted in a high negative correlation coefficient, indicating that an increasing content of primary macronutrients, such as nitrogen, phosphorus, potassium and sulfur, in leaves was correlated with a better health status of street trees.

Many studies suggest that small amounts of trace elements are dissolved in the soil solution and may be translocated to subsoil horizons. The most important chemical process that affects the solubility and mobility of trace elements is their sorption onto soil solid phases. Metal sorption depends, *inter alia*, on the nature of organic and inorganic soil constituents, as well as soil pH. The soil pH is the main factor which controls the solubility, mobility and transport of trace elements in a soil profile. Metal solubility and mobility decrease under a higher soil pH. The soil organic matter content is the second most important factor affecting the mobility of trace elements in the soil solution. An increase in the soil DOM concentration leading to the formation of organic and trace elements complexes may enhance the downward transport of these elements in a soil profile (Silvana, Rodrigo 2012). The vertical distribution of copper in the soil solution depends on the content of soil organic matter. Organic complexes of copper accounted for more than 90% of the copper solution in the surface horizon. This process of forming complexes increases the concentration of Cu in the mobile phase, which promotes Cu transport over long distance. The copper vertical distribution in the soil solution is associated with the total organic carbon and soil solution pH. Fulvic acids are the main organic compounds responsible for Cu mobility. The mobility of TOC and Cu is the lowest in a strongly acid soil solution but higher in a soil solution with neutral reaction. This suggests that Cu can be transported downward in a soil profile to the the 60-120 cm layer, which acted like a barrier to a further downward transport (Altaher et al. 2011).

The solubility and mobility of manganese, zinc and iron are generally low under a high pH and high organic matter content. The mobility of these elements in the soil solution of acid soils with a low organic matter content is high (Violante et al. 2010).

Beesley et al. (2010) showed that Zn can be transported downward in a soil profile (70-100 cm). The peak concentration of Zn in the soil solution was recorded at a 50 cm depth. There was no correlation between Zn and DOC in the soil solution.

Based on the correlation analysis, relationships were determined between elemental concentrations in the soil solution, and their content in leaves of linden trees (Figure 2). For most elements, there was no distinct, statistically significant correlation. The only positive correlation was found between the concentrations of Na and Cl in the soil versus the content of these elements in leaves. Alfani et al. (1996), who studied urban trees of the species *Quercus ilex,* found no correlation between the content of metals (Cu, Fe, Mn) in the soil and in tree leaves. The authors attributed this finding to high levels of airborne metals accumulated by leaves. The positive correlations were found only for Pb. Łukasik et al. (2002) also failed to verify a statistically significant relationship between concentration of metals (Pb, Cd, Zn) in the soil and their content in leaves of urban trees and shrubs (*Acer, Robinia, Symphoricarpos, Ligustrum*). The lack of statistical significance between soil and leaf content of metals was also reported by Samecka-Cymarman and Kempers (1999), who studied Zn and Pb in leaves of *Ilex aquafolium, Mahonia aquifolium* and *Rhododendron catawbiense*.

The regression analysis (Figure 2) revealed a relationships between the content of Na and Cl in the soil solution and in leaves. The regression

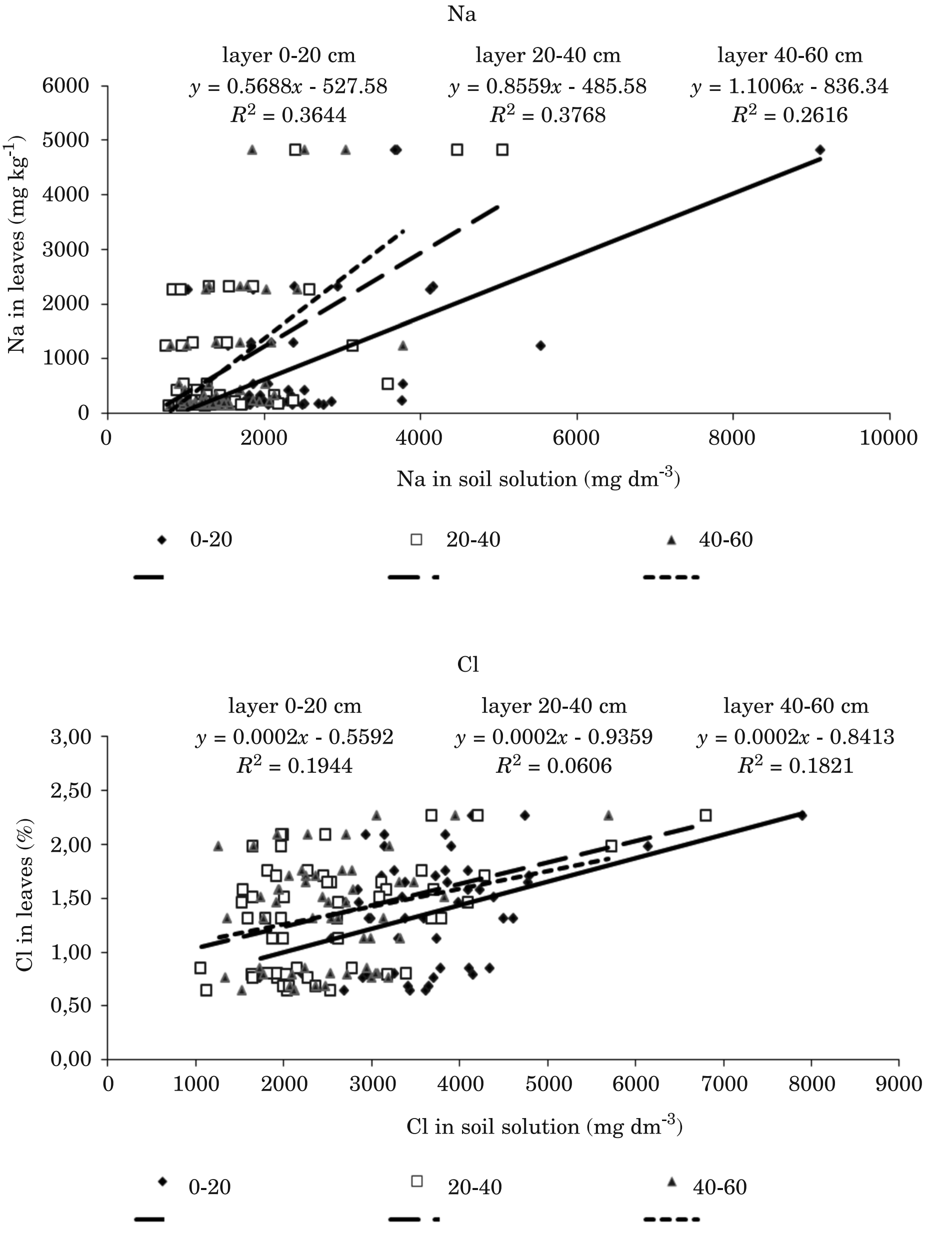

Fig. 2. Regressions between the content of Na and Cl in the soil solution and in leaves for different soil layers

function enabled us to estimate the content of Na and Cl in leaves, given the specific content of these elements in the soil on different levels. In the case of Cl, all regression functions are similar in the form, hence we can estimate that an increase in Cl in the soil solution by 1000 mg dm^{-3} caused an average increase of the Cl content in leaves by 0.2%. Regarding sodium, the regression functions for different Na levels in soil assumed different shapes, while demonstrating a similar power of the relationship between the content of Na in the soil and in leaves.

CONCLUSIONS

1. The soil from street side surface contained statistically significantly more Cl, Na, Cu, Fe, Zn and Mn than the park soil. Particularly large differences occurred in the contents of Cl and Na resulting from de-icing of the roads. The soil salinity also caused increase in soil alkalinity.

2. The street trees contained much higher amounts of Cl and Na than the park trees. Significant correlations were found between the content of Cl and Na in the leaves of trees and their state of health. With the increase in the content of these elements, the health condition of the leaves clearly deteriorated.

3. The content of Cu, Fe, Zn and Mn in the leaves of linden were at levels considered “normal”, no values were found to be indicative of a deficiency or toxic levels. The level of Fe and Zn in the leaves had no significant effect on the health of the trees. A statistically significant negative correlation was found between the index of leaf damage, and their content of Cu and Mn. This means that with an increase in the degree of leaf damage, the content of Cu and Mn decreased.

4. A positive correlation was found between Na and Cl content in the soil, and the content of these elements in the leaves. In the case of Cu, Zn, Fe and Mn., there were no clear statistically significant dependencies between their concentration in the soil, and the content in the leaves.

5. Based on regression analysis it was estimated that increasing the Cl content in the soil solution by 1000 mg·dm^{-3} causes an average increase in the leaf Cl content of approximately 0.2%.

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YIELD AND SELECTED INDICES OF GRAIN QUALITY IN SPRING WHEAT (*TRITICUM AESTIVUM* L.) DEPENDING ON FERTILIZATION

Krzysztof Gondek

Department of Agricultural and Environmental Chemistry
University of Agriculture in Krakow

Abstract

The aim of the research was to determine the effect of fertilization with manure, compost from biodegradable waste and municipal sewage sludge on the yield and certain indices of grain quality in spring wheat in comparison to fertilization with mineral fertilizers. The research was conducted as a three-year field experiment. The limited access of plants to nutrients (mainly nitrogen) introduced with waste organic materials and with manure strongly affected the crop yield and its quality, particularly in the first year of the research. A much better residual of fertilization with pig manure and compost from biodegradable waste on the spring wheat grain yield was observed in comparison to fertilization with municipal sewage sludge. Fertilization with waste organic materials, in doses based on plant requirements for nutrients, did not lead to a decrease in the biological value of yield. Irrespective of the applied fertilization, copper and manganese were the microelements that limited the fodder value of spring wheat grain, whereas the quality of protein was determined by the content of lysine.

Keywords: spring wheat, microelements, exogenous amino acids.

PLON I WYBRANE WSKAŹNIKI JAKOŚCI ZIARNA PSZENICY JAREJ (*TRITICUM AESTIVUM* L.) W ZALEŻNOŚCI OD NAWOŻENIA

Abstrakt

Celem badań było określenie wpływu nawożenia obornikiem, kompostem z odpadów biodegradowalnych oraz komunalnym osadem ściekowym na plon i niektóre wskaźniki jakości ziarna pszenicy jarej, w porównaniu z nawożeniem nawozami mineralnymi. Badania prowadzono w warunkach doświadczenia polowego przez 3 lata. Ograniczony dostęp roślin do składników pokarmowych (głównie do azotu) wprowadzonych z materiałami organicznymi pochodzenia odpadowego oraz z obornikiem w znacznym stopniu determinował wielkość i jakość plonów, zwłaszcza w pierwszym roku badań. Stwierdzono istotnie lepszy, następczy wpływ nawożenia

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dr hab. inż. Krzysztof Gondek, prof. UR, Al. Mickiewicza 21, 31-120 Kraków, Poland, e-mail: rrgondek@cyf-kr.edu.pl

obornikiem od trzody chlewnej oraz kompostem z odpadów biodegradowalnych na plon ziarna pszenicy jarej w porównaniu z nawożeniem komunalnym osadem ściekowym. Nawożenie materiałami organicznymi pochodzenia odpadowego, w dawkach zgodnych z zapotrzebowaniem roślin na składniki pokarmowe, nie spowodowało pogorszenia wartości biologicznej plonu. Niezależnie od zastosowanego nawożenia, mikroelementami ograniczającymi wartość paszową ziarna pszenicy jarej były miedź i mangan, natomiast jakość białka była determinowana zawartością lizyny.

Słowa kluczowe: pszenica jara, mikroelementy, aminokwasy egzogenne.

INTRODUCTION

Wheat plays an important role in human nutrition and has a high yielding potential, which makes it one of the most popular crops not only in Poland, but also worldwide. Globally, about 60% of grain production is used for nutrition. The technological value of wheat grain is, to the greatest extent, conditioned by genetics. However, environmental and agro-technical conditions are also significantly important (SIELING et al. 2005, CIU et al. 2006).

When analyzing the abundance of Polish soils in nutrients and humus, and in respect of acidification, signs of degradation of these soils are increasingly more frequent. It is mainly the result of insufficient amounts of applied natural and organic fertilizers as well as inadequate liming. The annual outflow of biogenic components with plant yields and losses of these components make it necessary to perform fertilization, which regulates levels of nutrients necessary for plants.

The delivery of adequate amounts of nutrients supplied to crops, while ensuring optimal soil conditions, is the main factor conditioning the expected yield characterized by a proper biological and technological value; and at the same time it may be a determinant for dosing manure, compost or sewage sludge.

Cereal grain has a diverse content of mineral components such as microelements, and organic components, including amino acids. In respect of nutrition, not all microelements or amino acids are equally important. Most often, the biological value of cereal grain is limited by the content of zinc, copper or manganese, and by the content of such amino acids as lysine and sulfur amino acids. The levels of these elements and compounds may be conditioned, for example, by fertilization (FLAETE et al. 2005).

The aim of the research was to determine the effect of fertilization with manure, compost from biodegradable waste and municipal sewage sludge on the yield and certain indices of grain quality in spring wheat in comparison to fertilization with mineral fertilizers.

MATERIAL AND METHODS

The research was conducted as a field experiment located 10 km west of Krakow (49°59' N; 19°41' E). Data from the meteorological station are presented in Table 1. Soil from the experimental site was classified to Stagnic Gleysol (IUSS Working Group WRB 2006). Table 2 presents selected soil properties prior to the commencement of the research.

Table 1

Monthly rainfall and average daily temperature at the site during 2005-2007 and long-term mean (1961-1999)

Month	Sum of monthly precipitation (mm)				Monthly average temperature (°C)			
	2005	2006	2007	mean 1961 - -1999	2005	2006	2007	mean 1961 - -1999
January	66	58	101	34	–1.2	–2.4	3.2	–3.3
February	33	49	42	32	–4.3	–3.0	1.2	–1.6
March	21	60	61	34	–0.2	0.2	6.0	2.4
April	49	57	15	48	6.8	5.6	8.5	7.9
May	61	52	52	83	11.4	10.9	15.2	13.1
June	41	89	72	97	14.4	15.0	18.4	16.2
July	113	14	71	85	17.6	18.6	19.4	17.5
August	103	104	76	87	15.4	15.6	19.0	16.9
September	27	17	180	54	12.5	13.4	12.4	13.1
October	8	32	48	46	7.1	9.1	7.7	8.3
November	30	21	90	45	3.9	6.3	0.8	3.2
December	47	16	21	41	–0.7	0.9	–1.1	–1.0

Table 2

Physical and chemical properties of the soil before experiment (0-20 cm layer)

Determination	Unit	Value
pH (KCl)	-	5.60
Organic C	g kg^{-1} d.m.	15.3
Total N	g kg^{-1} d.m.	1.59
Total Cu	mg kg^{-1} d.m.	15.8
Total Zn	mg kg^{-1} d.m.	132.8
Total Mn	mg kg^{-1} d.m.	2230
P available	mg kg^{-1} d.m.	71.8
K available	mg kg^{-1} d.m.	297.5
Mg available	mg kg^{-1} d.m.	367.7
Bulk density	g cm^{-3}	1.52
Total porosity	cm^{3} cm^{-3}	0.41
Fraction < 0.02 mm	g kg^{-1} d.m.	520

The experiment was set up with the randomized blocks method. The plot area was 30 m^2. The experimental design comprised 5 treatments in four replications: without fertilization (0), fertilization with mineral fertilizers (MF) [110.0 kg N ha^{-1}, 58,6 kg P ha^{-1} and 120.0 kg K ha^{-1}], fertilization with pig manure (PM) [dose of 3.23 t ha^{-1} d.m.], fertilization with compost from biodegradable waste (C) [dose of 2.83 t ha^{-1} d.m.], and fertilization with municipal sewage sludge (SS) [dose of 2.65 t ha^{-1} d.m.].

The field was limed before setting up the experiment (autumn 2004). The measure was conducted according to ˝ hydrolytic acidity value (962.0 kg CaO ha^{-1}) In the spring of the following year, after basic cultivation measures, manure, compost and sewage sludge were evenly distributed on surfaces of the plots and ploughed in. Two weeks later, supplementary mineral fertilization was applied; the fertilizers were mixed with the soil using a cultivator/harrow aggregate. The nitrogen dose supplied with organic materials was 110.0 kg N ha^{-1}. Phosphorus and potassium were supplemented with mineral fertilizers to an equal level in all treatments (except the control), [phosphorus to 58.6 kg P ha^{-1} as single superphosphate, and potassium to 120.0 kg K ha^{-1} as 60% potassium salt]. In the second and third year of the experiment, the same doses of components as in the first year were used, but only in the form of mineral fertilizers.

The experiment was performed on cv, Jagna spring wheat. The assumed sowing density was 485 plants per 1 m^2. Chemical measures were carried out during the vegetative growth to protect the plantation against weeds and fungal diseases.

The duration of a wheat vegetative period in individual years was weather-dependent. Wheat was harvested at grain maturity: on 13 August 2005 in the first year of the research, on 3 August 2006 in the second year, and on 31 July 2007 in the third year. In order to determine the wheat yield in field conditions, the plants were harvested from an area of 4 m^2, separately from each plot.

The swine manure used in the research had been stored in a manure heap for 6 months. The compost was made with the Mut-Kyberferm technology from plant waste and other biodegradable waste in the following proportions: 25% grass, 20% wood chips, 20% leaves, 10% organic waste from market squares, 5% tobacco dust, and 20% waste from coffee production. The compost came from a composting plant located in the city of Krakow. The stabilized sewage sludge (SS) originated from a municipal biological sewage treatment plant located in the Czernichów commune (Malopolska region).

The following assessments were made on fresh samples of manure, compost and sewage sludge: dry mass content (at 105°C for 12 h), pH by potentiometery, electrolytic conductivity with a conductometer, total nitrogen content by the Kjeldahl's method. The content of organic matter and ash was determined in dried and ground material after sample mineralization

Table 3

Physical and chemical properties of the organic materials

Determination	Unit	Pig manure (PM)	Compost (C)	Sewage sludge (SS)
N_{tot}	g kg^{-1} d.m.**	34.0	38.9	41.6
P_{tot}	g kg^{-1} d.m.	12.8	5.9	22.3
K_{tot}	g kg^{-1} d.m.	21.8	29.9	1.2
Cu_{tot}	mg kg^{-1} d.m.	156	34	80
Zn_{tot}	mg kg^{-1} d.m.	284	194	950
Mn_{tot}	mg kg^{-1} d.m.	355	280	112
EC*	mS cm^{-1}	2.89	2.62	0.47
pH (H_2O)		8.23	7.31	6.57
Organic matter	g kg^{-1} d.m.	831	531	726
Water content	g kg^{-1} f.m.***	774	563	742
Ash	g kg^{-1} d.m.	169	469	244

* EC – electrical conductivity, ** data are based on 105°C dry matter weight, *** f.m. – fresh matter

in a chamber furnace (at 450°C for 5 h). The phosphorus content was determined by colorimetry in a Beckman DU 640 spectrophotometer, and the potassium content was assessed by flame emission spectroscopy (FES) in a Philips PU 9100X apparatus. The content of copper, zinc and manganese was determined by the ICP-AES method in a JY 238 Ultrace apparatus. The analyses were performed according to the methodology described by Baran and Turski (1996), and Krzywy (1999), and the analytical results are presented in Table 3.

The content of selected microelements was determined in dried and ground wheat grain after sample mineralization in a chamber furnace (450°C, 5 h) (Ostrowska et al. 1991) by the ICP-AES method in a JY 238 Ultrace apparatus.

Concentrations of selected amino acids were assessed on an AAA-400 (Ingos) analyzer following protein hydrolysis in 6 mol dm^{-3} HCl (110°C, 24 h). The methionine content was determined after oxidation with formic acid.

On the basis of the results, the index of limiting amino acid CS (Chemical Score) was computed. It was introduced by Block and Mitchell (Bęza 1967) and consists of the ratio of an exogenous limiting amino acid in the analyzed protein to the content of the same amino acid in a standard protein. Two standards were used for the calculations: mature human protein (MH) and whole egg protein (WE) (FAO/WHO 1991).

The analysis of the plant material was conducted in four replications.

Table 4

Amounts (mean ± SD) of metals released for material NCS DC733448, as well as data for analytical precision and accuracy

Metal	The value obtained in current study (mg kg^{-1} d.m.)	Recommended value (mg kg^{-1} d.m.)	Accuracy	Accuracy
Zn	21.4±1.0	20.6±2.2	4.71	3.88
Cu	5.3±0.1	5.2±0.5	1.88	1.92
Mn	55±1	58±6	1.82	5.45

The accuracy of Zn, Cu and Mn determinations was assessed with reference material NCS DC733448 (China National Analysis Center for Iron & Steel). Data on the accuracy of performed determinations are presented in Table 4 (Fuentes et al. 2004).

A two-way analysis of variance (factors: fertilization x years) was conducted for spring wheat grain yield, and a one-way analysis of variance (factor: fertilization) in an entirely randomized design using f-Fisher test was conducted for weighted mean (from 3 years) content of the analyzed microelements, protein and of exogenous amino acids. The significance of differences between arithmetic means was verified on the basis of homogenous groups determined by the Duncan's test at the significance level $p < 0.05$. All statistical computations were conducted using a Statistica PL package (Stanisz 1998).

RESULTS

Grain yields

On treatments where pig manure (PF), compost from biodegradable waste (C), and municipal sewage sludge (SS) were applied, spring wheat grain yields in the first year of the research were smaller by 0.95 t, 1.23 t, and

Table 5

Dry-matter yields of grain spring wheat for different year of experiment

Year	Treatments				
	0	MF	PM	C	SS
	(t d.m. ha^{-1})				
2005	2.53^{b} ± 0.13	4.30^{ef} ± 0.23	3.35^{c} ± 0.16	3.07^{bc} ± 0.31	3.74^{c} ± 0.25
2006	2.00^{a} ± 0.18	4.97^{hi} ± 0.26	4.44^{efg} ± 0.27	4.31^{ef} ± 0.30	4.86^{gh} ± 0.30
2007	2.54^{b} ± 0.10	4.30^{ef} ± 0.35	5.39^{i} ± 0.31	5.41^{i} ± 0.11	4.42^{efg} ± 0.11

Means ± standard error. Different letters indicate significant differences ($\sum < 0.05$, Duncan's multiple range test).

0.56 t d.m. ha^{-1}, respectively, in comparison to the yield from the treatment fertilized with mineral fertilizers (MF) – Table 5. The statistical analysis of the results confirmed significance of the differences between arithmetic means from individual treatments.

In the second year, much smaller differences in wheat grain yields were found. The difference between the lowest grain yield, obtained after the application of compost from biodegradable waste (C), and the highest grain yield, obtained after fertilization with mineral fertilizers (MF), was 0.66 t d.m. ha^{-1}.

In the third year, higher spring wheat grain yields were gathered from the treatments where pig manure (PF), compost (C) and municipal sewage sludge (SS) were applied in the first year than from the one where wheat was fertilized exclusively with mineral fertilizers (MF). The results indicate a much better residual effect of fertilization with pig manure (PM) and compost from biodegradable waste (C) on the spring wheat grain yield in comparison to fertilization with municipal sewage sludge (SS).

Content of microelements in grain

The zinc content in spring wheat grain was varied, depending on the applied fertilization (Table 6). The lowest content of this element was obtained in wheat grain from the treatment fertilized with pig manure (SM), whereas

Table 6

Content of zinc, copper and manganese in grain of spring wheat

Treatments		(mg kg^{-1} d.m.)		
Fertilizer	Year	Zn	Cu	Mn
0	1st	41.1	4.56	30.7
	3rd	51.3	2.58	17.7
	average*	49.7^{b} ± 4.02	3.61^{a} ± 0.51	27.9^{a} ± 4.60
MF	1st	48.4	4.68	28.1
	3rd	53.0	3.57	23.4
	average*	51.7^{b} ± 1.68	3.37^{a} ± 0.72	28.3^{a} ± 2.49
PM	1st	34.1	4.11	23.9
	3rd	45.8	4.76	16.0
	average*	43.1^{a} ± 3.99	3.35^{a} ± 0.96	24.9^{a} ± 4.73
C	1st	36.8	4.28	32.1
	3rd	47,5	4.63	18.1
	average*	45.9^{ab} ± 4.59	3.57^{a} ± 0.77	27.2^{a} ± 4.59
SS	1st	35.1	3.92	27.4
	3rd	52.3	4.16	14.9
	average*	46.0^{ab} ± 4.81	3.26^{a} ± 0.71	24.7^{a} ± 4.37

* Average of three years ± standard error. Different letters in columns indicate significant differences ($\sum < 0.05$, Duncan's multiple range test).

Table 7

Content and selected indicators of the quality of grain protein of spring wheat

Treatments		Total protein (g kg^{-1} d.m.)	∑ EAA (g 100 g^{-1} protein)	CS (WE)$_{Lys}$	CS (MH)$_{Lys}$
Fertilizer	year				
0	1st	125.9	25.60	35.94	50.32
	3rd	162.9	22.12	40.22	56.31
	average*	142.8^{a} ± 9.38	25.2^{a} ± 1.34	31.3^{bc} ± 1.67	39.8^{bc} ± 2.13
MF	1st	153.7	26.33	42.63	59.68
	3rd	173.3	24.27	45.56	63.78
	average*	159.6^{b} ± 6.1	26.2^{a} ± 1.03	31.6^{c} ± 0.99	40.2^{c} ± 1.53
PM	1st	129.8	26.37	37.92	53.09
	3rd	176.0	22.26	43.42	60.79
	average*	154.6^{b} ± 11.7	25.1^{a} ± 1.28	31.1^{b} ± 1.58	39.6^{b} ± 2.02
C	1st	118.5	37.64	46.80	65.50
	3rd	174.3	23.35	42.11	59.00
	average*	149.6^{ab} ± 14.4	27.6^{b} ± 1.21	32.3^{c} ± 1.65	41.1^{c} ± 1.91
SS	1st	130.8	25.66	37.08	51.91
	3rd	184.3	20.72	41.40	57.95
	average*	157.8^{b} ± 13.5	24.0^{a} ± 1.44	29.3^{a} ± 1.75	37.3^{a} ± 2.23

CS – chemical score of limiting amino acid; WE – whole egg protein standards; MH – mature human;

* Average of three years ± standard error. Different letters in columns indicate significant differences (∑ < 0.05, Duncan's multiple range test).

the highest amount of zinc was found in wheat grain from the treatment where only mineral fertilizers were used (MF).

No significant differences in the content of copper in spring wheat grain after fertilization were found (Table 6). The weighted mean content of Cu was within the range of 3.26 mg kg^{-1} d.m. (grain from the treatment where municipal sewage sludge was used) to 3.61 mg kg^{-1} d.m. (grain from the unfertilized treatment).

Among the fertilized treatments, the highest content of manganese, was determined in grain of wheat fertilized with mineral fertilizers (MF) and after the application of compost from biodegradable waste (C) – Table 6.

Content of protein and exogenous amino acids in grain

The total protein content was significantly higher in wheat grain from fertilized treatments in comparison to the protein content in unfertilized wheat grain (Table 7). The highest weighted mean content of total protein was found in grain of wheat fertilized with mineral fertilizers (MF).

The highest weighted mean content of exogenous amino acids (∑ EAA) occurred in wheat grain from the treatment where fertilization with compost from biodegradable waste was applied (27.6 g 100 g^{-1} of protein) – Table 7. On treatments fertilized with pig manure (PM), municipal sewage sludge

Table 8

Composition of selected exogenous amino acids in protein of grain protein of spring wheat

Treatments		(g 100 g^{-1} protein)								
Fertilizer	year	Thr	Val	Ile	Leu	Phe	Lys	Met	His	Arg
0	1st	2.10	3.51	2.53	5.00	3.20	2.21	1.06	2.04	3.95
	3rd	1.87	2.87	2.06	4.31	2.76	1.90	0.61	1.71	3.52
	average*	2.14^{ab} ± 0.15	3.30^{a} ± 0.19	2.37^{ab} ± 0.14	4.96^{ab} ± 0.31	3.17^{ab} ± 0.20	2.19^{a} ± 0.14	0.71^{a} ± 0.16	2.14^{a} ± 0.05	4.04^{ab} ± 0.29
MF	1st	2.07	3.42	2.71	5.11	3.47	2.13	1.01	2.04	4.37
	3rd	2.01	3.10	2.34	4.76	3.17	2.03	0.66	1.88	4.09
	average*	2.19^{b} ± 0.14	3.37^{a} ± 0.14	2.55^{ab} ± 0.10	5.18^{b} ± 0.23	3.45^{b} ± 0.15	2.21^{a} ± 0.12	0.72^{a} ± 0.13	2.12^{a} ± 0.05	4.45^{b} ± 0.23
PM	1st	2.24	3.49	2.67	5.13	3.28	2.25	1.10	1.97	4.22
	3rd	1.89	2.89	2.32	3.69	2.90	1.91	0.65	1.71	3.70
	average*	2.17^{ab} ± 0.13	3.32^{a} ± 0.29	2.66^{b} ± 0.17	4.27^{a} ± 0.47	3.31^{ab} ± 0.22	2.18^{a} ± 0.13	0.76^{ab} ± 0.15	2.15^{a} ± 0.09	4.23^{ab} ± 0.29
C	1st	4.12	6.13	4.43	8.35	5.69	3.61	1.27	3.28	7.56
	3rd	3.57	5.30	3.86	7.63	5.10	3.24	1.10	2.92	6.56
	average*	2.49^{c} ± 0.43	3.70^{b} ± 0.65	2.69^{b} ± 0.46	5.29^{b} ± 0.77	3.55^{b} ± 0.55	2.26^{a} ± 0.35	0.76^{ab} ± 0.13	2.30^{b} ± 0.29	4.58^{b} ± 0.79
SS	1st	2.03	3.42	2.60	5.16	3.23	2.19	1.03	2.02	4.00
	3rd	1.71	2.57	1.91	4.01	2.64	1.73	0.60	1.58	3.32
	average*	2.01^{a} ± 0.15	3.05^{a} ± 0.22	2.27^{a} ± 0.18	4.75^{ab} ± 0.32	3.12^{a} ± 0.22	2.05^{a} ± 0.14	0.72^{a} ± 0.13	2.09^{a} ± 0.07	3.91^{a} ± 0.29

* Average of three years ± standard error. Different letters in columns indicate significant differences ($\sum < 0.05$, Duncan's multiple range test).

(SS), and mineral fertilizers (MF), the weighted mean content of amino acids was significantly lower, respectively by 9.1%, 13.1%, and 5.1%.

The content of amino acids in wheat protein was much lower than in standard protein (Hidvégi, Békés 1984, FAO/WHO 1991). Lysine, methionine and threonine are amino acids which are highly deficient in fodders. Fertilization with compost from biodegradable waste generally increased the content of the assayed exogenous amino acids to the highest degree (except for methionine). In comparison to the amino acid composition of mature, human protein or hen egg protein, it was stated that lysine was the amino acid limiting the quality of spring wheat grain (Table 8).

DISCUSSION

Numerous research results indicate a possibility of using organic waste material for biomass production (Jamil et al. 2006, Carbonell et al. 2011). The dynamics of biochemical processes occurring in soil, additionally intensified by environmental factors, may significantly affect the yield-forming efficiency of applied fertilization (Stewart, Hash 1982, Bonde et al. 1988) and chemical composition of plants, including the content of microelements and exogenous amino acids (Mallarino et al. 1999). In the first year of the research, the significantly lower wheat grain yield on treatments fertilized with organic materials resulted from limited possibilities of nutrient uptake by plants, particularly in the case of nitrogen, whose availability from organic materials is a result of weather conditions during the vegetative season, dose of applied materials and the value of carbon to nitrogen ratio (Barbarika et al. 1985, Agehara, Warncke 2004). Fertilization with organic material makes it difficult to synchronize the amounts of N released from organic substances with plant requirements for this component (Mikkelsen, Harrtz 2008). The present research results clearly indicate that the regress in wheat grain yield in the first year of the research might have been dictated by the spring term of organic materials application.

With time, the residual effect of applied organic materials becomes distinguishable, resulting in an increase in yield, which has been confirmed by a three-year scientific research. A beneficial effect of fertilization with sewage sludge and composted biodegradable waste on wheat biomass yield was shown by Tamrabet et al. (2009), Jamil et al. (2004), and Barzegar et al. (2002). However, Ibrahim et al. (2008), while studying the effect of compost fertilization on wheat yield, drew attention to the necessity of balancing the amounts of nutrients supplied with fertilization.

The microelement content in plant biomass is a result of the content of their assimilable forms in the soil, which is strongly modified by soil pH, organic matter content, soil sorptive capacity, and also by the cultivated plant species and fertilization (Kopeć, Przetaczek-Kaczmarczyk 2006). An insufficient copper content, in terms of fodder value, was found in the author's own research. The low copper content may have caused a decrease in the fodder value of grain, but also limited the amount and quality of yield, particularly as copper is included in the composition of enzymes and proteins participating in specific metabolic processes (Prasad 1995, Yruela 2005). Soil reaction and content of organic matter to which copper shows high affinity are the factors limiting the availability of this element. According to Gondek and Kopeć (2004), the formation of permanent bonds between humus and copper may be responsible for a low efficiency of soil fertilization with this element. The manure used in the research contained the largest amount of copper (among the applied organic materials), but despite this the content of this element in spring wheat grain did not increase markedly. The results imply no significant effect of compost from biodegradable waste or municipal sewage sludge on the zinc and manganese content in spring wheat grain in comparison to plants from the treatment fertilized with manure. It might have resulted from a relatively low content of these elements in the organic materials. The zinc content in grain was within the range considered optimal for animal nutrition, whereas the manganese content was deficient, regardless of the applied fertilization (Moller et al. 2000).

While using composts and municipal sewage sludge for fertilization one may expect their beneficial effect on plant yield, but also on its biological value. Taking into consideration the chemical composition, which determines the fodder nutritional value, it may be said that cereal grain is a carbohydrate fodder with a low or medium content of total protein (Brand et al. 2003). According to Dubetz and Gardiner (1980), the content of protein and amino acids in wheat grain changes considerably under the influence of increasing doses of nitrogen. In the own research, the nitrogen dose in all treatments (except the control) was the same, and the use of a dose of this element in compliance with the wheat nutritional requirements, both in the form of mineral fertilizers and organic materials (manure, compost, sewage sludge), did not cause any significant changes in the content of the analyzed amino acids in the protein of spring wheat grain. Also, according to Jasiewicz and Baran (2011), mineral fertilization diversifies the content of amino acids in plant biomass more than fertilization with organic materials. Cereal grain usually has a low content of lysine. Also in the own research, lysine was the amino acid limiting protein quality in grain with reference to both the whole egg protein standard (WE) and the mature human protein standard (MH), regardless of the applied fertilization. Sherry (2007) obtained similar results in his research. The current research results are not reflected in the report of Jasiewicz and Baran (2011), who found that methionine was the amino acid limiting the protein quality in maize. The differences in the research results may be attributed to the applied fertilization (type of organic material), the size of nitrogen dose and the cultivated plant species. It is crucial to maintain a proper balance of amino acids in the composition in feeds, matching the nutrient supply with animal requirements. Firstly, it enables animals to use most effectively one of the most valuable food ingredients, i.e. fodder protein. Secondly, it has an environmental aspect because of the decrease in nitrogen excreted with urine by avoiding excess of amino acids in relation to animal needs.

CONCLUSIONS

1. The limited access of plants to mineral components (mainly nitrogen) introduced with waste organic material and with manure determined the crop yield, particularly in the first year of the research.

2. A better residual effect of fertilization with pig manure and compost from biodegradable waste on the spring wheat grain yield was observed in comparison to fertilization with municipal sewage sludge.

3. Waste organic material used in moderate doses, based on plant requirements for nutrients, did not lead to a decrease in the biological value of yield.

4. Irrespective of the applied fertilization, copper and manganese were the microelements that limited the fodder value of spring wheat grain, whereas the quality of protein was determined by the content of lysine.

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COEXISTENCE OF *APHIS FABAE* SCOP. PREDATORS ON BROAD BEAN GROWING ON SOIL POLLUTION WITH HEAVY METALS*

Janina Gospodarek[1], **Alina Kafel**[2]

[1] Chair of Agricultural Environment Protection
University of Agriculture in Krakow
[2]Chair of Animal Physiology and Ecotoxicology
University of Silesia, Katowice

Abstract

The investigations were conducted to determine the effect of soil contamination with heavy metals (cadmium, copper, nickel, lead and zinc) on the level corresponding to 3rd pollution degree in the IUNG classification on interactions between individual aphidophagous groups and their stages of development occurring in *Aphis fabae* Scop. colonies on broad beans. The following were analysed: the occurrence and number of individual stages of predator development (eggs, larvae, pupae and adult specimens). The Agrell's index of coexistence were computed from the achieved results. The values of the Agrell's index for broad bean were low for individual predator groups and their development stages. Among the investigated predator groups, the best mutual tolerance was observed for Syrphidae and Coccinellidae. Co-existence of individual aphidophagous groups seems to be strongly conditioned by food resources, i.e. aphid availability. Heavy metals, by affecting the host plant, modified the degree of its colonization by aphids and their number, hence changes in the incidence of predators. Thus, soil pollution with zinc and nickel contributed to limiting the extent of broad bean colonization by aphids and predators, and consequently to a decrease in the values of their coexistence indices; on the other hand, soil contamination with cadmium and lead favours plant colonization by aphids and predators and therefore the presence of individual aphidophagous groups was noted more frequently on the same plants.

Keywords: heavy metals, broad bean, Ag index, aphidophagous.

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dr hab. inż. Janina Gospodarek, Chair of Agricultural Environment Protection, Agricultural University in Krakow, al. Mickiewicza 21, 31-120 Krakow, Poland, e-mail: rrjgospo@cyf-kr.edu.pl

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WSPÓŁWYSTĘPOWANIE DRAPIEŻCÓW MSZYCY BURAKOWEJ *APHIS FABAE* SCOP. NA BOBIE W WARUNKACH ZANIECZYSZCZENIA GLEBY METALAMI CIĘŻKIMI

Abstrakt

Celem badań było określenie wpływu skażenia gleby metalami ciężkimi (kadmem, miedzią, niklem, ołowiem i cynkiem) na poziomie odpowiadającym III stopniowi zanieczyszczenia wg klasyfikacji IUNG na wzajemne oddziaływanie między poszczególnymi grupami afidofagów oraz ich stadiami rozwojowymi występującymi w koloniach mszycy burakowej *Aphis fabae* Scop. na bobie. Analizowano występowanie i liczebność poszczególnych stadiów rozwojowych drapieżców (jaj, larw, poczwarek, postaci dorosłych). Na podstawie wyników obliczono wskaźniki współwystępowania Agrella. Wartości wskaźników Agrella na bobie dla poszczególnych grup drapieżców i ich stadiów rozwojowych były niskie. Spośród badanych grup drapieżców największą wzajemną tolerancję obserwowano w przypadku bzygowatych i biedronkowatych. Współwystępowanie poszczególnych grup afidofagów wydaje się być silnie uwarunkowane dostępnością pokarmu – mszyc. Zastosowane metale ciężkie, wpływając na kondycję rośliny żywicielskiej, modyfikowały stopień jej zasiedlenia przez mszyce oraz ich liczebność, a w rezultacie występowanie drapieżców. Skażenie gleby cynkiem oraz niklem przyczyniło się do ograniczenia stopnia opanowania bobu przez mszyce i drapieżców, a tym samym do zmniejszenia wartości wskaźników ich wzajemnego współwystępowania, natomiast skażenie gleby kadmem i ołowiem sprzyjało opanowaniu roślin przez mszyce i drapieżców, w związku z czym częściej notowano obecność poszczególnych grup afidofagów na tych samych roślinach.

Słowa kluczowe: metale ciężkie, bób, wskaźnik Ag, afidofagi.

INTRODUCTION

Heavy metals present in consumed food affect herbivores (e.g. aphid) as well as the subsequent food chain link, i.e. predators. Considerable accumulation of some heavy metals was assessed in bodies of *Aphis fabae* Scop. preying on polluted plants (CRAWFORD et al. 1995, KAFEL et al. 2012). Among aphis predators, Diptera, Syrphidae, which consume big quantities of these pests, are particularly exposed to heavy metals (WINDER et al. 1999). On the other hand, Coleoptera, Coccinellidae, when preying on *Sitobion avenae* aphids and at risk of a higher cadmium level in host plants, showed no signs of biomagnification of this element in their organisms (GREEN et al. 2003), in which they resembled larvae of Neuropetra, Chrysopidae, consuming *Rhopalosiphum padi* aphids (ALONSO et al. 2009). However, cadmium concentrations in the bodies of predators exposed to an elevated Cd content were much higher than in the control specimens. It should be remembered that accumulation of heavy metals in an organism does not equal its toxicity (CORTET et al. 1999). There is great diversification in invertebrates' response to an elevated level of heavy metals in the environment.

Certain antagonisms, which may weigh on the final effect of heavy metals in the environment, appear among individual representatives of a broad range of aphids' natural enemies (ALMOHAMAD et al. 2010). However behaviour strategies of individual aphidophagous groups may be conditioned by several factors, many of which still await explanation.

The aim of the investigations was to determine the influence of soil contamination with heavy metals (cadmium, copper, nickel, lead and zinc) on the level corresponding to the 3rd pollution degree in the IUNG classification on interactions between individual aphidophagous groups and their stages of development occurring in *Aphis fabae* Scop. colonies on broad beans.

MATERIAL AND METHODS

An experiment in pots containing 9.8 kg d.m. of soil was conducted in 2007 and 2008 in Zagaje Stradowskie located in the Świętokrzyskie Province, the commune of Kazimierz. Broad bean (*Vicia faba* L.) was chosen as a model plant because it is a preferred food of aphids (*Aphis fabae* Scop.). Observations were conducted on broad beans of the cultivar White Windsor, grown in the following treatments: unpolluted soil with the natural heavy metal content (C); unpolluted soil with the natural heavy metal content and receiving mineral fertilization (NPK); soil polluted with cadmium, soil polluted with lead, soil polluted with copper, soil polluted with zinc and soil polluted with nickel. The applied doses were: 4 mg Cd kg^{-1}d.m. of soil, 85 mg Cu kg^{-1} d.m., 110 mg Ni kg^{-1} d.m., 530 mg Pb kg^{-1} d.m. and 1000 mg Zn kg^{-1} d.m. of soil, corresponded to the 3rd level of pollution, i.e. medium pollution according to the classification developed by the IUNG in Puławy (KABATA-PENDIAS et al.1993). Heavy metals were supplied to the soil in spring 2007 as water solutions of the following salts: $3CdSO_4 \cdot 8H_2O$, $NiSO_4 \cdot 7H_2O$, $CuSO_4$, $ZnSO_4 \cdot 7H_2O$, $Pb(NO_3)_2$. The following fertilizer doses were applied: 0.7 g N (as NH_4NO_3); 0.8 g P_2O_5 (as KH_2PO_4); 1.2 g K_2O (as KCl) per pot. The doses were equal in all the treatments except the control (C). In the pots where soil was treated with $Pb(NO_3)_2$, in order to maintain the nitrogen quantity similar to that in the other groups, basic fertilization was diminished accordingly. Each pot contained separately prepared batches of soil. A thin layer of crumbled soil was put on plastic film and sprayed with an appropriate mixture of fertilizer and metal, afterwards it was thoroughly mixed. This procedure was repeated several times. In 2008, the soil contaminated in the previous year was used for the experiment. Prior to it, the soil was loosened and basic fertilization was applied, as in 2007. Five broad bean plants were grown in each pot. The experiment was set up in 8 replications, in a randomized block system. The experimental soil was degraded humus (chernozem) developed from loess. It had acid reaction (pH in 1 mol·dm^{-3} was 5.3 and in water 6.1) and contained 1.13% of organic carbon. The analysis of the texture proved that the soil was a clay silt deposit. The analyses of the soil heavy metal content before the experiment showed that their levels were generally below the threshold limits set for soils unpolluted with these metals. The soil pH was assessed in 1 mol dm^{-3} KCl solution with a potentiometer; organic carbon content was determined with the

Tiurin's method; grain size distribution was checked with the Bouyoucose-Casagrande's method in the Prószyński's modification. The heavy metal content in initial soil was assessed following digestion of a sample in aa mixture of nitric and perchloric acids (2:1). The heavy metal concentrations in the extracts were assessed using atomic absorption spectrometry (AAS).

Aphid fabae Scop. populations were monitored since the first winged migrants were settled on the plants, twice a week, until the end of their presence. All larvae, wingless females and winged migrants were counted. The presence of all pre-imago development stages of predators was observed within the aphid colonies (Diptera, Syrphidae; Coleoptera, Coccinellidae; Neuroptera, Chrysopidae; Heteroptera, Anthocoridae and Aranea). The following were analysed: the occurrence and number of individual stages of predator development (eggs, larvae, pupae and adult specimens) on all plants (not only settled by aphids) because aphidophagous insects could also appear on plants free from aphids. The incidence of so-called tarry black spots, which evidenced the presence of Diptera, Syrphidae larvae and mummies of parasited aphids, was also noted. The values of the Agrell's index of co-existence (GÓRNY, GRÜM 1981) were computed for both individual aphid natural enemies and selected development stages, well as for Formicidae, according to the following formula:

$$Ag = (N_{a,b}/N)\ 100,$$

where: $N_{a,b}$ – number of plants on which units a and b occurred jointly (a and b – comparable pairs of aphid natural enemies, their development stages, and Formicidae);

N – number of all plants.

Also, the percentages of plants on which aphid were observed as well as individual development stages of their natural enemies and Formicidae were computed.

Statistical calculations were made with Statistica 8.0PL software. The significance of differences between the means was assessed by the one-way Anova and means were differentiated using the Duncan's test on the significance level $p<0.05$.

RESULTS AND DISCUSSION

The most numerous aphid colonies were encountered on plants growing on the cadmium polluted soil (Table 1). On the broad bean plants growing on the soil contaminated with either lead or copper, the number of the aphid colonies was the same as in the control, but significantly lower on the plants growing on the soil contaminated either with zinc or nickel. In the previous experiment with the same level of pollution, and in the same location, an average number of aphids on plants growing on zinc contaminated soil con-

Table 1

Occurrence of *Aphis fabae* Scop. aphid, its natural enemies and Formicidae on broad bean plants cultivated on unpolluted soil (control) and soil contaminated with single heavy metals

Specification	*C* *	NPK	Cd	Cu	Pb	Ni	Zn
Mean number of aphids per plant	575.2 *b***	525.1 *b*	656.8 *b*	414.2 *b*	610.0 *b*	14.4 *a*	113.3 *a*
	274.0 *b*	408.3 c	711.4 d	258.6 *b*	359.7 *b* c	5.3 *a*	62.1 *a*
Mean number of *Syrphidae* eggs per plant	0.008 *a*	0.016 *a*	0.016 *a*	0.027 *a*	0.039 *a*	0.000 *a*	0.007 *a*
	0.043 *b*	0.044 *b*	0.019 *ab*	0.010 *a*	0.029 *ab*	0.000 *a*	0.004 *a*
Mean number of *Syrphidae* larvae per plant	0.041 *a*	0.041 *a*	0.024 *a*	0.009 *a*	0.032 *a*	0.000 *a*	0.015 *a*
	0.019 *ab*	0.043 *bc*	0.062 *c*	0.024 *ab*	0.038 *abc*	0.004 *a*	0.017 *ab*
Mean number of *Coccinellidae* eggs per plant	0.008 *a*	0.008 *a*	0.008 *a*	0.018 *a*	0.024 *a*	0.017 *a*	0.007 *a*
	0.010 *a*	0.000 *a*	0.014 *a*	0.000 *a*	0.010 *a*	0.000 *a*	0.008 *a*
Mean number of *Coccinellidae* larvae per plant	0.285 *b*	0.187 *ab*	0.312 *b*	0.186 *ab*	0.333 *b*	0.008 *a*	0.073 *a*
	0.236 *bc*	0.256 *b* c	0.344 c	0.175 *b*	0.196 *b*	0.008 *a*	0.050 *a*
Mean number of *Coccinellidae* pupae per plant	0.081 *abc*	0.098 *bc*	0.088 *bc*	0.071 *abc*	0.143 *c*	0.000 *a*	0.015 *ab*
	0.014 *a*	0.019 *a*	0.019 *a*	0.048 *b*	0.019 *a*	0.000 *a*	0.000 *a*
Mean number of *Coccinellidae* adults per plant	0.130 *bc*	0.081 *abc*	0.096 *abc*	0.142 *c*	0.040 *ab*	0.008 *a*	0.073 *abc*
	0.260 *bc*	0.135 *b*	0.364 *c*	0.228 *b*	0.139 *b*	0.000 *a*	0.017 *a*
Mean number of *Chrysopidae* eggs per plant	0.008 *a*	0.008 *a*	0.000 *a*	0.044 *ab*	0.071 *b*	0.000 *a*	0.007 *a*
	0.000 *a*	0.000 *a*	0.005 *ab*	0.015 *b*	0.005 *ab*	0.000 *a*	0.000 *a*
Mean number of *Formicidae* per plant	0.106 *ab*	0.211 *b*	0.400 *c*	0.106 *ab*	0.238 *b*	0.000 *a*	0.102 *ab*
	0.038 *ab*	0.217 c	0.062 *ab*	0.049 *ab*	0.091 *b*	0.000 *a*	0.059 *ab*
Mean number of *Aranea* per plant	0.049 *abc*	0.073 *c*	0.040 *abc*	0.062 *bc*	0.040 *abc*	0.000 *a*	0.007 *ab*
	0.029 *b*	0.014 *ab*	0.019 *ab*	0.019 *ab*	0.005 *a*	0.000 *a*	0.000 *a*
Mean number of *Anthocoridae* per plant	0.049 *a*	0.057 *a*	0.032 *a*	0.049 *a*	0.055 *a*	0.008 *a*	0.036 *a*
	0.000 *a*	0.000 *a*	0.005 *a*	0.000 *a*	0.010 *a*	0.000 *a*	0.000 *a*
Mean number of mummies of parasitized aphids per plant	0.000 *a*	0.415 *a*	1.232 *a*	0.000 *a*	0.000 *a*	0.000 *a*	0.000 *a*
	0.591 *a*	8.341 *b*	0.019 *a*	0.121 *a*	0.507 *a*	0.025 *a*	0.000 *a*

* *C* – control, NPK – soil with mineral fertilization, Cd – soil contaminated with cadmium, Cu – soil contaminated with copper, Pb – soil contaminated with lead, Ni – soil contaminated with nickel, Zn – soil contaminated with zinc.

** Means marked with the same letter in lines do not differ statistically at $p<0.05$. Upper and lower values relate to experiments from 2007 and 2008, respectively.

taminated in 2002-2005 was about 36 individuals per plant, whereas in the control objects there were 565 specimens per plant (GOSPODAREK 2012*a*).

Syrphiade eggs were absent from plants contaminated with zinc. No marked differences were noted in the number of this predator's eggs in aphid colonies on the control and lead or cadmium containing plants. Significantly fewer Syrphidae eggs were found in 2008 on plants growing on the nickel and copper polluted soil. Their number was below the level established on the control plants. In 2007, no statistically significant differences in the number of Syrphidae larvae were found between the analyzed objects. The larvae were absent from plants contaminated with zinc. In 2008, the most numerous Syrphidae larvae were noticed in aphid colonies on cadmium contaminated plants. They were markedly more numerous than on the control plants without mineral fertilization and on plants growing on soils contaminated with copper, nickel or zinc. Syrphidae larvae were most rarely spotted on plants growing on the zinc polluted soil. No differences were detected between the analyzed treatments in the number of eggs laid by Coccinellidae. Ladybird larvae were several times more numerous than Syrphidae larvae. In 2007, Coccinellidae larvae were most numerous in the aphid colonies on the plants growing in soils contaminated with cadmium and lead. Significantly fewer ladybird larvae were found in aphid colonies on plants growing on soils contaminated with nickel and zinc. In 2008, the most numerous ladybird larvae were found in aphid colonies on cadmium contaminated plants. There were markedly larger numbers of the larvae on these plants than in all the other treatments contaminated with heavy metals. Similarly as in 2007, Coccinellidae larvae were most rarely spotted on plants from soils contaminated with nickel and zinc, where their number was significantly lower than on the control plants. The number of Coccinellidae larvae on broad bean growing on the copper or lead polluted and on the control soil was similar. Adult Coccinellidae were most numerous in 2007 on the treatment where soil was polluted with copper and in 2008 on plants growing on soil contaminated with cadmium, where there were significantly more ladybirds than on the other treatments except for the control without mineral fertilizers. Adult Coccinellidae occurred most rarely on plants polluted with zinc. Markedly fewer adult ladybirds were also found in experimental group with nickel. The number of adult Coccinellidae on plants contaminated with lead remained on the control level.

The other aphid predators, e.g. Chrysopidae and Anthocoridae, occurred occasionally. The number of Anthocoridae did not depend on the analyzed stressors. In 2007, the Chrysopidae eggs were most numerous on plants contaminated with lead and in 2008 – on copper polluted plants. Chrysopidae larvae were not detected. In 2008, Aranea were most numerous on the control non-fertilized plants. Aranea was less common on plants polluted with zinc and nickel, and in 2008 additionally on lead polluted plants. Numerous Formicidae were observed on plants polluted with cadmium (2007) and on the fertilized control plants (2008), which might have helped to lower the number of some predators (e.g. adult ladybirds) in aphid colonies on the plants from this treatments.

Considering the analysed predators, the highest percentage of broad bean plants was settled by predatory Coccinellidae. Larvae of these preda-

Table 2

Mean infestation of broad bean grown on heavy metal contaminated soil with aphids (*Aphis fabae* Scop.), its natural enemies and Formicidae in 2007 and 2008 (infested plants in %)

Specification	*C* *	NPK	Cd	Cu	Pb	Ni	Zn
	2007						
Hover-fly eggs	0.81	1.63	1.60	2.65	1.59	0.72	0.00
Hover-fly larvae	3.25	3.25	2.40	0.88	2.38	1.45	0.00
Hover-fly pupae	0.81	0.81	0.80	0.00	2.38	0.00	0.00
Black spots	0.81	5.69	10.40	2.65	6.35	2.90	0.00
Ladybird eggs	0.81	0.81	0.80	1.77	2.38	0.72	0.84
Ladybird larvae	13.82	10.57	15.20	9.73	19.05	5.80	0.00
Ladybird pupae	7.32	8.13	7.20	7.08	9.52	1.45	0.00
Ladybird adults	10.57	5.69	8.00	7.96	3.97	5.07	0.84
Lacewings eggs	0.81	0.81	0.00	3.54	5.56	0.00	0.00
Ants	8.13	14.63	21.60	10.62	15.87	5.80	0.00
Spiders	4.07	6.50	2.40	5.31	3.97	0.72	0.00
Anthocorids	3.25	4.88	2.40	3.54	3.97	2.90	0.84
Parasitized aphids	0.00	2.44	4.00	0.00	0.00	0.00	0.00
Aphids	55.28	58.54	64.00	51.33	64.29	44.93	12.61
	2008						
Hover-fly eggs	3.85	3.38	1.91	0.97	2.39	0.83	0.00
Hover-fly larvae	1.92	3.38	5.74	1.94	3.35	1.66	0.39
Hover-fly pupae	0.00	0.00	0.48	0.00	0.48	0.00	0.00
Black spots	4.81	3.38	9.09	0.97	3.35	0.41	0.00
Ladybird eggs	0.96	0.00	1.44	0.00	0.96	0.83	0.00
Ladybird larvae	14.90	14.98	22.01	10.68	11.48	3.73	0.78
Ladybird pupae	1.44	1.93	1.91	4.37	1.91	0.00	0.00
Ladybird adults	13.94	10.63	16.75	10.19	10.05	1.66	0.00
Lacewings eggs	0.00	0.00	0.48	1.46	0.48	0.00	0.00
Ants	3.37	12.56	4.78	2.91	4.31	2.90	0.00
Spiders	2.88	0.97	1.91	1.94	0.48	0.00	0.00
Anthocorids	0.00	0.00	0.48	0.00	0.48	0.00	0.00
Parasitized aphids	3.85	26.09	1.44	0.49	5.74	0.83	0.00
Aphids	57.69	65.70	75.12	53.88	56.94	24.90	8.63

* Key: see Table 1

tors were found on about 10-15% of the analyzed plants unpolluted by heavy metals. Also adult forms were quite numerously spotted, i.e. they settled about 6-14% of unpolluted broad bean plants (Table 2). In previous research, the average colonization of aphid colonies on broad bean by these predators was 10-14% (Jaworska, Gospodarek 2003), whereas on *Syringa* plants the presence of adult ladybird beetles was noted in about 8% of analyzed aphid colonies (Gospodarek 2012*b*).

Soil contamination with cadmium in both years and with lead in 2007 contributed to increased plant colonization by Coccinelliade larvae. In all probability, this was the effect of a more extensive colonization of greater broad bean plants by aphids on these plants than on unpolluted plants. On the other hand, about 2.5-4-fold fewer plants with these predators' larvae were identified among broad beans growing on soil contaminated with nickel than among the control plants, although the percentage of plants settled by aphids was respectively twice as low. Also, far fewer plants settled by these predators were observed on soil contaminated with zinc than it might have been suggested by the percentage of plants settled by aphids. In this case, the number of aphids in colonies was also important. The appeal of aphid colonies as a source of food for aphidophagous insects, e.g. Diptera or Coccinelidae depends on the colony size (Wnuk, Starmach 1977, Honěk 1980). Typically, the more numerous the colony, the more numerous predators.

The presence of predatory Syrphidae was much less frequently observed in aphid colonies on broad bean. Larvae of these predators settled only about 2-3% of unpolluted plants. In some previous investigations conducted therein, the occurrence of these predators was recorded on *ca* 4% of control plants (Gospodarek 2012a). As herein, the percentage was much lower on soils contaminated with nickel and zinc (below 1%).

The presence of eggs of such aphidophages as Anthocoridae and Aranae or Neuroptera was rarely noted on broad bean. In 2007, a considerable percentage of aphid colonies was settled by Formicidae (on average about 8-14% of unpolluted plants and between 6 and 22% of plants cultivated in heavy metal polluted soil). Only the plants growing on the soil polluted with zinc were not visited by these insects.

The values of the Agrell's index obtained for individual predator groups and their stages of development were low (Tables 3-5). In 2007 and 2008, no co-occurrence of any predators was noticed on plants growing on zinc polluted soil polluted. On unpolluted soil and not fertilized with mineral salts, the Agrell's index reached the highest value (2.42) for Coccinellidae larvae and adults. It was also high for plants on unpolluted and NPK fertilized soil (Table 3). The co-occurrence of these development stages of predators was observed in almost 4% of plants on cadmium polluted soil (Table 4). On the other hand, on soil contaminated with copper, Coccinellidae larvae and adults occurred together on about 2.2% of plants, whereas on lead polluted soil only 1.19% of plants had these insects (Tables 4, 5). In the latter case, the concurrent presence of Coccinellidae larvae and pupae on the same plants was observed more frequently. Much lower values of this index (0.26%) – Table 5 were registered for plants polluted with nickel. There are reports about limited egg laying by Coccinellidae in colonies settled by other

Table 3

Comparison of Ag index value for individual pairs of aphids' natural enemies, their developmental stages and ants (%) (mean in the years 2007–2008) on broad bean grown on control soil (*C*) – green font and on uncontaminated soil with mineral fertilization (NPK) – black font

Specification	1*	2	3	4	5	6	7	8	9	10	11	12	13
Hover-fly eggs (1)		**0.00**	**0.00**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**	**0.00**	**0.00**	**0.30**
Hover-fly larvae (2)	0.30		**0.00**	**0.30**	**0.00**	**0.30**	**0.00**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**
Hover-fly pupae (3)	0.00	0.00		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Black spots (4)	0.30	0.61	0.30		**0.00**	**1.21**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**
Ladybird eggs (5)	0.00	0.00	0.00	0.00		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**	**0.00**	**0.00**
Ladybird larvae (6)	0.00	0.61	0.30	2.42	0.00		**0.91**	**2.42**	**0.00**	**0.00**	**0.91**	**0.30**	**0.61**
Ladybird pupae (7)	0.00	0.00	0.00	0.00	0.00	0.61		**0.91**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Ladybird adults (8)	0.00	0.30	0.00	0.30	0.00	3.03	0.91		**0.00**	**0.00**	**0.61**	**0.00**	**0.61**
Lacewings eggs (9)	0.00	0.00	0.00	0.30	0.00	0.30	0.00	0.00		**0.00**	**0.00**	**0.00**	**0.00**
Ants (10)	0.61	0.30	0.00	0.61	0.00	0.91	0.00	0.30	0.30		**0.00**	**0.00**	**0.00**
Spiders (11)	0.00	0.00	0.30	0.91	0.00	1.52	0.30	0.30	0.00	0.30		**0.00**	**0.00**
Anthocorids (12)	0.00	0.30	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.00	0.30		**0.00**
Parasitized aphids (13)	0.61	0.91	0.00	1.52	0.00	5.45	0.00	1.82	0.00	2.42	0.30	0.00	

* numbers (1 – 13) correspond to groups of invertebrates mentioned in the first column

predators (Hemptinne et al. 1992, Doumbia et al. 1998, Oliver et al. 2006). A very rare co-occurrence of Coccinellidae eggs with other predators was also observed in the present research on most of the treatments except the one where soil was contaminated with lead. Clusters of Coccinellidae eggs were spotted on the same plants that were settled by other development forms of these predators, as well as Chrysopidae eggs and Aranea (Table 4). This may be the effect of a high number of plants on which Coccinellidae eggs were found in this experimental variant, particularly in 2007 (Table 1). Soil pollution with lead favoured aphid occurrence on plants, thus the presence of predators, particularly ladybird beetles, was much more frequent.

Table 4

Comparison of Ag index value for individual pairs of aphids natural enemies, their developmental stages and ants (%) (mean in the years 2007–2008) on broad bean grown on cadmium contaminated soil (Cd) – green font, and on copper contaminated soil (Cu) – black font

Specification	1*	2	3	4	5	6	7	8	9	10	11	12	13
Hover-fly eggs (1)		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.60**	**0.00**	**0.00**	**0.00**
Hover-fly larvae (2)	0.00		**0.00**	**2.10**	**0.00**	**2.10**	**0.00**	**0.00**	**0.00**	**0.30**	**0.60**	**0.00**	**0.30**
Hover-fly pupae (3)	0.00	0.00		**0.00**	**0.00**	**0.60**	**0.30**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Black spots (4)	0.00	0.00	0.00		**0.00**	**5.99**	**0.30**	**0.90**	**0.00**	**0.30**	**0.60**	**1.20**	**0.00**
Ladybird eggs (5)	0.00	0.00	0.00	0.31		**0.60**	**0.00**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Ladybird larvae (6)	0.31	0.63	0.00	0.63	0.00		**1.20**	**3.89**	**0.00**	**1.50**	**0.30**	**1.50**	**0.60**
Ladybird pupae (7)	0.00	0.00	0.00	0.00	0.00	0.31		**0.90**	**0.00**	**0.30**	**0.00**	**0.00**	**0.00**
Ladybird adults (8)	0.00	0.31	0.00	0.31	0.00	2.19	1.57		**0.30**	**0.60**	**0.90**	**0.30**	**0.00**
Lacewings eggs (9)	0.00	0.00	0.00	0.00	0.00	0.63	0.94	0.31		**0.00**	**0.00**	**0.00**	**0.00**
Ants (10)	0.00	0.00	0.00	0.00	0.00	0.31	0.31	0.63	0.00		**0.00**	**0.30**	**0.30**
Spiders (11)	0.31	0.00	0.00	0.31	0.00	0.63	0.31	0.63	0.31	0.31		**0.00**	**0.00**
Anthocorids (12)	0.00	0.00	0.00	0.00	0.00	0.31	0.31	0.31	0.31	0.00	0.00		**0.00**
Parasitized aphids (13)	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.00	

* numbers (1 – 13) correspond to groups of invertebrates mentioned in the first column

Predatory Syrphidae revealed the best tolerance to ladybird beetle larvae. Syrphidae larvae were found on the same plants as Coccinellidae larvae in between 0.30 and 2.10% of cases, depending on the treatment. This index reached the highest value on soil contaminated with cadmium, the the same variant, in 2008, was found to comprise the highest number of plants colonized by the above predators. For comparison, on Syringa, both predatory stages were noted jointly in between 0.31 and 0.68% of the analyzed *A. fabae* aphid colonies (Gospodarek 2012b). Usually, tarry black spots evidenced Syrphidae larvae feeding on aphid colonies, and Coccinellidae larvae were found on the same plants (the Ag index ranged from 0.63% for the pot

Table 5

Comparison of Ag index value for individual pairs of aphids natural enemies, their developmental stages and ants (%) (mean in the years 2007–2008) on broad bean grown on lead contaminated soil (Pb) – green font, and on nickel contaminated soil (Ni) – black font

Specification	**1***	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Hover-fly eggs (1)		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**
Hover-fly larvae (2)	0.00		**0.00**	**0.60**	**0.00**	**0.90**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Hover-fly pupae (3)	0.00	0.00		**0.00**	**0.00**	**0.60**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Black spots (4)	0.00	0.00	0.00		**0.60**	**1.79**	**0.00**	**0.30**	**0.00**	**0.00**	**0.30**	**0.00**	**0.60**
Ladybird eggs (5)	0.00	0.00	0.00	0.00		**0.60**	**0.30**	**0.30**	**0.30**	**0.00**	**0.60**	**0.00**	**0.00**
Ladybird larvae (6)	0.00	0.53	0.00	0.79	0.26		**1.79**	**1.19**	**1.19**	**0.00**	**0.30**	**0.30**	**1.19**
Ladybird pupae (7)	0.00	0.00	0.00	0.26	0.00	0.26		**0.30**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**
Ladybird adults (8)	0.00	0.26	0.00	0.26	0.26	0.26	0.00		**0.30**	**0.30**	**0.00**	**0.00**	**0.60**
Lacewings eggs (9)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		**0.00**	**0.30**	**0.00**	**0.00**
Ants (10)	0.00	0.00	0.00	0.53	0.00	0.53	0.26	0.26	0.00		**0.00**	**0.30**	**0.30**
Spiders (11)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		**0.00**	**0.30**
Anthocorids (12)	0.00	0.00	0.00	0.26	0.00	0.26	0.00	0.26	0.26	0.26	0.00		**0.00**
Parasitized aphids (13)	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

* numbers (1 – 13) correspond to groups of invertebrates mentioned in the first column

with copper contaminated soil to 5.99% in the case of cadmium pollution). On the other hand, Syrphidae eggs were found on the same plants as tarry black spots only on unpolluted plants and only in 0.3% of the cases, despite the report by Alhmedi et al. (2007), noticing the presence of *Episyrphus balteatus* larvae in aphid colonies causing no decrease in the number of eggs laid by Syrphidae. Eggs and larvae of Syrphidae were also found frequently on the same aphid colonies on Syringa (Gospodarek 2012*b*).

Ants in aphid colonies, using honey-dew as food, often become antagonists towards predators such as Coccinellidae (Banks, Macaluay 1967, Styrski, Eubanks 2007, Powell, Silverman 2010), Anthocoridae, Chamaemyiidae

(Winiarska 1997), Chrysopidae (Powell, Silverman 2010). The competitive relationship is especially strong when the number of aphids is low. In their research, Crawford et al. (1995) also noted that *A. fabae* aphids excreted copper together with honey dew, leading to elevated exposure of ants to this element in food. Coexistence of ladybird eggs and ants was not found during the current experiment in any of the treatments, which confirms the results obtained by Sloggett and Majerus (2003), who stated that ladybirds more eagerly lay eggs in aphid colonies free from ants. On the other hand, ladybird larvae were spotted on the same plants as ants in the pots where the soil was contaminated with cadmium, copper and nickel and in the treatment with unpolluted soil fertilized with mineral salts; most frequently on plants growing on soil polluted with cadmium (the Ag index = 1.5%). Plants growing on soil contaminated with cadmium were heavily colonized by aphids, so food availability for both ladybirds and ants was high. Thus, the lack of co-occurrence of ants and ladybird larvae under conditions of soil contaminated with lead and control soil becomes more distinct, despite the considerable colonization of plants by the two insect groups. The index assumed the lowest values (0.26-0.63%) in our analysis of the coexistence of ants and adult ladybirds. In this case, no simultaneous occurrence of ants and adult ladybirds was registered on the control soil (C). On Syringa, the mutual presence of Coccinellidae eggs and Formicidae was noted in the same aphid colonies only in one season of investigations. Adult ladybirds were encountered in the same colonies as ants in just 0.00 to 0.51% of cases (Gospodarek 2012*b*). No co-existence of Syrphidae eggs, larvae and pupae or tarry black spots together with ants was registered on broad bean cultivated on soil contaminated with lead and copper. In the soil contaminated with cadmium, the value of the Ag index was 0.00-0.60%; on nickel polluted soil it varied from 0.00 to 0.53%. It was similar in the NPK pot, but in the unfertilized control, ants were spotted only with Syrphidae eggs. On Syringa, ants and Syrphidae eggs were noted in the same colonies quite frequently, but Syrphidae larvae occurred together with ants in the same colonies in just 0.00-0.52% of cases (Gospodarek 2012*b*). This is consistent with previous observations conducted by Winiarska (1997) on burdock and pigweed and by Gospodarek (2012*a*) on broad bean.

Neuroptera eggs and other predators were very rarely spotted on the same plants. Co-occurrence of these forms with various development stages of ladybirds was observed only on soil contaminated with copper and lead, which is undoubtedly connected with their more numerous occurrence on plants in these treatments.

The Ag index asumed low values also in the analysis of the coexistence of Aranea, Anthocoridae and aphids parasitized together with other predators in the majority of the experimental variants. It was only on the control soil fertilized with mineral salts that parasitized aphid mummies and predators were often observed on the same plants.

CONCLUSIONS

1. The values of the Agrell's index were low for individual predator groups and their development stages on broad bean, which demonstrates that predators may avoid competition when much food is available. Among the investigated predator groups, the best mutual tolerance was observed for Syrphidae and Coccinellidae.

2. Co-existence of individual aphidophagous groups seems to be strongly conditioned by food, i.e. aphid availability. The applied heavy metals, by influencing the host plant, modified the degree of its colonization by aphids and their number, consequently causing changed in the occurrence of predators. Thus, soil pollution with zinc and nickel contributed to limiting the extent of broad bean colonization by aphids and predators, hence decreasing the values of their coexistence indices, whereas soil contamination with cadmium and lead favoured plant colonization by aphids and predators and therefore the presence of individual aphidophagous groups was noted more frequently on the same plants.

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ACCUMULATION OF COPPER, ZINC, MANGANESE AND IRON BY SELECTED SPECIES OF GRASSLAND LEGUMES AND HERBS

Stefan Grzegorczyk, Marzenna Olszewska,
Jacek Alberski

Chair of Grassland Management
University of Warmia and Mazury in Olsztyn

Abstract

The aim of this study was to determine the accumulation of copper, zinc, manganese and iron by selected species of permanent grassland legumes and herbs. The study was conducted in 1998-2000 (June to the first ten days of July) in the Olsztyn Lakeland. A total of 444 plant samples were analyzed, including 123 collected in organic soils. The following legume and herb species were studied: *Trifolium pratense, Trifolium repens, Lotus corniculatus, Lathyrus pratensis, Lotus uliginosus, Vicia cracca, Taraxacum officinale, Achillea millefolium, Plantago lanceolata, Alchemilla vulgaris, Heracleum sibiricum* and *Cirsium oleraceum*.

The analyzed organic soils were characterized by moderate abundance of copper, zinc and manganese and low abundance of iron, whereas mineral soils contained medium levels of zinc, manganese and iron, and had a low copper content. The average copper content of plants ranged from 5.3 to 10.6 mg kg^{-1} d.m. The highest copper levels were noted in *Taraxacum officinale,* and the lowest in *Lotus corniculatus* grown on organic soils. The average zinc content of plants ranged from 31.0 to 71.0 mg kg^{-1} d.m. *Vicia cracca* grown on organic soils had the highest zinc content, while the lowest zinc concentrations were observed in *Trifolium repens*. The average manganese content of plants ranged from 45.0 to 233.0 mg kg^{-1} d.m. *Alchemilla vulgaris* grown on mineral soils accumulated the highest levels of manganese, and *Trifolium repens* had the lowest manganese content. The iron content of plants varied widely, from 116.0 to 223.0 mg kg^{-1} d.m.

Keywords: copper, zinc, manganese, iron, legumes, herbs.

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prof. dr hab. Stefan Grzegorczyk, prof. zw., Chair of Grassland Management University of Warmia and Mazury, pl. Łódzki 1, 10-727 Olsztyn, Poland, e-mail: stefan.g@uwm.edu.pl

GROMADZENIE Cu, Zn, Mn I Fe PRZEZ WYBRANE GATUNKI ROŚLIN MOTYLKOWATYCH I ZIÓŁ ŁĄKOWYCH

Abstract

Celem badań było pokazanie zdolności gromadzenia Cu, Zn, Mn i Fe przez pospolite gatunki roślin motylkowatych i ziół łąkowo-pastwiskowych występujących na trwałych użytkach zielonych. Badania prowadzono w latach 1998-2000 (czerwiec – pierwsza dekada lipca) na terenie Pojezierza Olsztyńskiego. Łącznie przebadano 444 próby roślinne, w tym 123 pochodzące z gleb organicznych. Badaniami objęto *Trifolium pratense, Trifolium repens, Lotus corniculatus, Lathyrus pratensis, Lotus uliginosus, Vicia cracca, Taraxacum officinale, Achillea millefolium, Plantago lanceolata, Alchemilla vulgaris, Heracleum sibiricum* i *Cirsium oleraceum*.

Analizowane siedliska gleb organicznych charakteryzowały się średnią zasobnością w miedź, cynk i mangan oraz niską w żelazo, zaś gleby mineralne odznaczały się średnią zasobnością w cynk, mangan i żelazo oraz niską w miedź. Średnia zawartość Cu w roślinach wahała się od 5,3 do 10,6 mg kg^{-1} s.m. Najwięcej Cu gromadził *Taraxacum officinale*, najmniej zaś *Lotus corniculatus* występująca na glebach organicznych. Średnia zawartość Zn mieściła się w granicach 31,0-71,0 mg kg^{-1} s.m. Istotnie najwięcej Zn gromadziła *Vicia cracca* występująca na glebach organicznych, najmniej zaś *Trifolium repens*. Zawartość Mn mieściła się w granicach 45,0-233,0 mg kg^{-1} s.m. Istotnie najwięcej Mn gromadził *Alchemilla vulgaris* występujący na glebach mineralnych, najmniej zaś *Trifolium repens*. Zawartość Fe w roślinach była bardzo zróżnicowana, a średnie wartości wahały się od 116,0 do 223,0 mg kg^{-1} s.m.

Słowa kluczowe: miedź, cynk, mangan, żelazo, rośliny motylkowate, zioła.

INTRODUCTION

The species composition of grasslands is an important consideration since it affects the quality of green forage and animal feedstuffs. Legumes and herbaceous plants are valuable components of grassland communities. The latter are a rich source of essential nutrients with therapeutic and medicinal properties as well as other compounds that affect feed intake and utilization by animals (TRZASKOŚ, CZYŻ 2000, WARDA, ĆWINTAL 2000). In Northern Europe, the most common legume species in grasslands are *Trifolium repens, Trifolium pratense, Trifolium hybridum*, *Medicago sativa* and *Lotus corniculatus*. In Western Europe, the most common species is *Trifolium repens,* accompanied by *Lotus corniculatus* and *Lotus uliginosus* which – in contrast to other legumes - can be grown under extreme conditions (NOVOSELOWA, FRAME 1992). The most common herb species are, among others, *Taraxacum officinale, Achillea millefolium, Plantago lanceolata* and *Alchemilla vulgaris.*

Mineral nutrients, in right proportions, are necessary for plant growth, development and maximal yield. Essential nutrients comprise macronutrients and micronutrients such as iron, manganese, zinc, copper, molybdenum and boron. Micronutrients regulate biochemical reactions and participate in numerous physiological processes including photosynthesis, respiration, maintaining hormonal balance and water balance, nitrogen metabolism and metabolism of organic compounds. Microelement deficiencies in plants may result not only from low micronutrient concentrations in soils but also from reduced levels of nutrients available for uptake. Adequate micronutrient concentrations in crop plants are an important criterion while evaluating their quality and suitability as livestock feed (Grzyś 2004).

The aim of this study was to determine the accumulation of copper, zinc, manganese and iron by selected species of permanent grassland legumes and herbs.

MATERIAL AND METHODS

The study was conducted in 1998-2000 (June to the first ten days of July) in the Olsztyn Lakeland. The analysis covered permanent grassland communities with at least 5% share of the following legume and herb species: *Trifolium pratense*, *Trifolium repens*, *Lotus corniculatus*, *Lathyrus pratensis*, *Lotus uliginosus*, *Vicia cracca*, *Taraxacum officinale*, *Achillea millefolium*, *Plantago lanceolata*, *Alchemilla vulgaris*, *Heracleum sibiricum* and *Cirsium oleraceum*. A total of 444 plant samples were analyzed, including 123 collected on organic soils. The chemical composition of soil samples was determined in 0.5 mol dm^{-3} HCl extract, by atomic absorption spectrometry (AAS). Plant material, mineralized in a mixture of nitric acid and perchloric acid (4:1 ratio), was assayed for the content of Cu, Fe, Mn and Zn by atomic absorption spectrometry (AAS).

RESULTS AND DISCUSSION

Copper is essential for the growth and development of plants and animals. Adequate quantities of copper in plants are necessary to meet the nutrient requirements of animals (Czekała 2004). Copper affects the chlorophyll content of plants; it is involved in the synthesis of proteins and carbohydrates and it stimulates the activity of nitrate reductase (Falkowski et al. 2000, Barczak et al. 2006). Copper is important for proper photosynthesis and maintaining water balance (Olszewska et al. 2008). The copper content of plants varies widely depending on the part of plant, growth stage, species and variety, as well as on copper concentrations in soil and climate conditions (Ruszkowska et al. 1996). In the present study, copper abundance was affected by soil type. Higher copper concentrations are generally observed in mineral soils than in organic soils, and in more acidic soils (Gondek 2009, Trąba, Wyłupek 1996). However, in our experiment lower copper levels were noted in mineral soil habitats (Figure 1), at 3.2-5.7 mg kg^{-1}. Organic soil habitats were richer in copper, where its average content ranged from 5.6 to 9.6 mg kg^{-1}, which is considered moderate abundance. Copper was present in the largest (although highly variable) quantities in the

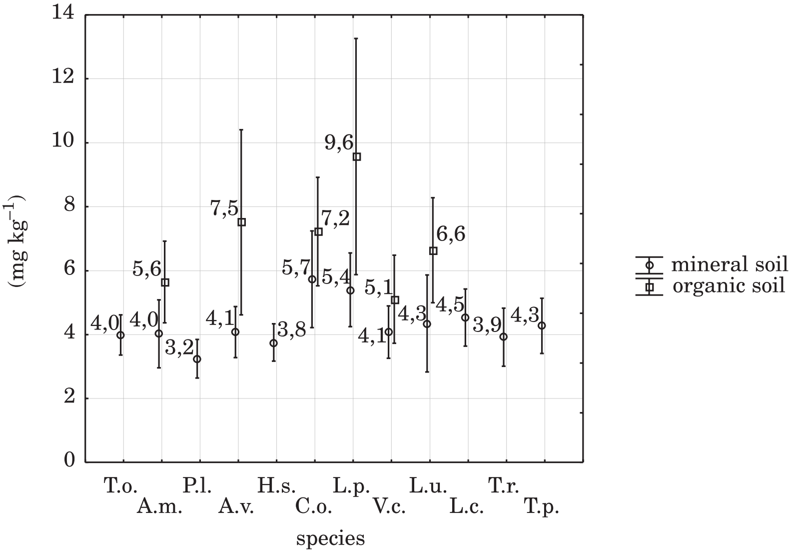

Fig. 1. Copper content of soil (means and 95.00% confidence interval)

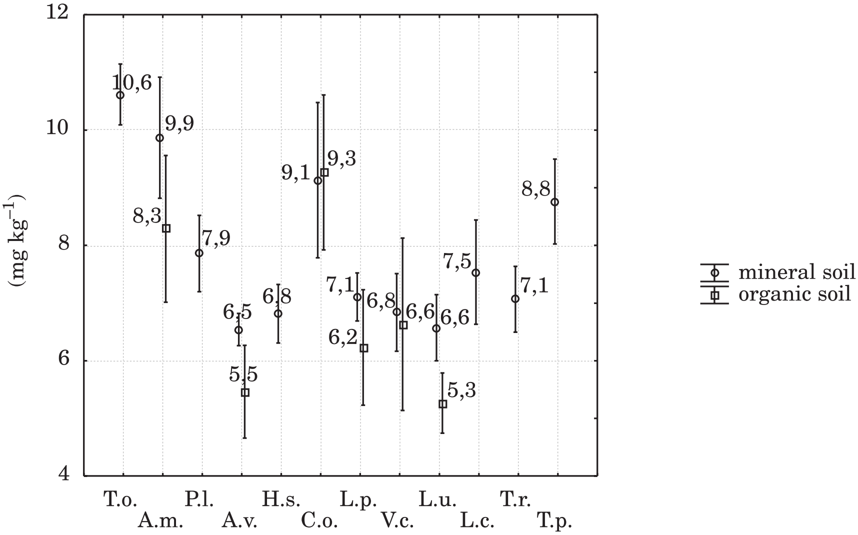

Fig. 2. Copper content of plants (means and 95.00% confidence interval)

habitats of *Lathyrus pratensis* and *Alchemilla vulgaris* on organic soils. The average copper content of plants ranged from 5.3 to 10.6 mg kg^{-1} d.m. (Figure 2). The highest copper levels were noted in *Taraxacum officinale,* and the lowest in *Lotus corniculatus* grown on organic soils. In view of the copper requirements of ruminants, only *Taraxacum officinale* had the optimal copper content. Soil abundance in copper was not directly correlated with the copper content of plants. According to Czekała (2004), copper in soils forms compounds of different solubility and availability to plants. The cited author demonstrated that copper accumulation in plants was significantly affected by water availability and plant species.

Zinc is important for both plants and animals. The zinc content of animal feedstuffs is often insufficient due to its low availability caused by high soil pH, high phosphorus concentrations and the specific biological properties of plant species (Bowszys et al. 2009, Kopeć, Gondek 2004, Trąba et al. 2000). Mineral soil habitats were characterized by moderate zinc abundance (Figure 3). Zinc concentrations remained in the narrow range of 12.0-18.0 mg kg^{-1}. A higher zinc content was noted only in the habitat of *Cirsium oleraceum*. Moderate but varying zinc abundance was observed in organic soil habitats (27.0-40.0 mg kg^{-1}). Zinc content was highest in the habitats of *Achillea millefolium* on organic soils. The average zinc content of plants ranged from 31.0 to 71.0 mg kg^{-1} d.m. (Figure 4). The majority of the examined plant species contained zinc concentrations which were too low, as 50 mg Zn per kg feed d.m. is necessary to meet the nutritional requirements of animals. *Vicia cracca* grown on organic soils had the highest zinc content, while the lowest zinc levels were observed in *Trifolium repens*. Such trends in zinc concentrations were also reported by Trąba and Wolański (2003), and Wyłupek (2003).

Manganese is a growth stimulator and an activator of numerous enzymatic processes. It also participates in nitrogen assimilation, the synthesis of proteins and vitamin C, respiration, as well as in the reactions of water

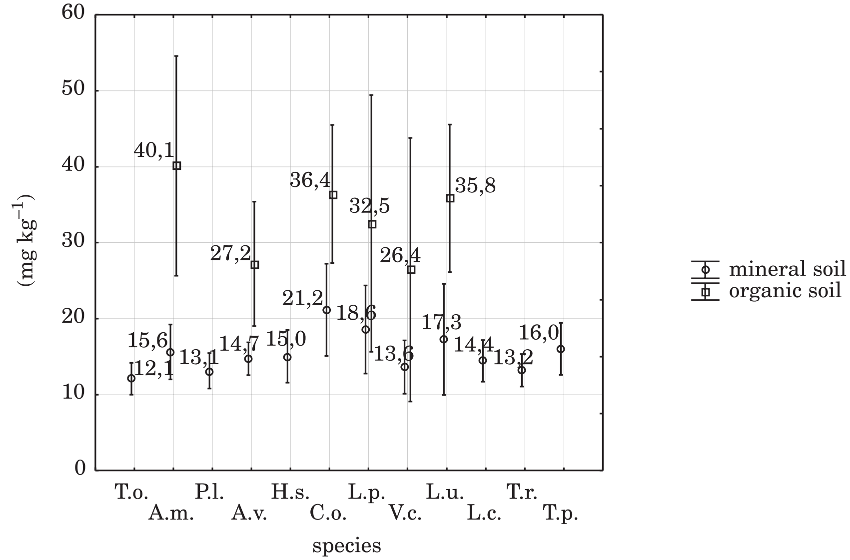

Fig. 3. Zinc content of soil (means and 95.00% confidence interval)

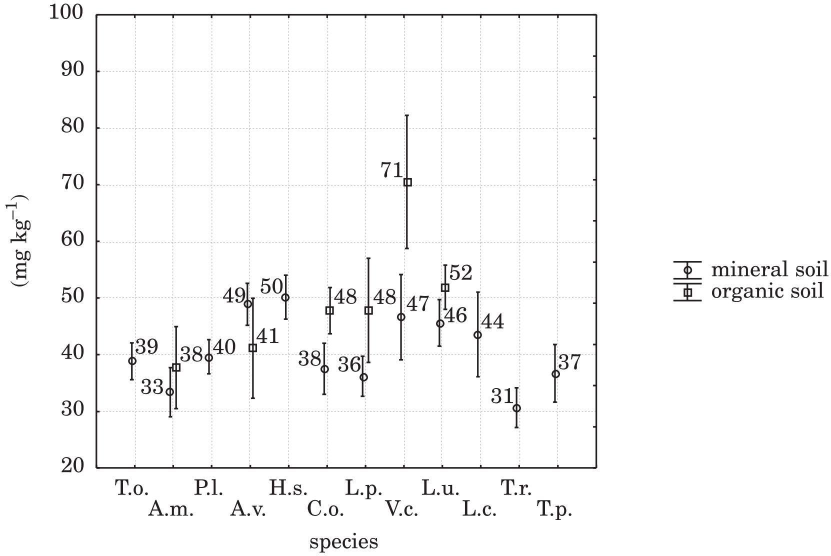

Fig. 4. Zinc content of plants (means and 95.00% confidence interval)

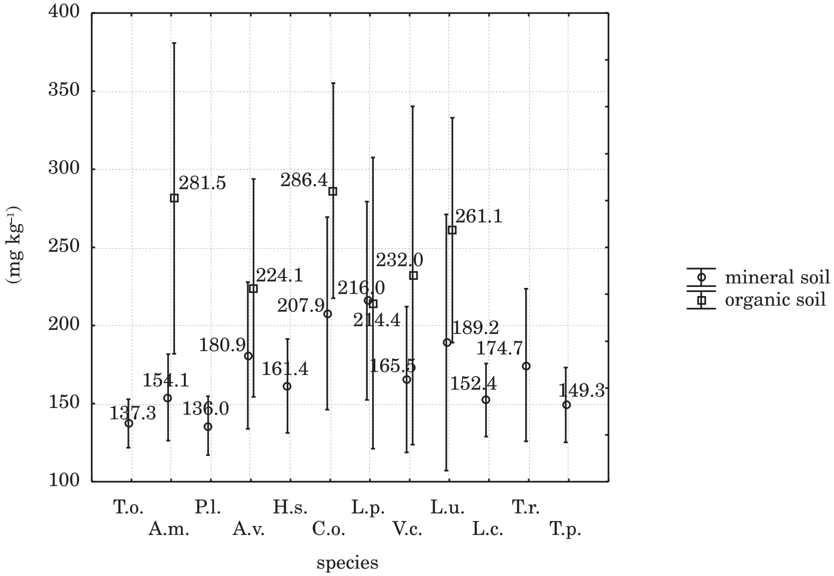

Fig. 5. Manganese content of soil (means and 95.00% confidence interval)

splitting to release oxygen during photosynthesis (GRZYŚ 2004). Manganese affects chlorophyll persistence, and its deficiency causes chlorophyll breakdown under the influence of intense light (OLSZEWSKA, GRZEGORCZYK 2008). Manganese deficiency occurs most often in crops grown on neutral carbonate soils and on soils with a high humus content (BOWSZYS et al. 2006, MALHI et al. 1999, Halasova et al. 2001, Kopittke, Menzies 2004). The analyzed soil habitats were characterized by moderate manganese abundance. Manganese concentrations in mineral and organic soils ranged from 136.0 to 216.0 mg kg^{-1} and from 214.4 to 286.4 mg kg^{-1}, respectively. Manganese levels varied widely across the studied habitats (Figure 5). Communities with *Achillea millefolium, Cirsium oleraceum* and *Lotus uliginosus* were richer in manganese. Chemical analyses of plant material revealed that in the majority of species, the manganese content determined on a dry matter basis exceeded the nutrient requirements of animals. The manganese content of plants ranged from 45.0 to 233.0 mg kg^{-1} d.m. (Figure 6). The biomass of plant species grown on mineral soils contained more manganese in comparison with those grown on organic soil. *Alchemilla vulgaris* and *Achillea millefolium* grown on mineral soils accumulated high levels of manganese, whereas *Cirsium oleraceum* and *Lotus uliginosus* grown on organic soils had the lowest manganese content. According to Harkot (2000), there is no close correlation between manganese concentrations in soil and hay since the supply of this micronutrient to grassland plants is largely dependent on its uptake (soil organic matter content, soil pH, water relations), a conclusion which is consistent with our findings.

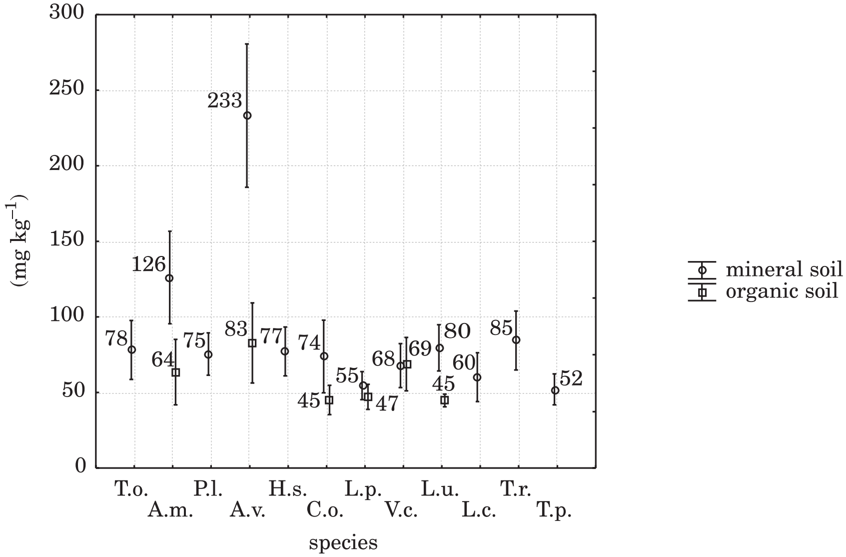

Fig. 6. Manganese content of plants (means and 95.00% confidence interval)

Iron stimulates the biosynthesis of chlorophyll pigments in plants and participates in the light phase of photosynthesis (Grzyś 2004, Zaharieva et al. 2004). Iron deficiency in animals can result in anemia, digestion disorders and impaired immunity (Kinal 2009). In the present study, iron abundance was moderate in mineral soils (1589.7-3178.8 mg kg^{-1}; Figure 7) and

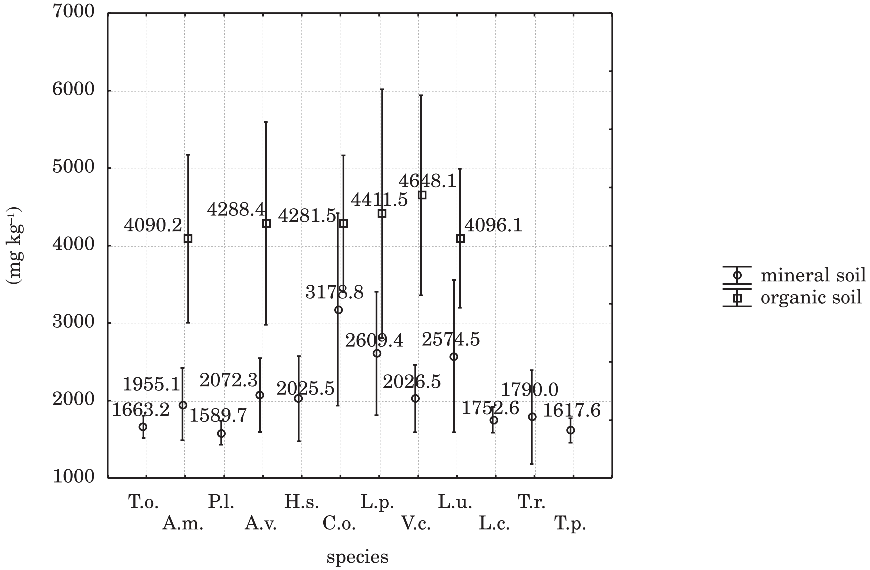

Fig. 7. Iron content of soil (means and 95.00% confidence interval)

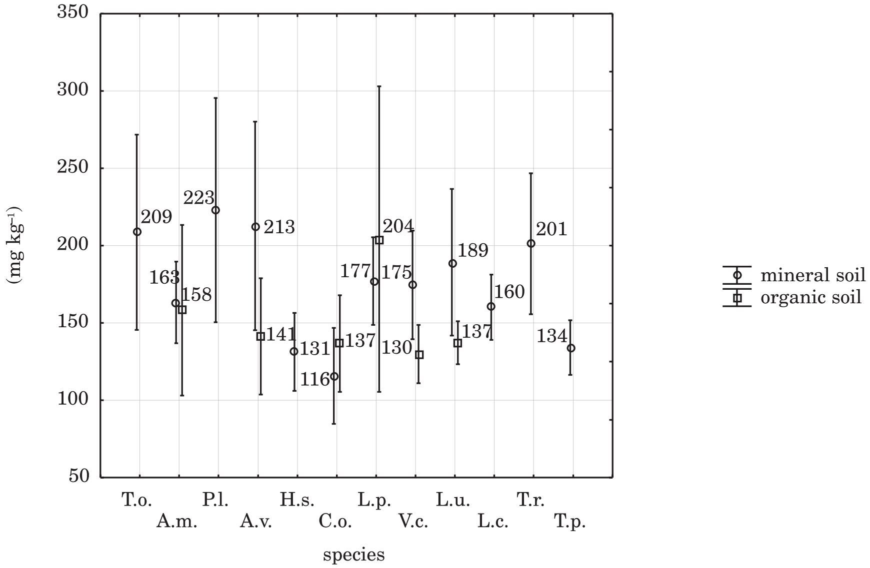

Fig. 8. Iron content of plants (means and 95.00% confidence interval)

low in organic soils (4096.1-4648.1 mg kg^{-1}), and it varied widely across the examined habitats. The highest iron levels were noted in communities with *Vicia cracca* on organic soils, and in communities with *Cirsium oleraceum* on mineral soils. High-quality animal feed should contain 50 to 100 mg

Fe kg^{-1} d.m. The analyzed plant species differed significantly with respect to average iron content, which ranged from 116.0 to 223.0 mg kg^{-1} d.m. (Figure 8). *Plantago lanceolata*, *Taraxacum officinale* and *Alchemilla vulgaris* accumulated the highest quantities of iron in their biomass. *Cirsium oleraceum* (cabbage thistle) grown on mineral soils that were richest in iron had the lowest iron content in dry matter. This indicates that the iron content of plants, similarly as manganese content, is not determined by iron concentrations in soil but by iron mobility and uptake by plants (Harkot 2000).

CONCLUSIONS

1. The analyzed organic soils were characterized by moderate abundance of copper, zinc and manganese and low abundance of iron, whereas mineral soils contained medium levels of zinc, manganese and iron, and had a low copper content.

2. In most cases, the abundance of micronutrients in soils was not directly related to the microelement content of plants, which confirms that concentrations of microelements in plants are determined primarily by their availability, uptake and plant species.

3. Among the studied plant species, *Vicia cracca* accumulated the highest levels of zinc, *Taraxacum officinale* had the highest copper content, *Alchemilla vulgaris* and *Achillea millefolium* were rich in manganese, while *Plantago lanceolata*, *Taraxacum officinale* and *Alchemilla vulgaris* had the highest iron content.

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LEAD AND CADMIUM CONTENT IN SOME GRASSES ALONG EXPRESSWAY AREAS

Kazimierz Jankowski, Jolanta Jankowska,
Grażyna Anna Ciepiela,
Jacek Sosnowski, Beata Wiśniewska-Kadźajan,
Roman Kolczarek, Janusz Deska

Institute of Agronomy
Siedlce University of Natural Sciences and Humanities

Abstract

Due to intense road traffic, plants are exposed to stress factors, mainly fumes, dust from rubber grinding and clutch dust. Among numerous chemicals deposited near roads, heavy metals, e.g. lead and cadmium, are the most dangerous. The aim of the study was to evaluate the effect of distance from a road on the content of lead and cadmium in some grasses and their influence on health and safety of livestock. The following grasses were collected from grasslands located near the S2, an international road running near Siedlce, a town in the east of Poland: orchard grass, meadow foxtail and tall oat grass. The plant material was taken 1 m, 5 m, and 10 m from a roadside ditch at sites approximately distant 100 m from one another. From individual batches, 5-7 samples were made for each species of plants and from each distance from the road edge. The content of lead and cadmium was estimated using the ASA method. The accumulation of lead and cadmium in grass growing along the expressway depended on the grass species and distance from the road. Regardless of the grass species, the highest level of heavy metals was found in the plant material collected 5 m from the road, and an average lead content was nearly 17-fold higher than that of cadmium. Irrespective of the distance from the road, lead was more readily accumulated in tall oat grass and meadow foxtail, whereas cadmium was mainly absorbed by orchard grass. The grass species recommended for sowing on soil contaminated with lead is orchard grass, but meadow foxtail is a better choice to sow on land polluted by cadmium.

Keywords: lead, cadmium, grasses, roadside strip.

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prof. dr hab. Kazimierz Jankowski, Institute of Agronomy, University of Natural Sciences and Humanities in Siedlce, e-mail: laki@uph.edu.pl

ZAWARTOŚĆ OŁOWIU I KADMU W WYBRANYCH GATUNKACH TRAW ROSNĄCYCH WZDŁUŻ DROGI SZYBKIEGO RUCHU

Abstrakt

Eksploatacja dróg powoduje narażenie roślin na czynniki stresogenne. Są to głównie składniki spalin oraz pyły powstające przy ścieraniu opon, tarcz sprzęgła oraz innych elementów ciernych pojazdów. Spośród wielu substancji chemicznych deponowanych obok drogi, największy wpływ na środowisko mają metale ciężkie, a wśród nich ołów i kadm. Celem badań była ocena wpływu odległości od drogi na zawartość ołowiu i kadmu w wybranych gatunkach traw w kontekście bezpieczeństwa sanitarnego w przypadkach spożycia przez zwierzęta hodowlane. Materiał roślinny w formie części nadziemnych pobrano w sierpniu 2011 r. wzdłuż 9 km odcinka trasy szybkiego ruchu S2 (Obwodnica Siedlec). Próbki pobierano losowo w odległości 1, 5, 10 i 15 m od skraju jezdni, po obu stronach drogi, na odcinkach długości ok. 100 m. Z pobranych próbek jednostkowych wykonano 5-7 próbek dla każdego gatunku roślin i każdej odległości od skraju jezdni. Zawartość ołowiu i kadmu oznaczono metodą AAS z użyciem absorcjometru Varian Spectra AA20. Kumulacja ołowiu i kadmu przez trawy rosnące wzdłuż drogi szybkiego ruchu zależała zarówno od gatunku trawy, jak i odległości od trasy. Niezależnie od badanego gatunku trawy, najwięcej oznaczonych metali ciężkich stwierdzono w materiale roślinnym zebranym 5 m od drogi, a średnia zawartość ołowiu była prawie 17 razy wyższa niż kadmu. Niezależnie od odległości od drogi szybkiego ruchu, ołów w największym stopniu był kumulowany przez wyczyniec łąkowy i rajgras wyniosły, a kadm głównie przez kupkówkę pospolitą. Gatunkiem trawy zalecanym do obsiewu terenów skażonych ołowiem jest kupkówka pospolita, a kadmem – wyczyniec łąkowy.

Słowa kluczowe: ołów, kadm, trawy, pasy przydrożne.

INTRODUCTION

Grasses are typical groundcovers in areas adjacent to a roadway. Roadside sward is composed of grasses which are most frequently sown there, dicotyledonous herbs and weeds. They are subject to continuous stress factors caused by traffic. Traffic-related metals originate mainly from combustion of heated fuel, e.g., Pb, lubricating motor oil, vehicle tire wear and brake pads, e. g.*,* Cd, Mn, Zn, Cu (Sadiq et al. 1989, Olajire, Ayodele 1997, Panayotova 2000). Motor vehicles release a number of pollutants into the environment. Roadsides receive considerable amounts of these traffic-generated pollutants (Sutherland, Tack 2000). Also, substances used to maintain roads passable in winter are a cause of pollution. Among many chemical substances deposited near roads there are heavy metals, including lead and cadmium, which have the strongest impact on the environment. Large quantities of heavy metals deposited in soil and those emitted by exhaust fumes lead to their increase in plants growing along roads. However, their actual accumulation in plants is mainly connected with the bioaccumulation potential, which is species-specific. The capacity of different plant species to accumulate metals, which reflects the environmental contamination, has been investigated by many researchers (Gondek, Filipek-Mazur 2006, Jankowska et al. 2007, Królak 2001, Mazur et al. 2007, Naszradi et al. 2004, Wieczorek et al. 2004).

On both sides of a highway there are often agricultural lands and livestock can be seen grazing (Filipek-Mazur et al. 2007, Olajire, Ayodele 1997, Garcia, Milan 1998). Thus, the aim of this study was to evaluate the effect of distance from a road on the content of lead and cadmium in some grasses and their influence on livestock's health.

MATERIAL AND METHODS

The plant material consisting of aerial grass biomass was taken in August 2011 along a 9-km section of the S2 Road (a bypass around Siedlce) – Figure 1. The whole road connects Cork in Ireland with Omsk in Russia and is therefore one of the most important transporation routes in Europe. The Polish section is called the S2. The area where samples were collected lies in the Province of Mazowsze (*województwo mazowieckie*), which is located in East-Central Poland, about 80 km east of Warsaw.

The following grasses were collected from grasslands located near the international road S2 near Siedlce: orchard grass, meadow foxtail and tall oat grass. The plant material was taken from the distance of 1 m, 5 m and 10 m from a wayside ditch, at approximately 100-meter intervals. Each batch served to make 5 to 7 samples of each plant species and each distance from the edge of the road.

First, the collected plant samples were dried at 105°C; next, the weighed samples were dry-mineralized at 450°C for over 24 h, and then solutions in 10% HCL were prepared. The concentrations of lead and cadmium in the

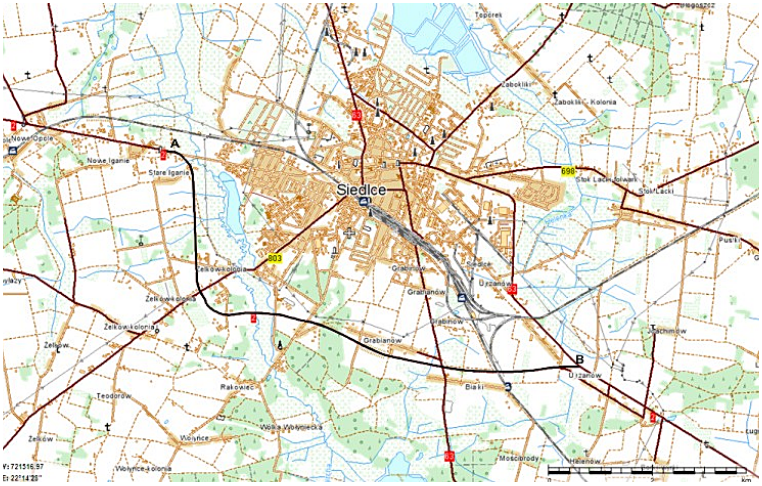

Fig.1. Map of sampling sites along the S2 Road

solutions was estimated using the ASA method. The results were statistically analyzed using Statistica 6.0. A detailed comparison between the mean values was conducted via the Tukey's test at $p \leq 0.05$. When the effect of the quantitative factor (distance) was found, orthogonal contrasts were used to estimate the character of the effect. Correlation coefficients were calculated according to the Sperman's rank between the content of lead and cadmium as well as between their harvest place.

RESULTS AND DISCUSSION

The content of lead and cadmium in the grass species depended on both the type of grass and the distance from the road. Among the examined grass species, the highest level of lead was found in meadow foxtail (5.18 mg g^{-1}) growing within 15 m from road. In both orchard grass and tall oat grass the largest lead accumulation occurred within 5 m from the road, where it reached 4.72 and 5.01 mg kg^{-1}, respectively. In both of these grass species, as the distance from the road grew, the accumulation of lead steadily declined, down to 2.20 at 10 m and 1.64 mg kg^{-1} at 15 m from the road. The results were compared to the limit imposed on the lead content in feeds for ruminants, which is 10 mg kg^{-1} d.m. according to Rengel (2004). The results suggest that the tested grasses can be used in animal nutrition. It should also be observed that the analyzed species of grasses looked healthy because their content of lead had not reached the toxic level (Goralach, Gambuś 2000). Plant poisoning with lead, according to Weglarz (2007), may occur when the Pb content is more than 15 mg kg^{-1} of dry matter. This concentration of lead can impair the process of photosynthesis by reducing chlorophyll biosynthesis (Baycu et al. 2003). With regard to Pb, the roadside concentration of this element found in our study was much lower than reported by other researchers (Filipek-Mazur et al. 2007, Garcia, Milan 1998). It is worth noting that the lead content in these grass species and at a distance of 1 m from the road was almost half of that found at 5 m distance from the road. Swaileh et al. (2004) found average metal concentrations in *Inula viscose* leaves distinctly below the range of metal concentrations in plant leaves considered phytotoxic. However, the concentrations of Pb in the roadside samples of *I. viscose* leaves were higher than those reported in leaves of the same plant from a control site (Swaileh et al. 2001).

Regardless of the grass type, the highest lead content was found in the plant material taken from the 5-meter distance from the road (4.35 mg kg^{-1}). This value differed significantly from the lead content in the plant material at the other distances from the road. Likewise, in his study, Swaileh et al. (2004) demonstrated that lead gradually decreased from a 0-m to a 15-m distance. Thereafter, no further significant decrease was observed.

In contrast to soil, no sharp decrease in the concentration of any metal in plants was observed. Chronopoulos et al. (1997) studied the reduction of Pb concentrations from a periphery to the interior (60 m) of some parks in Athens, Greece. Lead contamination was found up to about 40 m, beyond which little change in Pb concentration was observed in leaves of *N. oleander* and *Pittosporum sinensis.* Othman et al. (1997) observed longer distances for the lead pollution coverage along roads of the city of Damascus. They recommended that vegetables should be planted at least 200 m away from a roadside. According to the same reference, most lead particles emitted by vehicles are deposited on roadside soils (0-5 m), but some can be carried by winds over a long distance.

Another important metal in plants growing in areas adjacent to roads is cadmium. Frener (2001) claims that a critical cadmium content in plants which precludes their use for consumption should be less than 0.15 mg kg^{-1} d.m., but it should come to ≤ 0.5 mg kg^{-1} d.m. for feeding. The studied plant material did not exceed this value.

Among the examined grass species, most Cd was bio-accumulated by cocksfoot at a 5-metere distance from the road (0.45 mg kg^{-1}). At a larger distance from the road, the cadmium content steadily decreased to 0.15 mg kg^{-1} in plants grown at a distance of 15 m from the road. As in the case of lead, the cadmium content in cocksfoot growing within 1 m from the road was almost 50% lower than in plants at a 5 m distance. In turn, tall oat grass and meadow foxtail accumulated the highest levels of cadmium one meter form the road (0.19 mg kg^{-1}and 0.18 mg kg^{-1}). Regardless of the grass type, the lowest cadmium values were found in the plant material collected from a 15-m distance from the road (mean value 0.12 mg kg^{-1}). This content was significantly different from the heavy metal content found in the plant material taken from the other (closer) distances from the expressway.

As in the case of lead, the highest level of cadmium was accumulated by plants at a distance of 5 m from the road (0.26 mg kg $^{-1}$). Diatta et al. (2003) states that concentrations of metals in plant samples collected at different distances from a road edge exhibited statistically significant negative relationships with the distance from the road edge, except for Cd.

The results contained in Table 2 lead to the conclusion that in areas contaminated with lead, cocksfoot is a suitable species for sowing because the maximum amount of this heavy metal was found only in tall oat grass and meadow foxtail. With regard to the accumulation of cadmium by those three grass species, it was demonstrated that the most recommended species for such areas is meadow foxtail. This species, regardless of the distance from the roadway, did not reach the maximum content of cadmium at different distances from the road. According to Swaileh et al. (2004) and Nabulu et al. (2006), using plants to identify the roadside pollution seems to be a better solution than using soil concentrations of pollutants. In addition, plants reflect amounts of metals that are biologically available to plants.

It is very difficult to compare literature data concerning contamination levels with heavy metals in plants near highways is very difficult (VIARD et al. 2004). The levels of cadmium extracted from the plant material analysed in our study were lower than observed by many authors in *Graminaceae* (RODRIGUEZ et al.1982 or HO and TAI 1988), who studied *Chenchrus echinatus, Sarghum halopense, Paspalum paniculatum* or *Stipia ichu*. Nevertheless, they are similar to the values achieved in recent studies on tobacco leaves or herb leaves and vegetables (OTHMAN et al. 1997). Levels of cadmium were lower than those observed in earlier studies of HO and TAI (1988), although they were comparable to those observed by many other authors (CIEPIELA et al. 2009, GARCIA, MILAN 1998, SEGURA-MUNOZ et al. 2004, PANAYOTOVA 2000, OKUNOLA et al. 2007). In comparison to the values responsible for causing subchronic toxic symptoms in cattle through forage, which is 0.5 Cd mg kg $^{-1}$ d.m. (GARCIA, MILAN 1998), the concentrations of this metal in our study were lower.

Table 1

Content of lead and cadmium in plant samples (mg kg^{-1})

Distance from the road (m)	Plant species	Lead		Cadmium	
		mean	coefficient of variation (%)	mean	coefficient of variation (%)
1	*Dactylis glomerata*	2.66	24.7	0.234	45.1
	Arrhenatherum elatius	2.52	27.8	0.19	34.2
	Alopecurus pratensis	3.30	32.8	0.18	32.9
Mean for 1 m		2.72	14.7	0.40	45.9
5	*Dactylis glomerata*	4.72	23.1	0.45	25.8
	Arrhenatherum elatius	5.00	47.2	0,19	32.1
	Alopecurus pratensis	3,70	22.9	0,14	38.3
Mean for 5 m		3.15	44.3	0.13	103.6
10	*Dactylis glomerata*	3.42	32.9	0.28	37.9
	Arrhenatherum elatius	3.96	36.9	0.33	33.2
	Alopecurus pratensis	3.46	45.3	0.11	23.1
Mean for 10 m		2.93	36.5	0.17	64.5
15	*Dactylis glomerata*	2.20	23.9	0.15	21.9
	Arrhenatherum elatius	1.64	17.8	0.08	23.9
	Alopecurus pratensis	5.18	67.1	0.13	34.1
Mean for 15 m		2.45	66.5	0.16	53.9
Mean for all distances		2.84	44.4	0.22	78.5

Moreover, the levels of Cd observed in plants were lower than the EU limits and the background value given by RENGEL (2004).

Table 2

Grasses with the lowest and highest bioaccumulation of the investigated elements

Distance from the road (m)	Maximum		Minimum	
	Pb	Cd	Pb	Cd
1	*Alopecurus pratensis*	*Dactylis glomerata*	*Arrhenatherum elatius*	*Alopecurus pratensis*
5	*Arrhenatherum elatius*	*Dactylis glomerata*	*Alopecurus pratensis*	*Alopecurus pratensis*
10	*Arrhenatherum elatius*	*Arrhenatherum elatius*	*Dactylis glomerata*	*Alopecurus pratensis*
15	*Alopecurus pratensis*	*Dactylis glomerata*	*Arrhenatherum elatius*	*Arrhenatherum elatius*

The multiple linear regression analysis (Figures 1, 2) showed that the variability of the lead content depended on the distance from the road and can be described by the following equation: $y = -0.565x^2 + 2.78x + 0.743$; for cadmium the euquation was $y = -0.0\ 44x^2 + 0.193x + 0.051$.

The stages of the function described by the above equations show that the greatest accumulation of these elements in plants occurred in a distance of 5-10 m from the road. The ruminants which wander and graze along roadsides feed on these grasses, while birds and domestic fowls feed there on insects and earthworms (AWOFOLU 2005, BASGEL, ERDEMOGL 2006). The transport of metals through the food chain and their accumulation are likely to cause health problems, especially in animals in higher links in the food chain. Thus, accumulation and toxicity of this metal for ruminants grazing on plants along these roads is very important.

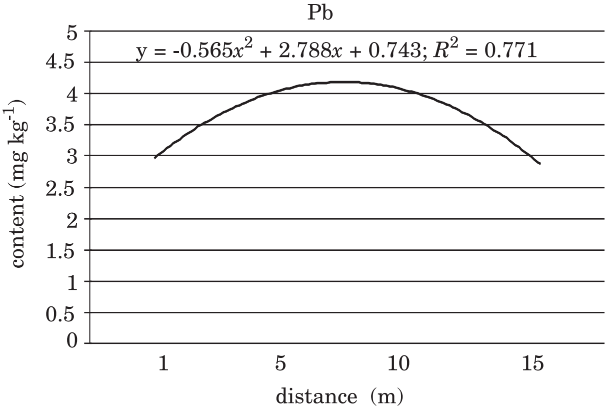

Fig. 1. Multiple regression equations describing the influence of the distance from the road on the content of lead in the analysed plants

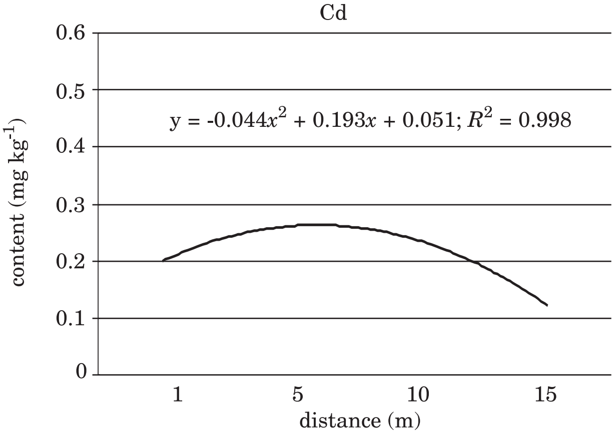

Fig. 2. Multiple regression equations describing the influence of the distance from the road on the content of cadmium in the analysed plants

As reported by Swaileh et al (2004), the average metal concentrations in scabwort leaves was clearly below the range of metal concentrations in plant leaves considered phytotoxic. However, the concentrations of Pb, Cd, and Zn in roadside samples of scabwort leaves were higher than reported in leaves of the same plant from a control site (Swaileh et al. 2001).

CONCLUSIONS

1. Lead and cadmium accumulation in grass growing along a road depended on the type of grass and the distance from the road.

2. Regardless of the grass species, the highest level of heavy metals was found in the plant material collected at a 5-metre distance from the road, and the average lead content was nearly 17-fold higher than that of cadmium.

3. Irrespective of the distance from the road, lead was largely accumulated by tall oat grass and meadow foxtail, while cadmium was absorbed mainly by orchard grass.

4. The grass species recommended for sowing on lead contaminated land is orchard grass while meadow foxtail is a better choice on the cadmium polluted soil.

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CHEMICAL AND ENZYMATIC CHANGES IN SOIL TREATED WITH AMMONIUM GLUFOSINATE*

Stefania Jezierska-Tys, Agata Rutkowska

Chair of Environmental Microbiology
University of Life Sciences in Lublin

Abstract

Intensive use of synthetic chemicals in crop cultivation ensures high crop yields. On the other hand, excessive use of agrichemicals disturbs the biological balance and leads to the contamination of soil environment. The resistance of soil to this type of pollution depends on its physical, chemical and biological properties. Persistence of chemical substances in soil plays a fundamental role in environmental pollution. Therefore, it is important to monitor how long a chemical agent applied to soil remains active. Soil microorganisms and their enzymatic activity are the best indicators of soil contamination with chemical compounds. The aim of this study was to evaluate the influence of the active ingredient (ammonium glufosinate) of the herbicide Basta 150 SL on the microbial activity and chemical properties of soil under winter rape cultivation.

The investigations on the herbicide Basta 150 SL (with ammonium glufosinate as the active ingredient) consisted of a three-year field experiment set up on soil representing the class of black soils. Basta 150 SL was applied to soil in a dose recommended by the producer (2.5 dm^3 ha^{-1}) and at a 10% higher dose (2.75 dm^3 ha^{-1}). Soil was sampled for analyses on 8 dates, i.e. immediately after winter rapeseed harvest and after 2, 10, 12, 14, 22, 24 and 26 months of the experimental period. The study demonstrated that the dose of the chemical agent substantially affected the rate of ammonification and nitrification. A higher dose resulted in the significantly lower rate of the above processes. The manufacturer's recommended dose caused in a significant decrease in the proteolytic activity of soil. None of the applied doses, however, had any significant effect on the soil activity urease. After the application of a recommended dose of Basta 150 SL, the pH_{KCl} values and C:N ratio were higher.

Keywords: soil, enzymatic activity, glufosinate ammonium, Basta 150 SL herbicide.

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prof. dr hab. Stefania Jezierska-Tys, Chair of Environmental Microbiology, University of Life Sciences in Lublin, Leszczyńskiego 7, 20-069 Lublin, Poland, e-mail: stefania_tys@op.pl

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ZMIANY CHEMICZNE ORAZ ENZYMATYCZNE GLEBY PODDANEJ DZIAŁANIU GLUFOSYNATU AMONOWEGO

Abstrakt

Intensywne stosowanie syntetycznych substancji chemicznych w uprawach polowych jest niezbędne ze względu na wysokość uzyskiwanych plonów. Nadmierne używanie tych substancji prowadzi do zachwiania równowagi biologicznej oraz zanieczyszczenia środowiska glebowego. Odporność gleby na te zanieczyszczenia zależy od jej właściwości fizycznych, chemicznych i biologicznych. Czas pozostawania substancji chemicznych w glebie ma duże znaczenie w zanieczyszczeniu środowiska naturalnego. Dlatego istotne jest monitorowanie czasu oddziaływania zastosowanego czynnika chemicznego na środowisko glebowe. Mikroorganizmy glebowe i ich aktywność enzymatyczna są najlepszymi wskaźnikami zanieczyszczenia gleby związkami chemicznymi.

Celem pracy była ocena wpływu substancji czynnej (glufosynatu amonowego) wchodzącej w skład herbicydu Basta 150 SL na aktywność mikrobiologiczną oraz chemiczne właściwości gleby pod uprawą rzepaku ozimego.

Badania nad czasem oddziaływania środka chemicznego Basta 150 SL (substancja aktywna – glufosynat amonowy) oparto na trzyletnim doświadczeniu polowym, założonym na glebie należącej do czarnych ziem właściwych. Do gleby wprowadzono środek chemiczny Basta 150 SL w dawce zalecanej przez producenta (2,5 dm^3 ha^{-1}) oraz w dawce zwiększonej o 10% (2,75 dm^3 ha^{-1}). Próbki gleby do analiz pobierano w 8 terminach, tj. bezpośrednio po zbiorze rzepaku ozimego, a następnie po 2, 10, 12, 14, 22, 24 i 26 miesiącach trwania doświadczenia. Wykazano, że na nasilenie procesów amonifikacji i nitryfikacji w glebie istotny wpływ miała dawka badanego środka chemicznego. Zwiększona dawka powodowała istotne osłabienie badanego procesu. Zaaplikowanie do gleby dawki zalecanej przez producenta spowodowało istotny spadek aktywności proteolitycznej. Ponadto stwierdzono, że zastosowane dawki nie wpływały istotnie na aktywność ureazy w badanej glebie. Zaobserwowano również wzrost wartości pH badanej gleby po zastosowaniu preparatu Basta 150 SL w dawce zalecanej oraz stosunku C:N.

Słowa kluczowe: gleba, aktywność enzymatyczna, glufosynat amonowy, herbicyd Basta 150 SL.

INTRODUCTION

Ammonium glufosinate is an active ingredient in the desiccant called Basta 150 SL, popular on winter rapeseed plantations. Ammonium glufosinate (Figure 1), a compound which belongs to aminophosphonates, is an inhibitor of glutamine synthetase and interferes with the photosynthetic process (Kidd, James 1991, Pieniążek et al. 2003, Jariani et al. 2010). Consequently, plant tissues become desiccated through dehydration, resulting in severe chlorosis and plant wilting. In soil, ammonium glufosinate is degraded by microorganisms to 3-methylphosphinicopropionic acid (MPP) and 2-methylphosphinicoacetic acid (MPA), and ultimately to carbon dioxide under aerobic conditions (Smith 1988).

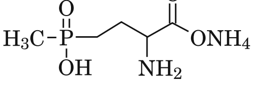

Fig. 1. Formula of ammonium glufosinate

The herbicide Basta 150 SL is a fast acting, contact herbicide, whose mode of action depends on natural biochemical processes occurring in plants. This agent is applied primarily to prepare rapeseed plantations for harvest by accelerating and equalizing plant maturation.

Through an appropriate use of chemical pesticides it is possible to control, either directly or indirectly, their negative impact. However, an inadequate and excessive application of synthetic chemical substances frequently leads to their accumulation in the environment (BEYER, BIZIUK 2007). This may result in the gradual degradation of ecosystems due to the impact of agrichemicals on the biological life of soil and yield quality (SEGHERS et al. 2004; WYSZKOWSKA, KUCHARSKI 2004b). The activity of soil microorganisms and its possible modification due to natural and anthropogenic environmental factors can be assessed via measurements of soil enzymatic activity. The activity of enzymes and biological nitrogen transformations may serve as sensitive indicators of the ecological status of soils (EMMERLING et al. 2002, NANNIPIERI et al. 2003).

The available literature deals with a wide arrays of issues related to the contamination of soil with chemical pesticides, providing data on the detrimental effect of agrichemicals on the microbial abundance and soil enzymatic activity (ROLA, KIELOCH 2001, WYSZKOWSKA 2002*ab*, KUCHARSKI, WYSZKOWSKA 2008). In their research, WYSZKOWSKA and KUCHARSKI (2004*a*) reported inhibitory effects of herbicides on the soil enzyme activity and microbial abundance as well.

The investigations carried out by WIBAWA et al. (2008) demonstrated that ammonium glufosinate applied at doses recommended by the producer did not accumulate in soil. However, PAMPULHA et al. (2007) indicated that application of ammonium glufosinate could stimulate as well as inhibit soil microorganisms, and the nature of specific interactions depended on the concentration of the substance and incubation period. The authors also found that glufosinate ammonium caused inhibition of dehydrogenase activity.

Prolonged and excessive use of synthetic chemical substances in crop cultivation should be looked at with care because of the risk of environmental contamination it creates. Briefly, the aim of the present three-year field experiment was to assess the impact of time- and dose-dependent activity of ammonium glufosinate, an active ingredient of a popular herbicide called Basta 150 SL, on the soil enzymatic activity, i.e. an indicator of soil richness and fertility.

MATERIAL AND METHODS

Characterisation of the soil

A field experiment was set up with the split-block method on soil classified as black soil (WRB-Mollic Gleysols) developed from light silty clay (2-0.5 mm – 65%, 0.05-0.002 mm – 22%, <0.002 mm – 7%). Table 1 presents the basic characteristics of the soil analysed in the study.

Table 1

Basic characteristics of the experimental soil

Parameter	Unit	Value
Reaction	pH_{KCl}	6.1
$C_{org.}$	g kg^{-1} d.m.	9.8
Total nitrogen	g kg^{-1} d.m.	1.3
C:N		7.5
Total phosphorus	g kg^{-1} d.m.	0.7
K	g kg^{-1} d.m.	0.1
Zn	mg kg^{-1} d.m.	34.0
Cd	mg kg^{-1} d.m.	0.15
Cu	mg kg^{-1} d.m.	10.8
Pb	mg kg^{-1} d.m.	9.6
Ni	mg kg^{-1} d.m.	7.5
Cr	mg kg^{-1} d.m.	14.4
Hg	mg kg^{-1} d.m.	0.1
Hh	$cmol^+$ kg^{-1} d.m.	1.38

Description of the experiment

An analysis of the impact of Basta 150 SL was based on a three-year field experiment (2010-2012) located at the Experimental Station for Cultivar Testing in Głębokie (52°38’41”N,18°26’18”E). The herbicide Basta 150 SL, containing 150 g of glufosinate ammonium (a compound from the aminophosphonate group) in 1 dm^3 of the agrichemical, was applied in the first year of the experiment. Two doses were tested: recommended by the manufacturer and 10% higher. The experimental model included the following objects:

K – control soil without herbicide;

B1 – soil treated with the optimal (2.5 dm^3 ha^{-1}) dose of Basta 150 SL;

B2 – soil treated with a 10% higher (2.75 dm^3 ha^{-1}) dose of Basta 150 SL.

The herbicide was applied with a knapsack sprayer 8 days before winter rapeseed harvest.

In the first year, seeds of the winter rapeseed cultivar Californium were sown in all objects; sugar beet and rye were cultivated in the second and third year, respectively. During the experiment, basic agrotechnical treatments were conducted and uniform fertilization was applied in accordance with recommendations for the cultivated plants. The surface area of the experimental plots was 12 m^2.

Soil for analyses was sampled from the topsoil layer of each plot eight times, i.e. immediately after winter rapeseed harvest (early August), and next after 2, 10, 12, 14, 22, 24, and 26 months of the experimental period. 20-30 soil samples were collected from each experimental object; after mixing, they formed a 6-kg composite sample.

Biochemical and chemical analyses

The soil was transported in heat-proof polyethylene bags at a low temperature. In a laboratory, the soil material was thoroughly sieved through 2-mm mesh. Soil from averaged samples was analysed and used for determination of protease activity with the method of LADD and BUTLER (1972) modified by ALEF and NANNIPIERI (1995), in which sodium caseinate served as a substrate; the activity of urease was assayed with the method of ZANTUA and BREMNER (1975) modified by ALEF and NANNIPIERI (1995), with urea as a substrate; ammonification rate was assessed with the Nessler's method while nitrification was analyzed with the brucine method (NOWOSIELSKI 1981). Chemical analyses included determination of the content of organic C by the Tiurin method; total N by the Kjeldahl method; total P spectrophotometrically; K by atomic emission spectrometry; Zn, Cd, Cu, Pb, Ni, Cr, Hg by atomic absorption spectrometry. The soil pH in 1M KCl was determined potentiometrically.

Statistical analyses

The results were analysed statistically with analysis of variance. The least significant differences were calculated with the Tukey's test at the significance level $\alpha = 0.05$. All statistical calculations were performed using Statistica 7.1 software.

RESULTS AND DISCUSSION

Intensity of ammonification and nitrification processes

Nitrogen is one of the major elements in the earth and its soil transformations are related to the level of C, which provides energy for microorganisms involved in these processes (EMMERLING et al. 2000). The content of nitrogen in soil is closely linked with organic matter (SCHNITZER 2001).

Figure 2 presents the effect of the herbicide Basta 150 SL applied during the field experiment on increasing the rate of ammonification in soil under rapeseed. The present study has shown considerable fluctuations in the process of ammonification in soil. The analysis of the results implies that initially the level of ammonification in soil treated with the recommended dose of the herbicide was significantly lower than in the control object. In turn, the 10% higher dose reduced ammonification to zero. Later, on the second sampling date, the rate of ammonification upon the application of the optimal herbicide dose declined substantially in comparison with the control object. Following an application of Basta 150 SL in a dose higher than recommended by the producer, the rate of ammonification was similar to the control. On the subsequent dates, periodic fluctuations in the intensity of ammonification were observed. Worth noticing was a significant increase

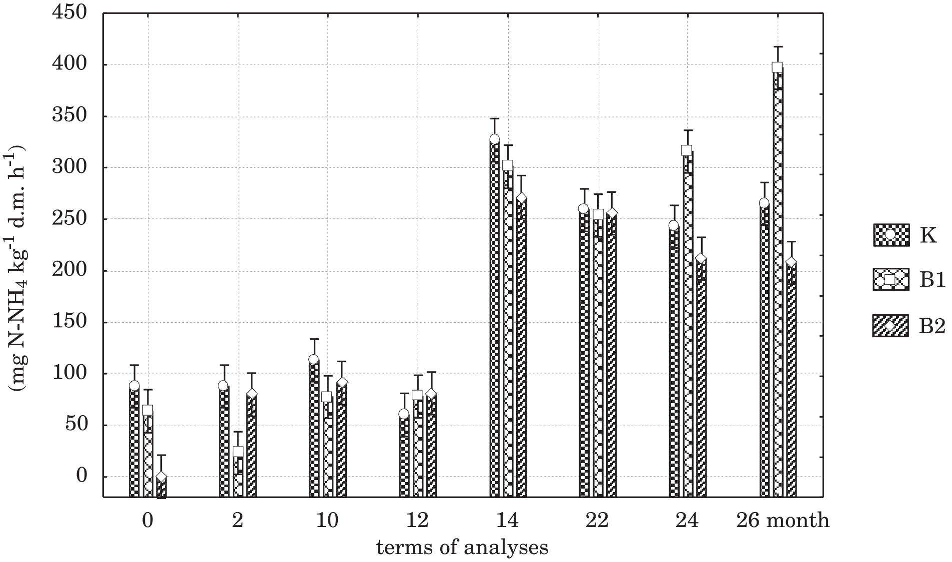

Fig. 2. Periodic intensification of the ammonification process in individual experimental objects: K – control soil without herbicide addition, B1 – soil treated with the optimal dose of Basta 150 SL herbicide, B2 – soil treated with the 10% higher dose of Basta 150 SL herbicide. Vertical bars indicate the 0.95 confidence interval

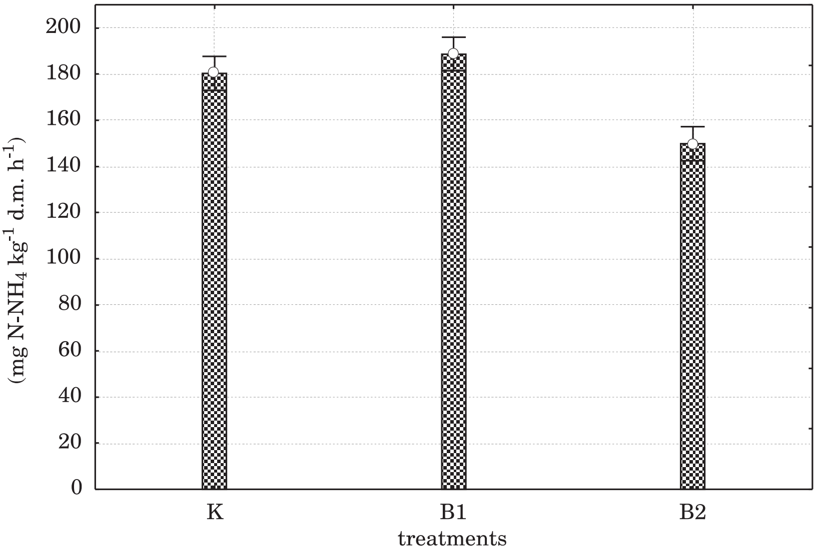

Fig. 3. Mean values of ammonification in individual experimental objects
Key: cf. Fig. 1

in the rate of ammonification 24 and 26 months after the application of the chemical agent in the manufacturer's recommended dose. Our analysis of the mean values from the entire experimental period and experimental objects (Figure 3) demonstrated a significant decrease in ammonification induced by Basta 150 SL applied in the higher dose; this may have been related to the $-NH_2$ group, which is a constituent of ammonium glufosinate. Investigations conducted by other authors (STRZELEC 1986, KRZYŚKO-ŁUPICKA 2008) revealed a stimulating effect of Roundup (containing glyphosate as an active ingredient) on the enhancement of ammonification in soil, which may indicate that the preparation is used up by microorganisms. KUCHARSKI et al. (2009) investigated the effect of a variety of herbicides (Harpun 500 S.C., Fawory 300 SL, Akord 180 OF, Mocarz 75 WG) on the course of ammonification in soil. They reported that Mocarz 75 WG caused the highest inhibition of ammonification in tested soil. Additionally, the authors showed that the type of herbicide, dosage and experiment's duration influenced the course of the process.

Figure 4 presents the results of determinations of the $N\text{-}NO_3$ content in the experimental objects. Comparing these results, it appears that changes in the rate of nitrification induced by the application of two different doses of Basta 150 SL were subject to periodic fluctuations. On the first sampling date, as well as 2, 22, and 24 months after the treatment, Basta 150 SL applied in the manufacturer's recommended dose significantly intensified nitrification versus the control object. In turn, the 10% higher dose significantly inhibited the process in 2nd, 10th, 12th and 26th month. The data presented in Figure 5 indicate significant stimulation by the recommended dose throughout the study period, and significant inhibition induced by the elevated

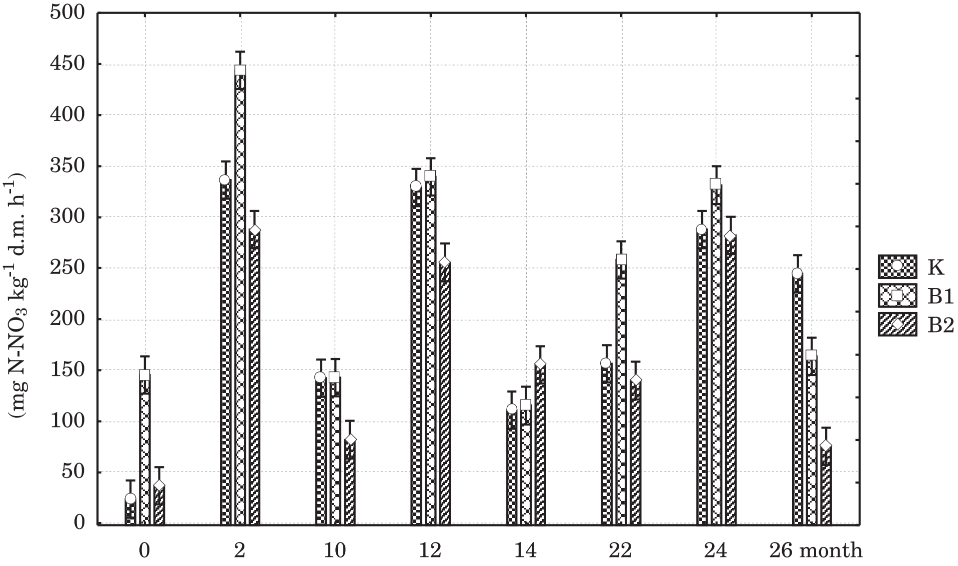

Fig. 4. Periodic intensification of nitrification in individual experimental objects

Key: cf. Fig. 1

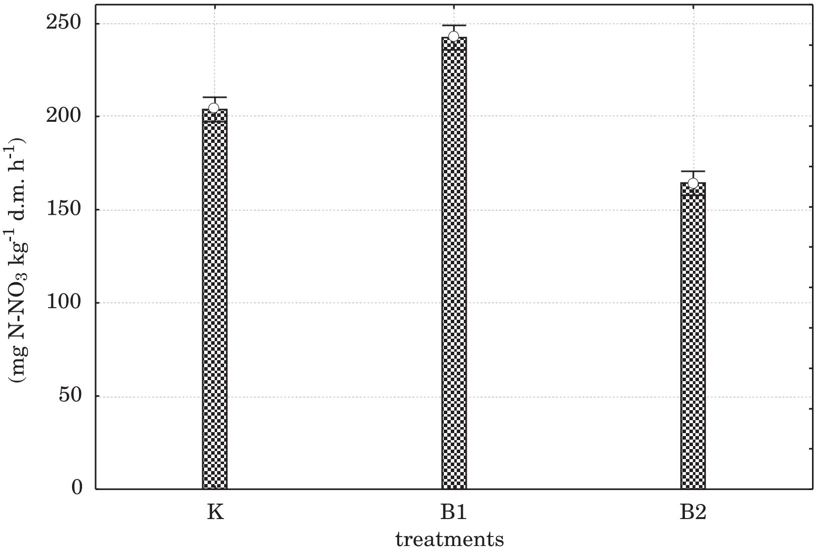

Fig. 5. Mean values of nitrification in individual experimental objects
Key: cf. Fig. 1

dose. Similarly, in their investigations on application of optimal doses of chemicals, Swoboda et al. (2007) reported an increased content of nitrate nitrogen compared with control objects.

Activity of protease and urease

The activity of protease measured with the hydrolytic activity towards casein represents the activity of extracellular enzymes (Ladd and Butler 1972). Figure 6 shows the activity of the enzyme in response to the two doses of Basta 150 SL. Changes in the activity of protease, which plays an essential role in mineralization of organic nitrogen, were found in individual soil objects. Based on the results, it was concluded the above process was stimulated in the first time period. A significant decline in the proteolytic activity was reported 2, 10, and 12 months after the application of the recommended dose, which had a considerable impact on the mean value from this object (Figure 7). The decrease in the activity was significant against both the control and the object treated with higher dose.

The activity of urease in soil treated with the different doses of the herbicide Basta 150 SL is illustrated in Figures 8 and 9. The data indicate that the two herbicide doses significantly increased the enzyme's activity compared to the control in the first study period. In contrast, 2 and 26 months after the application, both the optimal dose and 10% higher doses caused a significant decline in the ureolytic activity in soil. Throughout the experiment, periodic fluctuations in the enzyme's activity were observed. The mean values obtained during the entire experiment corresponded to the va-

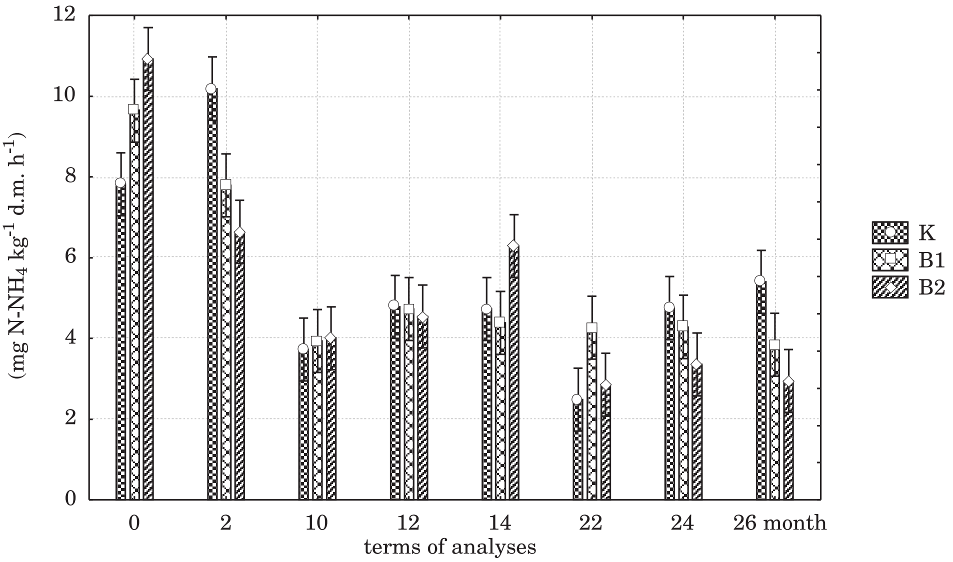

Fig. 6. Periodic activity of urease in individual experimental objects
Key: cf. Fig. 1

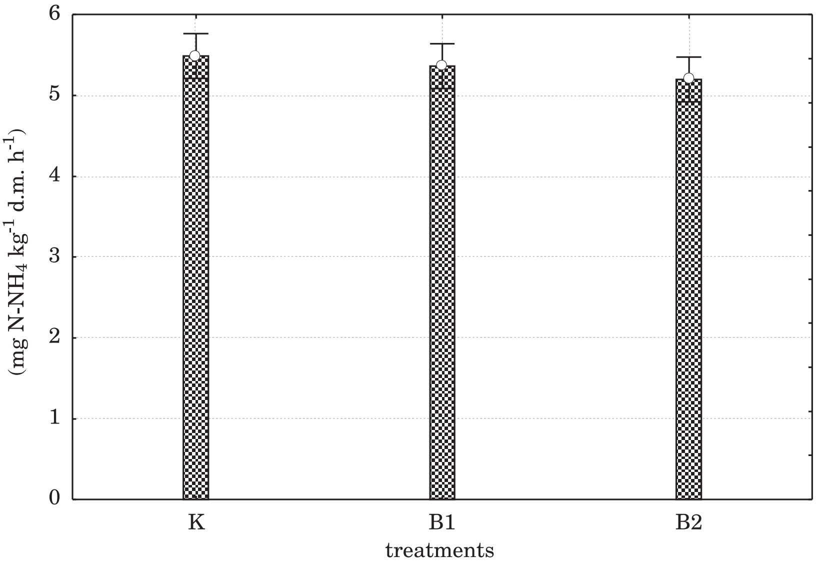

Fig. 7. Mean urease activity in individual experimental objects
Key: cf. Fig. 1

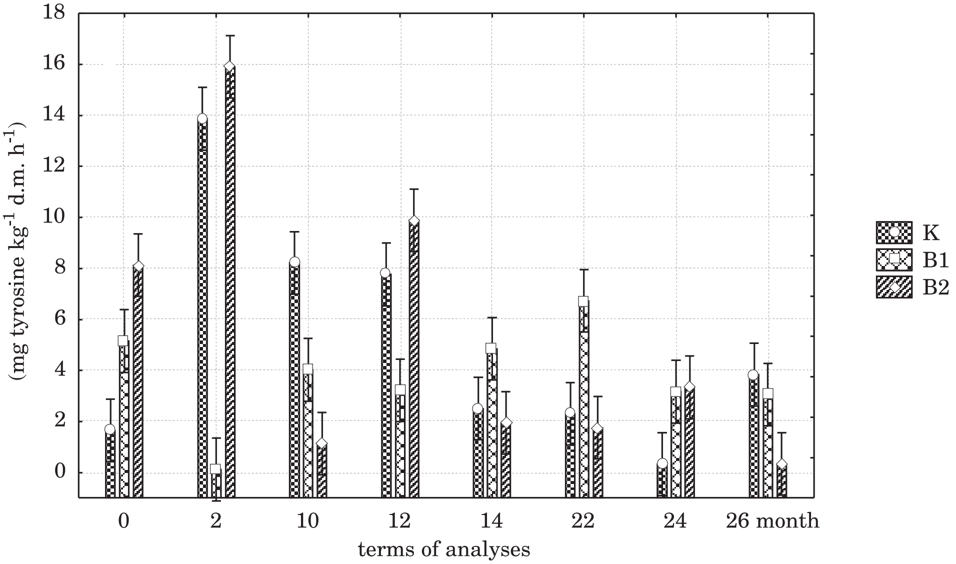

Fig. 8. Periodic activity of protease in individual experimental objects
Key: cf. Fig. 1

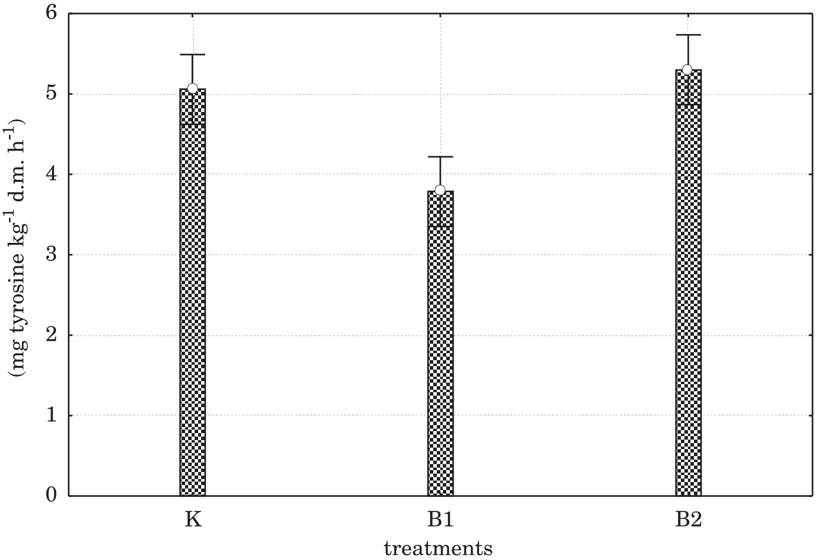

Fig. 9. Mean protease activity in individual experimental objects
Key: cf. Fig. 1

lues obtained in the control soil. Therefore, it can be concluded that neither the recommended nor the increased dose triggered significant changes in the activity of urease. Similar results were obtained by Wyszkowska (2002a) and Kucharski and Wyszkowska (2008) and Baćmaga et al. (2012), who found a negative effect of herbicides on urease in soil.

pH_{KCl} of soil

The results concerning pH are presented in Table 2. In general, throughout the experiment, Basta 150 SL did not have any significant effect on the pH value of soil, although a periodic decline or rise in the reaction was observed. It is noteworthy that the pH value increased immediately after application of the chemical agent at both the recommended and 10% higher dose.

Table 2

Periodic soil pH_{KCl} values in individual experimental objects during the experiment

Experimental objects	Analysis period month							
	0	2	10	12	14	22	24	26
Control soil without herbicide addition (K)	5.6	7.1	6.1	7.0	6.8	6.6	6.6	7.1
Soil treated with the optimal dose of Basta 150 SL herbicide (B1)	6.5	7.0	6.2	7.0	6.7	6.5	6.5	7.1
Soil treated with the 10% higher dose of Basta 150 SL herbicide (B2)	6.8	7.1	6.8	6.9	7.1	6.8	6.8	6.7

Content of organic carbon, total nitrogen, and C:N

Our analysis of the mean organic carbon content (Table 3) showed that C_{org} increased in response to the application of the recommended dose of Basta 150 SL (11.4 g kg^{-1} d.m.). A similar tendency was found for the nitrogen content. The highest N_{org} content (1.4 g kg^{-1} d.m.) was found in the soil treated with the recommended dose of Basta 150 SL. The lowest value of the C:N ratio was found in the experimental objects in 2010, when it reached 8.2 in the control soil and 8.8 in the soil treated with the increased Basta 150 SL dose. Furthermore, with time, the C:N ratio was found to have risen by 27% in the soil treated with the higher dose of the herbicide Basta 150 SL.

Table 3

Content of organic carbon, total nitrogen, and C:N in the individual experimental objects during the experiment

Experimental objects	Year	Parameter		C:N
		C_{org} (g kg^{-1} d.m.)	total N (g kg^{-1} d.m.)	
Control soil without herbicide addition (C)	2010	9.8	1.3	7.5
	2011	10.8	1.2	9.0
	2012	11.6	1.3	9.0
Mean		10.7	1.3	8.2
Soil treated with the optimal dose of Basta 150 SL herbicide (B1)	2010	10.4	1.4	7.4
	2011	11.8	1.3	9.1
	2012	12.0	1.4	8.6
Mean		11.4	1.4	8.1
Soil treated with the 10% higher dose of Basta150 SL herbicide (B2)	2010	9.4	1.2	7.8
	2011	10.5	1.1	9.5
	2012	11.9	1.2	9.9
Mean		10.6	1.2	8.8

CONCLUSIONS

1. The dose of the herbicide applied had a significant effect on the rate of ammonification in soil.
2. The proteolytic activity was significantly the highest after the application of Basta 150 SL in the dose recommended by the producer.
3. The applied doses did not significantly affect the activity urease in soil.
4. The herbicide Basta 150 SL used in the recommended and increased doses caused an increase in the pH reaction of soil and in the C:N ratio.

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MICROBIOLOGICAL ACTIVITY OF SOIL AMENDED WITH GRANULATED FERTILIZER FROM SEWAGE SLUDGE

Jolanta Joniec[1], Edyta Kwiatkowska[2]

[1] Department of Environmental Engineering
Catholic University of Lublin
[2] Chair of Environmental Microbiology
University of Life Science in Lublin

Abstract

The study was conducted on a model of a pot experiment in which grey-brown podzolic soil developed from weakly loamy sand was amended with granulated dry organic-mineral fertilizer prepared from municipal sewage sludge. Three levels of pre-sowing fertilization with the granulate were applied: I – a dose which brought nitrogen in the amount of 0.35 g kg^{-1} d.m. of soil, II – a 50% higher dose, and III – a 100% higher dose. Additionally, a treatment with average mineral fertilization (NPK) was run. The control treatment was non-fertilized soil. Soil prepared as above was placed in pots and sown with white mustard (cv. Borowska) in spring in the 1st and 2nd year of the experiment. During the experiment, the soil moisture was maintained at the level of *ca* 60% t.w.c. Microbiological and biochemical soil assays were conducted three times in the first and second years of the experiment, i.e. in May and July and 41 days from harvesting the crop and mixing of its fragmented residues with the soil. The scope of the analyses included numbers of bacteria with low and high nutrition requirements, total numbers of filamentous fungi, cellulolytic, proteolytic and lipolytic bacteria and fungi. Other analyses included determinations of the respiratory activity in soil, the rate of cellulose mineralization and the activity of dehydrogenases, lipase, protease and acid phosphatase. The study showed a stimulating effect of the sludge granulate on the above groups of bacteria and fungi, both in the 1st and in the 2nd years of the experiment. The effect generally weakened with time. The fertilization also stimulated the respiratory rate and activity of phosphatase. However, it inhibited the mineralisation of cellulose as well as the activity of dehydrogenases, lipase and protease in soil. The application of mineral fertilization in the form of NPK did not cause any changes in numbers of the analyzed bacterial groups, except a certain decreasing tendency in the second year. In both years, mineral fertilization contributed to the stimulation of the growth of most fungi. It also enhanced the respiratory activity, rate of cellulose mineralisation, and the activity of dehydrogenase and acid phosphatase, albeit only in the first year of the experiment. Finally, in the soil with mineral fertilization the activity of protease and lipase was observed to have declined.

Keywords: grey-brown podzolic soil, mustard, sludge granulate, NPK, numbers of bacteria and fungi, biochemical activity.

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dr Jolanta Joniec, Department of Environmental Engineering, Catholic University of Lublin, Off-Campus Faculty of Social Sciences in Stalowa Wola,ul. Ofiar Katynia 6a, 37-450 Stalowa Wola, Poland, e-mail: jolajoniec1@gmail.com

AKTYWNOŚĆ MIKROBIOLOGICZNA GLEBY NAWOŻONEJ GRANULATEM Z OSADU ŚCIEKOWEGO

Abstrakt

Badania przeprowadzono na modelu doświadczenia wazonowego, w którym glebę płową, wytworzoną z piasku słabo gliniastego, nawieziono granulowanym suchym nawozem organiczno-mineralnym, wykonanym na bazie osadu ścieków komunalnych. W doświadczeniu zastosowano przedsiewnie trzy poziomy nawożenia granulatem: I – dawka z którą wniesiono azot w ilości 0,35g kg^{-1} s.m. gleby; II – dawka zwiększona o 50%, III dawka zwiększona o 100%. Dodatkowo wprowadzono kombinację z przeciętnym nawożeniem mineralnym (NPK). Kontrolę doświadczenia stanowiła gleba nienawożona. Tak przygotowaną glebę umieszczono w wazonach i obsiewano wiosną w 1. i 2. roku badań gorczycą białą odmiany Borowska. Wilgotność gleby w czasie doświadczenia utrzymywano na poziomie ok. 60% c.p.w. Analizy mikrobiologiczne i biochemiczne gleby wykonano trzykrotnie w 1. i 2. roku doświadczenia, tj. w maju i lipcu oraz po 41 dniach od zbioru rośliny i wymieszaniu jej rozdrobnionych resztek z glebą. Określano liczebność: bakterii o małych i dużych wymaganiach pokarmowych, grzybów nitkowatych ogółem, bakterii i grzybów celulolitycznych, proteolitycznych oraz lipolitycznych. Ponadto w glebie analizowano aktywność oddechową, tempo mineralizacji celulozy oraz aktywność dehydrogenaz, lipazy, proteazy i kwaśnej fosfatazy.

Wykazano stymulujący wpływ zastosowanego granulatu osadowego na ww. grupy bakterii i grzybów, zarówno w 1., jak i w 2. roku doświadczenia. Obserwowane oddziaływanie na ogół słabło w miarę upływu czasu. Nawożenie to stymulowało również aktywność oddechową i aktywność fosfatazy. Natomiast hamowało tempo mineralizacji celulozy, a także aktywność dehydrogenaz, lipazy i proteazy w glebie. Zastosowanie nawozu mineralnego w postaci NPK w 1. roku badań nie spowodowało zmian w liczebności. badanych grup bakterii, a w kolejnym roku odnotowano nawet pewną tendencję spadkową w tym zakresie. W obu latach nawożenie mineralne przyczyniło się do pobudzenia rozwoju większości grup grzybów oraz tylko w 1. roku badań – aktywności oddechowej, tempa mineralizacji celulozy, aktywności dehydrogenaz i fosfatazy kwaśnej. W glebie z nawozem mineralnym odnotowano ponadto spadek aktywności proteazy i lipazy.

Słowa kluczowe: gleba płowa, gorczyca, granulat osadowy, NPK, liczebność bakterii i grzybów, aktywność biochemiczna.

INTRODUCTION

Owing its high fertilizing potential, sewage sludge is much more than just waste (BARAN et al. 2002). When stored, often on sites inadequately prepared for the purpose, it constitutes a hazard to the environment. In contrast, after introduction to soil it has a beneficial effect on numerous physical, chemical, physicochemical and biological properties, as reported by SASTRE et al. (1996), KSIĘŻOPOLSKA et al. (2002), FURCZAK and JONIEC (2007), PASCUAL et al. (2007), JONIEC and FURCZAK (2008), OCIEPA (2011) or SCHERER et al. (2011) and other researchers. Moreover, natural utilisation of sewage sludge means that large amounts of biogenic substances accumulated and immobilised in sewage sludge, primarily carbon, nitrogen and phosphorus, are returned into the cycling of elements. However, prior to using sewage sludge, a risk of its containing excessive levels of chemical pollutants should be considered, e.g. heavy metals, PAHs, pathogenic organisms, as well as of large amounts of organic matter and deficient levels of potassium (BARAN et al. 2002, OLESZCZUK 2007, WALCZAK, LALKE-PORCZYK 2009). Therefore, in order to improve the properties of sewage sludge, it is frequently subjected to various processes, e.g. composting (WOLNA-MARUWKA 2009, NICOLAS et al. 2012,) or phytoremediation with the use of alternative crop plants (LIPHADZI et al. 2006). One such plant is white mustard, characterised by phytosanitary and phytoremediation properties moreover; white mustard can also inhibit the migration of biogenic compounds to the environment and improve the soil structure (SAWICKA, KOTIUK 2007, VANEK et al. 2010). The production of granulated organic-mineral fertilizers from sewage sludge may turn to be another solution, ensuring better characteristics of sewage sludge and creating new possibilities of utilisation municipal sewage sludge (NOWAK et al. 2002).

In this study, an attempt was made to estimate the applicability of such fertilizer for the improvement of biological properties of grey-brown podzolic soil under cropped with white mustard. Additionally, the effect of the organic-mineral granulate was compared with conventional NPK mineral fertilization.

MATERIAL AND METHODS

The study was conducted on a model of a pot experiment set up in a greenhouse of the Faculty of Environmental Microbiology, University of Life Sciences in Lublin. Each of the pots contained 5 kg of grey-brown podzolic soil developed from weakly loamy sand (pH_{KCl} – 5.3; $C_{org.}$ – 9.12 g kg^{-1}), fertilized once with dry granulated organic-mineral fertilizer from municipal sewage sludge, containing: N – 61 g kg^{-1}; P – 21.4 g kg^{-1}; K – 108 g kg^{-1}; Ca – 28.6 g kg^{-1} and Mg – 6.03 g kg^{-1}. The granulate was composed of 42.5% of organic matter and 57.5% of inorganic components. The fertilizer was synthesised at the Department of Coke Engineering and Environmental Protection, Institute of Chemical Processing of Coal in Zabrze, and consisted of 70% of sewage sludge and 30% of potassium nitrate (KNO_3). Detailed characteristics of the fertilizer are given by Wiater and Żebranowicz (2005). Three levels of pre-sowing fertilization with the granulate were applied in the experiment: I – dose which brought nitrogen in the amount of 0.35 g kg^{-1} d.m. of soil, II – a 50% higher dose, and III – a 100% higher dose. In another experimental object, an average mineral fertilization treatment was applied in the form of NPK. The control treatment consisted of soil without any fertilization. The experimental design included three replications for each of the treatments. The soil in all the pots was stabilised at a moisture level of 60% t.w.c. and sown with white mustard (cv. Borowska). That level of soil moisture was maintained fairly stable throughout the experiment. After harvesting aerial parts of the plants, the residues were fragmented, mixed with the soil and left till the next year. White mustard was sown again into the same pots in spring the following year to observe the residual effect of the fertilization on the biological parameters of the soil.

Microbiological and biochemical analyses were performed three times (in May and July, i.e. during the growth of the plant, and 41 days after harvesting the crop and mixing fragmented plant residues with the soil) in the first and second years of the experiment.

The scope of the analyses included assays of the total number of oligotrophic bacteria, on a medium with soil extract or sludge extract (350 cm^3 dm^{-3}) and K_2HPO_4; total number of macrotrophic bacteria on the BUNT-ROVIRA medium (1955); so-called total number of filamentous fungi on the Martin medium (1950); numbers of cellulolytic bacteria on liquid medium accoring to POCHON and TARDIEUX (1962), the most probable number of those bacteria was read from McCrady's Tables; numbers of cellulolytic fungi on mineral agar, with an addition of antibiotics in the amount recommended by MARTIN (1950), covered with Whatman paper discs; numbers of bacteria decomposing proteins, on the Frazier gelatine medium (RODINA 1968); numbers of fungi decomposing proteins, on the Frazier gelatine medium (RODINA 1968) with an addition of antibiotics in the amount recommended by (MARTIN 1950); numbers of lipolytic bacteria on agar medium with tributyrate (BURBIANKA et al. 1971) in which yeast extract was replaced with broth (NOWAK et al. 1992); numbers of lipolytic fungi on the medium as above, with an addition of antibiotics in the amount recommended by (MARTIN 1950); respiratory activity with the method of RÜHLING and TYLER (1973); rate of cellulose mineralisation in 25-gram weighed portions of soil enriched with 0.5% of powdered Whatman cellulose, from the amount of CO_2 emitted from soil during 20 days assayed with the method of RÜHLING and TYLER (1973); activity of dehydrogenases with the method of THALMANN (1968); lipase activity with the method of POKORNA (1964) modified by KUHNERT-FINKERNAGEL and KANDELER (1996); protease activity with the method of LADD and BUTLER (1972); activity of acid phosphatase with the method of TABATABAI and BREMNER (1969).

All analyses were made in three replications. The results of the microbiological and biochemical analyses were processed statistically by analysis of variance. The significance of differences was determined with the Tukey's test at $p = 0.05$.

RESULTS AND DISCUSSION

The study indicated that the applied doses of the granulate caused an increase in the numbers of almost all of the bacterial groups in the 1st year, i.e. oligotrophic, macrotrophic, proteolytic and lipolytic bacteria (Tables 1, 2 and 3). The effect was the strongest in the case of proteolytic bacteria and

Table 1

Total numbers of bacteria in the soil

Fertilization	Oligotrophic bacteria (cfu 10^9 kg^{-1} d.m.of soil)		Macrotrophic bacteria (cfu 10^9 kg^{-1} d.m.of soil)	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	5.078	12.456	3.622	22.611
Soil + NPK	1.633	7.400	3.889	7.111
Soil + I dose of granulate	6.678	9.267	8.378	16.311
Soil + II dose of granulate	5.133	36.956	8.889	122.200
Soil + III dose of granulate	6.889	8.900	9.433	17.467
Mean	5.082	14.996	6.842	37.140
LSD year LSD year x fertilization	0.416 1.530		0.600 2.203	

Table 2

Numbers of proteolytic bacteria and fungi in the soil

Fertilization	Proteolytic bacteria (cfu 10^9 kg^{-1} d.m. of soil)		Proteolytic fungi (cfu 10^6 kg^{-1} d.m. of soil)	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	0.467	2.667	28.89	66.89
Soil + NPK	0.822	1.933	59.11	87.78
Soil + I dose of granulate	1.844	5.756	39.11	70.00
Soil + II dose of granulate	1.978	4.244	42.67	58.44
Soil + III dose of granulate	2.511	5.989	24.33	58.44
Mean	1.524	4.118	38.82	67.51
LSD year LSD year x fertilization	0.185 0.682		4.13 15.19	

Table 3

Numbers of lipolytic bacteria and fungi in the soil

Fertilization	Lipolytic bacteria (cfu 10^9 kg^{-1} d.m. of soil)		Lipolytic fungi (cfu 10^6 kg^{-1} d.m. of soil)	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	0.367	1.144	18.02	11.97
Soil + NPK	0.278	1.167	17.79	19.31
Soil + I dose of granulate	0.689	1.911	21.98	12.30
Soil + II dose of granulate	0.822	1.411	23.13	15.93
Soil + III dose of granulate	1.000	1.333	21.00	11.71
Mean	0.631	1.393	20.38	14.24
LSD year LSD year x fertilization	0.056 0.207		0.96 3.55	

the weakest regarding oligotrophic bacteria (Tables 1, 2 and 3). Moreover, the growth of proteolytic and lipolytic bacteria was more intensive under higher doses of the sludge granulate. It was only the number of cellulolytic bacteria that was slightly reduced in the first year of the experiment, and the decrease was the biggest under the effect of the largest dose of the granulate (Table 4).

Table 4

Numbers of cellulolytic bacteria and fungi in the soil

Fertilization	Cellulolytic bacteria (10^6 kg^{-1}· d.m. of soil)		Cellulolytic fungi (cfu·10^6 kg^{-1} d.m. of soil)	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	52.41	1.60	28.15	17.01
Soil + NPK	45.00	1.11	41.20	36.80
Soil + I dose of granulate	47.02	7.42	30.50	23.24
Soil + II dose of granulate	47.31	4.20	35.32	25.20
Soil + III dose of granulate	30.43	7.70	38.52	16.98
Mean	44.43	4.41	34.74	23.85
LSD year			1.17	
LSD year x fertilization			4.31	

In the second year, the growth of oligo- and macrotrophic bacteria was stimulated only by the middle dose of the granulate (Table 1) whereas the other two doses (I and III) caused a slight decreasing tendency in the numbers of those bacteria. On the other hand, the stimulated growth of proteolytic and lipolytic bacteria proceeded in the 2nd year of the experiment in all the treatments with the granulate (Tables 2, 3), although it was weaker than in the previous year. The growth of cellulolytic bacteria, as opposed to the first year, was distinctly stimulated by all doses of the granulate (Table 4).

In a comparison of the effects of fertilization with the granulate versus NPK, it was noted that the granulate had a much better effect on the growth of the analyzed bacterial groups (Tables 1-4). In the 1st year, numbers of bacteria from most of the groups in the soil amended with NPK alone were on levels similar to or below the control treatments. However, in the second year, a tendency for the inhibited growth of most of the bacterial groups was observed (except for lipolytic bacteria) – Tables 1-4.

As with bacteria, numbers of all analyzed fungi, i.e. total, cellulolytic, proteolytic and lipolytic fungi, increased in the first year in the soil amended with the granulate (Tables 2-5). This effect was the most distinct for the total number of fungi.

In the second year, a slight stimulation by the granulate was still observed in the growth of most of the groups of fungi (Tables 2-5). As for total fungi, however, the stimulating effect was weaker with time, disappearing completely in the case of proteolytic fungi.

Table 5

Total number of filamentous fungi in the soil

Fertilization	Filamentous fungi (cfu·10^6 kg^{-1}d.m. of soil)	
	1st year	2nd year
Non-fertilized soil	102.3	112.6
Soil + NPK	305.3	158.7
Soil + I dose of granulate	192.8	127.3
Soil + II dose of granulate	172.9	159.9
Soil + III dose of granulate	136.7	83.8
Mean	182.0	128.4
LSD year LSD year x fertilization	6.7 24.8	

The data in Tables 2, 4 and 5 show that the total number of filamentous, cellulolytic and proteolytic fungi increased also in the soil fertilized with NPK in the first year. As time passed, the positive effect on the total number of fungi and proteolytic fungi was weaker, although it continued also in the second year. Reversely, the stimulation of the growth of cellulolytic and lipolytic fungi intensified in later stages of the experiment (Tables 3 - 4). Populations of fungi in treatments with mineral fertilization were even larger than in treatments with the sewage sludge granulate. This indicates that mineral fertilization created more favourable conditions for fungi than sludge granulate. The application of mineral fertilization probably lowered the soil pH, an effect which is known to promote the growth of fungi. Lower soil reaction caused by mineral fertilizers is reported by KOPER and PIOTROWSKA (2003), among others. For soil quality, a more intensive growth of fungi is an adverse development. It may contribute to stronger acidification of soils and increased concentration of metabolites produced by fungi, including mycotoxins.

The stimulation of the growth of the analyzed bacterial and fungal groups by the sludge-mineral granulate was probably induced by the introduction of a certain amount of organic matter to soil because organic matter in sludge promotes the growth and activity of heterotrophic soil microorganisms (SASTRE et al. 1996, FURCZAK, JONIEC 2007, PASCUAL et al. 2007).

A review of the available literature shows absence of studies focused on the comparison of effects of sewage sludge granulate and mineral fertilization on soil microbiological parameters. On the other hand, there have been experiments on the effect of sewage sludge and mineral fertilization on microbial populations (SASTRE et al. 1996). The cited authors demonstrated stimulated growth of various groups of bacteria and fungi, both by sewage sludge and by mineral fertilization.

In the first year of the current study, soil amendment with the experimental doses of the granulate stimulated the respiratory activity and the activity of acid phosphatase in soil (Tables 6-7). This effect was stronger in response to higher doses of the granulate. The positive effect of this fertilizer was also visible albeit more weakly in the second year of the experiment (Tables 6-7).

Both in the first and second year, the granulate doses most frequently caused a slight inhibition of the cellulose mineralization rate and the activity of dehydrogenases, lipase and protease (Tables 6-8).

In the first year of the experiment, mineral fertilization (NPK) stimulated the respiratory activity of soil and slightly improved the activity of dehydrogenases and acid phosphatase activity as well as the rate of cellulose mineralization (Tables 6-8). However, the positive effect of NPK was weaker, especially with respect to the respiratory and phosphatase activity, than the granulate. This was probably due to lower levels of organic carbon and, regarding phosphatase activity, to higher amounts of mineral phosphorus in soil fertilized with NPK, the reason also implied by Madejón et al. (2001), Gianfreda et al. (2005), and Pascual et. al. (2007) and others. With time, this effect was disappearing, and lasted only in the case of cellulose mineralisation, although it was much weaker than in the preceding year (Tables 6-8).

Table 6

Biochemical activity in the soil

Fertilization	Respiratory activity (mg C-CO_2 kg^{-1} d.m. of soil d^{-1})		Cellulose mineralization (mg C-CO_2 kg^{-1} d.m. of soil 20 d^{-1})	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	130.9	95.5	196.6	205.7
Soil + NPK	174.8	92.3	220.8	213.5
Soil + I dose of granulate	168.1	105.4	197.0	214.3
Soil + II dose of granulate	198.0	130.1	188.5	200.1
Soil + III dose of granulate	195.5	114.8	180.8	186.3
Mean	173.4	107.6	196.7	204.0
LSD year LSD year x fertilization	5.0 18.3			

Table 7

Protease and acid phosphatase activity in the soil

Fertilization	Protease (mg tyrosine kg^{-1} d.m. of soil h^{-1})		Acid phosphatase (mg PNP kg^{-1} d.m. of soli h^{-1})	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	12.096	7.791	30.72	44.84
Soil + NPK	5.403	2.484	34.07	44.23
Soil + I dose of granulate	8.538	6.758	35.40	48.79
Soil + II dose of granulate	7.317	6.308	37.75	52.43
Soil + III dose of granulate	5.432	7.468	38.95	49.66
Mean	7.757	6.162	35.38	47.99
LSD year LSD year x fertilization	0.318 1.170		1.10 4.05	

Table 8

Dehydrogenases and lipase activity in the soil

Fertilization	Dehydrogenases (mg TPF kg^{-1} d.m. of soil d^{-1})		Lipase (units g^{-1} d.m. of soil)	
	1st year	2nd year	1st year	2nd year
Non-fertilized soil	0.909	0.876	0.633	1.068
Soil + NPK	1.050	0.886	0.516	0.930
Soil + I dose of granulate	0.708	0.897	0.550	1.038
Soil + II dose of granulate	1.132	0.686	0.644	0.834
Soil + III dose of granulate	0.962	0.813	0.580	0.867
Mean	0.952	0.831	0.585	0.947
LSD year LSD year x fertilization	0.057 0.208		0.020 0.073	

Analogously to our experiment, studies by some other authors demonstrated a better effect of sewage sludge on the respiratory and phosphatase activity in soil compared to NPK (Sastre et al. 1996, Pascual et al. 2007, Scherer et al. 2011).

Fertilization of soil with NPK caused a slight decrease of lipase activity, same as the sludge granuate, and a stronger decline of protease activity. These effects persisted throughout the whole experiment. Different results were obtained by Pascual et al. (2007), who only found a slight drop in protease activity in soil fertilized with NPK, and a significant increase of the same parameter under the effect of sewage sludge.

Based on the current results of the granulate affecting the microbiological and biochemical activity of soil, and also on the studies by Wiater and Żebranowicz (2005) on its fertilizer value, it should be concluded that sludge-organic granulate can be an alternative to and possibly a better fertilizer than NPK.

CONCLUSIONS

1. In the first year of the experiment, the applied granulate doses caused stimulated almost all of the bacterial and fungal groups, and the effects persisted in the second year, although it was weaker. Initially, a slight inhibition affected only cellulolytic bacteria, but with time it changed into distinct stimulation.

2. Application of the experimental doses of the granulate resulted in an increase of respiration and acid phosphatase activity in the 1st year. This effect continued also in the 2nd year, but on a lower level. Throughout the experimental period, the doses of the granulate most frequently caused a slight decrease in the rate of dehydrogenases, lipase and protease activity.

3. In the 1st year, no response of most of the bacterial groups to mineral fertilization was observed. In the 2nd year, there was even a slight tendency towards their inhibited growth was noted (except for lipolytic bacteria). However, the mineral fertilization had a favourable effect on the growth of most of the fungal groups during the two-year period.

4. In the 1st year, mineral fertilization (NPK) stimulated respiration, cellulose mineralization and, to some degree, activity of dehydrogenases and acid phosphatase in soil. With time, this effect disappeared, remaining only in the case of cellulose mineralization, but on a notably lower level than in the preceding year. NPK fertilization of soil led to a decrease in the activity of protease and lipase, which lasted throughout the whole experiment. The decline was most pronounced in the activity of protease, especially in the second year.

5. The observations during the two years of the experiment proved that fertilization of soil under white mustard with granulated organic-mineral fertilizer had a more beneficial effect on the growth of the analyzed bacterial groups, and on the respiratory and phosphatase activity of the soil, than mineral NPK fertilization. In turn, mineral fertilisation was more favourable for the growth of the fungal groups, which is an adverse development from the viewpoint of soil quality. Both types of fertilizers had a negative effect on lipase and protease activity in soil, but that effect was generally more pronounced in soil fertilized with NPK.

6. The results of biological analyses indicate that sludge granulate has a better effect on most of the soil biological parameters than mineral fertilization. Thus, the tested sludge granulate can be a valuable fertiliser in agricultural practice.

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BIOACCUMULATION OF SOME ELEMENTS IN THE MILLIPEDE *GLOMERIS HEXASTICHA* (BRANDT, 1833) (DIPLOPODA, GLOMERIDA)

Grzegorz Kania[1], Jerzy Lechowski[2]

[1]Department of Biology and Parasitology
Medical University of Lublin
[2]Department of Swine Breeding and Protection
University of Life Sciences in Lublin

Abstract

Millipedes are soil invertebrates participating in the decomposition of leaf litter. Previous studies indicated that soil invertebrates were useful in the environmental pollution research dedicated to the content and accumulation of heavy metals. The aim of the present study was to compare the content of some elements in bodies of adult millipedes *Glomeris hexasticha* (Brandt, 1833) collected from a more polluted town called Jaworzno in Upper Silesia, Southern Poland, and from the less polluted Lublin area, Eastern Poland. The content of elements was determined with the ICP method on a VISTA MPX Varian spectrometer. The concentrations of elements in the millipedes from Jaworzno appeared in the following decreasing order: Ca, P, S, Mg, K, Na, Fe, Al, Zn, Cu, Mn, Pb, B, Cr, Cd and Ni, compared to the Lublin area: Ca, P, K, Mg, S, Na, Fe, Al, Cu, Zn, Mn, Pb, B, Cr, Cd and Ni. It was confirmed that the elements such as Al, Zn, Mn, Cr, Cd and Ni in *Glomeris hexasticha* from Jaworzno were in higher concentrations than in specimens from the Lublin area. Reveresly, millipedes collected from the Lublin area contained higher concentrations of Ca, P, K, Mg, Na, Fe, Cu and B than those from Jaworzno. The results show that differences in the content and distribution of some elements at two locations with different levels of environmental pollution were clearly reflected in bodies of the millipede *G. hexasticha*.

Keywords: elements, contamination, millipedes, *Glomeris hexasticha*.

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dr Grzegorz Kania, Department of Biology and Parasitology, Medical University, Radziwiłłowska 11, 20-080 Lublin, Poland, e-mail: grzegorz.kania@umlub.pl

BIOAKUMULACJA NIEKTÓRYCH PIERWIASTKÓW U KROCIONOGA *GLOMERIS HEXASTICHA* (BRANDT,1833) (DIPLOPODA, GLOMERIDA)

Abstrakt

Krocionogi są bezkręgowcami glebowymi, biorącymi udział w dekompozycji ściółki liściowej. Wcześniejsze badania wykazały, że zawartość pierwiastków i akumulacja metali ciężkich u bezkręgowców glebowych stanowią użyteczne dane w badaniach nad zanieczyszczeniem środowiska. Celem pracy było porównanie zawartości niektórych pierwiastków u dorosłych osobników krocionogów *Glomeris hexasticha* (Brandt, 1833) zbieranych z obszaru bardziej zanieczyszczonego Jaworzna na Górnym Śląsku i z mniej zanieczyszczonego obszaru Lublina we wschodniej Polsce. Zawartość pierwiastków oznaczano metodą ICP na spektrofotometrze VISTA MPX VARIAN. U krocionogów z Jaworzna zawartość oznaczonych pierwiastków w kolejności stężeń od najwyższego do najniższego była następująca: Ca, P, S, Mg, K, Na, Fe, Al, Zn, Cu, Mn, Pb, B, Cr, Cd i Ni , a z Lublina: Ca, P, K, Mg, S, Na, Fe, Al, Cu, Zn, Mn, Pb, B, Cr, Cd i Ni. Takie pierwiastki, jak Al, Zn, Mn, Cr, Cd i Ni stwierdzono w większym stężeniu u *Glomeris hexasticha* z Jaworzna niż u osobników z Lublina. Natomiast u krocionogów zebranych z Lublina wykazano większe stężenie następujących pierwiastków: Ca, P, K, Mg, Na, Fe, Cu i B, w porównaniu ze stężeniem u krocionogów z Jaworzna. Wyniki wskazują, że różnice w rozkładzie zawartości niektórych pierwiastków w dwu miejscach Polski o różnym poziomie zanieczyszczenia środowiska, były wyraźnie odzwierciedlone u krocionogów *Glomeris hexasticha.*

Słowa kluczowe: pierwiastki, zanieczyszczenie, krocionogi, *Glomeris hexasticha.*

INTRODUCTION

Soil dwelling arthropods are distinguished by a great diversity of species. Millipedes are soil invertebrates which participate in the decomposition of leaf litter. Leaf litter decay is significantly reduced by heavy metal pollution (Mcenroe, Helmisaari 2001). Heavy metals have been found to cause reductions in the abundance of soil decomposers and in the diversity of animal communities (Haimi, Siira-Pietikainen 1996, Grelle et al. 2000). Environmental pollution field studies have been conducted on terrestrial isopods, centipedes, millipedes, earthworms, insects and snails (Czarnowska 1980, Hopkin et al. 1985, Dallinger et al. 1992, Dallinger 1994, Paoletti et al. 1991, Grelle et al. 2000, Hobbelen et al. 2004, Nakamura et al. 2005, Warchałowska-Śliwa et al. 2005, Kowalczyk-Pecka 2009, Kania 2010). It has been stated that heavy metal deposition in millipedes depends on environmental pollution (Hopkin et al. 1985, Kőhler et al. 1995, Nakamura, Taira 2005). *Glomeris hexasticha* is the south-eastern and central European millipede species of soil macrofauna. Specimens of *Glomeris hexasticha* (Brandt,1833) were collected from a more polluted town called Jaworzno, which lies in Upper Silesia (southern Poland), an area in the state of ecological emergency (Pawłowski 1990, www.zazi.iung.pulawy.pl/Images/metale.jpg) and from the less polluted Lublin area, in the eastern part of Poland (Dechnik et al. 1987, Kozak, Kozak 1987). Soils around Lublin do not indicate excessive concentrations of heavy metals, with their levels similar to natural ones (Bełz

2003, Lipiński 2003). The aim of the present study was to compare concentrations of some elements in bodies of adult millipedes *Glomeris hexasticha*, from two locations: Jaworzno and Lublin in Poland.

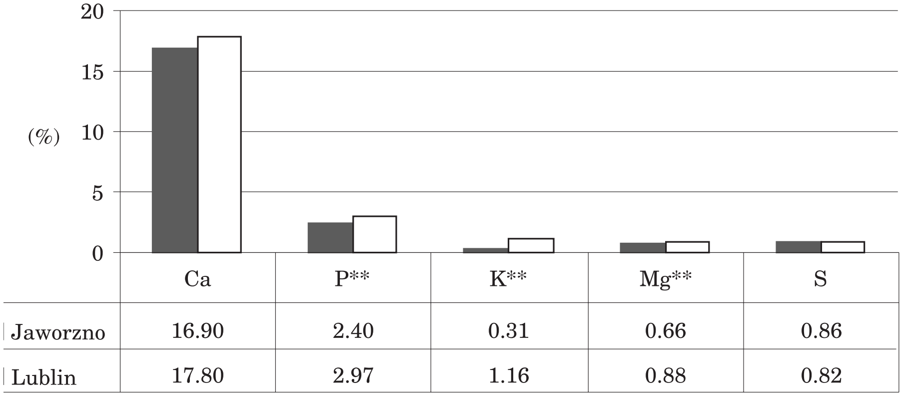
MATERIAL AND METHODS

Soils in Jaworzno are moderately, heavily and very heavily polluted. Soils with the content of Cd, Cu, Pb above the upper threshold and with a very high concentration of Zn have been found in there (Pawłowski 1990). It has been reported that Cd, Cu, Pb and Zn in soils in the south-eastern part of Jaworzno are up to 25.7 mg kg^{-1}, 119 mg kg^{-1}, 403 mg kg^{-1} and 1920 mg kg $^{-1}$, respectively [www.bip.jaworzno.pl]. The soil pH in the northern part of Jaworzno exceeds 8 but falls below 7 in southern areas of the town. Acid soils are mainly south of the town [www.bip.jaworzno.pl]. Soils in the Lublin area are characterized by very good properties, for example 92% of soils have a natural content of heavy metals (Bełz 2003, Lipiński 2003). The content of heavy metals in soils in allotment gardens in Lublin demonstrate their slight contamination (Bożek, Królik 2009). The soil pH (7.1-7.8) in Lublin is neutral and alkaline (Sobocińska 2003). The millipedes *Glomeris hexasticha* were collected from an ecotone of a pine forest (*Pinus silvestris*) and sandy grassland in the south-eastern part of Jaworzno (Upper Silesia), as well as from a mixed forest with pine and oak in Zemborzyce, south of in Lublin, Poland. The surface area for sampling was 500 m^2 at each location. In May and June 2006, millipedes were captured manually, three times and in three replicates, at ten-day intervals, both in Jaworzno and Lublin. In total, 18 samples, 9 from each region, were studied. In 2008, the content of the elements Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S and Zu was determined in the bodies of *Glomeris hexasticha*. In a laboratory, the millipedes were kept in glass vessels and fed with plant litter. For sample preparation, adult millipedes were kept for two days in Petri dishes on wet Whatman medium to defecate. Next, the specimens were placed in a sterile container and washed with distilled sterile water for 1 minute. The procedure was repeated four times. Then, the millipedes were euthanized by immersing in ether, after which they were dried to dry mass by keeping in an airy place at room temperature for a few days. Finally, they were preserved until analyses. Prior to analyses, the samples were dried to constant mass in a dryer set at 30°C for 72 hours, according to the method described in Falandysz et al. (2007). Samples of dry material (0.25 g-0.5 g) were added 10 ml concentrated HNO_3 and 1 ml of $H_2O_{2,}$ and mineralized in a MARS 5 microwave, according to instruction. Concentrations of the elements were determined with the ICP method on a VISTA MPX Varian spectrometer. They were measured in solutions and then recalculated to dry weight, as a percentage (%) of dry weight for Ca, P, K, Mg and S. However, Na, Fe, Al, Zn, Cu, Mn, Pb, B, Cr, Cd and Ni were given in mg kg $^{-1}$ dry weight. At the sites where millipedes were collected, measurements of soil pH were taken with a Takemura Soil Tester DM-15. Statistical differences in elemental concentrations found in bodies of millipedes from the two locations and in soil pH were checked by Student's *t* test.

RESULTS AND DISCUSSION

The content of the elements such as Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S and Zn was determined in whole bodies of the millipede *Glomeris hexasticha*, collected from Jaworzno and from the Lublin area. In the samples from Jaworzno the elements appeared in the following decreasing order: Ca, P, S, Mg, K, Na, Fe, Al, Zn, Cu, Mn, Pb, B, Cr, Cd, Ni; in millipedes from the Lublin area, the decreasing order was Ca, P, K, Mg, S, Na, Fe, Al, Cu, Zn, Mn, Pb, B, Cr, Cd, Ni. In all the specimens, the content of Ca was the highest, on average 16.9% (from Jaworzno) and 17.8% (from Lublin), respectively (Figure 1). Millipedes from Lublin had higher concentrations of the following elements: Ca, P, K, Mg, Na, Fe, Cu and B, than those from Jaworzno. *Glomeris hexasticha* from Upper Silesia showed higher quantities of Al, Zn, Mn, Cr, Cd and Ni, than individuals of the same species collected from the Lublin area. The differences were statistically significant in the case of P, K, Mg and Zn, Fe, Al, Cu (Student's *t* test) – Figures 1, 2.

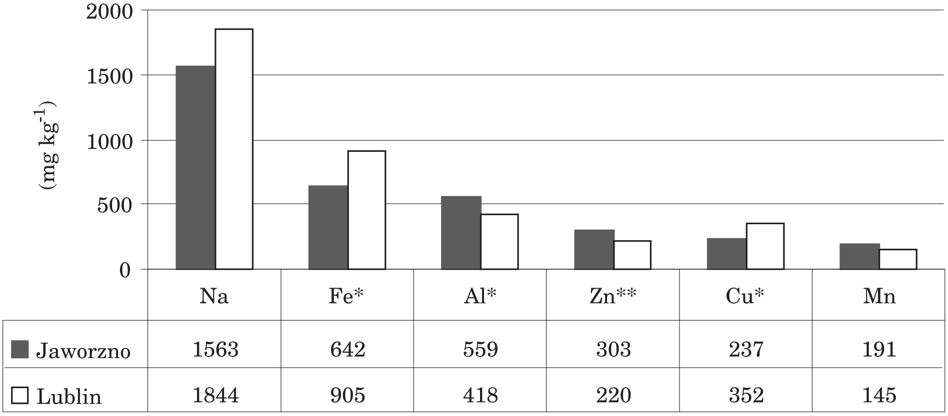
In particular, Cu was found in a remarkably high concentration in *G. hexasticha*. The Cu content was 352 mg kg $^{-1}$ dry weight in millipedes from the Lublin area and 237 mg kg^{-1}dry weight in specimens from Jaworzno.



	Ca	P**	K**	Mg**	S
Jaworzno	16.90	2.40	0.31	0.66	0.86
Lublin	17.80	2.97	1.16	0.88	0.82

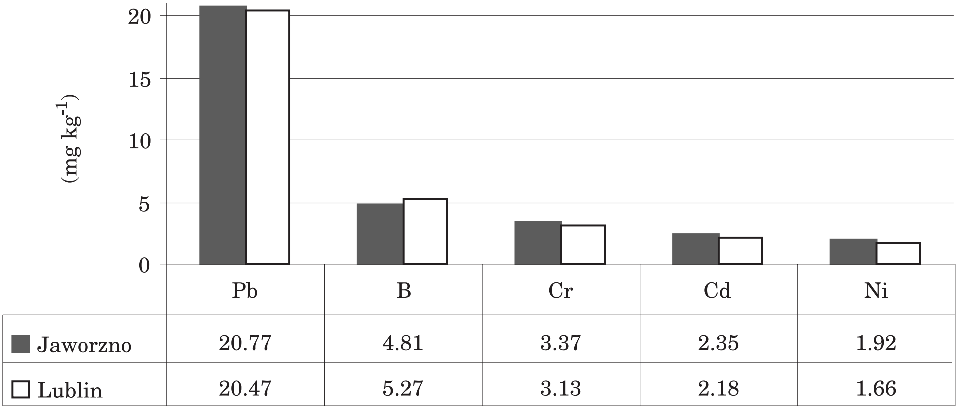
** differences statistically significant $p \leq 0.01$

Fig. 1. Content of elements (% dry weight) in *Glomeris hexasticha*



	Na	Fe*	Al*	Zn**	Cu*	Mn
■ Jaworzno	1563	642	559	303	237	191
☐ Lublin	1844	905	418	220	352	145

* differences statistically significant $p \leq 0.05$ ** differences statistically significant $p \leq 0.01$
Fig. 2. Content of elements (mg kg $^{-1}$ dry weight) in *Glomeris hexasticha*



	Pb	B	Cr	Cd	Ni
■ Jaworzno	20.77	4.81	3.37	2.35	1.92
☐ Lublin	20.47	5.27	3.13	2.18	1.66

differences are not statistically significant
Fig. 3. Content of elements (mg kg $^{-1}$ dry weight) in *Glomeris hexasticha*

Concentrations of hazardous metals determined in all the examined specimens of *G. hexasticha* were in the order of Cu> Pb> Cd (in mg kg^{-1} dry weight): 237 > 20.77 > 2.35 from Jaworzno and 352 > 20.47 > 2.18 from Lublin, respectively (Figure 2, 3). Interestingly, the levels of S and Pb were almost the same in the millipedes from both sampling sites, while the potassium concentration in specimens from the Lublin area was 3.74-fold higher than in the millipedes from Jaworzno (Figures 1, 3). The soil from Jaworzno was acidic (pH = 6.28) and the soil from the Lublin area was neutral (pH = 6.91) – Table 1.

Some contaminated areas may have unusual communities of fauna, shaped by their varied resistance to pollutants (HOPKIN et al. 1985). Previous studies indicated that elemental content and accumulation of heavy metals

Table 1

Soil pH at two locations

Location	Soil pH		
	range	average	SD
Jaworzno	5.62 - 6.68	6.28	0.46
Lublin	6.85 - 6.94	6.91	0.04

Differences are not statistically significant

in invertebrates were useful for studies on environmental pollution caused by harmful products from industries (Read et al. 1998, Niijma 1998, Heikens et al. 2001, Warchałowska-Śliwa et al. 2005). Excessive amounts of various chemicals interfere with complex processes occurring in soils and may lead to the degradation of soils, primarily because of two factors: a change in soil acidity or alkalinity and accumulation of trace elements, particularly metals (Pawłowski 1990). The soil properties such as reaction and elemental concentrations modify assimilation of some elements by soil organisms. According to Adrianek and Skowronek (2005), acidic soils prevail in Upper Silesia, the fact which is confirmed by our results on soil pH dterminations in Jaworzno; the mean pH was 6.28, within the range 5.62-6.68 (Table 1). South of Lublin, in Zemborzyce, the mean soil pH was 6.91, within the range 6.85-6.94 (Table 1), which supports previous data on soil pH in the Lublin area (Sobocińska 2003). In acidic soils, saturation with soil exchange ions with loss of Ca^{2+} and Mg^{2+} is lower, and potentially toxic metals Al^{3+} and Mn^{2+} travel to the soil solution (Siuta 1987, Turski 1987, Adrianek, Skowronek 2005).

Acidification of soils activates considerable amounts of Al, whose excess affects the plant growth and development as well as the human health (Michałek 1997). Al^{3+} ions, which appear in the soil solution at pH = 5.5-7.0, are accessible to plants. Absorbed by plants, Al^{3+} ions enter the trophic chain, showing links with with soil fauna. This explains why the specimens of *G. hexasticha* from Jaworzno showed a higher concentration of aluminum than millipedes from the Lublin area (Figure 2). Acidic soils of Upper Silesia are poor in absorbable forms of Ca, Mg, P and K, which translates into the inferior availability of elements to plants and soil fauna (Adrianek, Skowronek 2005). In contrast, medium and high concentrations of P, K, Mg and B were found in soils of the Lublin area (Dechnik et al. 1987), which manifested themselves in bodies of *Glomeris hexasticha*. Particularly, 3.74-fold more potassium was found in *G. hexasticha* individuals from Lublin than in the ones from Jaworzno (Figure 1). Such results indicated lesser environmental contamination of the Lublin area, because excessive concentration of potassium were indicated in plants from areas which are not industrially polluted (Siuta 1987). Millipedes *G. hexasticha* and *O. sabulosus* from Lublin (Kania 2010) contained more Fe than those from Jaworzno (Figure 2). High levels of Fe content are characteristic for most soils in the Lublin area (Kabata-Pendias 1981). This is confirmed by Kowalczyk-Pecka (2009), who showed high concentrations of Fe in samples of soil and in snail tissues from

Lublin. The lower Fe content in millipedes from Jaworzno may have resulted from the Fe metabolism being blocked by heavy metals (Adrianek, Skowronek 2005), due to the competition between heavy metals and iron for the same places in chelates. Heavy metals in soils of Upper Silesia appear in quantities above the norm (Pawłowski 1990, www.zazi.iung.pulawy.pl/Images/metale.jpg). The most severely contaminated with heavy metals are acidic soils in the south-eastern part of Jaworzno (www.bip.Jaworzno.pl), which is where the millipedes for our analyses were collected. The acid reaction of soils increases assimilation of some elements by soil organisms (Siuta 1987). The differences in the distribution of elements, particularly toxic metals, between the two locations with different soil pH were distincly manifested by differences in the elemental composition of millipedes. The elements such as Al, Zn, Mn, Cr, Cd and Ni in *G. hexasticha* from Jaworzno were in higher concentrations than in *G. hexasticha* from Lublin (Figres 2, 3).

The content of Zn in *G. hexasticha* reflected the environmental state of pollution, with an abnormally high concentration of Zn in the soils lying in thesouth-eastern part of Jaworzno (www.bip.Jaworzno.pl). The higher Zn content in *G. hexasticha* from Upper Silesia than from Lublin coincided with a higher Zn concentration in *Tetrix tenuicornis* (*Orthoptera*, Insecta) collected from Upper Silesia (Warchałowska-Śliwa et al. 2005) and with high Zn quantities in *Glomeris marginata* from a contaminated grassland in England (Hopkin et al. 1985). Differences in the Zn concentration between environmental habitats were also reflected in bodies of the millipede *Oxidus gracilis* (Nakamura et al. 2005). In contrast, Heikens et al. (2001) reported that Zn remained on a constant level in invertebrates.

Copper is an essential metal for invertebrates and, to a certain degree, can be regulated by several species, so that its concentration in animal bodies is rather constant over a range of soil concentrations (Heikens et al. 2001). High Cu concentrations have been indicated in millipedes (Hopkin et al. 1985, Hunter et al. 1987, Heikens et al. 2001, Nakamura et al. 2005, Kania 2010) which was confirmed by the present study (Figure 2). The higher content of Cu in the millipedes from Lublin than that from Jaworzno reflected its medium content in soils of Lublin (Sobocińska 2003), where the range of Cu was 1-191 mg kg^{-1} dry weight (Wiatr et al. 1997), compared to 1-119 mg kg^{-1} dry weight in soils of Jaworzno (www.bip.Jaworzno.pl). In *G. hexasticha* specimens from Lublin, the concentration of Cu was 1.8-fold higher than in soil, and in the millipede individuals from Jaworzno, the concentration of Cu was double the one in the soil. However, the concentration of Cu in the millipede *Chamberlinius hualienensis* was 4-11-fold higher than in soil (Nakamura et al. 2005).

Millipedes have been shown to accumulate cadmium (Hopkin et al. 1985, Hunter et al. 1987), which is comparable to the Cd content in *G. hexasticha* reported herein and in *O. sabulosus* (Kania 2010). Cadmium is one of the heavy metals which is potentially toxic at low levels (Kabata-Pendias 1981).

According to HOPKIN et al. (1985), the net assimilation of lead in millipedes during the passage through the gut is very small, for example the pill millipede *Glomeris marginata* did not assimilate significant amounts of lead even when it foraged on badly polluted sites. Therefore, the content of Pb in millipedes from both locations examined herein is nearly identical (Figure 3). However, according to POBOZSNY (1985), lead concentrations in millipedes differs from species to species. There is strong evidence that many soil animal species have adapted to heavy metals.

Different metal accumulation strategies of soil invertebrates are a consequence of vdiverse detoxification mechanisms (GRAFF et al. 1997). Diplopods possess effective mechanisms to bind and detoxify potentially toxic metals in tissues (KŐHLER et al. 1995). The detoxification mechanism of metals in millipedes may explain the absence of significant differences between concentrations of heavy metals in *G. hexasticha* collected from more (Upper Silesia) and less contaminated (Lublin area) locations in Poland.

The concentrations of hazardous heavy metal in millipedes such as *Brachyiulus pusillus, Cylindroiulus latestriatus, C. brittanicus, Brachydesmus superus, Julus scandinavius,, Polydesmus denticulatus, Oxidus gracilis* and *Ommatoiulus sabulosus* (HOBBELEN et al. 2004, NAKAMURA, TAIRA 2005, KANIA 2010) were in the order of Cu>Pb>Cd, which agrees with the results obtained in the current study on *Glomeris hexasticha.*

CONCLUSIONS

1. Because of the local environmental pollution, samples from Jaworzno had a higher content of toxic metals such as Al, Zn, Mn, Cr, Cd, Ni than the ones from Lublin.

2. Concentrations of the following elements: Ca, P, K, Mg, Na, Fe, Cu, and B in bodies of *G. hexasticha* reflected their concentrations in soils at the two locations.

3. The results show that differences in the distribution of some elements between the two locations, due to the differences in the degree of environmental pollution, were clearly reflected in *G. hexasticha.*

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DETERMINATION OF SPATIAL VARIABILITY OF SOME MAGNESIUM FORMS IN PHAEOZEM USING GEOSTATISTICAL METHODS*

Mirosław Kobierski[1], **Jacek Długosz**[1],
Anna Piotrowska-Długosz[2]

[1]Department of Soil Science and Soil Protection
[2]Department of Biochemistry
University of Technology and Life Sciences in Bydgoszcz

Abstract

The spatial variability analysis of soil properties facilitates the prediction of their contents across various sites, useful for design of site-specific farming practices. Intrapopulation and spatial variation of soil available (Mg-A), exchangeable (Mg-E) and water-soluble (Mg-H_2O) magnesium forms was evaluated in a field on Phaeozem soil, located in the village Orlinek near Mrocza, in the Province of Kujawy and Pomorze (*województwo kujawsko-pomorskie*), north-western Poland. Soil samples were collected from an area of 0.5 ha situated within an 80-hectare arable field. In April 2007, 50 soil samples were collected in a 10 x 10 m grid square pattern from the field cropped with winter wheat. The content of Mg forms was analyzed with descriptive statistics and the geostatistic modeling of semivariograms to plot variability maps. The Mg-A content ranged from 4.9 to 12.2 mmol kg^{-1}, while the Mg-E form varied from 5.6 to 16.4 mmol kg^{-1}. The average content of Mg-H_2O was 1.27 mmol kg^{-1}. All the properties revealed a normal content distribution, which coincided with similar values of means, medians and significantly lower values of standard deviations (SD) than the means. Moderate variability of all the Mg forms content was confirmed by the coefficient of variation (CV%), falling within 19.4-23.6%. The spatial dependence of the Mg forms content was evaluated by the use of semivariograms and krigged maps. The parameters of variogram models, except for the Mg-A content, revealed a share of random variance (a nugget) in total variability (sill). The content of Mg-E demonstrated high spatial dependence (the nugget effect <25%), while the content of Mg-H_2O and the percentage share of Mg-E in the sum of base cations (S) fell within the moderate class of spatial variability (the nugget effect between 25% and 75%). The spatial correlation of the properties studied was assessed by 11.1 to 21.3 m range. The spatial variation maps showed that the Mg-A and Mg-E contents had a similar distribution in the research area, while the Mg-H_2O content and the percentage of the Mg-E in (S) presented a different pattern of variability. The maps revealed that almost 80% of the field showed a very high Mg-A content (Class I abundance), which suggested that Mg fertilizer is unnecessary.

Key words: available Mg, exchangeable Mg, water-soluble Mg, spatial variability, geostatistics, Phaeozem.

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dr inż. Mirosław Kobierski, Department of Soil Science and Soil Protection, University of Technology and Life Sciences, Bernardyńska 6/8 St., 85-029 Bydgoszcz, Poland, e-mail: kobierski@utp.edu.pl

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ANALIZA ZMIENNOŚCI PRZESTRZERNNEJ WYBRANYCH FORM MAGNEZU W CZARNEJ ZIEMI Z WYKORZYSTANIEM METOD GEOSTATYSTYCZNYCH

Abstrakt

Analiza zmienności przestrzennej parametrów glebowych umożliwia oszacowanie ich zawartości między miejscami pobrania próbek glebowych, co jest podstawą stosowanego coraz częściej systemu rolnictwa precyzyjnego. Badano zmienność zarówno wewnątrzpopulacyjną, jak i przestrzenną dotyczącą zawartości Mg przyswajalnego (Mg-A), wymiennego (Mg-E) oraz wodnorozpuszczalnego (Mg-H_2O) w czarnej ziemi w okolicach wsi Orlinek k. Mroczy (województwo kujawsko-pomorskie). Próbki glebowe pobierano z obszaru 0,5 ha znajdującego się w obrębie 80-hektarowego pola produkcyjnego, na którym uprawiano pszenicę ozimą. W kwietniu 2007 r. pobrano 50 prób glebowych rozmieszczonych w siatce kwadratów o boku 10 m x 10 m. Zawartości badanych form magnezu analizowano z wykorzystaniem podstawowych statystyk opisowych oraz metod geostatystycznych, które posłużyły do wykreślenia map ich zmienności. Zawartość Mg-A wynosiła 4,9-12,2 mmol kg^{-1}, zawartość Mg-E od 5,6 do 16,4 mmol kg^{-1}, średnia zawartość Mg-H_2O 1,27 mmol kg^{-1}. Wszystkie badane parametry wykazywały normalny rozkład zawartości, co potwierdzone zostało przez zbliżone wartości średnich, median oraz istotnie niższe wartości odchylenia standardowego (SD), w porównaniu ze średnimi. Umiarkowaną zmienność zawartości badanych form magnezu potwierdzono za pomocą współczynnika zmienności (CV%), którego wartości mieściły się w granicach 19,4-23,6%. Zależność przestrzenną zawartości badanych form magnezu oszacowano za pomocą semivariogramów oraz wykreślonych na ich podstawie map rastrowych. Parametry modeli variogramów, z wyjątkiem zawartości Mg-A, wskazywały na udział zmienności losowej (samorodek) w kształtowaniu całkowitej zmienności tego parametru. Zawartość Mg-E wykazywała wysoką zależność przestrzenną (efekt samorodka <25%), natomiast zawartość Mg-H_2O oraz udział procentowy Mg-E w sumie zasadowych kationów (S) mieściły się w umiarkowanej klasie zmienności (efekt samorodka między 25% a 75%). Korelację przestrzenną badanych parametrów oznaczono z wykorzystaniem zakresu od 11,1 do 12,3 m. Mapy zmienności przestrzennej wykazały, że zawartości Mg-A i Mg-E charakteryzowały się podobnym do siebie rozmieszczeniem na powierzchni badanego obszaru, natomiast zawartość Mg-H_2O oraz udział procentowy Mg-E w (S) wykazywały odmienne rozmieszczenie w porównaniu z pozostałymi parametrami. Mapy rastrowe wykazały bardzo wysoką zawartość Mg-A (I klasa zasobności) na prawie 80% powierzchni badanego pola, co wskazywało, że nawożenie magnezem nie jest konieczne.

Słowa kluczowe: Mg przyswajalny, Mg wymienny, Mg wodnorozpuszczalny, zmienność przestrzenna, geostatystyka, czarna ziemia.

INTRODUCTION

Several primary and secondary minerals are original sources of the soluble and available forms of Mg in soil. Magnesium is taken up by plants in the form of cations (Mg^{2+}), which play a crucial role in photosynthesis as an essential element of chlorophyll molecules. Magnesium is also vital for animal and human metabolism (Grzebisz 2011), for example it is a constituent or activator of over 300 enzymes (Ebel, Gunter 1980, Cowan 2002). Magnesium also determines important metabolic reactions, such as the synthesis of proteins, nucleic acids. In addition, it participates in the transfer of energy within the plant. Available magnesium, together with calcium (Ca^{2+}), potassium (K^+), and sodium (Na^+), is a major cation related to the sum of base cations (S). Regarding plant nutrition, the most important forms of magnesium are exchangeable and water-soluble ones (Idriceanu et al. 1999). Both represent so-called available Mg form, which is used in agronomy to define the classes of magnesium abundance (PN-R-04020:1994/Az1).

Soil nutrients often vary significantly across a field, so that uniform fertilization may result in an over-application in some parts of the field and under-fertilization in others. Nutrient runoff and leaching from over-fertilized areas may contaminate groundwater, while in under-fertilized areas crop yield may be limited (Cahn et al. 1994). Magnesium deficiency in Polish soils as well as marked enrichment of groundwater with Mg have been recorded (Łabętowicz et al. 2004, Sapek 2007, 2008). Magnesium levels in soil decline over time due to crop removal, soil erosion and leaching (Kobierski et al. 2011, Kondratowicz-Maciejewska, Kobierski 2011). Low soil pH, and/or high levels of potassium (K^+) and calcium (Ca^{2+}), low temperatures and arid soil conditions can all contribute to Mg deficiency (Marschner 1995).

In order to control side-effects of inadequate fertilization and other mismanaged faming practice, spatial variability of soil nutrient concentrations needs to be identified for development of site-specific farming practice that will match agricultural input with the actual crop requirements. The spatial variability of soil properties is evaluated with the use of geostatistical analysis for detection, estimation and mapping spatial patterns of soil variables (Sebai et al. 2007). Geostatistics, originally applied in the mining industry (Matheron 1963), has been proven useful in soil science for defining and mapping the spatial variation of soil properties. The main techniques used in geostatistics are variography and krigging. Variography involves drawing semivariograms to define and model the spatial variability, whereas krigging uses the modeled variation to estimate values between sampling points (Burgess, Webster 1980). Finally, krigged values are used to develop maps for site-specific farming practices (Mulla 1989).

The objective of the study was to evaluate and compare the intrapopulation and spatial variability of different forms of magnesium using traditional statistics and geostatistical techniques.

MATERIAL AND METHODS

The spatial heterogeneity of soil magnesium forms was investigated on an 80-hectare arable field, located in the village Orlinek near Mrocza, (the province of Kujawy and Pomorze, *województwo kujawsko-pomorskie*; 53° 15’ 31” N, 17° 32’ 43” E). An area of 0.5 ha was selected for the research purposes. The soil was classified as Gleyic Phaeozem (IUSS Working Group WRB 2006). Winter wheat after winter oilseed rape was cultivated on the field. In total, 50 soil samples were collected in April 2007 from the topsoil (0-20 cm) using the point sampling method with a 10 x 10 m regular grid square pattern. The soil samples were air-dried and passed through a 2 mm mesh sieve.

The soil fractions <2 mm were analyzed to determine available Mg (Mg-A), after extraction in 0.0125 mol dm^{-3} $CaCl_{2,}$ according to the Schachtschabel method; exchangeable Mg (Mg-E) was determined in 0.1 M $BaCl_2$ (ISO 11260:1994), and water-soluble Mg (Mg-H_2O) was assayed after extraction in distilled water (soil /water – 1/5). The content of Mg^{2+} and Ca^{2+}, K^+ and Na^+ in extracts was determined by atomic absorption spectroscopy (AAS) on a Philips PU 9100X spectrometer. Basic physicochemical properties of soil were determined: particle-size by the Cassagrande method, modified by Prószyński; soil fraction >2 mm content applying the sieving method; pH in 1 M KCl – potentiometrically (PN-ISO 10390:1997); the organic carbon content – in a dry combustion CN analyzer (Vario Max CN). The assays were verified with the TILL-3 certificate. Based on the exchangeable cation content (Ca^{2+}, Mg^{2+}, K^+, Na^+), the sum of base cations (S) was calculated.

The data were evaluated with the use of traditional statistical methods (Statistica v. 10.0 Software). In order to characterize the differentiation in a whole population of data sets, arithmetic and geometric means, standard deviation, the coefficient of variation and skewness and kurtosis were calculated. For estimation and visualization of the spatial variability of Mg forms in the surface horizon of the field, geostatistical methods were used. Empirical and the best fitted model variograms were plotted as well as the sill, nugget, nugget effect and the range of the influence were calculated. The mean squared deviation ratio (MSDR) was used to verify the agreement of model variogram with the empirical variogram. The data were estimated with the punctual krigging method. To express the level of spatial dependence, the relative nugget effect $[Co/(Co+C)]\cdot 100$ was used, as described in CAMBARDELLA and KARLEN (1999). Raster maps showing the spatial variability of the magnesium forms were plotted based on the semivariograms. All geostatistical analyses were processed using the software Isatis of Geovariance.

RESULTS AND DISCUSSION

The texture of topsoil was represented by 21 sandy loam samples; 28 fine sandy loam and a single medium sandy loam sample. While identifying the agronomic category, it was noticed that the vast majority of samples were in the average category of soils and only 7 represented light soil. The Ap horizon grain size composition showed 57% to 77% of sand fraction, 11% to 23% of silt fraction and 7% to 20% of clay fraction (Table 1). Most soil samples had neutral reaction, and only in some samples the reaction was

Table 1

Basic statistics of selected properties (n=50)

Parameters	Fraction percentage			pH	C_{org}	Ca^{2+}	K^+	Na^+	Hh	(S)	CEC
	sand	silt	clay	1M KCl	(g kg^{-1})	(mmol kg^{-1})					
Min.	57.0	11.0	7.0	6.48	13.1	137.8	2.5	0.6	3.0	150.1	156.1
Max.	77.0	23.0	20.0	7.19	25.1	350.1	7.4	1.7	10.5	368.2	371.9
Arithmetic mean	66.4	18.4	15.2	6.78	18.7	251.8	4.7	0.9	6.3	268.8	275.1
SD	4.77	2.91	2.92	0.18	2.83	53.57	1.17	0.26	2.20	55.0	54.7

SD – standard deviation, Hh – hydrolytic acidity, (S) – sum of base cations, CEC – cation exchange capacity

Table 2

Basic statistics of selected properties (n=50)

Parameters	Mg-A	Mg-E	Mg-H_2O	%Mg-E in (S)
	(mmol kg^{-1})			
Min.	4.88	5.60	0.82	2.80
Max.	12.2	16.40	1.96	7.00
Arithmetic mean	8.43	11.34	1.27	4.27
Median	8.44	11.70	1.25	4.35
SD	1.75	2.68	0.25	0.84
Kurtosis	-0.511	-0.300	0.326	0.808
Skewness	-0.135	-0.333	0.537	0.453
CV(%)	20.8	23.6	19.4	19.8

Mg-A – available magnesium; Mg-E – exchangeable magnesium;
Mg-H_2O – water soluble magnesium; SD – standard deviation
CV (%) – coefficient of variation; (S) – sum of base cations

slightly acid (pH <6.6). The organic carbon content ranged from 13.1 to 25.1 g kg^{-1}, with the average value of 18.7 g kg^{-1} (Table 1). The soil sorption complex was mostly saturated by alkaline cations.

The cation content varied in respective soil samples. The base cations were dominated by Ca^{2+} (137.8-350.1 mmol kg^{-1}), followed by Mg^{2+} (5.6-16.4 mmol kg^{-1}) and K^+ (2.5-7.4 mmol kg^{-1}) and Na^+ (0.6-1.7 mmol kg^{-1}). The share of magnesium cations in the cation exchange capacity (CEC) ranged from 2.67% to 6.66%. The hydrolytic acidity ranged from 3.0 to 10.5 mmol kg^{-1}. The data sets of selected magnesium forms, derived from soil analysis, were initially evaluated using basic statistical parameters. The means and medians were used as primary estimates of the central tendency, and the standard deviation and CV were used as estimates of the population variability. The results summarized in Table 2 show the available Mg average from 4.88 to 12.2 mmol kg^{-1}. The average values of exchangeable Mg ranged from 5.61 to 16.4 mmol kg^{-1} and water-soluble Mg from 0.82 to 1.96 mmol kg^{-1}. The properties showed similar values of means and medians but a significantly lower SD than the means, which is an important condition for normal frequency distribution (MULLA, MCBRATNEY 2000). Additionally, all the properties demonstrated a low degree of skewness: from –0.333 to 0.537 (Table 2), which suggests that the data distribution was approximately symmetric. Another measure of the data distribution shape is the kurtosis, which defines the height and sharpness of the peak relative to the rest of the data (BALANDA and MACGILLIVRAY 1988). Kurtosis values calculated for available and exchangeable Mg were –0.551 and –0.300, respectively, suggesting that those properties were slightly platykurtic, the peaks were just a bit shallower than the normal distribution peak. The water soluble Mg content and the percentage of Mg-E in the sum of cations (S) revealed the positive kurtosis below one, which indicated a slightly leptokurtic distribution. The exchangeable Mg content in the study of STUTTER et al. (2004) showed marked deviation from normality with consistent positive skewness (0.87-2.52) and kurtosis (0.30-9.18). According to these authors, the differences were related to the dynamics of organic material decomposition as a result of varying litter decomposition rate and vegetation types. The same Mg form achieved the values of skewness and kurtosis closer to zero in the study of BUSCAGLIA and VARCO (2003). One of the most frequent measures of spreading about the mean is the coefficient of variation – CV (MULLA, MCBRATNEY 2000). The data of magnesium forms showed moderate variability, which was confirmed by the coefficient of variation (CV) ranging from 19.4 to 23.6% (Table 2). Those moderate class values established for CVs (WILDING 1985) were consistent with the results by DAMPNEY et al. (1997), and ŠTÍPEK et al. (2004). A moderate and high variability range (CV = 18.2-43.7%) was reported for exchangeable Mg by BUSCAGLIA and VARCO (2003) and BREJDA et al. (2000). A lower CV (10%) value of the exchangeable Mg content was noted by YANAI et al. (2003) in an onion field for more than 20 years, and that of available Mg by CAVIGELLI et al. (2005) in a no-till corn field (15.2%). On the other hand, COBO et al. (2010) found that exchangeable Mg was the most variable chemical parameter, with the coefficients of variation ranging from 60 to 120%. According to MULLA and MCBRATNEY (2000), the properties such as soil pH, texture or porosity are the least variable, while those connected to water or solute transport are more variable.

The variability in statistical parameters of Mg forms across different literature reports, including the present one, was probably the result of the influence of climate, plants, soil type and depth, landscape features as well as the management history (e.g. crop rotation, fertilization, tillage) (BUSCAGLIA, VARCO 2003, STUTTER et al. 2004).

Since the normality of the data was the general observation, we did not transform values before geostatistical analyses. Geostatistical parameters are presented in Table 3 and Figures 1-4. Experimental semivariograms of available and exchangeable Mg content and the share of Mg-E in (S) were well-described by the Gaussian model while the semivariograms of water-soluble Mg data could only be described by a spherical model. All the semivariograms, except for the available Mg, revealed the percentage of

Table 3

Parameters of variogram models (n=50)

Parameters	Model	Sill (Co+C)	Nugget (Co)	Nugget effect Co/(Co+C)	Range	MSDR	Spatial depen-dence
		(mmol kg^{-1})^2		(%)	(m)		
Mg-A	G	3.16	-	-	21.3	1.0005	-
Mg-E	G, NE	5.97	0.37	6.2	21.3	0.9997	S
Mg-H_2O	Sf, NE, L	0.054	0.02	37.0	11.1	1.0002	M
%Mg-E in (S)	G, NE	0.75	0.19	25.3	18.0	1.0004	M

Mg-A – available magnesium; Mg-E – exchangeable magnesium;
Mg-H_2O – water soluble magnesium; (S) – sum of base cations
G – Gaussa, Sf – spherical, L – linear, NE – nugget effect
MSDR – mean squared deviation ratio, S – strong, M – moderate

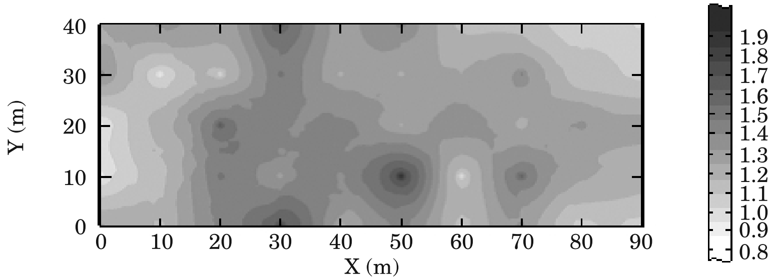

Fig. 1. Spatial variability of water-soluble Mg content (Mg-H_2O), mmol kg^{-1}

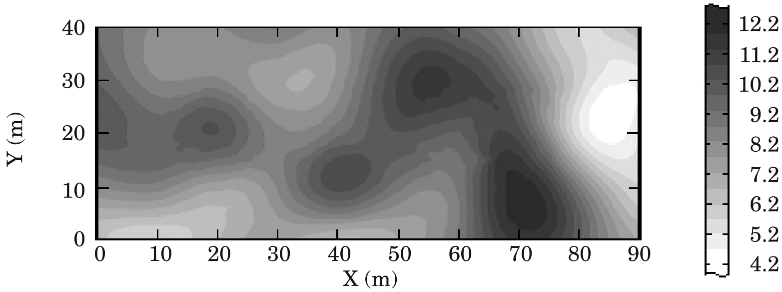

Fig. 2. Spatial variability of available Mg content (Mg-A), mmol kg^{-1}

random variability (nugget, Co) in total variability (sill, $Co+C$), which can be explained by sampling error, or random and inherent variability (SUN et al., 2003). To determine the grade of spatial dependence of each Mg form, the nugget-to-sill ratios from all semivariograms were calculated. As reported by CAMBARDELLA et al. (1994), HUANG et al. (2006), ROSSI et al. (2009), WANG et al. (2009), if the ratio is lower than 25%, the spatial variability is considered strong; if the ratio falls within 25 and 75%, the dependence is moderate; and if the ratio is higher than 75%, the variability is considered weak. A similar

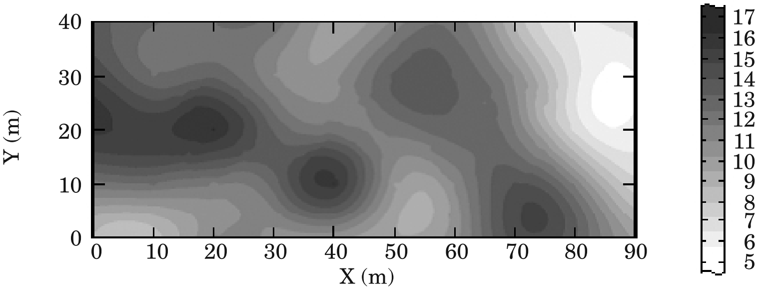

Fig. 3. Spatial variability of exchangeable Mg content (Mg-E), mmol kg^{-1}

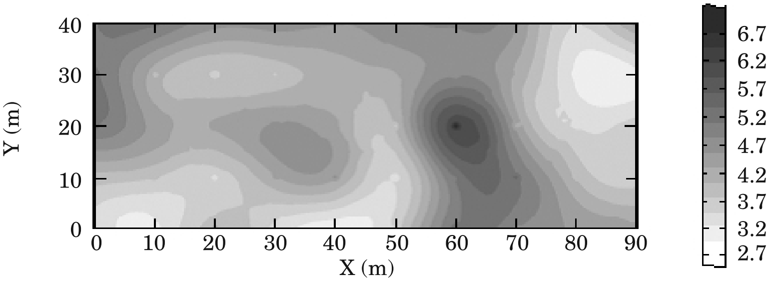

Fig. 4. Spatial variability of Mg-E share in sum of base cations (S), %

approach was used in this study. The exchangeable Mg content in the soil samples revealed strong variability (the nugget effect – 6.2%), while the water-soluble Mg content and the percentage share of Mg-E in (S) values were moderately variable (the nugget effect – 37.0% and 25.3%) – Table 3. The relative nugget effect in the total variance of available Mg (4-23%) reported by ŠTÍPEK et al. (2004), or moderate (32-70%) – by TABI and OGUNKUNLE (2007) did not emerge in our study, which revealed no share of random variability. The lack or a low share of the nugget effect in total variability of Mg forms suggest that the variables were spatially dependent and they were mainly influenced by internal factors, e.g. the soil type, texture, topography, while the external factors (e.g. fertilization, tillage) were less essential.

The range of influence is considered as the distance beyond which the observations are not spatially dependent (SUN et al. 2003) and it is a very important parameter for planning the sampling scheme. In our study, the distance ranged from 11.1 to 21.3 m (Table 2). These values were higher than the sampling distance (10 m), which proved that the sampling scheme was proper and all the variables were spatially correlated. Additionally, the sampling distance for Mg-A and Mg-E contents could be even longer if researched further. The range values for the same property assume different values in different studies, mainly due to the size of an area studied and sampling intervals. The range of available Mg was between 40 and 50 m when soil was sampled every 10 m (Tabi, Ogunkunle 2007), exchangeable Mg ranged 459-522 m when soil samples were collected in the 750 x 750 m grid (Cobo et al. 2010) and 322-660 m when the grid was established with regular intervals of 50 m with 129 sampling points (De Leão et al. 2011).

The water-soluble Mg content (Figure 1) was irregularly distributed in the soil surface horizon and did not show any similarities with the map representing Mg-A and Mg-E distribution, which suggested that additional factors may control the distribution of this form of magnesium. A comparison of the krigged maps of spatial distribution of Mg forms revealed that Mg-A and Mg-E showed comparable spatial variation (Figures 2, 3). Indeed, both maps showed four nests of higher results distributed irregularly, yet in the same way, among a lower data set. The long-term intensive use of Phaeozem must have resulted in the variation of respective parameters, especially the organic carbon content, which was significantly positively correlated with respective magnesium forms (Table 4). The content of clay fraction demonstrated a significant positive effect on the content of exchangeable and available magnesium forms (r = 0.680, and r = 0.554, $p<0.05$). The above relationship was additionally confirmed by a high coefficient of correlation (r = 0.845; $p<0.05$) between Mg-A and Mg-E contents (Table 4). The content of these magnesium forms was significantly positively correlated with the sum of base cations (S). The map showed that almost 80% of the area revealed a very high Mg-A abundance class, while 14% of the field – a high amount of this Mg form and only 6% fell within the moderate Mg-A abundance class (Figure 2). The water-soluble Mg data (Figure 1) was irregularly distributed in the soil surface horizon and did not show any similarities with the map representing Mg-A and Mg-E distribution, which suggests that additional factors may control the distribution of that form. The highest share of Mg-E in (S) was detected at 60-70 m of length and all across the width of the area (Figure 4). Relatively high values of this ratio were also recorded along the northern perimeter of the field (0-70 m of length) and along the western edge of the field (15-40 m of the width).

Table 4

Correlation coefficients significant at $p<0.05$ (n=50)

Parameters	Mg-A	Mg-E	Mg-H_2O
C_{org}	0.494	0.458	0.301
Clay fraction	0.554	0.680	
(S)	0.410	0.643	
Mg-E	0.845		

Mg-A – available magnesium; Mg-E – exchangeable magnesium;
Mg-H_2O – water soluble magnesium; (S) – sum of base cations

CONCLUSIONS

1. The results of soil magnesium forms in the experimental area showed moderate intrapopulation and spatial variability, which was confirmed by the values of the coefficients of variation and semivariogram parameters.

2. The spatial range values indicated that with the sampling interval established in the study (10 m) the sampling design was proper and all the Mg forms were spatially correlated. The sampling distance for Mg-A and Mg-E contents could be even longer and fewer samples would need to be collected.

3. The nugget-to-sill ratios calculated to determine the level of spatial dependence of each Mg form showed a strong and moderate share of random variance (nugget*, Co*) in total variability (sill, *Co+C*) of Mg-E and Mg-H_2O, while Mg-A was defined by the structural variance only. This suggests that structured variance was dominant over the nugget effect/random component. The results of nugget-to-sill ratios can facilitate the evaluation of the contribution of geologic and pedologic soil forming factors in the total variability, which can camouflage the influence of external factors, such as tillage or other management practices.

4. The spatial variability map of Mg-A displayed most of the analyzed area (almost 80%) having a very high available magnesium level, despite the fact that although no Mg fertilizer had been applied there over the last several years. This indicates high native soil Mg content, which means that application of mineral Mg fertilization is not recommended.

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CONTENT OF SELECTED TRACE ELEMENTS AND EXCHANGEABLE CATIONS IN SOILS OF THE BARYCZ RIVER VALLEY*

Beata Łabaz, Adam Bogacz

Institute of Soil Science and Environmental Protection
Wroclaw University of Environmental and Life Sciences, Poland

Abstract

In many ways, the Barycz Valley is a unique region. For one thing, it contains the highest number of fish ponds in Poland. Secondly, it is free from heavy industry and large cities. Thus, this region may be considered considerably clean, and the observed concentrations of trace elements are typical for non-degraded environments. There is, however, a threat of excessive accumulation of trace elements such as Zn, Pb and Cu in waters of the rivers in the Barycz drainage basin, received from point sources, and especially supplied with wastewater. These rivers play a very important role; namely, they are the source of water for filling up numerous ponds (Marek 1989). On the other hand, any seepage of river waters into the groundwater may cause excessive concentrations of trace elements in soils near the ponds.

The objective of the study was to analyse and assess sorption properties of sandy soils used as hay meadows. As the study was conducted in a special bird protection area (SBP) included in the European Ecological Network Nature 2000, special attention was paid to the content of selected trace elements. The following analyses were performed on the collected soil samples: particle size distribution, pH in 1 mol KCl dm^{-3}, TOC, N total, content of exchangeable base cations (Ca^{+2}, Mg^{+2}, K^{+}, Na^{+}) and total content of the metals Fe, Mn, Zn, Pb and Cu.

The genesis of the examined soils seems closely linked to the very wet moistened local environment, which significantly influenced the physical and physicochemical soil parameters. These soils were formed in a region crisscrossed by a dense network of watercourses of the Barycz River drainage basin, with strong ominant aquaculture traditions. The sum of base exchange cations (S) and cation exchange capacity (CEC) in the examined soils are strongly affected by the particle size distribution, the fact confirmed by significant positive relationships with the silt fraction and colloidal fraction, and significant negative correlations with the sand fraction. Higher content of Zn, Pb and Cu in surface genetic horizons of the examined soil profiles may point to the anthropogenic origin of the metals. Despite the dominant sandy texture, the soils are characterised by a thick humus horizon, abundant in TOC and TN, which increases their capacity for trace element accumulation. Our assessment of the contamination

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dr inż. Beata Łabaz, Institute of Soil Science and Environmental Protection, Wroclaw University of Environmental and Life Sciences, 50-357 Wrocław, Grunwaldzka 53, e-mail: beata.labaz@up.wroc.p

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of these soils with Zn, Pb and Cu did not demonstrate any excess in terms of the permissible levels of the trace elements.

Keywords: soil physicochemical properties, soil chemical properties, concentration of trace elements in soils.

ZAWARTOŚĆ WYBRANYCH METALI CIĘŻKICH ORAZ WYMIENNYCH KATIONÓW ZASADOWYCH W GLEBACH POŁOŻONYCH W DOLINIE BARYCZY

Abstrakt

Dolina Baryczy stanowi pod wieloma względami region unikatowy. Występuje tam największe w Polsce skupisko stawów rybnych i brak jest większego przemysłu oraz dużych ośrodków miejskich. Region ten można więc uznać za mało zanieczyszczony, a stwierdzone stężenia pierwiastków śladowych typowe dla środowiska niezdegradowanego. Istnieje jednak niebezpieczeństwo nadmiernego nagromadzenia się pierwiastków śladowych, takich jak Zn, Pb i Cu, w wodach rzek zlewni Baryczy wnoszonych ze źródeł punktowych, szczególnie wraz ze ściekami. Rzeki te pełnią bardzo ważną funkcję, są źródłem wody dla licznych stawów w okresie ich napełniania (Marek 1989), a ich przesiąki dostają się do wód gruntowych i mogą powodować nadmierną koncentrację pierwiastków śladowych również w sąsiadujących ze stawami glebach.

Przedmiotem badań była analiza i ocena właściwości sorpcyjnych piaszczystych gleb czarnoziemnych użytkowanych jako łąki kośne. Ponieważ badania prowadzono na obszarze specjalnej ochrony ptaków (OSO) objętym Europejską Siecią Ekologiczną Natura 2000, zwrócono szczególną uwagę na zawartość wybranych pierwiastków śladowych. Badania wykonano w 6 profilach glebowych usytuowanych na terenie Doliny Baryczy i jednocześnie na obszarze specjalnej ochrony ptaków (OSO). Po opisaniu ich morfologii, z poziomów genetycznych pobrano próbki glebowe, w których wykonano następujące oznaczenia: skład granulometryczny, C-organiczny, pH w H_2O oraz 1mol KCl, zawartość $CaCO_3$, kwasowość wymienną (Hw), zawartość N ogółem, wymienne kationy zasadowe (Ca^{+2}, Mg^{+2}, K^+, Na^+) oraz całkowitą zawartość metali: Fe, Mn, Zn, Pb i Cu.

Genezę powstawania badanych gleb należy wiązać ze środowiskiem silnie uwilgotnionym, które w istotny sposób wpłynęło na ich parametry fizyczne i fizykochemiczne. Gleby te wykształciły się bowiem na obszarach zdominowanych przez hodowlane stawy rybne oraz pokrytych gęstą siecią cieków wodnych zlewni rzeki Baryczy. Zawartość kationów wymiennych zasadowych (S) oraz pojemność kompleksu sorpcyjnego (CEC) w badanych glebach jest ściśle związana z udziałem poszczególnych frakcji granulometrycznych, czego potwierdzeniem są istotne dodatnie zależności z frakcją pyłu i frakcją koloidalną oraz istotne ujemne zależności z frakcją piasku. Większa zawartość Zn, Pb i Cu w wierzchnich poziomach genetycznych analizowanych profilów glebowych może wskazywać na ich antropogeniczne pochodzenie. Badane gleby, mimo dominującego piaszczystego uziarnienia, charakteryzują się dużą miąższością poziomu próchnicznego zasobnego w C-org. i N-og., co zwiększa ich możliwości do akumulowania pierwiastków śladowych. Ocena stopnia zanieczyszczenia analizowanych gleb pod względem zawartości Zn, Pb i Cu nie wskazuje na przekroczenie w nich dopuszczalnych zawartości wymienionych pierwiastków śladowych.

Słowa kluczowe: właściwości fizykochemiczne gleb, właściwości chemiczne gleb, zawartość pierwiastków śladowych w glebach.

INTRODUCTION

Under natural conditions, the amount of trace elements observed in soils is mainly determined by their abundance in the bedrock, intensity of weathering processes and the course of soil formation processes (Kabata-Pendias 1981, Czarnowska, Gworek 1987, Czarnowska 1996, Gworek, Jeske 1996). Analysis of soluble forms of trace elements in soil allows one to foresee threats to the environment due to the incorporation of such elements into biotic elements of the food chain and their migration to groundwater and surface waters (Chojnicki, Kowalska 2009). Abundance of nutrients, in turn, is one of the factors which determine soil fertility. The amount and distribution of nutrients in soil profiles are mostly influenced by the type of bedrock, soil texture and the course of soil formation processes.

A distinguishing feature of the natural environment in the Barycz Valley, resulting from human activity, is the presence of numerous breeding ponds, which were first created in that area in the early 13th century. The development of aquaculture was encouraged by the shallow declines of the River Barycz and its tributaries, the flatness of the area and the presence of numerous earth pits after bog iron exploitation. The early 19th century witnessed expansion of the embankments along the Barycz River, increasing water shortages and a growing demand for arable soils, which all led to a considerable drainage of water from the whole area. Many large fish ponds were liquidated and much of the area was transformed into forests, meadows and arable fields (Ranoszek 1999). Human interference with the natural environment of the Barycz Valley evidently affected the formation of soil types in the region. Next to the sandy sandur hills with overlying podzols, there are land depressions where sandy chernozem soils with very thick humus horizons dominate. In the past, they were the bottoms of fish breeding ponds, and currently they are directly adjacent to ponds and therefore constantly exposed to pond waters. In many respects, the Barycz Valley is a unique region. For one thing, it contains the highest number of fish ponds in Poland. Secondly, it is free from heavy industry and large cities. Thus, this region may be considered considerably clean, and the observed concentrations of trace elements are typical for non-degraded environments. There is, however, a threat of excessive accumulation of trace elements such as Zn, Pb and Cu in waters of the rivers in the Barycz drainage basin, received from point sources, and especially supplied with wastewater. These rivers play a very important role; namely, they are the source of water for filling up numerous ponds (Marek 1989). On the other hand, any seepage of river waters into the groundwater may cause excessive concentrations of trace elements in soils near the ponds.

The objective of the study was to analyse and assess sorption properties of sandy soils used as hay meadows. As the study was conducted in a special bird protection area (SBP) included in the European Ecological Network

Nature 2000, special attention was paid to the content of selected trace elements.

MATERIAL AND METHODS

The study was conducted on 6 soil profiles situated in the Barycz Valley, within a special bird protection area (SBP). According to the World Soil Resources classification (WRB 2006), the analysed soils may be rated as Mollic Gleysols (Arenic), Mollic Phaeozems (Arenic) and Endogenic Phaeozems (Arenic). Having identified and described their morphology, soil samples were collected from genetic horizons and the following analyses were conducted: particle size distribution using the Bouyoucos aerometric method modified by Casagrande and Prószyński, total organic carbon (TOC) using the Tiurin's oxidometric method, pH in H_2O and 1mol KCl dm^{-3} using the potentiometric method, the $CaCO_3$ dm^{-3} content using the Scheibler's method, exchange acidity (Ea) using the Sokołow's method, total N (Nt) content using the Kjeldahl's method, exchangeable base cations (Ca^{+2}, Mg^{+2}, K^+, Na^+) in an extract of 1mol CH_3COONH_4 dm^{-3} of pH 7 using the Pallman's method, and total content of the metals Fe, Mn, Zn, Pb and Cu dissolved in 70% $HClO_4$ and determined using the ASA method. The results of the study were elaborated statistically using correlation coefficients at significance levels of $p = 0.05$ and $p = 0.01$ with Statistica 9 software. An assessment of the degree of contamination with metals was performed according to the Regulation of the Ministry of the Environment of 2002, on standards on soil quality and standards of earth quality (Journal of Law no 165, item 1358 and 1359).

RESULTS AND DISCUSSION

Surface humus horizons A, marked as A1 horizons of the thickness of 6-11 cm, were distinguished in the morphological structure of the analysed soil profiles. Deeper lying humus horizons with visible gley or altered colour were marked as horizons A2 and A3 (Table 1). Humus horizons with transient horizons A/C reached the depth of 50 cm (profile no 1). The features of gley and traces of ferruginous and manganese participation in the form of stains, pimentos and vertical seepages were noted in most of the analysed soils. The gley contribution in particular soil profiles was differentiated and dependent on the intensity of reduction processes induced by the activity of stagnant groundwater lying at a depth from 28 cm (profile no 3 and 5) to 70 cm (profile no 2 and 6). The level of groundwater table determined the depth of soil sample collection for laboratory analysis.

Table 1

Grain size composition of soils according to the classification of PTG-2008 (2009)

Pro-file No.	Soil horizon	Depth (cm)	Skeletic parts	Sum of fraction			Texture classes according to USDA (2009)
			(%) content of fraction ∅ in mm				
			> 2.0	2.0 - 0.05	0.05 - 0.002	< 0.002	
Endogenic Phaeozems (Arenic)							
1	A1	0-10	0	90	9	1	sand
	A2	10-20	0	88	11	1	sand
	A3	20-34	0	89	9	2	sand
	A/Cgg	34-50	0	91	7	2	sand
	Cgg	>50	0	98	1	1	sand
Mollic Phaeozems (Arenic)							
2	A1	0-10	0	84	11	5	loamy sand
	A/C	10-30	1	79	12	9	loamy sand
	C	30-50	2	49	21	30	sandy clay loam
	IIC	50-70	1	97	1	2	sand
	IIIC	>70	0	99	0	1	sand
Mollic Gleysols (Arenic)							
3	A1	0-11	0	90	10	0	sand
	A2	11-17	0	82	18	0	loamy sand
	A3gg	17-28	0	93	6	1	sand
	Cgg	>28	0	92	7	1	sand
Mollic Gleysols (Arenic)							
4	A1	0-10	0	81	19	0	loamy sand
	A2	10-22	0	75	25	0	sandy loam
	A3gg	22-32	0	60	38	2	sandy loam
	A/Cgg	32-38	0	61	36	3	sandy loam
	Cgg	>38	0	86	9	5	sand
Mollic Gleysols (Arenic)							
5	A1	0-6	0	84	16	0	loamy sand
	A2	6-18	0	81	19	0	loamy sand
	A/Cgg	18-28	0	76	23	1	loamy sand
	Cgg	>28	0	69	29	2	loamy sand
Mollic Gleysols (Arenic)							
6	A1	0-10	0	85	14	1	sand
	A2gg	10-20	0	81	16	3	loamy sand
	A/Gox	20-45	0	79	13	8	loamy sand
	Goxr	45-70	0	91	3	6	sand
	Gr	>70	0	84	10	6	loamy sand

In sandy soils, the particle size distribution, excluding organic matter content, strongly determines the range of physical and physicochemical features (Drozd, Licznar 1996). In earthy parts of the analysed soil profiles, the sand fraction had the highest contribution (2-0.05 mm) and its content was at a level of 49 to 99%. The content of the silt fraction (0.05-0.002 mm) was considerably lower, usually less than 38%. A very small contribution of clay fraction (<0.002 mm), in the range of 1 to 30%, was observed in the

Table 2

Chemical properties of the soils

Profile No.	Soil horizon	Depth (cm)	pH		TOC	TN	TOC/TN
			H_2O	KCl	(g kg^{-1} soil)		
Endogenic Phaeozems (Arenic)							
1	A1	0-10	4.31	3.41	40.52	2.85	14.22
	A2	10-20	5.53	4.32	5.77	0.68	8.49
	A3	20-34	5.14	4.14	5.46	0.55	9.92
	A/Cgg	34-50	6.26	5.43	2.90	0.33	8.78
	Cgg	>50	6.53	6.04	1.50	n.d.	n.d.
Mollic Phaeozems (Arenic)							
2	A1	1-10	6.05	5.37	36.13	3.90	9.26
	A/C	10-30	6.42	5.53	22.09	2.40	9.20
	C	30-50	7.34	5.82	3.20	n.d.	n.d.
	IIC	50 -70	7.91	6.66	2.90	n.d.	n.d.
	IIIC	>70	7.14	6.44	2.70	n.d.	n.d.
Mollic Gleysols (Arenic)							
3	A1	0-11	5.23	4.32	34.14	3.10	11.01
	A2	11-17	4.71	3.84	62.11	4.40	14.12
	A3gg	17-28	5.03	4.27	20.29	1.00	20.29
	Cgg	>28	5.75	4.71	0.50	n.d.	n.d.
Mollic Gleysols (Arenic)							
4	A1	0-10	4.65	3.83	103.45	6.10	16.96
	A2	10-22	4.51	4.02	124.89	8.70	14.36
	A3gg	22-32	4.84	3.86	99.61	6.10	16.33
	A/Cgg	32-38	4.53	3.72	61.40	2.80	21.93
	Cgg	>38	5.22	4.21	6.40	n.d.	n.d.
Mollic Gleysols (Arenic)							
5	A1	0-6	4.42	3.63	79.72	5.60	14.24
	A2	6-18	4.38	3.66	85.50	5.40	15.84
	A/Cgg	18-28	4.52	3.71	50.67	6.70	7.56
	Cgg	>28	4.86	3.94	4.70	n.d.	n.d.
Mollic Gleysols (Arenic)							
6	A1	0-10	6.71	6.27	29.91	3.19	9.38
	A2gg	10-20	6.83	6.19	19.36	2.22	8.72
	A/Gox	20-45	6.95	6.32	4.12	0.57	7.23
	Goxr	45-70	7.34	6.43	0.89	n.d.	n.d.
	Gr	>70	7.52	6.38	0.50	n.d.	n.d.

Key: n.d. – not determined

samples (Table 1). Wide ranges of particular texture fractions prove the genetic diversity of particular soil horizons. The particle size distribution typical for loose sands, slightly loamy sands and loamy sands predominated in the examined soils (USDA 2009). In terms of soil heaviness, the examined soil profiles may be classified as very light and light.

Table 3

Coefficient of correlations between selected properties of soils

Variable	Fe (g kg^{-1})	Mn	Zn	Pb	Cu
		(mg kg^{-1})			
2.0-0.05 mm	-0.57**	-0.08	-0.11	-0.63**	-0.02
0.05-0.002 mm	0.19	-0.02	-0.06	0.65**	-0.03
<0.002 mm	0.84**	0.19	0.32	0.19	0.10
Depht (cm)	-0.01	-0.21	-0.11	-0.50**	0.01
pH KCl	0.40*	0.46*	0.16	-0.57**	0.10
TOC (g kg^{-1} soil)	-0.12	-0.18	-0.07	0.71**	-0.03
TN (g kg^{-1} soil)	-0.07	-0.06	-0.04	0.69**	-0.02
Ea (cmol(+) kg^{-1})	-0.10	-0.33	0.02	0.59**	0.08
S (cmol(+) kg^{-1})	0.74**	0.53**	0.41*	0.49**	0.22
CEC (cmol(+) kg^{-1})	0.67**	0.41*	0.38*	0.62**	0.23
BS (%)	0.44*	0.46*	0.17	-0.11	0.07

Statistically significant at $p < 0.05$*, $p < 0.01$**, $n = 28$.

The above soils were formed from carbonate-free formations, which was certainly conditioned by their sandy texture composition (Table 2). The values for pH in 1 mol KCl dm^{-3} were in the range of 3.41-6.66, indicating strongly acidic to neutral reaction. Also, the tendency for an increase in pH values in deeper genetic horizons was observed and confirmed statistically (Table 3). The pH values demonstrated, a significant positive relationship with the degree of the sorption complex saturation with base cations (BS), and negative relationships with the 0.05-0.002 mm fraction, exchange acidity (Ea), and the content of TOC and TN.

The humus horizons A were characterised by differentiated content of TOC (5.46-124.89 g kg^{-1}) and TN (0.55-8.70 g kg^{-1}), which distinctly decreased in deeper soil horizons (Table 2). A higher content of TOC and TN was determined in samples more abundant in the silt fraction (0.05-0.002 mm) and characterised by higher value of exchange acidity (Ea) and cation exchange capacity (CEC) – Table 4. The value of TOC/TN was in the range from 7.23 to 21.93, with some increasing tendency in deeper horizons.

Regarding the above properties, the analysed soils are similar to chernozems formed from sands found on the Tarnobrzeska plain and studied by KLIMOWICZ (1980), ground-gley soils discussed by GIEDROJĆ et al. (1990, 1992), chernozems and ground-gley soils of the Kampinos Forest analysed by KONECKA-BETLEY et al. (1999), chernozems of the Poznańskie Lake District analysed by MARCINEK and KOMISAREK (2004), soils of the Milicko-Głogowskie Depression (ŁABAZ et al. 2010*a*), soils of the Barycz Valley Landscape Park (BOGACZ et al. 2008, ŁABAZ et al. 2008, 2010*b*, 2011) and chernozems of the Milicz area examined by BOGACZ et al. (2010).

Table 4

Coefficient of correlations between selected soil properties

Variable	pH KCl	TOC (g kg^{-1})	TN (g kg^{-1})	Ea (cmol(+) kg^{-1})	S (cmol(+) kg^{-1})	CEC (cmol(+) kg^{-1})	BS (%)
2.0-0.05 mm	0.27	-0.37	-0.32	-0.42	-0.55**	-0.63**	0.02
0.05-0.002 mm	-0.54**	0.63**	0.58**	0.62**	0.42*	0.55**	-0.23
<0.002 mm	0.38*	-0.33	-0.33	-0.21	0.41*	0.33	0.35
Depth (cm)	0.60**	-0.59**	-0.66**	-0.56**	-0.37	-0.49**	0.36
pH KCl	-	-0.63**	-0.56**	-0.76**	0.16	-0.05	0.72**
TOC (g kg^{-1})	-0.63**	-	0.94**	0.64**	0.37	0.51**	-0.17
TN (g kg^{-1})	-0.57**	0.94**	-	0.63**	0.44*	0.58**	0.15
Ea (cmol(+) kg^{-1})	-0.76**	0.64**	0.63**	-	0.11	0.37	-0.72**
S (cmol(+) kg^{-1})	0.16	0.37	0.44*	0.11	-	0.97**	0.46*
CEC (cmol(+) kg^{-1})	-0.05	0.51**	0.58**	0.37	0.97**	-	0.24
BS (%)	0.72**	-0.17	-0.15	-0.72**	0.46*	0.24	-

Statistically significant at $p < 0.05$*, $p < 0.01$**, $n = 28$

Magnesium, calcium, potassium and sodium are present in soil in various forms, of which their exchangeable forms are the most important for plant nutrition. Cations adsorbed on soil colloids are a specific pool of nutrients (Kobierski et al. 2011). In arable soils, the sorption properties depend mainly on their particle size distribution, organic matter content and applied fertilization (Askegaard et al. 2005, Ersahin et al. 2006).

The sorption complex of the analysed soils was highly saturated with base cations, mostly calcium, followed by magnesium (Table 5). The contribution of other cations, e.g. potassium and sodium, was often in the range of trace amounts. Both the content and distribution of exchange base cations of in particular profiles of the examined soils were strongly differentiated and strictly connected to the content of particular particle size fractions, which was confirmed by the calculated correlation coefficients (Table 4). Higher sums of base cations (S) and cation exchange capacity (CEC) were noted in samples characterised by higher contributions of silt and clay fractions. This relationship was also confirmed in earlier studies by Błaszczyk (1998) and Kobierski et al. (2011). The exchange acidity was in the range of 0.11 to 5.15 cmol(+) kg^{-1}, and its value decreased significantly in deeper genetic horizons (Table 5). The Ea values demonstrated a positive relationship with the content of the silt fraction, TOC and TN, while having a significant, negative relationship with the degree of sorption complex saturation (BS) – Table 4. The sum of exchangeable base cations (S) ranged from 1.19 to 19.74 cmol(+) kg^{-1}, while the cation exchange capacity (CEC) was within the range of 1.57 to 23.20 cmol(+) kg^{-1}. The degree of the saturation of the sorption complex with base cations (BS), following a wide range of the results on sorption

Table 5

Sorption properties of the soils

Profile No.	Soil horizon	Depth (cm)	Ca^{+2}	Mg^{+2}	K^{+}	Na^{+}	Ea*	S**	CEC***	BS****
			(cmol(+) kg^{-1})							(%)
Endogenic Phaeozems (Arenic)										
1	A1	0-10	1.20	1.14	0.31	0.18	5.15	2.83	7.98	35.5
	A2	10-20	1.14	0.48	0.05	0.15	3.43	1.82	5.25	34.7
	A3	20-34	1.50	0.46	0.06	0.13	1.31	2.15	3.46	62.1
	A/Cgg	34-50	2.00	0.50	0.02	0.14	0.61	2.66	3.27	81.3
	Cgg	>50	1.46	0.02	0.02	0.13	0.21	1.63	1.84	88.6
Mollic Phaeozems (Arenic)										
2	A1	1-10	16.80	2.10	0.20	0.50	3.60	19.60	23.20	84.5
	A/C	10-30	17.60	1.20	0.10	0.50	3.00	19.40	22.40	86.6
	C	30-50	16.80	1.50	0.20	0.60	1.00	19.10	20.10	95.0
	IIC	50-70	2.40	0.40	0.10	0.30	0.50	3.20	3.70	86.5
	IIIC	>70	1.20	0.20	0.10	0.20	0.50	1.70	2.20	77.3
Mollic Gleysols (Arenic)										
3	A1	0-11	5.12	0.68	0.11	0.17	1.14	6.08	7.22	84.2
	A2	11-17	7.36	0.58	0.12	0.18	2.54	8.24	10.78	76.4
	A3gg	17-28	3.04	0.34	0.05	0.14	0.82	3.57	4.39	81.3
	Cgg	>28	0.80	0.28	0.03	0.08	0.38	1.19	1.57	75.8
Mollic Gleysols (Arenic)										
4	A1	0-10	8.40	1.26	0.18	0.29	3.19	10.13	13.32	76.1
	A2	10-22	14.00	1.61	0.19	0.38	2.15	16.18	18.33	88.3
	A3gg	22-32	12.00	1.65	0.11	0.38	4.95	14.14	19.09	74.1
	A/Cgg	32-38	6.64	1.16	0.07	0.26	4.22	8.13	12.35	65.8
	Cgg	>38	2.40	0.56	0.05	0.14	0.98	3.15	4.13	76.3
Mollic Gleysols (Arenic)										
5	A1	0-6	5.76	0.98	0.27	0.31	4.03	7.32	11.35	64.5
	A2	6-18	5.12	0.76	0.19	0.29	5.04	6.36	11.40	55.8
	A/Cgg	18-28	5.14	0.60	0.09	0.19	4.22	6.02	10.24	58.8
	Cgg	>28	2.40	0.44	0.05	0.14	3.47	3.03	6.50	46.6
Mollic Gleysols (Arenic)										
6	A1	0-10	16.80	2.24	0.21	0.49	0.11	19.74	19.85	99.4
	A2gg	10-20	14.40	1.00	0.06	0.31	0.05	15.77	15.82	99.7
	A/Gox	20-45	8.40	0.78	0.05	0.27	0.61	9.50	10.11	94.0
	Goxr	45-70	4.01	0.54	0.04	0.17	0.11	4.76	4.87	97.7
	Gr	>70	3.60	0.56	0.04	0.17	0.11	4.37	4.48	97.5

* Exchangeable acidity; ** Sum of exchangeable cations; *** Cation exchange capacity; **** Base saturation

properties, was within 34.7 to 99.7%. Both the sum of base cations (S) and cation exchange capacity (CEC) demonstrated lower values in samples more abundant in the sand fraction. Moreover, the cation exchange capacity (CEC) demonstrated higher values in samples more abundant in the silt fraction, TOCorg and TN, which was confirmed statistically.

Table 6

Content of Fe and trace elements in the soils

Profile No.	Soil horizon	Depth (cm)	Fe	Mn	Zn	Pb	Cu
			(g kg^{-1})	(mg kg^{-1})			
Endogenic Phaeozems (Arenic)							
1	A1	0-10	1.97	93.63	66.00	5.83	4.39
	A2	10-20	1.86	99.82	42.53	5.83	17.48
	A3	20-34	1.87	76.58	30.46	2.66	24.00
	A/Cgg	34-50	1.62	42.81	22.03	1.00	57.72
	Cgg	>50	2.78	36.67	10.64	1.45	37.65
Mollic Phaeozems (Arenic)							
2	A1	1-10	26.10	293.68	64.55	22.71	44.41
	A/C	10-30	23.60	294.93	42.59	31.17	32.73
	C	30-50	51.40	232.11	47.03	38.33	15.38
	IIC	50-70	4.70	42.68	8.20	4.20	2.80
	IIIC	>70	1.70	28.37	5.30	4.10	3.70
Mollic Gleysols (Arenic)							
3	A1	0-11	3.90	352.28	21.84	26.52	5.70
	A2	11-17	2.30	48.54	11.13	29.04	2.20
	A3gg	17-28	1.40	50.66	7.10	18.02	1.30
	Cgg	>28	0.80	21.83	3.00	11.00	0.50
Mollic Gleysols (Arenic)							
4	A1	0-10	5.50	36.76	18.14	30.03	10.11
	A2	10-22	5.90	36.34	20.23	32.67	13.53
	A3gg	22-32	8.60	41.90	17.56	31.52	24.63
	A/Cgg	32-38	8.40	46.77	12.93	26.04	14.56
	Cgg	>38	2.60	28.56	5.70	14.45	1.10
Mollic Gleysols (Arenic)							
5	A1	0-6	4.70	62.73	14.54	36.53	5.50
	A2	6-18	5.50	49.80	17.63	33.46	5.70
	A/Cgg	18-28	4.40	39.88	11.24	26.04	3.30
	Cgg	>28	4.20	37.72	8.48	18.02	0.60
Mollic Gleysols (Arenic)							
6	A1	0-10	16.70	815.32	20.22	6.67	4.90
	A2gg	10-20	19.10	923.75	17.30	5.67	4.10
	A/Gox	20-45	30.70	930.09	10.18	4.00	2.59
	Goxr	45-70	7.63	114.38	3.65	2.00	1.40
	Gr	>70	3.86	61.52	7.23	1.50	2.18

The levels of iron (Fe) and manganese (Mn) in the examined soil depended above all on the changing red-ox conditions, especially in profiles with high levels of groundwater. Both elements very easily undergo oxidation and reduction processes, which results in their increased solubility and mobility (Orzechowski, Smólczyński 2010). The content of Fe was in the range of 0.80 to 51.40 g kg^{-1}, while the Mn amounts varied from 21.83-930.09 g kg^{-1} (Table 6). Both elements were significantly positively

Table 7

Coefficient of correlations between selected trace elements

Variable	Fe	Mn	Zn	Pb
Fe	-	0.57**	0.42*	0.27
Mn	0.57**	-	0.08	-0.20
Zn	0.42*	0.08	-	0.10
Pb	0.27	-0.20	0.10	-
Cu	0.16	-0.10	0.91**	-0.02

Statistically significant at $p < 0.05$*, $p < 0.01$**, $n = 28$

correlated with each other and with the pH and sorption properties (Tables 3 and 7). Iron (Fe) additionally demonstrated a strong positive relationship with the clay fraction and a strong negative relationship with the sand fraction (Table 3). The determined amounts of Fe and Mn and their relationships with soil texture and sorption properties were similar to the results obtained by KONECKA-BETLEY et al. (1996, 1999), ORZECHOWSKI and SMÓLCZYŃSKI (2010), ŁABAZ et al. (2011).

The other examined elements, i.e. Zn, Pb and Cu, were observed in considerably smaller amounts than Fe and Mn. The zinc (Zn) content was in the range of 3.00 to 66.00 mg kg^{-1}, lead (Pb) from 1.00 to 38.33 mg kg^{-1}, and copper (Cu) from 0.50 to 44.41 mg kg^{-1} (Table 6). The decreasing tendency in the Zn, Pb and Cu content in deeper genetic horizons appeared in all the examined soil profiles, although was confirmed statistically only in the case of Pb (Table 3). The determined amounts of Zn were very similar to the data presented by ANDRUSZCZAK and CZUBA (1984), who assessed the Zn content in Polish chernozems on a level of 13-150 mg kg^{-1}. Zinc had significant positive correlations with the sum of base cations (S) and cation exchange capacity (CEC), as well as with the Fe and Cu content. Total Zn content was the highest in humus horizons and was subject to a decrease in deeper situated genetic horizons. This is probably caused by biological accumulation in humus horizons which was observed previously in the study by DOMAŃSKA (2009). The Pb content was close to the one observed in mucky chernozems and ground-gley soils in the Kampinos Forest area (KONECKA-BETLEY et al. 1996, 1999). In our research, the lead content was found in the highest number of significant correlations with the examined soil parameters, namely it was positively correlated with the silt fraction, TOC, TN, S, CEC, while negatively correlated with an increasing depth of the soil profile, sand fraction and pH value. The total Cu content was similar to that found by KABATA-PENDIAS (1981) and ANDRUSZCZAK and CZUBA (1984) in Polish chernozems and in ground-gley soils of the Kampinos Forest, but higher when compared to the results in degraded and mucky chernozems in the same area (KONECKA-BETLEY et al. 1996, 1999). Copper (Cu), except for the strong positive relationship with zinc (Zn), did not demonstrate any significant correlations with the analysed parameters of the soils.

Our assessment of the degree of heavy metal contamination in the examined soils, referred to to the Regulation of the Ministry of the Environment on standards on soil quality and standards of earth quality (Journal of Law no 165, item 1358 and 1359), showed to excess of the permissible levels of Zn, Pb and Cu.

CONCLUSIONS

1. The genesis of the examined soils can be linked to the very wet environs, which significantly manner influenced their physical and physicochemical parameters. These soils were formed in an area dominated by breeding fish ponds and crisscrossed with a dense network of watercourses in the Barycz River drainage basin.
2. The sum of base exchange cations (S) and the cation exchange capacity (CEC) in the examined soils are strongly connected to the particle size distribution, the finding confirmed by significant positive relationships with the silt fraction and colloidal fraction, and significant negative correlations with the sand fraction.
3. Higher content of Zn, Pb and Cu in surface genetic horizons of the examined soil profiles may point to their anthropogenic origins. The examined soils, apart from the dominant sandy texture, are characterised by a thick humus horizon abundant in TOC and TN, which increases soil capacity for trace elements accumulation.
4. Our assessment of the contamination of the soils with respect to the content of Zn, Pb and Cu does not demonstrate any excess of the permissible levels of these trace elements.

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CALCIUM AND MAGNESIUM IN ATMOSPHERIC PRECIPITATION, GROUNDWATER AND THE SOIL SOLUTION IN LONG-TERM MEADOW EXPERIMENTS

Barbara Sapek

Department of Water Quality Protection
Institute of Technology and Life Sciences in Falenty

Abstract

At present, atmospheric precipitation is considered a cause of environmental pollution. Less attention is paid to nutrients in rainfall that might supply plant requirements. Hence, there is a need to assess relationships between rainfall, groundwater and the soil solution in the context of their mineral content (including magnesium and calcium) essential for soil fertility as well as proper plant growth and development. Such an attempt was made based on long-term meadow experiments run in the vicinity of the village Falenty, the Province of Mazowsze (*województwo mazowieckie*). The relationships between the Ca and Mg content in wet precipitations in Falenty, and in groundwater and the soil solution under long-term meadow experiments were evaluated. Moreover, concentrations and loads of these elements were compared between wet precipitation, groundwater and the soil solution. The positive effect of rainfall Ca and Mg on soil water is differentiated by the significant variability of both concentrations and loads of Ca in precipitation. The fact that Ca binds more strongly to soil than Mg may result in the lack of a positive effect of Ca on the soil solution. The beneficial influence of Ca concentrations on soil solutions depend on the acid reaction of soil. No such relationship was observed for Mg. This confirms the influence of differences in soil pH, soil abundance and different solubility of the two elements on the chemical composition of soil waters.

Key words: atmospheric precipitation, calcium, groundwater, long-term meadow experiment, magnesium, relationship, soil solution.

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prof. dr hab. Barbara Sapek, Department of Water Quality Protection, Institute of Technology and Life Sciences in Falenty, Falenty, al. Hrabska 3, 05-090 Raszyn, Poland, phone: (22) 735-75-66, e-mail: b.sapek@itep.edu.pl

WAPŃ I MAGNEZ W OPADZIE ATMOSFERYCZNYM, WODZIE GRUNTOWEJ I ROZTWORZE GLEBOWYM W DŁUGOLETNICH DOŚWIADCZENIACH ŁĄKOWYCH

Abstrakt

Obecnie szczególną uwagę zwraca się na opad atmosferyczny, jako jedną z możliwych przyczyn zanieczyszczenia środowiska. Natomiast znacznie mniejszą poświęca się zawartości w nim składników pokarmowych uzupełniających potrzeby roślin. Skłania to do próby oceny wzajemnych powiązań między opadem, wodą gruntową i roztworem glebowym w aspekcie zawartości w nim składników mineralnych, w tym wapnia i magnezu, niezbędnych dla żyzności gleby oraz rozwoju i wzrostu roślin. Oceny dokonano w warunkach rejonu Falent, w województwie mazowieckim, na przykładzie długoletnich doświadczeń łąkowych. Badano i oceniono zależności między Ca i Mg w mokrym opadzie z Falent oraz w fazie wodnej gleby – wodzie gruntowej i roztworach glebowych spod doświadczeń łąkowych. Ponadto oszacowano oddziaływanie stężenia, a także ładunku tych składników między mokrym opadem, wodą gruntową i roztworem glebowym. Dodatnie oddziaływanie Ca i Mg zawartych w opadzie na wody glebowe różnicuje znaczna zmienność zarówno stężenia, jak i ładunku Ca w opadzie. Natomiast silniejsze w porównaniu z Mg wiązanie Ca przez glebę może skutkować brakiem wpływu tego składnika z opadu na jego stężenie w roztworze glebowym. Dodatni wpływ stężenia Ca w wodzie gruntowej na jego stężenie w roztworach glebowych warunkuje kwaśny odczyn gleby. W przypadku Mg nie obserwowano takiej zależności. Potwierdza to wpływ różnic odczynu, zasobności gleb oraz różnej rozpuszczalności związków omawianych składników na skład chemiczny wód glebowych.

Słowa kluczowe: długoletnie doświadczenia łąkowe, magnez, opad atmosferyczny, roztwór glebowy, wapń, woda gruntowa.

INTRODUCTION

Our care of the environment, justified and reasonable, now turns people's attention to atmospheric precipitation as a potential source of pollution. This problem was discussed for example in a review by SAPEK (2011), which dealt with nitrogen, and in another article by SAPEK and SAPEK (2011), concerned with phosphorus. Calcium and magnesium are contained in atmospheric precipitation, both wet like rain and snow, and dry such as dew, fog, cloud drops and aerosols (VAN LOON, DUFFY 2007). The main source of these elements in the atmosphere is the dispersion of soil and fragments of biological matter, wherefrom both elements are dissolved in the atmospheric water (RUBIO et al. 2008, TSUKUDA et al. 2006). Apart from industrial regions with excessive alkaline dust emissions, calcium and magnesium surplus is a rare problem, while the shortage of these elements is a much more common issue (SAPEK, SAPEK 2011). The presence of both elements in atmospheric precipitation is a factor aiding neutralization of acid rains. The content of calcium and magnesium in wet precipitation as well as their presence in particulate matter contained in dry deposits determine the pH of acid rain (SCHROEDER 1989, ANSTASIO 2000, FISAK et al. 2002, HLAWLICZKA et al. 2002). Rainfall is often seen as a source of water, but much less attention is paid to its content of nutrients, which supply plants' demands. Atmospheric precipitation may be an important source of nutrients provided they are deficient in natural ecosystems (Schlesinger et al. 1982, Arianoutsou 1989, Chadwick et al. 1999, Kacorzyk et al. 2012). As a source of calcium and magnesium, which control its pH, rainfall may play a significant role in maintaining the fertility of natural meadow ecosystems.

Precipitation is the main source of water to the liquid phase of soil, comprising groundwater and the soil solution, which is the milieu for plant nutrition. The availability of calcium and magnesium, which ensures good plant growth, depends *inter alia* on the ca and Mn concentrations in groundwater and particularly in the soil solution (Prusińkiewicz 2011, Szperliński 2002).

Atmospheric precipitation is an additional source of plant nutrients, although its exact contribution depends on relationships between rainwater, groundwater and the soil solution. Another factor to be considered is the mineral composition of deposition. Sapek, Kalińska and Nawalany (2006) tried to assess the effect of mineral components in atmospheric precipitation on their content in groundwater. They evaluated the relationship between the ionic composition of wet deposition and groundwater, which should be viewed in the context of the antagonistic behaviour between calcium and magnesium in the environment (Gorlach, Mazur 2002). The content of minerals in groundwater and in surface waters also depends on the type of soil and its use. Positive relations between concentrations of magnesium, potassium and sodium have been observed in waters from agricultural areas (Cymes, Szymczyk 2005, Orzepowski, Pulikowski 2008). An important factor in the cycling of nutrients, including Ca and Mg, in the water phase is the presence of particulate matter (PM) and organic compounds in atmospheric precipitation (Anastasio 2000, Hlawliczka et al. 2002, Schroeder 1989). The above considerations have inspired the current assessment of relationships between calcium and magnesium in the water phase (groundwater and soil solution) of soil covered with grassland.

In brief, the aim was to assess changes in the content of calcium and magnesium during the year as well as their mutual relationships in wet precipitation falling in the village Falenty and in the groundwater and soil solution under long-term meadow experiments. Another objective was to evaluate relationships between ca and Mn concentrations and loads in wet precipitation and the water phase of soil.

MATERIAL AND METHODS

Atmospheric precipitation samples collected in Falenty represented wet deposition, i.e. rain and snow (Sapek 2011). Samples were collected on a meadow maintained as a long-term experiment at the Experimental Station of the Institute of Technology and Life Science in Falenty, 13 km south of Warsaw. The description of the sampling apparatus and protocol can be found in Sapek et al. (2003). The atmospheric precipitation data were obtained from a meteorological station set near the experimental site in Falenty (Sapek and Nawalany 2006). Both experiments were located 2 and 3 km from the meteorological station and precipitation sampling site. Groundwater samples were collected from soil under long-term meadow experiments set up in 1981/1982 on acid (pH_{KCL} – 4.5; 4.3) mineral soil in Janki (J) and Laszczki (L), situated 2 km south from meteorological station. A map showing the exact location of all experimental sites was attached to the former paper (Sapek 2006).

In 1981 and 1982, two long-term meadow experiments were set up according to the random blocks method with four replications. The aim of these trials was to assess the impact of single liming on soil acidity, nutrient dynamics and yields from meadows; another goal was to study the mineralization of nitrogen and phosphor compounds during successive years. The experimental fields and a regulated stream were used to investigate the groundwater, soil solution and surface water chemistry (Sapek, Sapek 2011). Soils differed in pH, content of organic carbon content (J – 19, L – 38 g kg^{-1}), Ca (J – 1.44, L – 2.58 g kg^{-1}), Mg (J – 0.47, L – 0.89 g kg^{-1}), particles <0.02 mm (J – 18.4, L – 22.4%) and moisture (J – 22.1, L – 26.7% vol.).

Groundwater was collected from control wells installed in strips of land separating the experiments from surroundings. The mean groundwater depth was 120 cm in experiment J (max. 85 cm, min. 156 cm) and 107 cm (max. 91 cm, min.124 cm) in experiment L (Sapek et al. 2003). Moreover, water was sampled from a nearby stream flowing from springs near the village Laszczki (Rossa, 2003). Soil solutions originated from the experiment in Janki, where suction apparatuses were installed in four fertilisation objects of the experiment: Ca_0N_2AN, Ca_2N_2AN, Ca_0N_2CN, Ca_2N_2CN, i.e. from soil fertilised with 240 kg N supplied as ammonium saltpetre (AN) and calcium saltpetre (CN), not limed (Ca_0) or limed (Ca_2) once at the onset of the experiment with calcium carbonate in an amount according to the hydrolytic acidity 2 Hh. Details of the sampling method with suction apparatuses fitted with porcelain cups and the calculations of component outwash from soil are given in Sapek and Pietrzak (1996) and Pietrzak, Urbaniak and Sapek (2006). A precise description of the experiments, including characteristics of fertilisation variants, is presented in Sapek and Nawalany (2006). Calcium and magnesium concentrations in samples of precipitation, groundwater and the soil solution were determined with atomic absorption spectrophotometry, while pH was measured in KCl by potentiometry (Sapek, Sapek 1997). The results of concentrations of the analyzed components cover the following periods: 1988-2007 for wet precipitation, 1995-2007 for groundwater and 1996-2007 (excluding 1999 and 2004) for the soil solution. Samples of wet precipitation were collected from subsequent rainfalls provided the volume was sufficient for chemical analyse. The sampling of the other types of water kinds produced an annual average of 23 samples of groundwater, 9 samples of surface water from the stream, from 4 to 7 samples of the soil solution in 1996-1997, and from 15 to 31 soil solution samples in 2000-2002. Mean values of determinations from the above years of investigations as well as variability coefficients were calculated.

The collected data on wet precipitation, groundwater and the soil solution were numerous and diverse, which is why nonparametric correlations of the Spearman's rank order and adequate coefficients r_s were calculated. The Pearson's linear correlation coefficient (r) was used to evaluate changes in the wet precipitation volume.

RESULTS AND DISCUSSION

Concentrations and loads of calcium and magnesium; mutual relationships between precipitation and the soil water phase

Precipitation. In 1988-2007, the average calcium concentration in wet precipitation was 3.24 mg dm^{-3} and that of magnesium 0.64 mg dm^{-3}, both with a similar range of variability: 23.1 and 23.4%, respectively. The concentrations of both elements were similar to the mean concentrations in rainfall calculated from various literature data (Ca – 3.44 mg dm^{-3}, Mg – 0.81 mg dm^{-3}) (Sapek, Sapek 2011). The rainfall pH was acidic and rather stable over the years (mean pH – 5.2 at a variability coefficient of 6.9%). The mean annual precipitation was 544 mm, varying within 13% (Table 1). Changes in the annual precipitation in 1988-2007 showed a statistically significant increasing trend ($r = 0.56^*$) – Figure 1*a*. The dynamics of calcium and magnesium is illustrated by changes in the annual concentrations and their direction. Fluctuations of the Ca and Mg concentrations over the years translated into non-significant values of the Spearman's rang correlation coefficients (Ca – $r_{s1} = 0.14$, Mg – $r_{s1} = 0.15$), although the increasing Ca and Mg concentrations were accompanied by their higher loads (Ca – $r_{s3} = 0.54^{**}$, Mg – $r_{s3} = 0.51^*$) – Figure 1*b*,*c*. Moreover, as concentrations of Ca in atmospheric precipitation increased, so did the Mg levels ($r_s = 0.46^*$) – Figure 1*d*.

The mean annual calcium loads (17.4 kg ha^{-1}) delivered with wet precipitation to the soil surface in 1988-2007 showed high variability (CV= 42.0%), especially compared with much smaller loads of magnesium: 2.5 kg ha^{-1} (CV = 21.2%) – Table 2. The latter resembled the mean loads of Mg (3.4 kg ha^{-1}) calculated from a reviewed publication (Sapek, Sapek 2011). However, loads of calcium in Falenty were much higher than the mean value (8.2 kg ha^{-1}), calculated from the same source as magnesium. Unlike the high changeability of Ca loads, the loads of magnesium significantly increased with time ($r_{s2} = 0.52^*$) – Figure1*c*. the absence of a significant relationship between loads of the two elements, Ca and Mg, might be the result of the aforementioned changes (r_s ns) – Figure1*e*.

Table 1

Mean pH and concentrations of calcium and magnesium in wet precipitation from Falenty and in groundwater and the soil solutions

Sample type	Sampling site/experiment	Fertilisation object	Years	pH***		Ca (mg dm^{-3})		Mg (mg dm^{-3})	
				mean	CV (%)	mean	CV (%)	mean	CV (%)
Precipitation*	Falenty		1988-2007	5.2	6.9	3.24	23.1	0.64	23.4
Ground water	Janki		1995-2007	7.5	4.6	64.2	17.8	6.34	21.1
Ground water	Laszczki		-„-	7.6	3.7	95.1	18.4	13.3	14.7
Stream water	stream** (Janki)		-„-	7.42	4.2	62.5	16.2	9.77	24.8
Stream water	stream** (Laszczki)		-„-	7.52	3.6	63.4	14.7	9.74	23.3
Soil solution	Janki	Ca_0N_2AN	1996-2007	8.01	2.9	74.8	20.7	17.5	53.1
Soil solution	Janki	Ca_2N_2AN	-„-	8.01	3.3	94.9	16.0	25.8	43.0
Soil solution	Janki	Ca_0N_2CN	-„-	7.96	6.2	87.8	37.1	17.7	52.0
Soil solution	Janki	Ca_2N_2CN	-„-	8.13	3.0	71.8	38.7	31.8	53.5

* Mean annual precipitation from the studied period = 544 mm (CV = 13%);

** Stream delivering water from springs in the field; Ca_0N_2AN, Ca_2N_2AN, Ca_0N_2CN, Ca_2N_2CN – soil objects fertilised with 240 kg nitrogen (N_2) in the form of ammonium saltpetre (AN) or calcium saltpetre (CN), not limed (Ca_0) or limed (Ca_2) according to hydrolytic acidity criterion 2 Hh;

*** pH of soil solutions - mean from 1997-2002

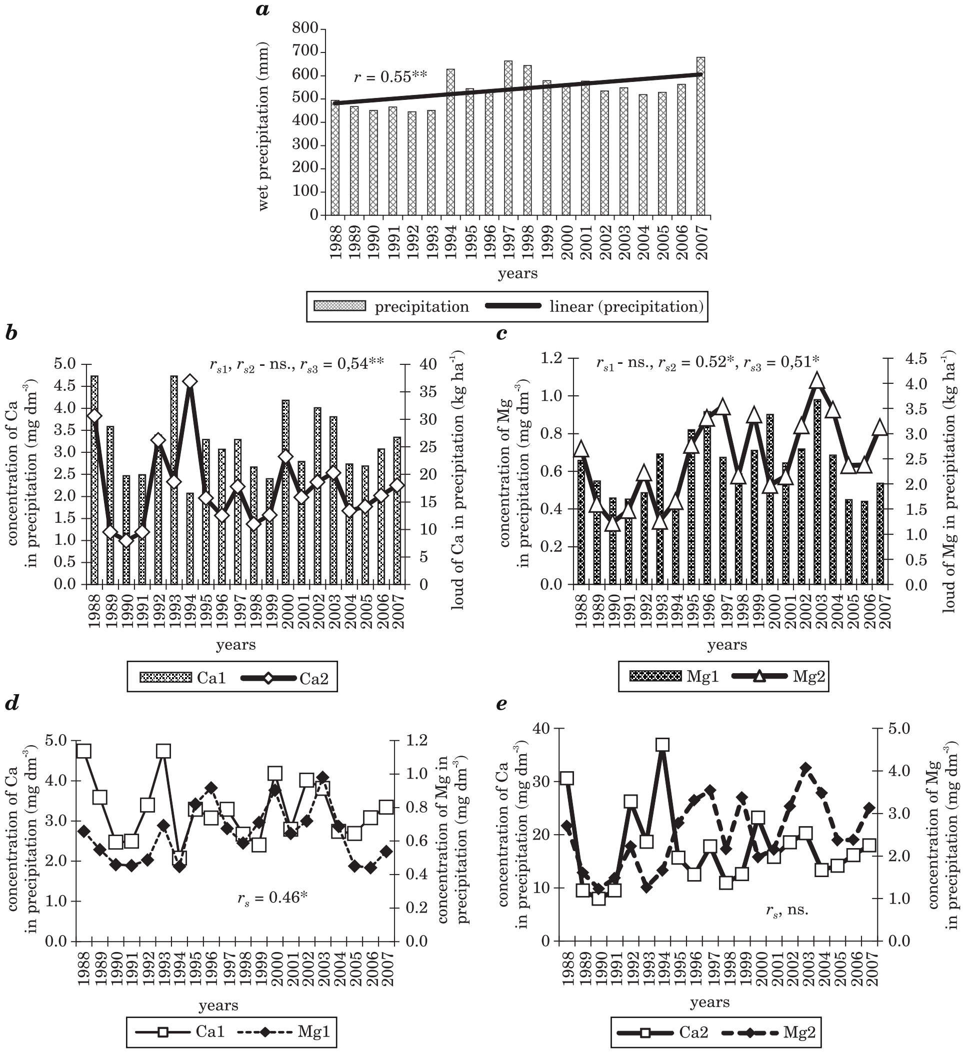

Fig. 1. Annual precipitation in Falenty and the concentration and load of calcium (Ca_1, Ca_2 and magnesium (Mg_1, Mg_2) in wet precipitation in 1995-2007 – relationships between precipitation volume – *a*, concentration – *b*, as well load – *c*, and years; relationships between concentration and load of calcium (Ca_1 x Ca_2) and magnesium (Mg_1 x Mg_2), concentration of calcium and magnesium (Ca_1 x Mg_1) – *d*, and their load (Ca_2 x Mg_2) – *e*, in wet precipitation; *r* – Pearson's linear correlation coefficient, r_s, r_{s1}, r_{s2}, r_{s3} – spearman's rank order correlation coefficients

Groundwater. Groundwater from the experiment in Laszczki was richer in calcium (95.2 mg dm^{-3}) than groundwater from experiment in Janki (64.2 mg dm^{-3}), with similar variability of element concentrations in both waters (c. 18%) during the study. Groundwater in the former site showed twice as much magnesium, weakly alkaline pH (7.6) and better stability

Table 2

Mean loads of calcium and magnesium introduced with wet precipitation on the soil surface in Falenty and mean calcium and magnesium leaching from soil to soil solution in the Janki experiment

Sample type	Sampling site/ experiment	Fertilisation object	Years	Ca ($kg\ ha^{-1}$)		Mg ($kg\ ha^{-1}$)	
				mean	CV (%)	mean	CV (%)
Precipitation	Falenty		1988-2007	17.4	42.0	2.50	21.2
Soil solution	Janki	Ca_0N_2AN	2000-2003	13.4	45.5	1.85	35.7
Soil solution	Janki	Ca_2N_2AN	-„-	13.6	60.3	2.38	73.5
Soil solution	Janki	Ca_0N_2CN	-„-	14.5	52.4	2.94	81.0
Soil solution	Janki	Ca_2N_2CN	-„-	10.7	63.6	5.40	70.6

Ca_0N_2AN, Ca_2N_2AN, Ca_0N_2CN, Ca_2N_2CN – as in Table 1

of both parameters compared to the other location (Table 1). The dynamics of changes in the concentrations showed a time-dependent increasing trend for Ca and decreasing one for Mg in both experimental locations. Thus, the Ca concentrations in groundwater in experiment J significantly increased during the study (r_{s1}(J) = 0,53*) – Figure 2*a*,*b*. Since 2004, the meadow in Lszczki has not been harvested, which encouraged the author to analyse concentrations of Ca and Mg in 2004-2007. Despite the lack of statistical significance, there was a clear tendency for decreasing Ca and Mg concentrations in groundwater from this experiment (Figure 2*b*,*d*). No correlations between the concentrations of the analysed elements were found in groundwater from both experiments (Figure 2*c*,*d*). The mean calcium concentrations determined in 1995-2007 in the stream which delivers water to fields and meadows in both Janki and Laszczki were similar to their concentrations in groundwater from Janki. Concentrations of magnesium (9.75 mg dm^{-3}) in the stream water were intermediate to Mg concentrations in groundwater from two experiments (Table 1).

Soil solutions. Soil solutions from the experiment in Janki contained more calcium and magnesium than groundwater, which was reflected in their mean concentrations from the years 1996-2007. The pH of the soil solutions from four different fertilisation treatments was close to 8.0, despite the big differences in the soil pH (pH_{KCl} 3.6-6.6) and differentiated Mg and Ca concentrations in the soil solution (Tables 1, 3). The highest mean Ca concentration (94.9 mg dm^{-3}) was found in the solution from very acidic soil (pH_{KCl} = 4.3) fertilised with acid-forming ammonium saltpetre but limed once at the onset of the experiment in 1981 (variant Ca_2N_2AN) – Tables 1, 3. This confirms re-acidification, that is higher soil acidification after liming, also demonstrated in earlier studies (SAPEK 2009). Most magnesium (31.8 mg dm^{-3}) was contained in soil solutions under soil with nearly neutral pH (pH_{KCl} = 6.6) from the treatment limed and fertilised with calcium saltpetre (Ca_2N_2CN), which indicates that mg was more intensively leached than

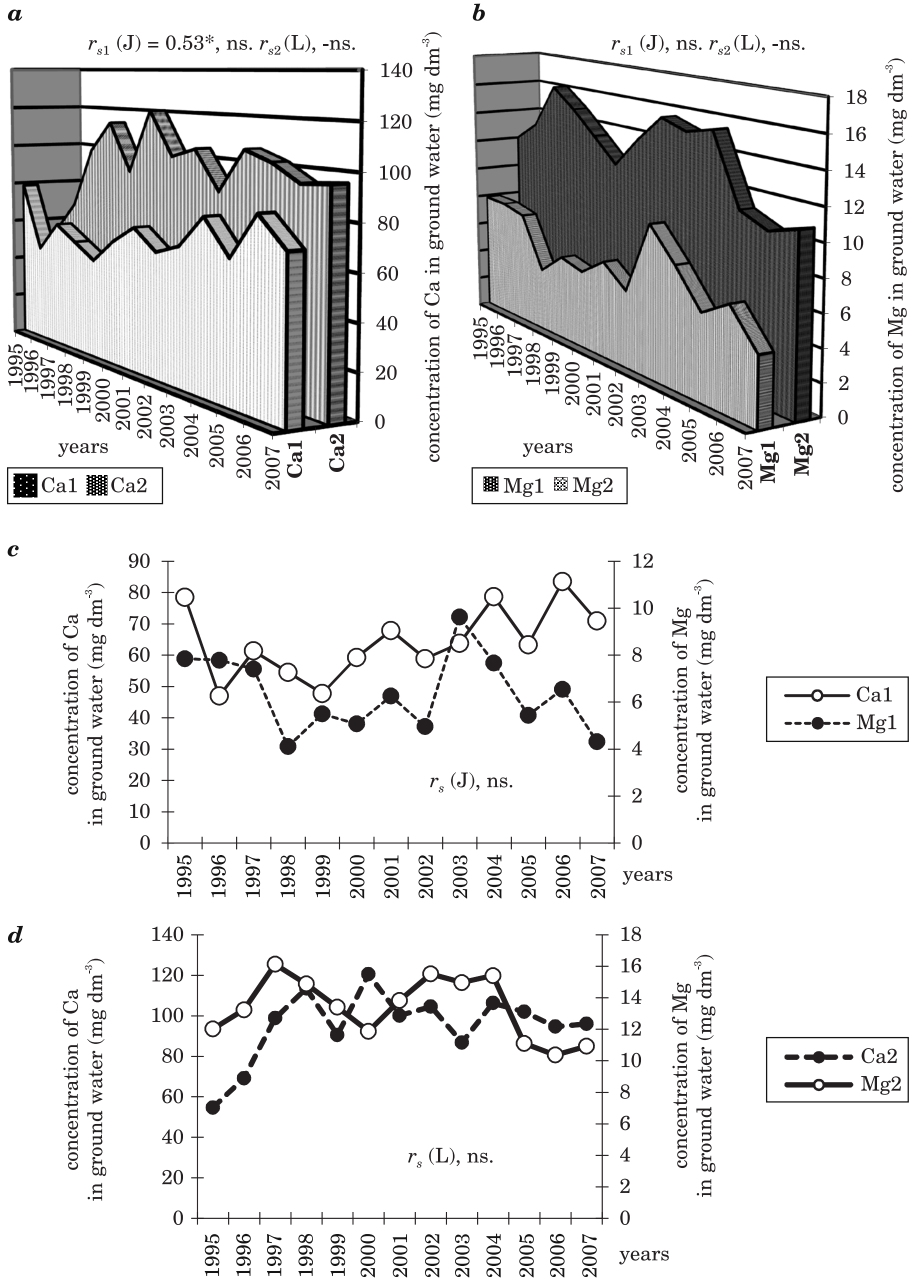

Fig. 2. Concentrations of calcium – *a*, and magnesium – *b*, in groundwater from the experiments in Janki (J) and Laszczki (L) in 1995-2007 – relationships between concentration and years; relationships between the concentration of calcium and magnesium in groundwater from experiment J (Ca_1-Mg_1) – *c*, and experiment L (Ca_2-Mg_2) – *d*; r_s, r_{s1}, r_{s2} – spearman's rank order correlation coefficients

Table 3

Mean pH_{KCl} in the 0-10 cm soil layer from four objects in the meadow experiment in Janki (1995-2007)

Value	pH_{KCl} of soil from fertilisation objects			
	Ca_0N_2AN	Ca_2N_2AN	Ca_0N_2CN	Ca_2N_2CN
Mean	3.6	4.3	5.0	6.6
Coefficient of variability, CV (%)	5.1	11.67	12.0	3.6

Ca_0N_2AN, Ca_2N_2AN, Ca_0N_2CN, Ca_2N_2CN – as in Table 1

Ca (GLIŃSKI 1999). The remarkable variability of elemental concentrations (CV%), especially magnesium, in soil solutions from particular fertilisation objects can be explained by different soil reaction and by the different uptake of these nutrients by plants (Tables 1, 3, Figure 3*a*,*b*). The effect of Ca and Mg uptake by plants on their content in the soil solution has been dealt with in another paper (SAPEK, 2011).

The Ca concentrations in the soil solutions showed just a tendency to increase in consecutive years, which originated from their great variability (Figure 3*a*). Concentrations of Mg in the solution from soils fertilised with calcium nitrate (Ca_0N_2CN, Ca_2N_2CN) were significantly decreasing in years (r_{s3} = -0.68*, r_{s4} = -0.83**) – Figure 3*b*. A comparison between the Ca and Mg concentrations in the soil solution from soils with an extreme pH showed a significant decrease in the Mg concentrations and an increase in the Ca content (r_{s4} = -0.83**) in the soil solution from acidic (Ca_0N_2AN) and neutral soil (Ca_2N_2CN) (Figure 3c and d), but only in the case of neutral soil a decrease in the Mg concentrations was significant;y correlated with the increase in the Ca concentrations (r_s = -0.68*) – Figure 3*c*,*d*. A further decrease in soil pH in the following fertilisation treatments was accompanied by a higher amount of leached Mg, but not Ca, which is demonstrated by the trend lines (Tables 2, 3, Figure 4*a*,*b*). The changes confirm the antagonistic behaviour between these elements in the environment (GORLACH, MAZUR 2002).

The smallest loads of Ca (13.4 kg ha^{-1}) and Mg (1.85 kg ha^{-1}) were leached from very acidic soil (pH_{KCl} = 3.6) of the variant Ca_0N_2AN, which is certainly attributable partly to their uptake by plants from soil, which was very poor in these elements. The high variability of Ca and Mg loads (compared to their concentrations) among particular fertilisation objects in the subsequent years was probably an effect of the less available information, but also of the differentiated Ca and Mg uptake by plants (SAPEK 2011) – Tables 1, 2.

Impact of calcium and magnesium concentrations and loads in wet precipitation on the soil water phase

The results on concentrations and loads of the two elements in wet precipitation and their concentrations in groundwater and the soil solutions did not enable the author to assess their relationships in 1995-2007. Due to

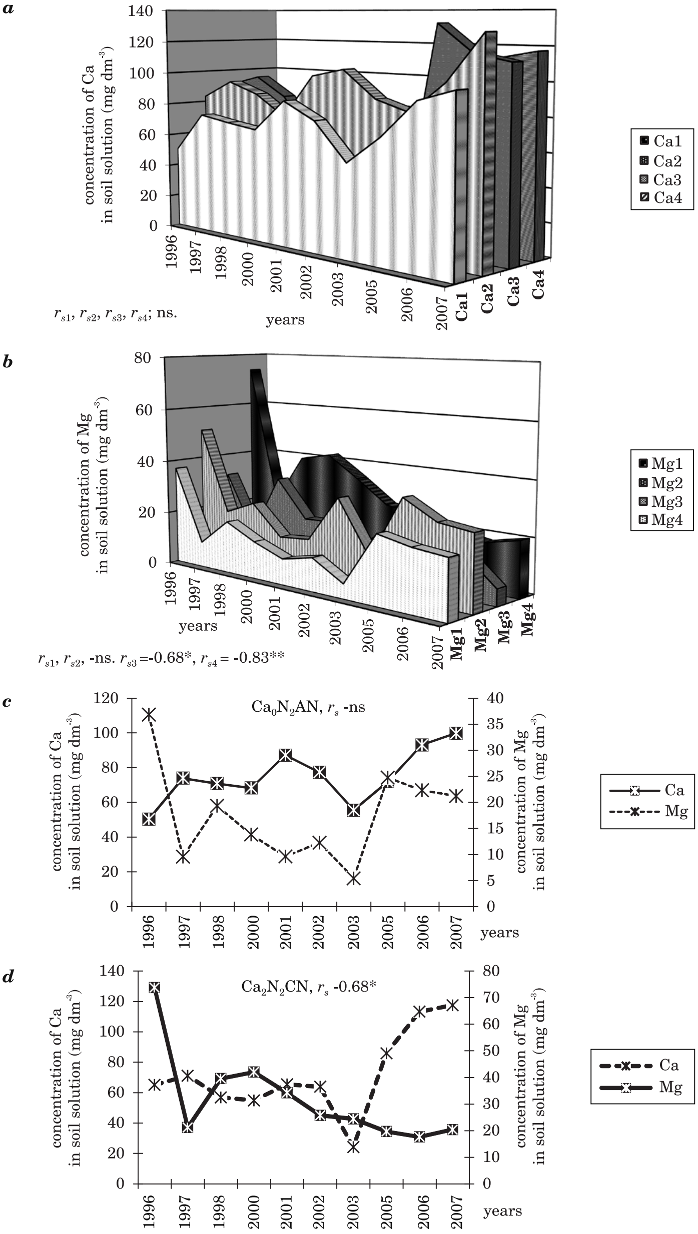

Fig. 3. Changes in the concentration of calcium (Ca_1, Ca_2, Ca_3, Ca_4) – *a*, and magnesium (Mg_1, Mg_2, Mg_3, Mg_4) – *b*, in soil solutions from 1-4 fertilisation objects (Ca_0N_2AN, Ca_2N_2AN, Ca_0N_2CN, Ca_2N_2CN) in the Janki experiment in 1996-2007 – relationships between concentration on 104 objects and years; relationships between the concentration of calcium and magnesium in solutions from the objects Ca_0N_2AN – *c*, and Ca_2N_2CN – *d*; r_s, r_{s1}, r_{s2}, r_{s3}, r_{s4} – spearman's rank order correlation coefficients

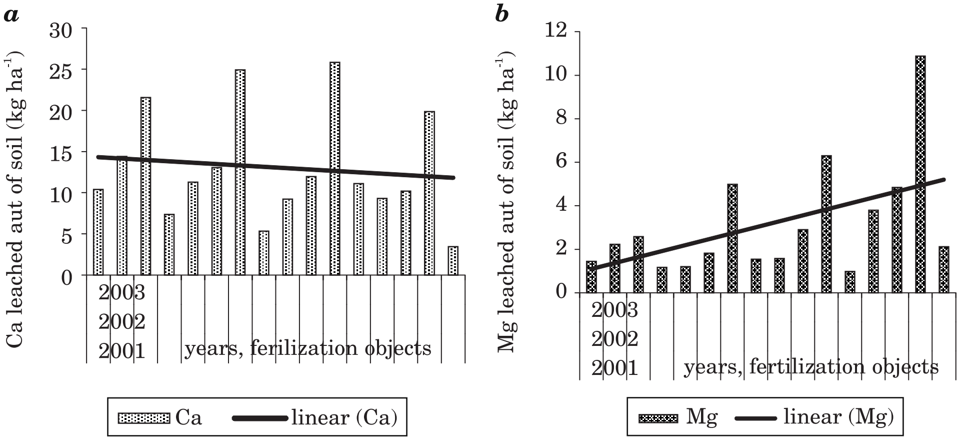

Fig. 4. Changes of calcium – *a,* and magnesium – *b*, loads released from soil to soil solutions on 4 fertilisation objects: Ca_0N_2AN, Ca_2N_2AN, Ca_0N_2CN, Ca_2N_2CN in the Janki experiment in 2000-2003

the insufficient number of data on Ca and Mg loads leached from soil to the soil solution (2000-2003) the relationships of these parameters with other variables were not analysed (Tables 1, 2).

Precipitation – groundwater. The concept of precipitation as a source of plant nutrients inspired the author to continue similar investigations, previously performed on the relationship between Ca and Mg concentrations in groundwater from meadow experiments, in water from a nearby stream and their loads delivered on the surface of land (Sapek et al. 2005). The Spearman's rank order correlations showed both positive and significant impact of Ca from precipitation on concentrations of this element in groundwater under less fertile and calcium poor soil from experiment J (r_{s1} = 0.67**). Similar effects were determined in water from the regulated stream (r_{s3} = 0.77**). No such influence was observed in groundwater under Ca richer soil from experiment L (r_{s2} ns.) – Figure 5*a*. Moreover, bigger loads of Mg in precipitation enriched groundwater with this element in both experiments (r_{s1} = 0.81**, r_{s2} = 0.80**). Besides, in such conditions, their concentrations in the stream water in experiment L increased significantly (r_{s3} = 0.90**) – Figure 5*b*. A positive impact of the Ca and Mg concentrations in precipitation on their levels in groundwater was verified.

Precipitation – the soil solution. The presence of Ca and Mg in wet precipitation has no significant relationship with the Ca concentrations in the soil solution from soils with the extreme pH, i.e. from 3.6 to 6.6, particularly the latter one (Table 3, Figure 6*a*). Changes in the Ca concentrations in 1996-2007 showed some tendency towards a decrease in the soil, although the content of calcium in precipitation increased between 1996 and 2007 (Figure 6*a*). That could have been caused by Ca binding to soil particles

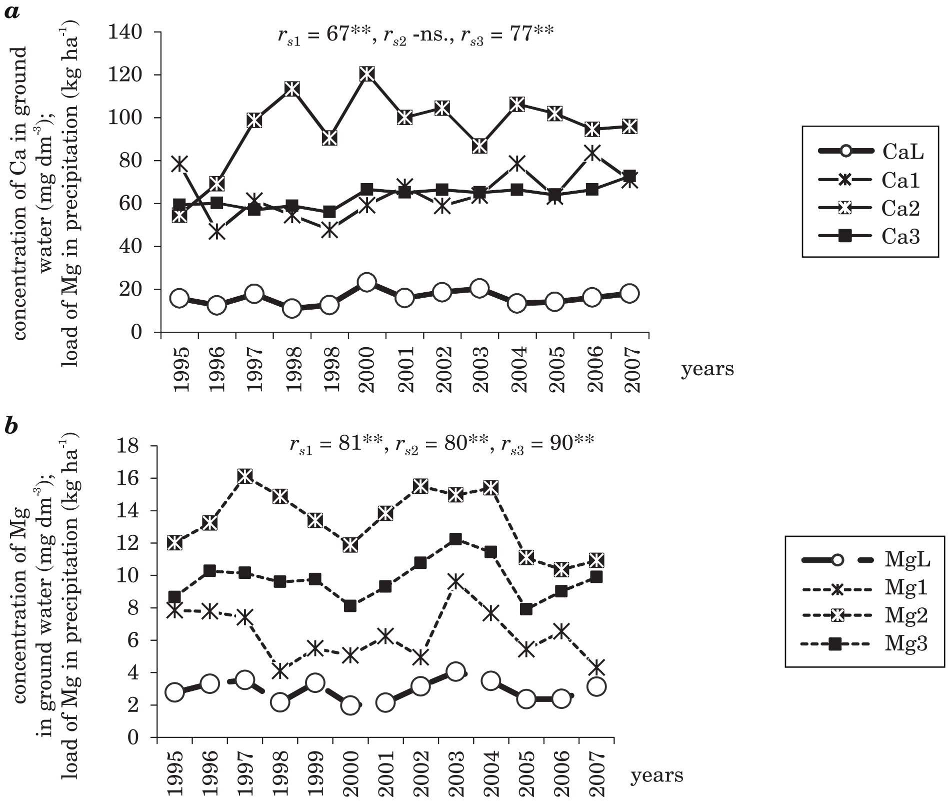

Fig. 5. Concentrations of calcium and magnesium in groundwater from experiment in Janki (Ca_1, Mg_1) and Laszczki (Ca_2, Mg_2) and in water from the stream (Ca_3, Mg_3), as well as loads of calcium (Ca_L) and magnesium (Mg_L) in precipitation in 1995-2007; relationships between calcium – *a*, and magnesium – *b*, in groundwater and loads in precipitation; r_{s1}, r_{s2}, r_{s3}, – spearman's rank order correlation coefficients

more readily Mg, and by the soil sorption capacity (GLIŃSKI, 1999), as manifested by an increase of Ca concentrations in precipitation being accompanied by higher Mg concentrations in the soil solution (r_{s2} = 0.68*) – Figure 6*b*. Changes in Mg loads in wet precipitation had no impact on Ca concentrations in the soil solution.

Groundwater – the soil solution. Calcium concentrations in groundwater were related to Ca concentrations in the soil solution (r_{s1} = 0,70*), but only from the acid soil (pH_{KCl} - 3.6) in the Ca_0N_2AN variant, where the leaching of this element was more intensive (SAPEK 2011) – Figure 7*a*. No such relation was assessed in the case of Mg (Figure 7*b*).

Chemical composition of the soil water phase (soil water), originating from the infiltration of precipitated waters, depends on the chemical composition of precipitation, its intensity and the rate of infiltration downwards

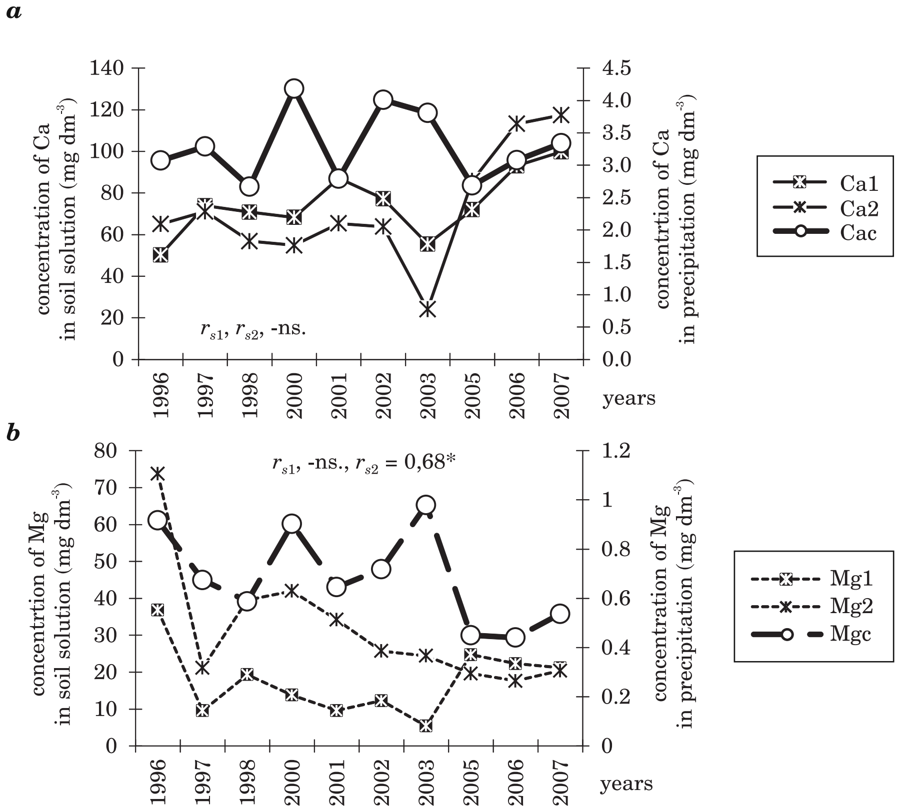

Fig. 6. Relationships between calcium and magnesium concentration in soil solution from the fertilisation objects Ca_0N_2AN ($Ca_{1,}$ Mg_1) and Ca_2N_2CN (Ca_2, Mg_2) in the Janki experiment and concentration of calcium (Ca_c) – *a*, and magnesium (Mg_c) – *b*, in precipitation; r_{s1}, r_{s2} – spearman's rank order correlation coefficients

the soil profile, but it is also conditioned by soil properties, chiefly chemical characteristic and grain size structure (Katz 1989, Raczyk 2008). The effect of soil properties on Ca and Mg concentrations in groundwater were confirmed by the substantial Ca and Mg enrichment of groundwater from the experiment in Laszczki, carried out on soil which was more compact and richer in organic carbon than soil from the experiment in Janki. Cymes and Szymczyk (2005) also found that the type of soil and its use are the most important factors differentiating concentrations of Ca and Mg. Due to their depth (on average 107-120 cm), the groundwater resources analysed herein may be perceived as soil-ground water. Therefore, their chemical composition largely depends on atmospheric precipitation and particularly on its Ca and Mg content (Szperliński 2002). Concentrations and loads of Ca and Mg in wet precipitation around Falenty made the pH of rainfall acidic (pH = 5.2), quite close to the pH = 5.63, which is typical of non-polluted precipitations (Zwoliński 1996). For comparison, the mean annual pH of atmospheric precipitation near Olsztyn was 4.47-4.87 (Szwejkowski et al. 2007). The environs of Falenty may be considered rural, as there are no large industrial companies nearby. On the other hand, the village lies in the vicinity of a large urban agglomeration (Warsaw) and near a motorway, which leads to the enrichment of wet precipitation in both acidifying components and in particulate matter containing calcium and magnesium. Calcium compounds are invariably less soluble, which is why - despite their greater abundance - their effect on soil and soil solutions may be more complex and less visible than that of magnesium (Hawliczka et al. 2003). In the conditions med during the current investigation, a positive and significant relation between concentrations of soluble forms Ca and Mg in precipitation, and the lack of a significant increase of Ca loads, unlike Mg loads, support the conclusions proposed by Hawliczka et al. (2003) about the effect of forms of both ele-

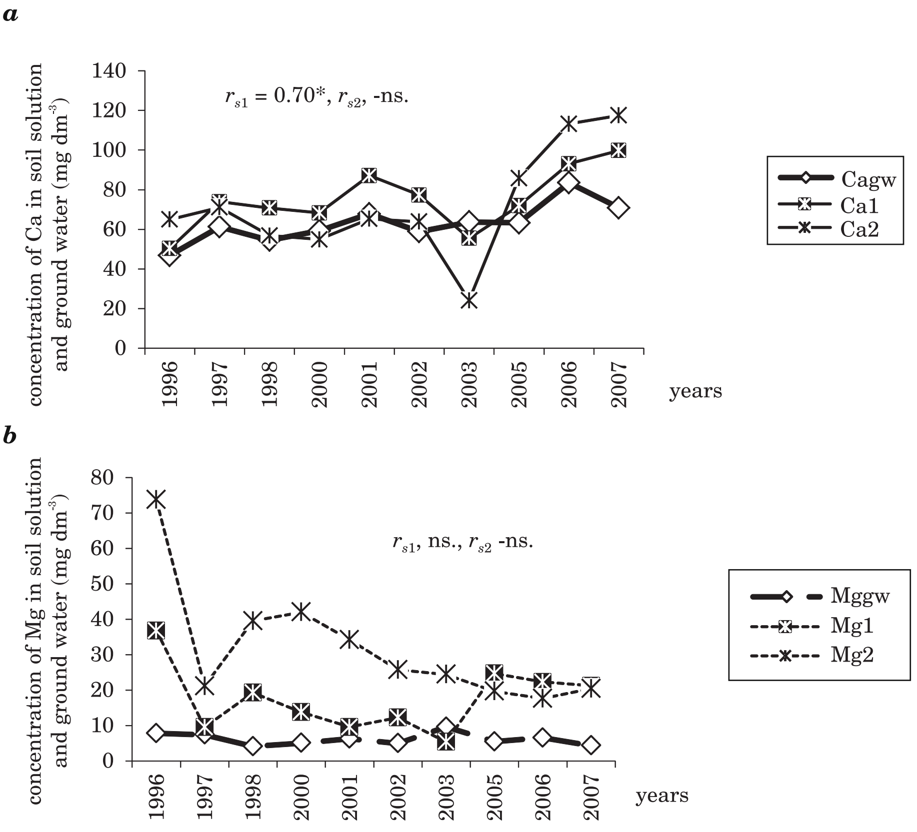

Fig.7. Relationships between the concentration of calcium – *a*, and magnesium – *b*, from objects: in soil solution Ca_0N_2AN (Ca_1, Mg_1) and Ca_2N_2CN (Ca_2, Mg_2) and concentration of calcium (Ca_{gw}) and magnesium (Mg_{gw}) in groundwater in the Janki experiment; r_{s1}, r_{s2} – spearman's rank order correlation coefficients

ments on their environmental cycling. The present determinations of Ca and Mg in the stream water imply that the soil properties from the experiments and agro-technical treatments had no effect on concentrations of either of the elements. As shown in Sapek et al. (2003), these concentrations largely depend on the runoff from nearby fields and crops.

Precipitation permeating downwards the soil profile affects both groundwater and the soil solution. Concentrations of Ca and Mg measured in the soil solutions are higher than in groundwater from the same experiment, which is also caused by the method of their acquisition and sampling depth (Ligęza, Smal 2004). In theseinvestigations, samplers collecting the soil solutions were installed 10 cm below the ground. Calcium or magnesium leached from this layer did not travel to deeper soil layers, neither was it adsorbed or diluted. However, the said concentrations were affected by the uptake by plants, for which the soil solution is one of the main mineral resources (Prusińkiewicz 2011, Sapek 2011). Differences in concentrations of minerals, including Ca and Mg, in the soil solutions collected from the fertilisation treatments with extremely different soil pH confirmed the effect of type of soil and its use, suggested for example by Ligęza and Smal (2004).

CONCLUSIONS

1. Increase in calcium concentrations in wet precipitation in and around Falenty was accompanied by higher concentrations of magnesium, although no such correlation was were observed for their loads.

2. Concentrations of calcium in groundwater from soil under experimental meadows increased in the subsequent years, but only in the case of soil richer in calcium and magnesium as well as organic carbon.

3. Concentrations of magnesium were deceasing in the soil solution from objects fertilised with calcium nitrate, but increasing in the soil solution from soils of neutral pH.

4. Amounts of magnesium transferred from soil to the soil solution increased at a pH ranging from 3.6 to 6.6, but analogous quantities of calcium remained constant.

5. Increase in calcium loads in precipitation resulted in its higher concentrations in groundwater from soil poor in calcium and organic carbon as well as in water from the stream. No such effect was detected for magnesium.

6. Increase in magnesium concentrations in precipitation lead to its higher concentrations in the soil solution from soils with the pH close to 7.

7. Increase in calcium concentrations in groundwater from soils with low pH was accompanied by higher calcium concentrations in the soil solution from acid soil. The absence of such relationships in the case of magnesium could have been due to the difference in solubility of both compounds.

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EFFECT OF THE EXTRACT FROM *ECKLONIA MAXIMA* ON SELECTED MICRO-AND MACROELEMENTS IN AERIAL BIOMASS OF HYBRID ALFALFA

Jacek Sosnowski, Kazimierz Jankowski,
Beata Wiśniewska-Kadżajan, Jolanta Jankowska,
Roman Kolczarek

Chair of Meadow Science and Grassland Management
Siedlce University of Natural Sciences and Humanities

Abstract

In order to determine the effect of a phytohormonal biostimulator on the evolution of morphological traits of hybrid alfalfa, a field experiment consisting of 6 m^2 plots cropped with this plant was set up in 2009. Each experimental variant was carried out in a triplicate. The experiment was run for three years (2009-2011). The experimental factor was an extract from *Ecklonia maxima*, sold under the commercial name of Kelpak SL. This product contains 11 mg dm^{-3} of auxin and 0.03 mg dm^{-3} of cytokines. In the experiment, it was used on all re-growths as a spray in a dose of 2 dm^{-3} ha^{-1} of the formula diluted in 350 dm^3 of water. The analyzed factors were the concentrations of macroelements: phosphorus, potassium, calcium, magnesium (in g kg^{-1} d.m.), and trace elements: manganese, zinc, copper, molybdenum (in mg kg^{-1} d.m.) in the aerial biomass of alfalfa plants. The ratios of Ca: P and K: (Ca + Mg) were also calculated. The results were analyzed statistically using the analysis of variance and the mean values were compared with the Tukey's test. The application of the seaweed extract led to an increase in phosphorus, potassium, zinc and manganese in alfalfa aerial biomass. The content of magnesium, calcium, copper and molybdenum did not change significantly in response to the analyzed extract. The modifications in the content of macroelements led to a decrease in the calcium to phosphorus ratio and an increase in the potassium-calcium ratio.

Keywords: seaweed extract, microelements, macroelements, macroelemental ratio, aerial biomass, alfalfa.

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dr Jacek Sosnowski, Chair of Meadow Science and Grassland Management, Siedlce University of Natural Sciences and Humanities, B. Prusa 14, 08-110 Siedlce, Poland, e-mail: laki@uph.edu.pl

WPŁYW EKSTRAKTU Z *ECKLONIA MAXIMA* NA ZAWARTOŚĆ WYBRANYCH MIKRO- I MAKROELEMENTÓW W BIOMASIE NADZIEMNEJ LUCERNY MIESZAŃCOWEJ

Abstrakt

W celu określenia wpływu biostymulatora fitohormonalnego na kształtowanie się cech morfologicznych lucerny mieszańcowej, założono doświadczenie polowe w 2009 roku. Powierzchnia poletka wynosiła 6 m^2. Każdą kombinację doświadczalną wykonano w trzech powtórzeniach. Doświadczenie użytkowano przez trzy lata (2009-2011). Czynnikiem doświadczenia był ekstrakt z *Ecklonia maxima* występujący pod nazwą Klepak SL. Preparat zawierał 11 mg dm^{-3} auksyn i 0,03 mg dm^{-3} cytokinin. Był on stosowany w na wszystkie odrosty w formie oprysku w dawce 2 dm^{-3} ha^{-1} preparatu rozcieńczonego w 350 dm^3 wody. Badane cechy to: zawartość makroelementów – fosforu, potasu, wapnia, magnezu (g kg^{-1} s.m.) i mikroelementów – manganu, cynku, miedzi, molibdenu (mg kg^{-1} s.m.) w biomasie nadziemnej roślinnej lucerny. Wyliczono również stosunek Ca:P oraz K:(Ca+Mg). Wyniki opracowano statystycznie, stosując analizę wariancji oraz NIR$_{0,05}$ wg testu Tukeya. Oprysk ekstraktem z wodorostów wpłynął na wzrost zawartości fosforu, potasu, cynku i manganu w biomasie nadziemnej lucerny. Zawartość wapnia, magnezu, miedzi i molibdenu nie zmieniła się znacząco pod wpływem czynnika badawczego. W konsekwencji zróżnicowania zawartości makroelementów pod wpływem ekstraktu nastąpił spadek wartości stosunku wapnia do fosforu i wzrost stosunku potasu do wapnia i magnezu.

Słowa kluczowe: ekstrakt z wodorostów, mikroelementy, makroelementy, stosunek makroelementów, biomasa nadziemna, lucerna.

INTRODUCTION

According to Bai et al. (2007) and Abou El-Yazied et al. (2012), seaweeds are one of the most important marine resources of the world. They are used as human food, animal feed and raw materials in many industries. They are also used as fertilizers for agricultural and horticultural crops (Temple et al. 1988, Beckett et al. 1994, Ouedraogo et al. 2001, Bai et al. 2007, Zodapea et al. 2009, Nour et al. 2010, Zodape et al. 2010, Abou El-Yazied et al. 2012). Better production results were obtained in production of agricultural crops after applying these extracts than mineral fertilizers (Aitken, Senn 1965). Owing to the presence of minerals and hormonal substances (Moller, Smith 1998, 1999), they cause an increase in plant resistance to stress and disease (Verkleij 1992). The chemical composition of extracts from seaweeds is complex and largely dependent on the method of seaweed harvest. According to Bai et al. (2007), the industrial production of extracts relies on the following species: *Enteromorpha intestine*, *Cladophora dalmatica*, *Ulva lactuca*, *Corralina mediterranea*, *Ascophyllum nodosum* and *Ecklonia maxima*. Studies on the use of extracts from seaweeds have been carried out since 1965 (Aitken, Senn 1965). Today, these preparations are seen as a new generation, natural organic fertilizer (Zodape et al. 2010, Abou El-Yazied et al. 2012). Plant protection based on extracts from seaweeds is gaining popularity, which has stimulated the development of a large number of products now available on the European and American markets (Singh, Chandela 2005, Thirumaran et al. 2009, Nour et al. 2010, Sridhar, Rengasamy 2010, Zodape et al. 2010, Abou El-Yazied et al. 2012). It has been proven that plants treated with seaweed extract are more resistant to drought, soil deficiency of nutrients and salinity. In addition, it effectively reduces the stress associated with plant pruning. The beneficial effect of biostimulator phytohormones is primarily reflected by increased yields (Temple, Bomke 1989, Liu 1991, Verkleij 1992, Mostafa, Zheekh 1999, Zodape 2001, Matysiak, Adamczewski 2006, Matysiak et al. 2012). The application of these products also affects the content of micro- and macronutrients in plant material (Rathore et al. 2009, Zodapea et al. 2009, Abou El-Yazied et al. 2012). In literature, the effect of extracts on cereals and vegetables has already been presented but there are no studies on the application of such formulas on hybrid alfalfa.

This study was undertaken to determine the effect of extract from *Ecklonia maxima* on the content of selected micro- and macroelements in aerial biomass of hybrid alfalfa. The experiment was to elucidate whether an annual application of seaweed extract on alfalfa for three years would affect the content of P, K, Mg, Ca, Mn, Zn, Cu and Mo in the plant material.

MATERIAL AND METHODS

In 2009, a field experiment with hybrid alfalfa (variety Tula) was set up on an experimental field of the Department of Meadow Science and Grassland Management. The soil belonged to the type Anthrosols, and sub-type Hortisole type, developed formed from weak loamy sand. A soil analysis, performed at the Regional Chemistry Station in Wesoła, showed that the soil was neutral (pH KCl = 7.2), very rich in humus (3.78%), and contained the following amount of available phosphorus (P_20_5) 900 mg kg^{-1} and magnesium (Mg) 84 mg kg^{-1}, while the average amount of total nitrogen N was 1.8 g kg^{-1} and absorbable potassium (K_20) 190 mg kg^{-1}. The plot size was 6 m^2.

Before sowing and in the second and third year of crop cultivation, fertilization was applied introducing to soil 45 kg P ha^{-1} and 100 kg K ha^{-1}. In April 2009, alfalfa seeds were sown in the amount of 12 kg ha^{-1} (600 units m^{-2} assuming 100% germination), at a depth of about 1 cm. During the shoot formation stage, the biostimulator Kelpak SL, containing natural plant hormones such as auxin (11 mg dm^{-3}) and cytokines (0.03 mg dm^{-3}), was applied. Kelpak Sl is made from the brown alage *Ecklonia maxima* (Temple et al. 1988). The formula was applied sprayed on all re-growths. The experimental variants were as follows: A1 – control (no extract), A2 – extract applied in a dose of 2 dm^{-3} ha^{-1} of the formula diluted in 350 dm^3 of water. Each treatment was carried out in a triplicate. During the whole experiment (2009-2011), three cuts of alfalfa were harvested in the early flowering stage.

The content of macroelements in the biomass was determined in all the cuts over the three years. The following analytical methods were used: P – flow spectroscopy, K – emissions by flame spectroscopy, Ca and Mg – atomic absorption spectroscopy. Furthermore, based on the the content of macroelements, the following ratios were calculated: Ca: P and K: (Ca + Mg). Additionally, Mn, Zn, Cu and Mo were assayed via atomic absorption spectrometry.

The results were evaluated statistically with analysis of variance for multivariate experiments. Differences between mean values were verified with the Tukey's test at $p \leq 0.05$.

The meteorological data documenting the research period were obtained from the Hydrological and Meteorological Station in Siedlce. In addition, for determination of the temporal variability of meteorological elements and their influence on plant growth, the Sielianinov's hydrothermic rate was calculated.

The data presented in Table 1 show that the most favorable rainfall distribution, with optimum air temperatures, occurred in 2009 and 2011. There were no months with drought and severe drought in none of these years.

Table 1

Values of Sielianinov's hydrothermical index of (*K*) in individual months of vegetation

Year	Month						
	Apr	May	June	July	Aug	Sept	Oct
2009	1.03	2.24	1.03	1.26	1.36	1.01	1.73
2010	0.40	2.21	1.19	1.18	1.79	2.81	0.53
2011	1.10	0.89	0.72	2.19	0.84	0.78	0.94

$K < 0.5$ – severe drougth; 0.51-0.69 – drought; 0.70-0.99 – weak drought; $K > 1$ – no drought

RESULTS AND DISCUSSION

Application of the extract from *Ecklonia maxima* in the cultivation of hybrid alfalfa resulted significantly increased the phosphorus content (by over 10%) and potassium (by over 15%) – Table 2. The statistical analysis showed that the content of these elements also depended on the year. Regardless of the experimental factor, phosphorus was the lowest in the second year of cultivation (2.8 g kg^{-1} d.m.) and the highest one in the first year (3.1 g kg^{-1} d.m.). However, the potassium content increased with plant age. It is noteworthy that the annual application of the extract did not influence significantly the content of magnesium or calcium in the tested plant material. This is supported by results reported by Abou El-Yazied et al. (2012),

Table 2

Effect of the extract from *Ecklonia maxima* on the content of selected macroelements ($g\ kg^{-1}$ d.m.) in aerial biomass of hybrid alfalfa in each year (means for cuts)

Macroelements	Years	Extract		Mean
		A1 (control)	A2 (factor)	
P	2009	2.70 *Bb*	3.40 *Aa*	3.10 *A*
	2010	2.70 *Bb*	2.90 *Ba*	2.80 *B*
	2011	3.00 *Aa*	3.00 *Ba*	3.00 *AB*
	mean	2.80 *b*	3.10 *a*	
K	2009	13.4 *Bb*	16.5 *Ba*	15.0 *B*
	2010	17.4 *Ab*	18.6 *Aa*	18.0 *A*
	2011	16.3 *Ab*	19.5 *Aa*	17.9 *A*
	mean	15.7 *b*	18.2 *a*	
Mg	2009	2.80 *Aa*	3.00 *Aa*	2.90 *A*
	2010	2.70 *Aa*	2.70 *Aa*	2.70 *A*
	2011	2.60 *Aa*	2.60 *Aa*	2.60 *A*
	mean	2.70 *a*	2.80 *a*	
Ca	2009	14.3 *Aa*	14.2 *Aa*	14.3 *A*
	2010	14.7 *Aa*	14.8 *Aa*	14.8 *A*
	2011	14.8 *Aa*	14.2 *Aa*	14.5 *A*
	mean	14.6 *a*	14.4 *a*	

mean values marked with the same small letter do not differ significantly
mean values marked with the same capital letter do not differ significantly

but does not correspond to the results obtained by ZODAPEA et al. (2009), who found a significant increase in the content of these macronutrients in cereal grains.

Many authors (FEATONBY-SMITH, VAN STADEN, 1983, VERKLEIJ 1992, GALBIATTIA et al. 2007) have shown beneficial effects of seaweed extracts as natural regulators, which increased yield, improved plant vigor and the strengthened the ability to resist unfavourable environmental conditions. Application of the extract as an organic biostimulator has quickly become common practice in horticulture because of the beneficial production effects (VERKLEIJ 1992, CROUCH, VAN STADEN 1993). According to SANDERSON and JAMESON (1986) or STIRK and VAN STADEN (1997), the main ingredients of extracts affecting the plants are cytokinins and auxins, which are found in the composition of most seaweed concentrates.

These hormones induce many processes connected with cytological and histological aspects of plants and have an influence on the content of some macronutrients (WIERZBOWSKA, BOWSZYS 2008). The research of ABOU EL-YAZIED et al. (2012) on effects of seaweed extracts on the quality of beans showed that their application during two growing seasons resulted in higher phosphorus and potassium concentrations in leaves against the control.

The same trend is observed in the magnesium content. Similar results are presented by PISE and SABALE (2010). An increase in the phosphorus and potassium content in plant material after spray application of seaweed extract was found by Shehata et al. (2011). Also Nour et al. (2010), who studied the effect of seaweed extract spray on the chemical composition of tomato, showed that most K and P occurred in vegetables grown on the treated plots.

Studies on soybean (Rathore et al. 2009) regarding the response of this plant to different concentrations of seaweed extracts showed a significant increase in the P and K content after extract application, regardless of the concentration. Zodapea et al. (2009), who used 1% spray from an extract of *Kappaphycus alvarezii* on *Triticum aestivum,* found the grains to contain higher concentrations of K (over 15% increase), P (18%), ca (45%) and Mg (28%). Higher levels of macronutrients in plants after application of extracts were also reported by Beckett et al. (1994) and zahid (1999).

The references on the nutritional value of feeds emphasize the importance of quantitative ratios between minerals as a quality parameter (Staniak 2004, Jankowska-Huflejt, Wróbel 2008, Nowak et al. 2008). According to Staniak (2004) or Wierzbowska and Bowszys (2008), it is important to determine the ratios of Ca:P and K:(Ca+Mg). Herein, the application of *Ecklonia maxima* extract significantly narrowed the calcium to phosphorus ratio from 5.22 to 4.67 (Table 3), regardless of the research year. However, it was still above the norm, which should be from 1.8 to 2.1 in feed for ruminants, as given by Jankowska-Huflejt and Wróbel (2008).

Table 3

Macronutrient ratios in aerial biomass of hybrids alfalfa depending on the extract of *Ecklonia maxima* and the growing year (mean for cut)

Ratio	Years	Extract		Mean
		A1 (control)	A1 (control)	
Ca : P	2009	5.30 *Aa*	4.18 *Ba*	4.74 *B*
	2010	5.44 *Aa*	5.10 *Ab*	5.27 *A*
	2011	4.93 *Ba*	4.73 *Aa*	4.84 *B*
	mean	5.22 *a*	4.67 *b*	-
K : (Ca + Mg)	2009	0.78 *Bb*	0.97 *Ca*	0.87 *B*
	2010	1.00 *Aa*	1.05 *Ba*	1.03 *A*
	2011	0.94 *Ab*	1.16 *Aa*	1.05 *A*
	mean	0.91 *b*	1.06 *a*	-

mean values marked with the same small letter do not differ significantly
mean values marked with the same capital letter do not differ significantly

The biomass examined in the experiment had a very high content of potassium in relation to animal nutrition standards (Jankowska-Huflejt, Wróbel 2008). It largely influenced the K: (Ca+Mg) ratio, which reached 0.91 in the plant material collected from the control crops. Moreover, spraying alfalfa with the extract caused a statistically significant increase by over 16%. In the subsequent years an increase in potassium content (Table 2) was observed, which further increased the K:(Ca+Mg) ratio to a value above 1 (Table 3).

Spraying hybrid alfalfa plants with the seaweed extract has led to a significant increase in the content of Mn and Zn in aerial plant biomass (Table 4).

Table 4

Effect of the *Ecklonia maxima* extract on the content of selected microelements (mg kg^{-1} d.m.) in aerial biomass of hybrid alfalfa in each year (means for cuts)

Microelements	Years	Extract		Mean
		A1 (control)	A2 (factor)	
Mn	2009	50.1 *Aa*	51.2 *Aa*	50.7 *A*
	2010	49.7 *AB*a	50.8 *Aa*	50.3 *A*
	2011	45.5 *Bb*	53.1 *Aa*	49.3 *A*
	mean	47.8 *b*	51.7 *a*	
Zn	2009	22.6 *Ab*	28.0 *Aa*	25.3 *A*
	2010	24.1 *Aa*	26.3 *Aa*	25.2 *A*
	2011	24.4 *A*	28.1 *Aab*	26.3 *A*
	mean	23.7 *b*	27.5 *a*	
Cu	2009	7.00 *Aa*	6.95 *Aa*	6.98 *A*
	2010	6.01 *Ba*	5.79 *Ba*	5.90 *B*
	2011	6.16 *Ba*	5.83 *ABa*	6.00 *AB*
	mean	6.39 *a*	6.19 *a*	
Mo	2009	0.51 *Aa*	0.48 *Aa*	0.50 *A*
	2010	0.47 *Ab*	0.56 *Aa*	0.52 *A*
	2011	0.49 *Aa*	0.52 *Aa*	0.51 *A*
	mean	0.49 *a*	0.52 *a*	

mean values marked with the same small letter do not differ significantly
mean values marked with the same capital letter do not differ significantly

The manganese content increased from 47.5 on control plots to 53.1 mg kg^{-1} on the treatment with the formula, which mean an over 7.5% increase. For zinc, an analogous increase was as high as over 13%. Regardless of the years the statistical evaluation did not find significant differentiation among the mean values after the application of the seaweed extract with respect to the content of copper and molybdenum. In addition, only one of the analyzed micronutrients, namely copper, was significantly differentiated in the biomass of alfalfa. The highest amount of this element was observed in the material collected in the first year. Higher content of some microelements was also found in the study of Zodape et al. (2009). According to these authors, application of the extract from *Kappaphycus alverezii* on cereals, regardless of the preparation concentration, resulted in an increase of zinc by 4.9%, and manganese by 9.42%, as well as molybdenum by16% in wheat grain. However, in their experiment, the copper content did not change under the influence of the extract.

CONCLUSIONS

1. Application of an extract from *Ecklonia maxima* in the cultivation of hybrid alfalfa resulted in a statistically significant increase in the content of phosphorus and potassium in aerial parts of this plant.

2. The content of calcium and magnesium in the biomass of alfalfa did not undergo significant differentiation under the influence of the seaweed extract during the research years.

3. Both the Ca : P and K : (Ca + Mg) ratios changed significantly as a result of the extract application. The calcium to phosphorus ratio was reduced by 10%, while that of potassium to calcium and magnesium increased by 16%.

4. Spraying with the seaweed extract resulted in an increase in the content of manganese and zinc in dry matter of alfalfa. Copper and molybdenum did not change significantly in repsponse to the extract.

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CHANGES IN THE CONTENT OF ZINC AND COBALT IN PLANTS AND SOIL, ABSORPTION OF THESE ELEMENTS BY GOAT'S RUE (*GALEGA ORIENTALIS* LAM.) BIOMASS AND BIOACCUMULATION FACTORS INDUCED BY PHOSPHORUS AND POTASSIUM FERTILIZATION

Barbara Symanowicz, Stanisław Kalembasa, Elżbieta Malinowska, Andrzej Wysokiński

Soil Science and Plant Nutrition Department
Siedlce University of Natural Sciences and Humanities

Abstract

This paper discusses changes in the content of zinc and cobalt in plants and soil under the influence of fertilization with phosphorus and potassium. The absorption of Zn and Co with biomass yield and the factors of their bioaccumulation were calculated. In 2005-2007, goat's rue (*Galega orientalis* Lam.), also known as eastern galega, was cultivated on an experimental field owned by the University of Natural Sciences and Humanities in Siedlce. The study included six fertilized objects (control, P_{50}, $K_{100,}$ $P_{50}K_{150}$, $P_{50}K_{200}$, $P_{50}K_{250}$). In each experimental year, three cuts of the test plant were harvested during the budding phase. The total content of zinc and cobalt in the plants and soil was determined with the ICP-AES method on a plasma-induced emission spectrophotometer. The fertilization with phosphorus and potassium significantly increased the Zn content in goat's rue biomass. The highest concentrations of zinc and cobalt were detected in goat's rue fertilized with the K_{100} dose. The successively higher doses of K with P decreased the content of the two elements in goat's rue biomass. The highest absorption of zinc and cobalt by goat's rue during the vegetation season was induced by the doses of $P_{50}K_{200}$ and $P_{50}K_{150}$, respectively. The highest concentrations of zinc and cobalt were detected in the soil fertilized with $P_{50}K_{250}$ and $P_{50}K_{150}$, respectively. The Zn and Co bioaccumulation factors were on an optimal level.

Keywords: PK fertilization, goat's rue, zinc, cobalt, absorption, bioaccumulation factors.

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dr hab. inż. Barbara Symanowicz, prof. nzw. UPH, Soil Science and Plant Nutrition Department, Siedlce University of Natural Sciences and Humanities, Prusa 14, 08-110 Siedlce, Poland, e-mail: bsymanowicz@uph.edu.pl

ZMIANY W ZAWARTOŚCI CYNKU I KOBALTU W ROŚLINIE I GLEBIE, POBRANIU PRZEZ BIOMASĘ RUTWICY WSCHODNIEJ (*GALEGA ORIENTALIS* LAM.) I WSPÓŁCZYNNIKACH BIOAKUMULACJI POD WPŁYWEM NAWOŻENIA FOSFOROWO-POTASOWEGO

Abstrakt

W pracy przedstawiono zmiany w zawartości cynku i kobaltu roślinie i glebie pod wpływem nawożenia fosforowo-potasowego. Obliczono pobranie Zn i Co z plonem biomasy rośliny testowej i współczynniki ich bioakumulacji. Rutwicę wschodnią (*Galega orientalis* Lam.) uprawiano w latach 2005–2007. Doświadczenie polowe prowadzono na polach doświadczalnych Uniwersytetu Przyrodniczo-Humanistycznego w Siedlcach. W badaniach uwzględniono sześć obiektów nawozowych (obiekt kontrolny, P_{50}, $K_{100,}$ $P_{50}K_{150}$, $P_{50}K_{200}$, $P_{50}K_{250}$). W każdym roku badań zbierano trzy pokosy rośliny testowej w fazie pąkowania. Zawartość całkowitą cynku i kobaltu w roślinie i glebie oznaczono metodą ICP-AES na spektrofotometrze emisyjnym z plazmą wzbudzaną indukcyjnie. Nawożenie fosforowo-potasowe wpłynęło istotnie na zwiększenie zawartości cynku w biomasie rutwicy wschodniej. Najwięcej cynku i kobaltu oznaczono w roślinie testowej nawożonej dawką K_{100}. Kolejne dawki nawożenia PK powodowały zmniejszanie zawartości analizowanych pierwiastków w biomasie rutwicy wschodniej. Największe pobranie cynku przez rutwicę wschodnią w ciągu okresu wegetacyjnego uzyskano pod wpływem nawożenia $P_{50}K_{200}$, a kobaltu – $P_{50}K_{150}$. Najwięcej cynku oznaczono w glebie nawożonej dawką $P_{50}K_{250}$, a kobaltu – $P_{50}K_{150}$. Współczynniki bioakumulacji Zn i Co kształtowały się na optymalnym poziomie.

Słowa kluczowe: nawożenie PK, rutwica wschodnia, cynk, kobalt, pobranie, współczynniki bioakumulacji.

INTRODUCTION

Goat's rue (*Galega orientalis* Lam.) is a perennial fabaceae, rich in essential elements and microelements (Raig et al. 2001 Symanowicz et al. 2004, Kalembasa, Symanowicz 2009). It can be used as a fodder ingredient in such feeds as green forage, straw, dry forage, silage and protein concentrate. After perennial cultivation, it may serve as a good preceding crop for winter wheat (Ignaczak, Szczepanek 2005). In studies on ^{15}N isotope carried out in Podlasie, a significant potential for the biological reduction of N_2 by goat's rue was detected during the vegetation season (Kalembasa, Symanowicz 2010), which occurred after the introduction of *Rhizobium galegae* bacteria into soil (Reichel et al. 1984, Peoples et al. 1995, Vanace 1998). The following factors are decisive for the yield and chemical composition of goat's rue biomass: soil, weather conditions, fertilization, growth stage, year of cultivation and strong resistance to fungal and viral diseases (Virkajärvi, Varis 1991, Valkonen 1993).

Zinc and cobalt are trace elements which must be monitored in fodder plants because their excess negatively influences the health of animals as well as the growth and development of plants (Spiak 2000). The literature provides evidence that the presence of cobalt in soil and plants is associated with the biological reduction of N_2 (Ruszkowska et al. 1996). However, there are no data on the qualitative and quantitative changes in the content of these elements in goat's rue biomass and in soil under the influence of different mineral fertilization regimes.

The objective of this study was to determine the impact of fertilization with phosphorus and potassium on changes in zinc and cobalt content in goat's rue (*Galega orientalis* Lam.) biomass and in soil. Another aim was to analyse the absorption of these elements by the biomass of the tested plant and to calculate the factors of Zn and Co bioaccumulation.

MATERIAL AND METHODS

The experiment was carried out in 2005-2007 on a plantation established in 1997 on an experimental field owned by the University of Natural Sciences and Humanities in Siedlce (52°17′N, 22°28′E). The soil on which eastern galega was cultivated was loamy sand (LS) and had neutral reaction. The abundance in absorbable phosphorus and potassium (determined with the Egner-Riehm's method) was medium, whereas the abundance in absorbable magnesium (measured with the Schachtschabel's method) was low. The study included the following fertilization treatments: control, P_{50}, $K_{100,}$ $P_{50}K_{150}$, $P_{50}K_{200}$, $P_{50}K_{250}$. Phosphorus fertilizers as triple superphosphate were applied in autumn while potassium fertilizer in the form of 60% potassium salt was split into two doses (applied in early spring and after the first cut).

The average monthly temperature in the consecutive vegetation seasons was comparable (15.0°C to 15.8°C) and significantly higher compared to the multi-annual records. The average precipitation during the vegetation seasons was lower than the multi-annual sum, except for 2006, when it was slightly higher (by 15.5 mm) because of heavy rainfalls in August, which were three-fold higher than the multi-annual sum (SYMANOWICZ, KALEMBASA 2010).

Three cuts of goat's rue at budding were harvested in each year of the study. During the harvesting of subsequent cuts of goat's rue (*Galega orientalis* Lam.), samples of whole plants were collected, dried and ground. Each year in autumn, soil samples were taken, dried and sieved through a 1mm mesh. Analytical solutions were prepared by dry mineralization of the tested plant's biomass and soil in a muffle furnace with a progressive increase in the temperature up to 450°C. Following complete oxygenation of organic compounds in the tested samples, 5 cm^3 HCl (1:1) was poured onto the ash left in a pot in order to degrade carbohydrates, extract silica and produce anions of inorganic acids and chlorides of tested cations. The excess of HCl was evaporated on a sand bath until dry. The content of the pot was again mixed with HCl (10%) and this solution was filtered into a 100 cm^3 measuring flask by a solid filter in order to separate silica. The content left on the filter was washed three times with diluted HCl and the content in the flask was filled up with water to the line representing analytical solution. The total content of Zn and Co in the tested plant and soil was determined with the ICP-AES method on a plasma-induced emission spectrophotometer (Szczepaniak 2005). The absorption of zinc and cobalt by the biomass of eastern galega and the factors of bioaccumulation of the analysed elements were determined.

The results were statistically processed with a three-way analysis of variance (ANOVA) and significant differences were calculated with the Tukey's test at α = 0.05. The Pearson's simple correlation r coefficients were calculated (Statistica 9.1; StatSoft) in order to identify correlations between the content of Zn and Co in the tested plant and soil and the absorption of these elements with the biomass yield. The significance of correlations was determined by comparing empirical values with critical values r at α =0.05.

RESULTS AND DISCUSSION

The average content of zinc in the DM of goat's rue harvested at budding was 53.89 mg kg^{-1} (Table 1), being significantly differentiated by the tested factors and their interrelations. The lowest content of zinc was detected in the biomass of eastern galega harvested in the first cut and in the second year of the experiment. The fertilization with phosphorus and potassium did not have any impact on the content of zinc in DM of goat's rue. The successively higher doses of potassium (K_{100}, $P_{50}K_{150}$) resulted in an increase in the Zn content in the biomass of goat's rue. The highest content of zinc was detected in the tested plant fertilized with 100 kg K ha^{-1}. These

Table 1

The content of zinc in biomass of goat's rue (mg kg^{-1} d.m.)

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	43.10	57.88	48.93	69.57	37.75	42.59	49.97
P_{50}	52.09	60.36	56.77	77.33	43.72	48.11	56.39
K_{100}	52.50	62.88	53.89	71.75	45.82	51.70	56.42
$P_{50}K_{150}$	53.31	56.24	55.51	71.15	43.61	50.30	55.02
$P_{50}K_{200}$	55.36	57.89	48.77	77.15	38.42	46.46	54.01
$P_{50}K_{250}$	51.11	57.14	46.33	58.28	43.86	52.44	51.53
Mean	51.24	58.73	51.70	70.87	42.20	48.60	53.89
$LSD_{0.05}$: cuts (C) – 1.33; years (Y) – 1.33; fertilization (F) – 2.29; YxC – 2.30; CxY – 2.30; FxC – 2.80; CxF – 2.30; FxY – 3.97; YxF – 3.25							

Table 2

The content of cobalt in biomass of goat's rue (mg kg^{-1} d.m.)

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	0.22	0.22	0.20	0.21	0.22	0.20	0.21
P_{50}	0.24	0.25	0.26	0.26	0.25	0.23	0.25
K_{100}	0.27	0.26	0.23	0.31	0.25	0.21	0.26
$P_{50}K_{150}$	0.28	0.25	0.25	0.27	0.27	0.24	0.26
$P_{50}K_{200}$	0.23	0.21	0.20	0.17	0.26	0.21	0.21
$P_{50}K_{250}$	0.26	0.25	0.20	0.23	0.23	0.24	0.23
Mean	0.25	0.24	0.22	0.24	0.24	0.22	0.24
$LSD_{0.05}$: cuts (C) – 0.01; years (Y) – 0.01; fertilization (F) – 0.01; YxC – 0.01; CxY – 0.01; FxC – 0.02; CxF – 0.01; FxY – 0.02; YxF – 0.02							

results were consistent with the studies carried out by Symanowicz et al. (2004), where changes in the content of heavy metals in goat's rue biomass were tested depending on the type of infection with different microbial strains (*Nostoc* cyanobacteria, *Rhizobium* galegae). The content of zinc in goat's rue biomass harvested in consecutive cuts in the experimental years from the objects fertilized with PK was within the permissible limits set for the content of trace elements in feedstuffs. According to Gorlach (1991), this was an optimal content.

The content of cobalt in the tested plant averaged 0.24 mg kg^{-1} d.m. (Table 2) and was significantly differentiated by the analysed factors and their interrelations. The highest concentration of cobalt was detected in the biomass of the tested plant harvested in the first cut (0.25 mg kg^{-1} d.m.) and also in 2005 and 2006 (0.24 mg kg^{-1} d.m.). The highest content of Co was recorded in goat's rue fertilized with K_{100} and $P_{50}K_{150.}$ These results are within the low range of cobalt concentrations in feedstuffs (Gorlach 1991, Ruszkowska 1996). Presumably, the soil reaction close to neutral explains the lower content of cobalt in the biomass of the tested plant. This conclusion is further supported by the results reported by Kalembasa, Symanowicz (2009) in another study on goat's rue, in which the content of cobalt ranged from 0.24 to 0.32 mg kg^{-1} d.m. with the pH of soil at 6.6.

The average absorption of zinc and cobalt with the yield of goat's rue (*Galega orientalis* Lam.) in 2005-2007 was 454.8 g Zn ha^{-1} and 1.95 g Co ha^{-1} (Tables 3 and 4). The plant absorbed most of the elements in yield after the first cut harvested at budding. The highest total amount of zinc and cobalt in three cuts was detected in the first year (651.3 g Zn ha^{-1} and 2.20 g Co ha^{-1}). Regarding the PK fertilization, the highest amounts of zinc and cobalt were accumulated by goat's rue fertilized with the dose of $P_{50}K_{200}$ and $P_{50}K_{150}$, respectively. These calculations were performed according to the publication by Symanowicz, Kalembasa (2010). The calculated correlation

Table 3

Uptake of zinc in the yield of goat's rue (g Zn ha^{-1})

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	150.8	120.4	58.2	518.3	283.9	227.4	343.2
P_{50}	203.1	176.8	95.4	796.5	369.9	321.4	495.9
K_{100}	195.8	139.0	100.2	625.7	355.6	358.3	446.5
$P_{50}K_{150}$	218.0	176.0	121.6	761.3	396.0	422.0	526.4
$P_{50}K_{200}$	298.9	167.9	115.1	918.1	407.2	441.4	588.9
$P_{50}K_{250}$	174.8	112.0	69.5	353.7	313.2	388.0	351.6
Mean	205.5	148.6	93.1	651.3	354.9	358.2	454.8

Table 4

Uptake of cobalt in the yield of goat's rue (g Co ha^{-1})

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	0.77	0.46	0.24	1.56	1.65	1.07	1.43
P_{50}	0.94	0.73	0.44	2.68	2.11	1.54	2.11
K_{100}	1.01	0.57	0.43	2.70	1.94	1.45	2.03
$P_{50}K_{150}$	1.14	0.78	0.55	2.89	2.45	2.01	2.45
$P_{50}K_{200}$	1.24	0.61	0.47	2.02	2.76	1.99	2.26
$P_{50}K_{250}$	0.89	0.49	0.30	1.40	1.64	1.78	1.61
Mean	1.00	0.61	0.40	2.20	2.02	1.62	1.95

coefficients demonstrated significant correlation (r = 0.92) between the absorption of zinc in goat's rue biomass fertilized with PK and the amount of absorbed cobalt. The content of absorbable forms of compounds in soil substantially affects their absorption by plants (SIENKIEWICZ et al. 2011).

The average total content of zinc in the humus horizon was 164.1 mg kg^{-1} (Table 5). Statistical calculations demonstrated significant differentiation of the Zn content in the soil. In the third year, the content of zinc in the soil decreased significantly in relation to the first year. In 2007, the concentration of zinc dropped by 12% compared to 2005. The zinc content in the soil decreased steadily in the consecutive years of the study, which was due to the removal of this element together with yields of the test plant. Having analysed the impact of increasing PK doses on the content of Zn in the soil, it was concluded that the concentration of zinc in the soil increased significantly although not steadily versus the control object. The highest content (174.8 mg kg^{-1}) was detected in the soil sampled from the object fertilized with $P_{50}K_{250}$. It is assumed that phosphorus and potassium fertilizers as well as some anthropogenic factors affecting the experimental field could

Table 5

The content of zinc in soil (mg kg^{-1} d.m.)

Fertilization	Years			Mean 2005-2007
	2005	2006	2007	
0	141.4	161.9	143.1	148.8
P_{50}	172.3	169.2	143.2	161.6
K_{100}	173.2	176.1	145.1	164.8
$P_{50}K_{150}$	175.0	175.6	158.7	169.7
$P_{50}K_{200}$	174.8	173.8	146.0	164.8
$P_{50}K_{250}$	181.7	174.4	114.0	174.8
Mean	169.7	171.8	150.7	164.1
LSD $_{0.05}$: years (Y) – 3.2; fertilization (F) – 5.6; FxY – 9.8; YxF – 7.9				

Table 6

The content of cobalt in soil (mg kg^{-1} d.m.)

Fertilization	Years			Mean 2005-2007
	2005	2006	2007	
0	1.27	1.44	1.52	1.41
P_{50}	1.45	2.04	1.55	1.68
K_{100}	1.40	2.04	1.60	1.68
$P_{50}K_{150}$	1.60	2.03	1.77	1.80
$P_{50}K_{200}$	1.49	2.10	1.44	1.68
$P_{50}K_{250}$	1.65	1.93	1.80	1.80
Mean	1.48	1.93	1.61	1.67
LSD$_{0.05}$: years (Y) – 0.10; fertilization (F) – 0.17.; FxY – 0.30.; YxF – 0.24				

have been sources of zinc. In pot experiments carried out by SYMANOWICZ and KALEMBASA (2012) and ŻARCZYŃSKI et al. (2011), the content of zinc was several times lower.

The average content of cobalt in the soil was low: 1.67 mg kg^{-1} (Table 6). The statistical analysis demonstrated the highest amount of this element in the soil sampled after the second year of the study (1.93 mg kg^{-1}). The subsequent doses of phosphorus and potassium fertilizers resulted in an increase in the cobalt concentration, which reached the highest value (1.80 mg kg^{-1}) under the fertilization treatment of $P_{50}K_{150}$. The experiment revealed a significant (r = 0.97) correlation between the content of zinc and cobalt in the soil.

Figures 1 and 2 show factors of Zn and Co bioaccumulation. The mean value of the zinc bioaccumulation factor was 0.33. The lowest value of this factor (0.22) was calculated for the object fertilized with $P_{50}K_{200}$ in the second

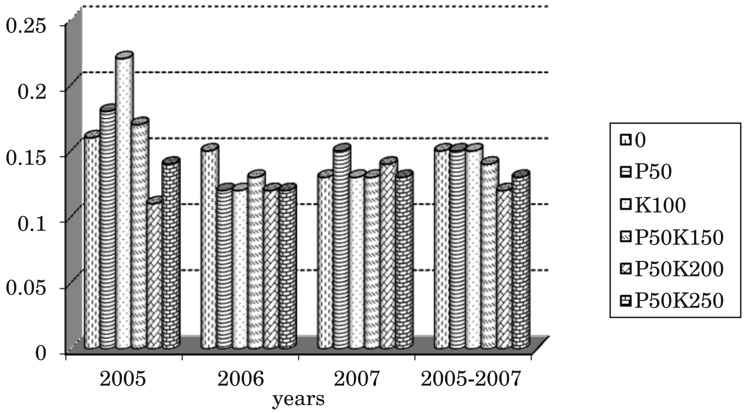

Fig. 1. Bioaccumulation factors of zinc

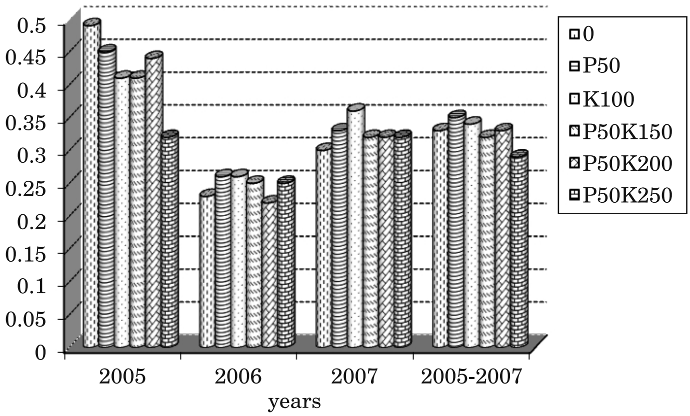

Fig. 2. Bioaccumulation factors of cobalt

year of the experiment. In the following years, the values of the Zn bioaccumulation factor varied (0.42-0.24-0.32). In a study by BARAN and JASIEWICZ (2009), the zinc bioaccumulation factor was approximately ten-fold higher, which most probably was connected with the acid reaction of soil and other test plants.

The calculated factors of cobalt bioaccumulation were on a comparable level of 0.12-0.14 for individual objects and within the range of 0.12-0.16 for the experimental years.

CONCLUSIONS

1. The incremental doses of phosphorus and potassium fertilizers had a significant impact on the zinc content in goat's rue biomass, increasing it versus the control object. The highest content of zinc was detected in the tested plant fertilized with K_{100}. Goat's rue accumulated the highest amounts of cobalt under the fertilization treatments of K_{100} and $P_{50}K_{150}$.

2. The recorded concentrations of zinc and cobalt in goat's rue biomass were within the optimal values in respect of the permissible limits of trace elements in feedstuffs.

3. The highest absorption of zinc and cobalt by goat's rue during the vegetation season was recorded for the fertilization treatments of $P_{50}K_{200}$ and $P_{50}K_{150}$, respectively.

4. The highest content of zinc was detected in the soil fertilized with $P_{50}K_{250}$, and the highest concentration of cobalt was measured in the soil fertilized with $P_{50}K_{150}$ and $P_{50}K_{250}$.

5. The highest values of factors of Zn and Co bioaccumulation were on an optimal level.

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Abstrakt

W pracy przedstawiono zmiany w zawartości cynku i kobaltu roślinie i glebie pod wpływem nawożenia fosforowo-potasowego. Obliczono pobranie Zn i Co z plonem biomasy rośliny testowej i współczynniki ich bioakumulacji. Rutwicę wschodnią (*Galega orientalis* Lam.) uprawiano w latach 2005–2007. Doświadczenie polowe prowadzono na polach doświadczalnych Uniwersytetu Przyrodniczo-Humanistycznego w Siedlcach. W badaniach uwzględniono sześć obiektów nawozowych (obiekt kontrolny, P_{50}, K_{100}, $P_{50}K_{150}$, $P_{50}K_{200}$, $P_{50}K_{250}$). W każdym roku badań zbierano trzy pokosy rośliny testowej w fazie pąkowania. Zawartość całkowitą cynku i kobaltu w roślinie i glebie oznaczono metodą ICP-AES na spektrofotometrze emisyjnym z plazmą wzbudzaną indukcyjnie. Nawożenie fosforowo-potasowe wpłynęło istotnie na zwiększenie zawartości cynku w biomasie rutwicy wschodniej. Najwięcej cynku i kobaltu oznaczono w roślinie testowej nawożonej dawką K_{100}. Kolejne dawki nawożenia PK powodowały zmniejszanie zawartości analizowanych pierwiastków w biomasie rutwicy wschodniej. Największe pobranie cynku przez rutwicę wschodnią w ciągu okresu wegetacyjnego uzyskano pod wpływem nawożenia $P_{50}K_{200}$, a kobaltu – $P_{50}K_{150}$. Najwięcej cynku oznaczono w glebie nawożonej dawką $P_{50}K_{250}$, a kobaltu – $P_{50}K_{150}$. Współczynniki bioakumulacji Zn i Co kształtowały się na optymalnym poziomie.

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Zinc and cobalt are trace elements which must be monitored in fodder plants because their excess negatively influences the health of animals as well as the growth and development of plants (SPIAK 2000). The literature provides evidence that the presence of cobalt in soil and plants is associated with the biological reduction of N_2 (RUSZKOWSKA et al. 1996). However, there are no data on the qualitative and quantitative changes in the content of these elements in goat's rue biomass and in soil under the influence of different mineral fertilization regimes.

The objective of this study was to determine the impact of fertilization with phosphorus and potassium on changes in zinc and cobalt content in goat's rue (*Galega orientalis* Lam.) biomass and in soil. Another aim was to analyse the absorption of these elements by the biomass of the tested plant and to calculate the factors of Zn and Co bioaccumulation.

MATERIAL AND METHODS

The experiment was carried out in 2005-2007 on a plantation established in 1997 on an experimental field owned by the University of Natural Sciences and Humanities in Siedlce (52°17′N, 22°28′E). The soil on which eastern galega was cultivated was loamy sand (LS) and had neutral reaction. The abundance in absorbable phosphorus and potassium (determined with the Egner-Riehm's method) was medium, whereas the abundance in absorbable magnesium (measured with the Schachtschabel's method) was low. The study included the following fertilization treatments: control, P_{50}, $K_{100,}$ $P_{50}K_{150}$, $P_{50}K_{200}$, $P_{50}K_{250}$. Phosphorus fertilizers as triple superphosphate were applied in autumn while potassium fertilizer in the form of 60% potassium salt was split into two doses (applied in early spring and after the first cut).

The average monthly temperature in the consecutive vegetation seasons was comparable (15.0°C to 15.8°C) and significantly higher compared to the multi-annual records. The average precipitation during the vegetation seasons was lower than the multi-annual sum, except for 2006, when it was slightly higher (by 15.5 mm) because of heavy rainfalls in August, which were three-fold higher than the multi-annual sum (SYMANOWICZ, KALEMBASA 2010).

Three cuts of goat's rue at budding were harvested in each year of the study. During the harvesting of subsequent cuts of goat's rue (*Galega orientalis* Lam.), samples of whole plants were collected, dried and ground. Each year in autumn, soil samples were taken, dried and sieved through a 1mm mesh. Analytical solutions were prepared by dry mineralization of the tested plant's biomass and soil in a muffle furnace with a progressive increase in the temperature up to 450°C. Following complete oxygenation of organic compounds in the tested samples, 5 cm^3 HCl (1:1) was poured onto the ash left in a pot in order to degrade carbohydrates, extract silica and produce anions of inorganic acids and chlorides of tested cations. The excess of HCl was evaporated on a sand bath until dry. The content of the pot was again mixed with HCl (10%) and this solution was filtered into a 100 cm^3 measuring flask by a solid filter in order to separate silica. The content left on the filter was washed three times with diluted HCl and the content in the flask was filled up with water to the line representing analytical solution. The total content of Zn and Co in the tested plant and soil was determined with the ICP-AES method on a plasma-induced emission spectrophotometer (SZCZEPANIAK 2005). The absorption of zinc and cobalt by the biomass of eastern galega and the factors of bioaccumulation of the analysed elements were determined.

The results were statistically processed with a three-way analysis of variance (ANOVA) and significant differences were calculated with the Tukey's test at α = 0.05. The Pearson's simple correlation r coefficients were calculated (Statistica 9.1; StatSoft) in order to identify correlations between the content of Zn and Co in the tested plant and soil and the absorption of these elements with the biomass yield. The significance of correlations was determined by comparing empirical values with critical values *r* at α =0.05.

RESULTS AND DISCUSSION

The average content of zinc in the DM of goat's rue harvested at budding was 53.89 mg kg^{-1} (Table 1), being significantly differentiated by the tested factors and their interrelations. The lowest content of zinc was detected in the biomass of eastern galega harvested in the first cut and in the second year of the experiment. The fertilization with phosphorus and potassium did not have any impact on the content of zinc in DM of goat's rue. The successively higher doses of potassium (K_{100}, $P_{50}K_{150}$) resulted in an increase in the Zn content in the biomass of goat's rue. The highest content of zinc was detected in the tested plant fertilized with 100 kg K ha^{-1}. These

Table 1

The content of zinc in biomass of goat's rue (mg kg^{-1} d.m.)

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	43.10	57.88	48.93	69.57	37.75	42.59	49.97
P_{50}	52.09	60.36	56.77	77.33	43.72	48.11	56.39
K_{100}	52.50	62.88	53.89	71.75	45.82	51.70	56.42
$P_{50}K_{150}$	53.31	56.24	55.51	71.15	43.61	50.30	55.02
$P_{50}K_{200}$	55.36	57.89	48.77	77.15	38.42	46.46	54.01
$P_{50}K_{250}$	51.11	57.14	46.33	58.28	43.86	52.44	51.53
Mean	51.24	58.73	51.70	70.87	42.20	48.60	53.89
$LSD_{0.05}$: cuts (C) – 1.33; years (Y) – 1.33; fertilization (F) – 2.29; YxC – 2.30; CxY – 2.30; FxC – 2.80; CxF – 2.30; FxY – 3.97; YxF – 3.25							

Table 2

The content of cobalt in biomass of goat's rue (mg kg^{-1} d.m.)

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	0.22	0.22	0.20	0.21	0.22	0.20	0.21
P_{50}	0.24	0.25	0.26	0.26	0.25	0.23	0.25
K_{100}	0.27	0.26	0.23	0.31	0.25	0.21	0.26
$P_{50}K_{150}$	0.28	0.25	0.25	0.27	0.27	0.24	0.26
$P_{50}K_{200}$	0.23	0.21	0.20	0.17	0.26	0.21	0.21
$P_{50}K_{250}$	0.26	0.25	0.20	0.23	0.23	0.24	0.23
Mean	0.25	0.24	0.22	0.24	0.24	0.22	0.24
$LSD_{0.05}$: cuts (C) – 0.01; years (Y) – 0.01; fertilization (F) – 0.01; YxC – 0.01; CxY – 0.01; FxC – 0.02; CxF – 0.01; FxY – 0.02; YxF – 0.02							

results were consistent with the studies carried out by Symanowicz et al. (2004), where changes in the content of heavy metals in goat's rue biomass were tested depending on the type of infection with different microbial strains (*Nostoc* cyanobacteria, *Rhizobium* galegae). The content of zinc in goat's rue biomass harvested in consecutive cuts in the experimental years from the objects fertilized with PK was within the permissible limits set for the content of trace elements in feedstuffs. According to Gorlach (1991), this was an optimal content.

The content of cobalt in the tested plant averaged 0.24 mg kg^{-1} d.m. (Table 2) and was significantly differentiated by the analysed factors and their interrelations. The highest concentration of cobalt was detected in the biomass of the tested plant harvested in the first cut (0.25 mg kg^{-1} d.m.) and also in 2005 and 2006 (0.24 mg kg^{-1} d.m.). The highest content of Co was recorded in goat's rue fertilized with K_{100} and $P_{50}K_{150.}$ These results are within the low range of cobalt concentrations in feedstuffs (Gorlach 1991, Ruszkowska 1996). Presumably, the soil reaction close to neutral explains the lower content of cobalt in the biomass of the tested plant. This conclusion is further supported by the results reported by Kalembasa, Symanowicz (2009) in another study on goat's rue, in which the content of cobalt ranged from 0.24 to 0.32 mg kg^{-1} d.m. with the pH of soil at 6.6.

The average absorption of zinc and cobalt with the yield of goat's rue (*Galega orientalis* Lam.) in 2005-2007 was 454.8 g Zn ha^{-1} and 1.95 g Co ha^{-1} (Tables 3 and 4). The plant absorbed most of the elements in yield after the first cut harvested at budding. The highest total amount of zinc and cobalt in three cuts was detected in the first year (651.3 g Zn ha^{-1} and 2.20 g Co ha^{-1}). Regarding the PK fertilization, the highest amounts of zinc and cobalt were accumulated by goat's rue fertilized with the dose of $P_{50}K_{200}$ and $P_{50}K_{150}$, respectively. These calculations were performed according to the publication by Symanowicz, Kalembasa (2010). The calculated correlation

Table 3

Uptake of zinc in the yield of goat's rue (g Zn ha^{-1})

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	150.8	120.4	58.2	518.3	283.9	227.4	343.2
P_{50}	203.1	176.8	95.4	796.5	369.9	321.4	495.9
K_{100}	195.8	139.0	100.2	625.7	355.6	358.3	446.5
$P_{50}K_{150}$	218.0	176.0	121.6	761.3	396.0	422.0	526.4
$P_{50}K_{200}$	298.9	167.9	115.1	918.1	407.2	441.4	588.9
$P_{50}K_{250}$	174.8	112.0	69.5	353.7	313.2	388.0	351.6
Mean	205.5	148.6	93.1	651.3	354.9	358.2	454.8

Table 4

Uptake of cobalt in the yield of goat's rue (g Co ha^{-1})

Fertilization	Cuts – means of 3 years			Years – means of 3 cuts			Mean 2005-2007
	I	II	III	2005	2006	2007	
0	0.77	0.46	0.24	1.56	1.65	1.07	1.43
P_{50}	0.94	0.73	0.44	2.68	2.11	1.54	2.11
K_{100}	1.01	0.57	0.43	2.70	1.94	1.45	2.03
$P_{50}K_{150}$	1.14	0.78	0.55	2.89	2.45	2.01	2.45
$P_{50}K_{200}$	1.24	0.61	0.47	2.02	2.76	1.99	2.26
$P_{50}K_{250}$	0.89	0.49	0.30	1.40	1.64	1.78	1.61
Mean	1.00	0.61	0.40	2.20	2.02	1.62	1.95

coefficients demonstrated significant correlation (r = 0.92) between the absorption of zinc in goat's rue biomass fertilized with PK and the amount of absorbed cobalt. The content of absorbable forms of compounds in soil substantially affects their absorption by plants (SIENKIEWICZ et al. 2011).

The average total content of zinc in the humus horizon was 164.1 mg kg^{-1} (Table 5). Statistical calculations demonstrated significant differentiation of the Zn content in the soil. In the third year, the content of zinc in the soil decreased significantly in relation to the first year. In 2007, the concentration of zinc dropped by 12% compared to 2005. The zinc content in the soil decreased steadily in the consecutive years of the study, which was due to the removal of this element together with yields of the test plant. Having analysed the impact of increasing PK doses on the content of Zn in the soil, it was concluded that the concentration of zinc in the soil increased significantly although not steadily versus the control object. The highest content (174.8 mg kg^{-1}) was detected in the soil sampled from the object fertilized with $P_{50}K_{250}$. It is assumed that phosphorus and potassium fertilizers as well as some anthropogenic factors affecting the experimental field could

Table 5

The content of zinc in soil (mg kg^{-1} d.m.)

Fertilization	Years			Mean 2005-2007
	2005	2006	2007	
0	141.4	161.9	143.1	148.8
P_{50}	172.3	169.2	143.2	161.6
K_{100}	173.2	176.1	145.1	164.8
$P_{50}K_{150}$	175.0	175.6	158.7	169.7
$P_{50}K_{200}$	174.8	173.8	146.0	164.8
$P_{50}K_{250}$	181.7	174.4	114.0	174.8
Mean	169.7	171.8	150.7	164.1
LSD $_{0.05}$: years (Y) – 3.2; fertilization (F) – 5.6; FxY – 9.8; YxF – 7.9				

Table 6

The content of cobalt in soil (mg kg^{-1} d.m.)

Fertilization	Years			Mean 2005-2007
	2005	2006	2007	
0	1.27	1.44	1.52	1.41
P_{50}	1.45	2.04	1.55	1.68
K_{100}	1.40	2.04	1.60	1.68
$P_{50}K_{150}$	1.60	2.03	1.77	1.80
$P_{50}K_{200}$	1.49	2.10	1.44	1.68
$P_{50}K_{250}$	1.65	1.93	1.80	1.80
Mean	1.48	1.93	1.61	1.67
LSD$_{0.05}$: years (Y) – 0.10; fertilization (F) – 0.17.; FxY – 0.30.; YxF – 0.24				

have been sources of zinc. In pot experiments carried out by Symanowicz and Kalembasa (2012) and Żarczyński et al. (2011), the content of zinc was several times lower.

The average content of cobalt in the soil was low: 1.67 mg kg^{-1} (Table 6). The statistical analysis demonstrated the highest amount of this element in the soil sampled after the second year of the study (1.93 mg kg^{-1}). The subsequent doses of phosphorus and potassium fertilizers resulted in an increase in the cobalt concentration, which reached the highest value (1.80 mg kg^{-1}) under the fertilization treatment of $P_{50}K_{150}$. The experiment revealed a significant (r = 0.97) correlation between the content of zinc and cobalt in the soil.

Figures 1 and 2 show factors of Zn and Co bioaccumulation. The mean value of the zinc bioaccumulation factor was 0.33. The lowest value of this factor (0.22) was calculated for the object fertilized with $P_{50}K_{200}$ in the second

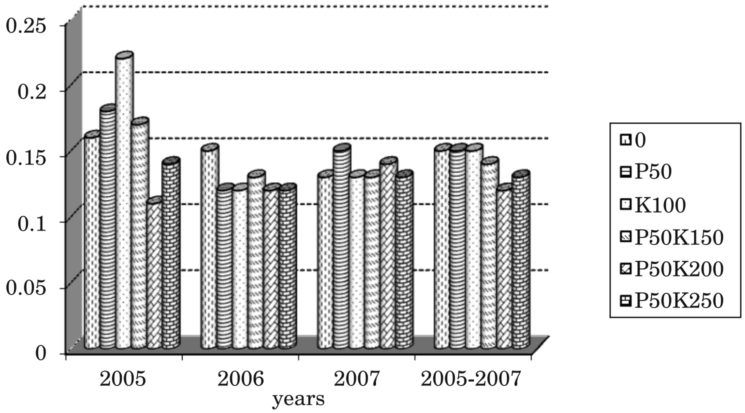

Fig. 1. Bioaccumulation factors of zinc

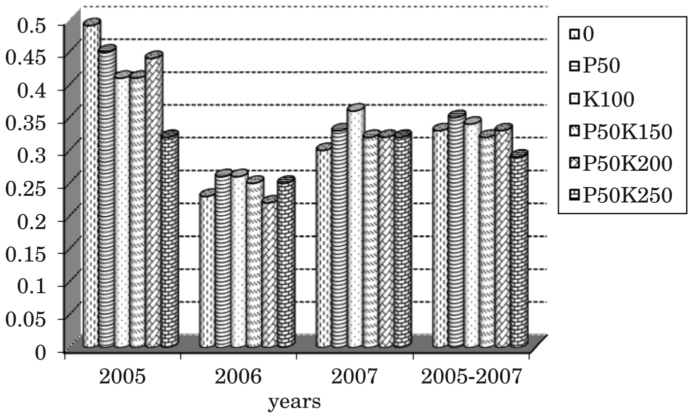

Fig. 2. Bioaccumulation factors of cobalt

year of the experiment. In the following years, the values of the Zn bioaccumulation factor varied (0.42-0.24-0.32). In a study by Baran and Jasiewicz (2009), the zinc bioaccumulation factor was approximately ten-fold higher, which most probably was connected with the acid reaction of soil and other test plants.

The calculated factors of cobalt bioaccumulation were on a comparable level of 0.12-0.14 for individual objects and within the range of 0.12-0.16 for the experimental years.

CONCLUSIONS

1. The incremental doses of phosphorus and potassium fertilizers had a significant impact on the zinc content in goat's rue biomass, increasing it versus the control object. The highest content of zinc was detected in the tested plant fertilized with K_{100}. Goat's rue accumulated the highest amounts of cobalt under the fertilization treatments of K_{100} and $P_{50}K_{150}$.

2. The recorded concentrations of zinc and cobalt in goat's rue biomass were within the optimal values in respect of the permissible limits of trace elements in feedstuffs.

3. The highest absorption of zinc and cobalt by goat's rue during the vegetation season was recorded for the fertilization treatments of $P_{50}K_{200}$ and $P_{50}K_{150}$, respectively.

4. The highest content of zinc was detected in the soil fertilized with $P_{50}K_{250}$, and the highest concentration of cobalt was measured in the soil fertilized with $P_{50}K_{150}$ and $P_{50}K_{250}$.

5. The highest values of factors of Zn and Co bioaccumulation were on an optimal level.

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EFFECT OF FERTILISATION TECHNIQUE ON SOME INDICES OF NUTRITIONAL VALUE OF SPRING TRITICALE GRAIN

Katarzyna Wojtkowiak[1], Arkadiusz Stępień[2], Małgorzata Warechowska[1], Iwona Konopka[3], Andrzej Klasa[4]

[1]Chair of Safety Foundations
[2]Chair of Agricultural Systems
[3]Chair of Food Plant Chemistry and Processing
[4]Chair of Agricultural Chemistry and Environment Protection
University of Warmia and Mazury in Olsztyn

Abstract

Triticale is a cereal which may compete with other species of crop plants in terms of the nutritional value. This grain crop enjoys an increasing popularity among farmers as well as food and animal feed manufacturers, hence there is a growing interest in foliar fertilisation with micronutrients in order to ensure fast and effective nutrition.

This study discusses the effect of soil fertilisation or soil and foliar fertilisation with nitrogen (with or without multi-component fertilisers) on the content of macronutrients (nitrogen, phosphorus, potassium, magnesium and calcium), total protein and protein fractions in the grain of spring triticale of the Andrus cultivar grown in north-eastern Poland. The fertilisation of cv. Andrus spring triticale involved a pre-sowing application of ammonium nitrate, an application of 46% urea in the period of tillering, (with and without the fertiliser Azofoska) and an application of ammonium nitrate as well as a soil and the foliar application of urea (10% solution, with and without an addition of Ekolist Z) in the period of stem shooting. Differentiation of the experimental results on the content of the analysed macronutrients as well as the protein composition was caused by differences of habitat conditions in the two experimental seasons. Higher nitrogen doses applied as a split urea dose resulted in a higher concentration of phosphorus, calcium and magnesium in grain from cv. Andrus spring triticale. The dose of 120 kg N ha^{-1} in ammonia nitrate and urea applied to soil or as foliar spray resulted in an increase of total protein. The accumulation of glutenins in triticale grain usually increased in response to the higher dose of nitrogen (120 kg ha^{-1}) applied both with and without the multi-component fertilisers. The foliar supplementation with urea and micronutrients in multi-component fertilisers contributed to a reduced accumulation of alpha/beta-type gliadins, regardless of the nitrogen dose.

Keywords: spring triticale, fertilization, macroelements, protein composition.

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dr inż. Katarzyna Wojtkowiak, Chair of Safety Foundations, University of Warmia and Mazury in Olsztyn, Heweliusza 10, 10-718 Olsztyn, Poland, phone (+48) 895245614, e-mail: katarzyna.wojtkowiak@uwm.edu.pl

WPŁYW TECHNIKI NAWOŻENIA NA WYBRANE WSKAŹNIKI WARTOŚCI ODŻYWCZEJ ZIARNA PSZENŻYTA JAREGO

Abstrakt

Pszenżyto uznawane jest za zboże, które może konkurować pod względem wartości odżywczej z innymi gatunkami zbóż i cieszy się coraz większym zainteresowaniem rolników, przetwórców żywności i paszy. Obserwuje się wzrost zainteresowania nawożeniem dolistnym mikroelementami, które zapewnia efektywne i szybkie dostarczenie potrzebnych składników pokarmowych.

W pracy omówiono wpływ nawożenia azotem doglebowo lub doglebowo i dolistnie, bez i z udziałem nawozów wieloskładnikowych, na zawartość makroskładników (azotu, fosforu, potasu, magnezu, wapnia), białka ogółem i frakcji białka w ziarnie pszenżyta jarego odmiany Andrus uprawianego w warunkach północno-wschodniej Polski.

W nawożeniu pszenżyta jarego odmiany Andrus zastosowano przedsiewnie saletrę amonową oraz w okresie krzewienia mocznik 46% bez azofoski lub z azofoską, a w okresie strzelania w źdźbło saletrę amonową, mocznik doglebowo i dolistnie (roztwór 10%) bez dodatku lub z dodatkiem ekolistu Z. Na zróżnicowanie wyników badań, zarówno zawartości makroelementów, jak i białka, w dużym stopniu wpłynęły warunki siedliskowe w latach realizowanego doświadczenia. Wyższy poziom nawożenia azotem z zastosowaniem części dawki mocznika w formie oprysku wpłynął na większą zawartość fosforu, wapnia i magnezu w ziarnie pszenżyta jarego odmiany Andrus. Dawka 120 kg ha^{-1} N w formie saletry amonowej i mocznika wprowadzona doglebowo i dolistnie wpłynęła na zwiększenie zawartości białka ogółem. Pod wpływem większych dawek azotu (120 kg ha^{-1}) zastosowanych bez dodatku i z dodatkiem nawozów wieloskładnikowych zwiększało się przeważnie nagromadzenie glutenin w ziarnie pszenżyta. Dolistne dokarmianie mocznikiem oraz mikroelementami w nawozach wieloskładnikowych, niezależnie od dawki azotu, przyczyniło się do zmniejszenia nagromadzenia gliadyn α i β.

Słowa kluczowe: pszenżyto jare, nawożenie, makroelementy, frakcje białek.

INTRODUCTION

The nutritional value of triticale grain from spring cultivars in comparison to winter ones is distinguished by a higher protein content (Petkow et al. 2000). Cultivars with a high nutritional value can be bred through the development and appropriate choice of parental forms, selection during the cultivation (Makarska et al. 2010) and implementation of suitable cultivation and agronomic practices.

The content of nutrients is an important quality feature of edible and fodder grains (Rachoń, Szumiło 2009). This factor significantly determines the supply of a daily food ratio of nutrients, thus ensuring production of wholesome grain is a high priority in maintaining human and animal health (Konopka et al. 2012).

The current situation on the Polish market has stimulated food producers' demand for good quality cereal grain. Triticale is a cereal which may compete with other cereal species in terms of nutritional value. Despite its advantages, this species is grown mainly for animal feeds (Harmoney, Thompson 2005). None of triticale cultivars is recommended for bread baking, although numerous studies have demonstrated its suitability for this purpose (Tohver et al. 2005). Some research has been carried out to show that triticale grain (like grain of other cereal species) can also be used in the food industry as raw material for flour and bread production (Amiour et al. 2002).

In recent years, there has been an increasing interest in foliar fertilisation with micronutrients, its superiority to soil fertilisation and benefits (particularly in the case of intensive nitrogen fertilisation) (Domska et al. 2009, Knapowski et al. 2012). Since foliar supplementation ensures a fast and effective supply of necessary nutrients, it appears highly appealing and can be recommended in agricultural practice as a cost-effective agricultural factor (Johansson et al. 2001, Wojtkowiak, Domska et al. 2009).

This study discusses the effect of soil or soil and foliar fertilisation with nitrogen, with or without addition of multi-component fertilisers, on the content of macronutrients (nitrogen, phosphorus, potassium, magnesium, and calcium), total protein and protein fractions in grain of spring triticale of the Andrus cultivar grown in the north-eastern Poland.

In the context of the research objectives, a zero hypothesis (H_0) claiming the lack of significant differences among levels of experimental factors was verified. Should the zero hypothesis (H_0) be rejected, a new alternative hypothesis (H_A) would be tested, stating that there were significant differences between the analyzed nitrogen fertilisation techniques, affecting selected quality indices of spring triticale grain.

MATERIAL AND METHODS

In 2010-2011, a field experiment was conducted in the Educational and Research Station of the University of Warmia and Mazury, in Tomaszkowo (53°72′N; 20°42′E). It was set up on typical brown soil with the texture of light loam, developed on the subsoil composed of very fine soil. In the Polish soil valuation system, the soil belonged to class III b. It was characterised by acid reaction, a low content of organic carbon (C_{org}.), a high content of phosphorus and potassium and a medium content of magnesium and calcium.

The experiment was established in a random block design with three replications. The size of a plot was 6.25 m^2, including 4.0 m^2 for harvest. The sowing rate of cv. Andrus grains was 282.1 kg of seeds ha^{-1}, with inter-row spacing of 10.5 cm. The preceding crop was winter triticale.

Fertilisation with phosphorus and potassium was the same for all treatments (30.2 kg ha^{-1} P in the form of triple superphosphate 46% and 83.1 kg ha^{-1} K in potassium salt). Nitrogen fertilisation was differentiated (Table 1). Two doses of nitrogen, 80 and 120 kg N ha^{-1}, were applied and

Table 1

Field experiment design in 2010-2011

Objects	Total N fertilisation (kg N ha^{-1})	Fertiliser type and application time (kg N ha^{-1} dose)		
		before sowing	tillering phase (BBCH 23-29)	stem shooting phase (BBCH 31-32)
1	80	-	urea (40)	urea (40)
2	80	-	urea (20) Azofoska (20)	urea (40)
3	80	-	urea (40)	urea 40*
4	80	-	urea (40)	ammonium nitrate (32) + Ekolist* (8)
5	120	ammonium nitrate (40)	urea (40)	urea (40)
6	120	ammonium nitrate (40)	urea (40) Azofoska (20)	urea (40)
7	120	ammonium nitrate (40)	urea (40)	urea (40)*
8	120	ammonium nitrate (40)	urea (40)	ammonium nitrate (32) + Ekolist*(8)

*foliar fertilisation

each dose were split into 40 kg N ha^{-1} sub-doses. Ammonium nitrate was applied before sowing, and 46% urea (both with and without Azofoska in a dose corresponding to 20 kg of nitrogen) was applied in the tillering period. Fertilisation during the stem shooting phase included an application of ammonium nitrate, urea to soil and sprayed over leaves in 10% solution, without or with Ekolist Z in a dose of 2.0 dm^{-3} ha^{-1}.

The experiment (in treatments 2, 4, 6, 8) was supplemented with multi-component fertilisers containing macronutrients and a set of properly selected micronutrients, as recommended in agricultural practice (Table 2).

Agronomic treatments were performed according to the requirements of spring triticale.

Temperature and precipitation were monitored during the experiment (Figure 1). The mean temperature in both years was similar, and the monthly distribution of temperatures did not differ from the means recorded

Table 2

Composition of the applied multi-fertilisers

Type of multi-fertiliser	N	P	K	Mg	S	Cu	Zn	Mn	Fe	Mo	B
Ekolist (g dm^{-3})	120.0	0.00	65.0	20.00	5.0	5.00	2.500	0.50	1.00	0.02	5.000
Azofoska (%)	13.6	1.83	15.9	2.71	9.2	0.18	0.045	0.27	0.17	0.04	0.045

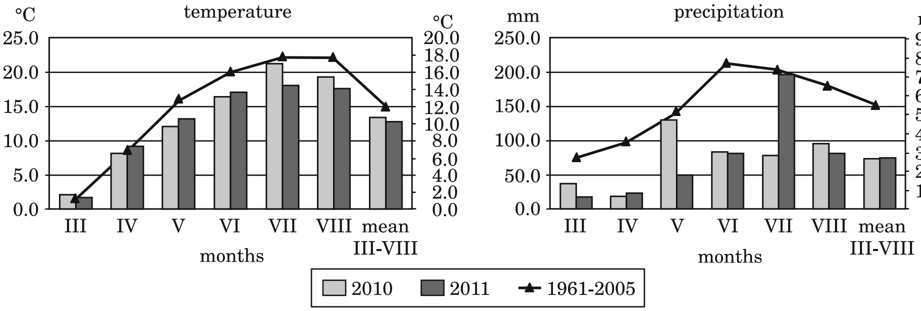

Fig. 1. Meteorological conditions during the investigations

in a multi-year period. Precipitation during the triticale vegetative period was varied. Noteworthy was the scanty rainfall in April. In May 2010, the amount of precipitation was close to the multi-annual mean (51.1), while in 2011, it more than doubled the mean sum of precipitation in the long-term period (131.9 mm). Between July and August, a higher rainfall sum was recorded than in the multi-year period, particularly in July 2011, when precipitation was very high (202 mm).

Each year, after harvest, grain samples were collected and subjected to chemical analyses, using methods applied in agricultural chemistry. Grain samples were wet mineralised in order to determine macronutrients in H_2SO_4 with H_2O_2 added as an oxidant. The following elements were determined: nitrogen – with the hypochlorite method, phosphorus – with the vanadium-molybdenum method, potassium, calcium – by ESA atomic spectroscopy, and magnesium – by ASA, using an AA-6800 Schimadzu apparatus.

Protein fractions were determined according to the method proposed by Wieser at al. (1998). Albumins were extracted with distilled water, globulins with a mixture of NaCl and $HKNaPO_4$, prolamins with 60% ethanol and glutenins in a mixture of 50% propanol-1+2 m urea + tris HCl and 1+ DTE under nitrogen. The determinations were performed on a Hewlett Packard series 1050 instrument. The results were analysed with the use of the HPLC software according to HP 3D ChemStation in mAU·s units. They were converted and presented as percentage values. All determinations were made in two replications.

The statistical calculations consisted of a two-factor variance analysis, compliant with the mathematical model of an experimental layout in random blocks. Mean values and the standard error of the means were determined for individual experimental objects. The calculations took into account the variability of a given feature depending on the year of cultivation, the fertilising technique and the interaction of the factors mentioned. Apart from the basic statistical parameters, statistically homogenous groups were distinguished (groups with no differences between means) using the Tukey's test at the significance level of α=0.05. Excel and Statistica software packages were used to perform the calculations and statistical analyses.

RESULTS AND DISCUSSION

Triticale enjoys an increasing popularity among farmers as well as food and animal feed manufacturers. The research conducted by KAMYAB et al. (2012) confirms a high nutritive value of this cereal species relative to its lower environmental requirements in comparison to wheat.

The spring triticale cultivar called Andrus was entered in the Polish national register of cultivars in 2009, classified into the group of very good, high yielding cultivars.

The content of such nutrients as nitrogen, potassium, phosphorus, calcium or magnesium proves the nutritive value of a cultivated variety (ŚCIGALSKA et al. 2000, KNAPOWSKI et al. 2010). In grain from cv. Andrus grown in 2010-2011 on light soil, fertilised with nitrogen in doses of 80 and 120 kg ha^{-1} applied into soil or into soil and on leafage, with and without an addition of micronutrients (Azofoska and Ekolist), no significant differences were found in the selected mineral components. In general, the chemical composition of the grain varied only slightly, which precludes any firm conclusion about which of the fertilisation technologies was the most effective. This was confirmed by the statistical analysis in both vegetative seasons and on the annual means.

The mean nitrogen content in cv. Andrus triticale grain ranged from 19.40 to 20.87 g kg^{-1} (Table 3). In the first year of cultivation, the highest value of nitrogen was found after the application of urea in two doses of 40 kg ha^{-1} into the soil and foliar application (21.27 g kg^{-1}). Nitrogen fertilisation (80 kg ha^{-1}) in the form of urea (BBCH 23-29) and ammonium nitrate, together with Ekolist (object 4) in the two years of cultivation contributed to an increase in the nitrogen accumulation in grain compared to the higher level of fertilisation (object 8). An increase in the nitrogen fertilisation to 120 kg and supplementation with a multiple-component fertilisers did not lead to an increase in the nitrogen content in grain, unlike in the research conducted by other authors (SZPUNAR-KROK et al. 2006, KNAPOWSKI et al. 2010). This could have been caused by the unfavourable weather conditions in the two years. In the current experiment, no significant differences were found in the content of potassium, magnesium and, in most cases, phosphorus and calcium between the experimental variant fertilisation treatments. According to ŚCIGALSKA et al. (2000), the concentration of macronutrients is a cultivar-specific characteristic. In our research, the levels of potassium, calcium and magnesium were higher and the level of phosphorus was lower than given in the charts showing nutritive values of cereals produced in Poland (IZ PIB – INRA 2009). Moreover, in the research conducted by KNAPOWSKI (2010), the values for potassium and calcium in spring triticale cultivar Kargo were lower. In our experiment, the grain of cultivar Andrus was characterised by a higher level of potassium and calcium and a lower level of phosphorus than grain of spring wheat Torka and Tonacja cultivars

Table 3

Content of macronutrients in grain of cv. Andrus spring triticale

Years	Objects	(g kg^{-1} of d.m.)				
		nitrogen	potassium	phosphor	calcium	magnesium
2010	1	20.93 ^{bc} ± 0.82	5.97 ^{c} ± 0.55	3.56 ^{bc} ± 0.13	1.48 ^{ab} ± 0.09	1.44 ^{abc} ± 0.02
	2	20.81 ^{abc} ± 0.65	5.42 ^{abc} ± 0.12	3.5 ^{b} ± 0.15	1.35 ^{a} ± 0.10	1.44 ^{abc} ± 0.08
	3	21.27 ^{c} ± 0.19	5.3 ^{ba} ± 0.05	3.69 ^{bcde} ± 0.11	1.35 ^{a} ± 0.06	1.59 ^{bc} ± 0.14
	4	20.80 ^{abc} ± 0.24	5.63 ^{bc} ± 0.15	3.81 ^{c} ± 0.20	1.35 ^{a} ± 0.08	1.59 ^{bc} ± 0.07
	5	20.10 ^{abc} ± 0.70	5.97 ^{c} ± 0.03	3.97 ^{de} ± 0.03	2.09 ^{d} ± 0.02	1.73 ^{c} ± 0.12
	6	19.40 ^{ab} ± 0.40	4.98 ^{a} ± 0.12	3.01 ^{a} ± 0.12	1.41 ^{ab} ± 0.11	1.44 ^{abc} ± 0.19
	7	20.57 ^{abc} ± 0.77	5.63 ^{bc} ± 0.20	3.85 ^{bcde} ± 0.16	1.48 ^{ab} ± 0.05	1.44 ^{abc} ± 0.09
	8	19.60 ^{ab} ± 0.40	5.30 ^{abc} ± 0.22	2.91 ^{a} ± 0.09	1.60 ^{abc} ± 0.20	1.44 ^{abc} ± 0.15
2011	1	20.41 ^{abc} ± 0.59	5.63 ^{bc} ± 0.09	3.69 ^{bcde} ± 0.11	1.72 ^{bc} ± 0.04	1.44 ^{abc} ± 0.06
	2	20.27 ^{abc} ± 0.07	5.63 ^{bc} ± 0.16	3.97 ^{de} ± 0.13	1.72 ^{bc} ± 0.04	1.44 ^{abc} ± 0.10
	3	20.33 ^{abc} ± 0.18	5.63 ^{bc} ± 0.08	3.93 ^{cde} ± 0.07	1.60 ^{abc} ± 0.10	1.44 ^{abc} ± 0.09
	4	20.62 ^{abc} ± 0.38	5.97 ^{c} ± 0.08	3.56 ^{bcde} ± 0.05	1.54 ^{abc} ± 0.06	1.33 ^{ab} ± 0.11
	5	20.22 ^{abc} ± 0.14	5.80 ^{bc} ± 0.20	3.77 ^{bcde} ± 0.08	1.84 ^{bc} ± 0.06	1.22 ^{a} ± 0.01
	6	19.77 ^{abc} ± 0.23	5.97 ^{c} ± 0.13	3.56 ^{bc} ± 0.14	1.60 ^{abc} ± 0.10	1.30 ^{ab} ± 0.01
	7	20.87 ^{abc} ± 0.14	5.97 ^{c} ± 0.08	4.05 ^{e} ± 0.05	2.12 ^{d} ± 0.12	1.73 ^{c} ± 0.03
	8	19.35 ^{a} ± 0.06	5.63 ^{bc} ± 0.13	3.65 ^{bcd} ± 0.06	1.60 ^{abc} ± 0.07	1.15 ^{a} ± 0.04
Mean for fertilisation × years	1	20.67 ^{c} ± 0.44	5.80 ^{a} ± 0.25	3.62 ^{b} ± 0.08	1.60 ^{a} ± 0.08	1.44 ^{ab} ± 0.03
	2	20.54 ^{bc} ± 0.31	5.52 ^{a} ± 0.10	3.74 ^{bc} ± 0.16	1.54 ^{a} ± 0.12	1.44 ^{ab} ± 0.05
	3	20.80 ^{c} ± 0.29	5.46 ^{a} ± 0.10	3.81 ^{bc} ± 0.09	1.48 ^{a} ± 0.09	1.51 ^{ab} ± 0.08
	4	20.71 ^{c} ± 0.19	5.80 ^{a} ± 0.11	3.68 ^{b} ±0.11	1.45 ^{a} ± 0.07	1.46 ^{ab} ± 0.09
	5	20.16 ^{abc} ± 0.29	5.89 ^{a} ± 0.10	3.87 ^{bc} ± 0.07	1.97 ^{b} ± 0.08	1.48 ^{ab} ± 0.15
	6	19.59 ^{ab} ± 0.21	5.48 ^{a} ± 0.29	3.28 ^{a} ± 0.17	1.51 ^{a} ± 0.08	1.37 ^{ab} ± 0.09
	7	20.72 ^{c} ± 0.33	5.80 ^{a} ± 0.13	3.95 ^{c} ± 0.09	1.80 ^{b} ± 0.19	1.59 ^{b} ± 0.09
	8	19.48 ^{a} ± 0.18	5.48 ^{a} ± 0.13	3.27 ^{a} ± 0.21	1.60 ^{a} ± 0.06	1.30 ^{a} ± 0.11
Mean for years	2010	20.43 ^{a} ± 0.24	5.53 ^{a} ± 0.12	3.53 ^{a} ± 0.17	1.51 ^{a} ± 0.07	1.51 ^{b} ± 0.06
	2011	20.22 $^{\mathrm{a}}$ ± 0.21	5.78 ^{b} ± 0.09	3.77 ^{b} ± 0.08	1.72 ^{b} ± 0.09	1.38 ^{a} ± 0.04

± standard error of the mean (SEM)
a, *b*, *c*... - with the same letter are not significantly different ($p < 0.05$)

examined by Rachoń et al. (2012) and Rachoń and Szumiło (2009). Fertilisation with a dose of 120 kg ha^{-1} nitrogen in the form of ammonium nitrate and urea applied into soil (object 5) in the first year contributed raised the phosphorus content in triticale grain by 11.52% and calcium by 41.22% in comparison to soil fertilisation with urea in a dose of 80 kg ha^{-1} (object 1). The highest content of phosphorus (4.05), calcium (2.12) and magnesium (1.73) was observed in triticale grain in the second year of its cultivation under the higher dose of nitrogen and with urea applied in the form of a spray at stage BBCH 31-32. Nogalska et al. (2012) found greater differentiation of nitrogen, phosphorus, potassium, calcium and magnesium uptake by spring triticale of cv. Wanad in each year of the research after application of Amofosmag 4, which ensured better assimilation of nutrients from multiple-component fertilisers. Particular attention should be paid to the high content of calcium in triticale grain of cultivar Andrus (ranging on average from 1.45 to 1.97). Its concentrations are much higher than detected by Knapowski et al. (2010), who determined 0.26 g ha^{-1} of calcium in grain from cv. Kargo spring triticale. In an experiment carried out by Wojtkowiak and Domska (2009), cultivar Gabo grown under different fertilisation regimes was also characterised by less calcium in grain (from 0.4 to 0.6 g ha^{-1}). A high level of calcium (about 1.0 g·ha^{-1}) was found by Nogalska et al. (2012) in grain of cultivar Wanad cultivated on light soil, but only in the second year of triticale cultivation.

The positive effect of nitrogen fertilisation can be observed in the analysis of total protein (nitrogen x 5.7) in cereal kernels. Spring triticale grain is a good source of proteins with a favourable amino acid composition (Pisulewska et al. 2000). The results of our research demonstrated a growing trend caused by fertilisation applied in the experiment only in the protein content (Figure 2). Evaluation of the fertilisation effect on the grain protein content is ambiguous and results published in literature are often contradictory. Domska et al. (2009) confirmed the effect of fertilisation with higher doses of nitrogen fertilisers on changes in the grain quality parameters. Makarewicz et al. (2012) recorded the highest amount of total protein in winter wheat grain in objects fertilised with a dose of 10% urea solution, which was not confirmed by Jarecki et al. (2012). On the other hand, according to Lestingi et al. (2010), a dose of 50 kg ha^{-1} is sufficient to ensure good quality of triticale grain, including a satisfying total protein content. Foliar supplementation with urea did not have a significant effect on the total protein content. Orlik et al. (2005) report that the content of protein in objects fertilised with ammonium nitrate applied to the soil and with urea sprayed over leaves was very similar, which is why - while comparing these forms of fertilisation - it is difficult to establish which had a favourable effect on the chemical composition of grain, the difficulty that also emerged in our exper-

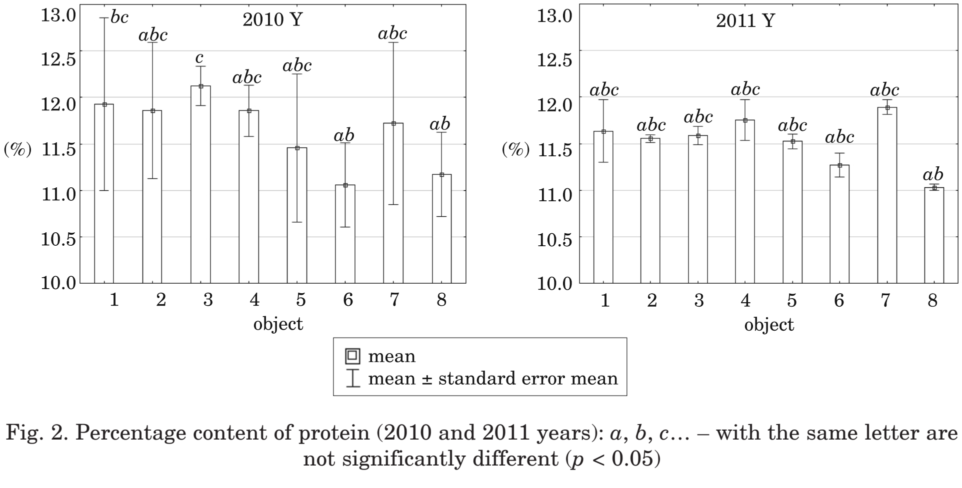

Fig. 2. Percentage content of protein (2010 and 2011 years): *a*, *b*, *c*… – with the same letter are not significantly different ($p < 0.05$)

iment. The absence of differences between the analysed fertilisation objects may stem from the genetic traits of the cultivar and from the changeable weather conditions in individual seasons. The course of weather changes not only yields of grains but also the grain protein content (Erekul, Köhn 2006). Jaskulski et al. (2011) also noted an increase in the protein content in grain under less rainfall in April and May, and more rainy June and July. Additionally, the protein concentration increased along with a decreasing calcium content, but grew as the phosphorus content increased. During our experiment, the season of 2010 was characterised by low precipitation in April and relatively high precipitation in May. The amount of protein in spring triticale grain did not increase in response to higher water supply, analogously to the research by Rakowski (2003).

An increase in the nitrogen fertilisation doses results in an increase in grain yield and in protein accumulation, but reduces grain quality (Johansson et al. 2001). Intensive fertilisation is not without significance for the protein fraction ratios. Research carried out in recent years indicates that splitting up nitrogen fertilisation into partial applications during the vegetative period and an application technique method results in changes in the protein fraction composition (Johnanson et al. 2004). The content of structural proteins (albumins and globulins) in cereal grain ranged from 21.13 to 24.01% in 2010-2011 (Table 4). The level of building protein and storage protein (glutenins) accumulation was lower in the relation to ratios of individual groups of proteins. Similar dependencies between protein fractions were found by Domska et al. (2002) in winter triticale grain from cultivar Malno. The highest percentage of the albumin and globulin fraction (24.01%) was found in the first year of cultivation under the dose 80 kg ha^{-1} nitrogen in the form of urea together with Azofoska at stage BBCH 23-29 (object 2). An increase in the nitrogen fertilisation to 120 kg ha^{-1} in the form of soil applied urea (23.47% object 5) contributed to a significant increase in albumins and globulins in triticale grain. In the second year (2011), a higher amount of structural proteins was observed in grain fertilised with 80 kg ha^{-1} nitrogen in the form of urea applied to leafage at stage BBCH 31-32 (object 3) and with the higher dose of nitrogen (120 kg ha^{-1}) applied to soil, or applied to soil and over leaves in the form of urea (object 5 and 7). The share of gliadin fraction in the examined triticale cultivar grain of was varied, being the highest in the grain from plants fertilised by the soil application of urea in a dose of 80 kg ha^{-1} (object 1), both in the first and in the second year of the experiment (56.69 and 57.93%). A 1.95% increase was caused by the higher level of fertilisation applied in the form of urea together with Azofoska at stage BBCH 23-29 (object 2 and 6) in 2010. On the other hand, in most cases (except for object 8), grain of triticale cultivated under the lower fertilisation level in the second year of contained significantly more storage protein of the gliadin fraction.

In 2010-2011, the content of storage protein of the glutenin fraction ranged from 20.65 to 24.66%. Fertilisation generally increased the share of

Table 4

True protein content (%) of grain from cv. Andrus spring triticale

Years	Object	Albumins and globulins	Gliadins	Glutenins
2010	1	21.13^{a} ± 0.53	56.69^{gh} ± 0.43	22.18^{bc} ± 0.10
	2	24.01^{d} ± 0.34	53.26^{ab} ± 0.80	22.73^{cdef} ± 0.46
	3	23.08^{bcd} ± 1.55	54.08^{bcd} ± 0.78	22.84^{cdef} ± 0.77
	4	22.92^{bcd} ± 0.05	53.28^{ab} ± 0.14	23.79^{fg} ± 0.09
	5	23.47^{cd} ± 0.09	53.69^{bc} ± 0.14	22.84^{cdef} ± 0.05
	6	22.20^{abc} ± 0.13	54.30^{bcde} ± 0.29	23.50^{def} ± 0.43
	7	22.79^{abcd} ± 0.71	54.24^{bcde} ± 0.12	22.97^{cdef} ± 0.59
	8	23.04^{bcd} ± 0.70	52.30^{a} ± 0.69	24.66^{g} ± 0.01
2011	1	21.43^{ab} ± 0.13	57.93^{h} ± 0.39	20.65^{a} ± 0.52
	2	21.99^{abc} ± 0.32	55.63^{efg} ± 0.61	22.38^{bcd} ± 0.30
	3	22.30^{abcd} ± 0.04	56.37^{fg} ± 0.34	21.34^{ab} ± 0.38
	4	22.22^{abc} ± 0.05	55.25^{def} ± 0.04	22.52^{bcde} ± 0.01
	5	22.55^{abcd} ±0.05	55.35^{defg} ± 0.34	22.10^{bc} ± 0.38
	6	22.44^{abcd} ± 0.30	53.91^{bcd} ± 0.23	23.65^{efg} ± 0.07
	7	22.80^{abcd} ± 0.13	54.76^{cde} ± 0.01	22.45^{bcde} ± 0.14
	8	21.97^{ab} ± 0.13	55.36^{defg} ± 0.23	22.67^{cdef} ± 0.10
Average for fertilisation × years	1	21.28^{a} ± 0.24	57.31^{c} ± 0.43	21.42^{a} ± 0.49
	2	23.00^{b} ± 0.61	54.44^{ab} ± 0.80	22.56^{bc} ± 0.24
	3	22.69^{b} ± 0.67	55.22^{b} ± 0.75	22.09^{ab} ± 0.56
	4	22.57^{b} ± 0.20	54.27^{ab} ± 0.57	23.16^{cd} ± 0.37
	5	23.01^{b} ±0.27	54.52^{ab} ± 0.50	22.47^{bc} ± 0.27
	6	22.32^{ab} ± 0.15	54.11^{a} ± 0.19	23.57^{d} ± 0.18
	7	22.79^{b} ± 0.30	54.50^{ab} ± 0.16	22.71^{bc} ± 0.29
	8	22.50^{b} ± 0.42	53.83^{a} ± 0.93	23.66^{d} ± 0.58
Average for years	2010	22.83^{b} ± 0.17	53.98^{a} ± 0.41	23.19^{b} ± 0.33
	2011	22.21^{a} ± 0.22	55.57^{b} ± 0.29	22.22^{a} ± 0.50

± standard error of the mean (SEM)
a, *b*, *c*... - with the same letter are not significantly different ($p < 0.05$)

this protein when a higher dose of nitrogen was applied, both in the first and in the second year of cultivation. A higher accumulation of the glutenin fraction (24.66 and 23.79%) was observed in response to the fertilisation with nitrogen as urea and ammonium nitrate together with Ekolist at stage BBCH 31-32 (object 4 and 8) in the first year and fafter the application of urea together with Azofoska at stage BBCH 23-29 in the first and the second year of cultivation (23.50% and 23.65, object 6). According to DOMSKA and RACZKOWSKI (2009), fertilisation of Maja cultivar spring triticale with micronutrients results in an increased share of one fraction of storage proteins, prolamins or glutenins. However, KONOPKA et al. (2007) demonstrated that the accumulation of individual fractions of proteins in wheat grain was largely affected by water shortage. Under water stress conditions, the accumulation of albumins, globulins, γ-gliadins and glutenins decreased. The content of gliadins and glutenins is important in the protein composition of grains used in the baking industry. The above proteins play a key role in securing unique rheological properties of wheat grain dough, and their variable concentrations depend on the genotype, growth conditions and technological processes (WIESER 2007).

The specific effect of nitrogen fertilisation on quantitative and qualitative changes in the composition of glutamin protein fraction in triticale grain remains unknown. As shown by several studies (DOMSKA et al. 2002, BARCZAK, KNAPOWSKI 2008), fertilisation with nitrogen is conductive to an increase in the content of gliadin storage proteins in various cereal species. Although the technological quality of grain improves, the content of toxic α/β gliadins can also rise (Figure 3). Proper cultivation techniques may lead to obtaining grain with a lower content of harmful gliadin fractions – gluten fractions. In the present experiment, the content of α/β gliadin fractions ranged from 28.71 to 31.66% of the protein content in the first year of cultivation and from 30.38 to 31.92% in the subsequent year. Soil application off 80 kg ha^{-1} nitrogen in the form of urea contributed to the highest accumulation of this protein fraction in the context of the two years of the field experiment. An increase in fertilisation from 80 to 120 kg ha^{-1}, applied to soil as urea together with Azofoska at stage BBCH 23-29 contributed to a significant growth of this protein fraction (object 6) only in the first year of cultivation.

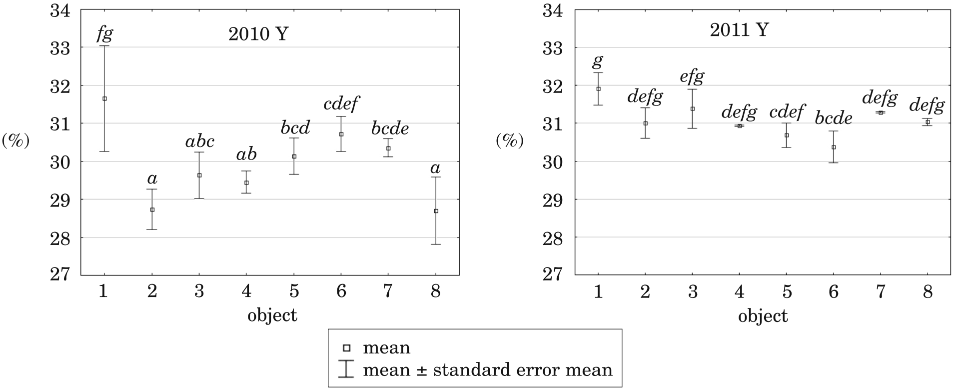

Fig. 3. Percentage part of α and β gliadins in total protein content in grain from cv. Andrus spring triticale (2010 and 2011): *a*, *b*, *c*... – with the same letter are not significantly different ($p < 0.05$)

CONCLUSIONS

1. Differences in the experimental results on concentrations of macronutrients and protein content were caused by differences of habitat conditions in the two examined seasons.

2. A higher content of phosphorus, calcium and total protein was found in the grain of triticale fertilised with a dose of 120 kg ha^{-1} nitrogen in the form of ammonium nitrate and urea applied to soil and on leaves.

3. The accumulation of glutenins in triticale grain usually rose in response to higher doses of nitrogen (120 kg ha^{-1}), applied with and without multi-component fertilisers.

4. The introduction of foliar supplementation of urea and micronutrients in multi-component fertilisers contributed to reduced accumulation of α and β gliadins regardless of the nitrogen dose.

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CHANGES IN THE CONTENT OF SOME MICRONUTRIENTS IN SOIL CONTAMINATED WITH HEATING OIL AFTER THE APPLICATION OF DIFFERENT SUBSTANCES*

Mirosław Wyszkowski, Veranika Sivitskaya

Chair of Environmental Chemistry University of Warmia and Mazury in Olsztyn

Abstract

Petroleum substances enter the environment, where they cause its degradation. Contamination has an adverse effect on the protective function of soil and soil physicochemical properties, leading to disorders in the soil enzymatic activity, depressing soil fertility and impairing the growth, development and chemical composition of plants. We should therefore look for methods which will enable us to reduce the effect of petroleum substances or even to recover the original properties of soil. The objective of the present study has been to determine the effect of substances applied to soil on the content of cadmium, lead, chromium and manganese in soil contaminated with incremental doses of heating oil (0-20 g kg^{-1} of soil). The effect of incremental doses of heating oil on the content of analyzed trace metals in soil was varied and depended on the application of alleviating substances to soil. The increasing levels of heating oil contributed to a decrease in the content of cadmium and chromium in soil, but had no such regular influence on the content of lead or manganese. None of the applied alleviating substances had a reducing effect on the content of manganese, cadmium, chromium or lead in the tested soil. Zeolite was an exception in that it reduced the content of cadmium and lead in soil. The content of heavy metals in soil, even when contaminated with the highest doses of heating oil, did not exceed the permissible levels defined in the Regulation of the Ministry of the Environment of 9 September 2002 and therefore allowed us to classify the soil as a zero contamination degree according to the IUNG Puławy classification.

Keywords: heating oil contamination, nitrogen, compost, bentonite, zeolite, calcium oxide, soil, trace elements.

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prof. dr hab. Mirosław Wyszkowski, Chair of Environmental Chemistry, University of Warmia and Mazury, Plac Łódzki 4, 10-727 Olsztyn, Poland, e-mail: miroslaw.wyszkowski@uwm.edu.pl

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ZMIANY ZAWARTOŚCI NIEKTÓRYCH MIKROPIERWIASTKÓW W GLEBIE ZANIECZYSZCZONEJ OLEJEM OPAŁOWYM PO APLIKACJI RÓŻNYCH SUBSTANCJI

Abstrakt

Substancje ropopochodne przedostają się do środowiska, powodując jego degradację. Zanieczyszczenia wpływają negatywnie na funkcję ochronną gleby i jej właściwości fizykochemiczne, zakłócają jej aktywność enzymatyczną, zmniejszają żyzność oraz oddziałują na wzrost, rozwój i skład chemiczny roślin. Należy zatem poszukiwać metod, które umożliwią ograniczenie wpływu substancji ropopochodnych lub nawet odtworzenie pierwotnych właściwości gleb. Celem badań było określenie wpływu zaaplikowanych do gleby substancji na zawartość kadmu, ołowiu, chromu i manganu w glebie zanieczyszczonej wzrastającymi dawkami oleju opałowego (0-20 g kg^{-1} gleby). Wpływ wzrastających dawek oleju opałowego na zawartość badanych pierwiastków śladowych w glebie był zróżnicowany i zależał od aplikacji substancji łagodzących do gleby. Wzrastające dawki oleju opałowego przyczyniły się do obniżenia zawartości kadmu i chromu w glebie oraz nie miały ukierunkowanego wpływu na zawartość ołowiu i manganu. Żadna z zastosowanych substancji łagodzących nie miała ograniczającego wpływu na zawartość manganu, kadmu, chromu i ołowiu w badanej glebie. Wyjątkiem był zeolit, który spowodował zmniejszenie zawartości kadmu i ołowiu w glebie. Zawartość metali ciężkich w glebie, nawet zanieczyszczonej najwyższymi dawkami oleju opałowego, nie przekraczała dopuszczalnych norm zawartych w Rozporządzeniu Ministra Środowiska z dnia 9 września 2002 r. oraz pozwoliła na ich zaklasyfikowanie do 0 stopnia zanieczyszczenia gleb wg wytycznych IUNG w Puławach.

Słowa kluczowe: zanieczyszczenie olejem opałowym, azot, kompost, bentonit, zeolit, tlenek wapnia, gleba, pierwiastki śladowe.

INTRODUCTION

Heating oil is produced by distillation and used for heating residential houses, greenhouses and small production plants. Some spillage can occur during the recovery, transport, storage and finally consumption of heating oil (NADIM et al. 2000, SYRYGAŁA, ŚLIWKA 2004). As a result, petroleum substances enter the environment, causing its degradation (SURYGAŁA 2006). As the environmental contamination with heating oil is becoming a pressing problem, inexpensive, quick and effective solutions are being searched for to clean the soil and water environment of the contaminant (SURYGAŁA 2006). Contamination has an adverse effect on the protective function of soil and soil physicochemical properties, causing disorders in the soil enzymatic activity (WYSZKOWSKA, WYSZKOWSKI 2010, ZIÓŁKOWSKA, WYSZKOWSKI 2010), depressing soil fertility (WYSZKOWSKI, ZIÓŁKOWSKA 2007, 2008, 2009*c*, WYSZKOWSKI, SIVITSKAYA 2012, 2013) and impairing the growth, development and chemical composition of plants (WYSZKOWSKI et al. 2004, WYSZKOWSKA, WYSZKOWSKI 2006, WYSZKOWSKI, ZIÓŁKOWSKA 2009*ab*, 2011, ZIÓŁKOWSKA, WYSZKOWSKI 2010). We should therefore look for methods which - under *in situ* conditions - will enable us to reduce the effect of petroleum substances or even to recover the original properties of soil.

The objective of the present study has been to determine the effect of substances applied to soil on the content of cadmium, lead, chromium and manganese in soil contaminated with incremental doses of heating oil.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse at the University of Warmia and Mazury in Olsztyn (north-eastern Poland), on soil whose texture corresponded to loamy sand (fractions in mm: <0.002 – 1.89%, 0.002-0.005 – 2.46%, 0.005-0.010 – 2.87%, 0.010-0.020 – 4.39%, 0.020-0.050 – 8.89%, 0.050-0.100 – 14.08%, 0.100-0.250 – 36.90%, 0.250-0.500 – 22.78%, 0.500-1.000 – 5.74%, 1.000-2.000 – 0%) and which had the following properties: pH in 1 mol KCl dm^{-3} – 4.52, hydrolytic acidity (Hh) – 25.4 mmol(+) · kg^{-1}, sum of the exchange capacity of cations Ca^{++}, Mg^{++}, K^{+} and Na^{+} (S) – 29.1 mmol(+) kg^{-1}, total exchangeable bases (T) – 54.5 mmol(+) kg^{-1}, percent base saturation (V) – 53%, $C_{org.}$ content – 11.3 g kg^{-1}, the content of available forms of phosphorus – 71.9 mg kg^{-1}, potassium – 118.6 mg kg^{-1} and magnesium – 104.2 mg kg^{-1} of soil, and the content of total forms of copper – 3.66 mg kg^{-1}, zinc – 14.86 mg kg^{-1}, nickel – 5.08 mg kg^{-1} and iron – 6.07 g kg^{-1}. The experiment was conducted in three replications, comprising six series: without alleviating substances, with nitrogen as urea (200 mg N kg^{-1} of soil), compost (3%), bentonite and zeolite (2% in relation to the soil mass) and 50% calcium oxide in a dose corresponding to one full hydrolytic acidity (10.93 g Ca kg^{-1} of soil). In each series, soil was contaminated with heating oil in doses of 0, 5, 10, 15 and 20 g kg^{-1} of soil. Additionally, mineral fertilizers were added to each treatment in the following amounts: 100 mg N, 30 mg P, 100 mg K, 50 mg Mg, 0.33 mg B, 5 mg Mn and 5 mg Mo per kg of soil. Nine-kilogram batches of the soil, carefully mixed with heating oil, alleviating substances and supplementary mineral fertilizers, were placed in polyethylene pots. The test crop was cv. Reduta maize (*Zea mays* L.), grown as 8 plants per pot. During the experiment, the soil moisture was maintained at 60% water saturation.

During the harvest, soil samples were taken. They were dried, ground and submitted to flame atomic absorption spectrophotemric assays (FAAS) in order to determine their content of cadmium, lead, chromium and manganese. The equipment was calibrated using certified standard solutions by Fluke and Merc. Soil was wet mineralized in Teflon HP500 vessels placed in a MARS 5 type oven, according to the US-EPA3051 methodology (1994). The soil samples underwent the following determinations: soil reaction (pH) with the potentiometric method in KCl aqueous solution of the concentration of 1 mol dm^{-3} (LITYŃSKI et al. 1976), hydrolytic acidity (Hh) and sum of the exchange capacity of cations (S) with the Kappen method (LITYŃSKI et al.

1976). Based on the hydrolytic acidity (Hh) and the exchange capacity of cations (S), the total exchangeable bases (T) and percent of base saturation (V) were calculated from the following formulas: T = Hh + S, V = S T^{-1} 100 (Lityński et al. 1976). Some soil determinations were completed before the experiment, namely the content of organic carbon (C_{org}) with the Tiurin method (Lityński et al. 1976), available phosphorus and potassium with the Egner-Riehm method (Lityński et al. 1976) and available magnesium with the Schachtschabel method (Lityński et al. 1976) as well as the granular composition of the soil with the laser method using a Mastersizer 2000 meter. The results were analysed statistically using a three-factor analysis of variance ANOVA from Statistica software (STATSOFT, INC. 2010).

RESULTS AND DISCUSSION

The increasing doses of heating oil caused different effects on the content of the analysed macronutrients in soil (Table 1). In the treatments without any alleviating substance, a significant decrease in the content of cadmium (r=-0.865) and chromium (r=-0.855) appeared in soil contaminated with heating oil (versus the treatment without this petroleum substance), with cadmium being depressed more strongly than chromium. Despite the reducing effect of heating oil on the content of chromium, its lowest dose (5 g kg^{-1}) raised this element in soil by 14%. The effect of heating oil on the content of lead and manganese in soil was not so evidently negative, and modified the content of manganese by less than 10%. However, it should be added that the highest dose of heating oil in soil (20 g kg^{-1}) caused a high increase in the content of lead.

Heavy metals contribute to a decrease in the biomass of microorganisms and inhibit their activity in soil; even when they do not diminish microbial counts, they depress the biological diversity of soil microorganisms (Wyszkowska et al. 2008). The concentrations of the analysed trace elements in soil contaminated with heating oil presented in this paper are within the tolerance ranges for toxic elements worked out by the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy, and the degree of soil contamination with cadmium and lead was low enough to classify the soil as zero contamination degree soil. According to the Regulation of the Ministry of the Environment of 9 September 2002 on soil quality standards and earth quality standards in soils contaminated with heating oil, the determined content of the trace elements did not exceed the permissible thresholds set up for any of the three groups of land.

The alleviating substances applied to non-contaminated soil did not cause any significant changes in the content of manganese in soil, but all of them, except nitrogen, raised the level of chromium (Table 1). Calcium oxide

Table 1

Content of trace elements in soil after plants harvest (mg kg^{-1} d.m. of soil)

Dose of heating oil (g kg^{-1} of soil)	Type of substance neutralizing the effect of heating oil						
	without additions	nitrogen	compost	bentonite	zeolite	CaO	average
	Manganese						
0	212.5	220.6	214.8	231.3	207.7	218.7	217.6
5	223.4	211.8	229.6	225.9	235.8	215.5	223.7
10	206.0	235.8	212.6	225.1	208.6	213.2	216.9
15	227.7	228.1	209.9	228.9	209.2	221.4	220.9
20	204.6	232.8	210.0	214.2	213.0	224.7	216.5
Average	214.8	225.8	215.4	225.1	214.9	218.7	219.1
r	-0.176	0.662*	-0.564	-0.752**	-0.215	0.616*	-0.255
LSD	*a* - n.s., *b* - n.s., *a b* - n.s.						
	Chromium						
0	2.49	2.32	3.55	3.45	3.89	4.74	3.41
5	2.83	1.98	3.58	3.52	4.23	4.54	3.45
10	2.29	2.80	3.21	3.58	4.47	5.43	3.63
15	1.95	2.87	3.69	4.03	4.40	4.98	3.65
20	1.84	2.87	3.65	4.20	4.33	5.12	3.67
Average	2.28	2.57	3.54	3.75	4.27	4.96	3.56
r	-0.855**	0.783**	0.254	0.947**	0.735**	0.553	0.931**
LSD	*a* - n.s., *b* - 0.28**, *a b* - 0.63*						
	Cadmium						
0	0.31	0.06	0.29	0.22	0.04	0.00	0.15
5	0.17	0.22	0.29	0.16	0.01	0.07	0.15
10	0.05	0.23	0.20	0.20	0.06	0.07	0.14
15	0.10	0.20	0.18	0.30	0.04	0.11	0.16
20	0.05	0.17	0.21	0.20	0.04	0.12	0.13
Average	0.14	0.18	0.23	0.22	0.04	0.07	0.15
r	-0.865**	0.461	-0.791**	0.280	0.258	0.934**	-0.586
LSD	*a* - n.s., *b* - 0.06**, *a b* - 0.13*						
	Lead						
0	4.81	5.20	5.48	7.51	6.78	4.00	5.63
5	5.63	10.18	17.04	6.97	4.03	8.90	8.79
10	2.65	9.23	15.32	14.09	3.22	9.54	9.01
15	4.10	9.88	11.66	5.20	4.52	10.33	7.62
20	12.94	11.77	12.44	8.33	5.11	5.39	9.33
Average	6.03	9.25	12.39	8.42	4.73	7.63	8.08
r	0.580	0.829**	0.305	-0.006	-0.337	0.240	0.651*
LSD	*a* - n.s., *b* - 4.17*, *a b* - n.s.						

LSD (least squares deviation) for: *a* – heating oil dose, *b* – kind of neutralizing substance, *a b* – interaction; significant for: * -*P* = 0.05, ** -*P* = 0.01, n.s. – non-significant; *r* – correlation coefficient was the most stimulating soil amendment as its application nearly doubled the content of chromium in soil under maize. The influence of the neutralizing substances on the content of cadmium in soil was strongly reducing. The content of cadmium fell to almost zero in the treatment with calcium oxide; the cadmium level was five-fold lower when nitrogen had been applied to soil and eight-fold lower under the influence of zeolite in soil compared to the non-amended soil. In the soils not contaminated with heating oil, it was only calcium oxide that reduced the content of lead, whereas all the other substances contributed to an increase in the content of this element. In soil under maize, bentonite and zeolite particularly stimulated the increasing levels of lead.

All the applied substances contributed to an increase in the average content of cadmium, chromium and lead in the soil contaminated with heating oil compared to the series without soil amendments (Figure 1). Zeolite and calcium oxide acted differently. Zeolite depressed the content of cadmium in soil by two-fold, while calcium oxide did not cause any changes in the soil content of this element. In that soil, nitrogen, compost and bentonite doubled the content of cadmium. Zeolite also reduced the content of lead in soil by 33% on average. The effect of the other substances on lead was stimulating, increasing its content in soil. Nitrogen raised the content of lead in soil by 62%, compost by 123%, bentonite by 37% and calcium oxide by 35%. The biggest changes in the soil content of trace elements were demonstrated with respect to chromium. The content of this element doubled in the soil amended with calcium oxide. Nitrogen and compost raised its soil content by 18 and 59%. Bentonite and zeolite raised its soil content by an average of 72 and 95%, respectively. The content of manganese in soil did not change much under the influence of the tested substances compared to the series without amending substances, except nitrogen (+5%) and bentonite (+4%), which slightly stimulated the increase in the content of manganese relative to the series without soil amendments.

Filipek-Mazur (2004) claims that while it is impossible to remove heavy metals from soil completely, it is possible to alleviate negative effects of their excessive amounts by using chemical methods: liming, application of organic matter, zeolites or phosphates; biological methods, such as phytoremediation, stimulation of crop yields and dilution of elements in yields, selection of cultures tolerant to the uptake of metals; and physical methods, e.g. deep extraction and electrokinetic techniques.

Baran et al. (2009) observed that the content of lead in soil increases as the amounts of sewage sludge and rockwool added to soil for rehabilitation increased. In the authors' own research, the content of trace elements increased as the dose of heating oil introduced to soil rose. Cadmium, a component of diesel oil, is characterized by high mobility, especially in light and acid soils (Buczkowski et al. 2002). Lead belongs to the so-called 'death metals'. The metals introduced to soil with fertilizers do not usually pose such

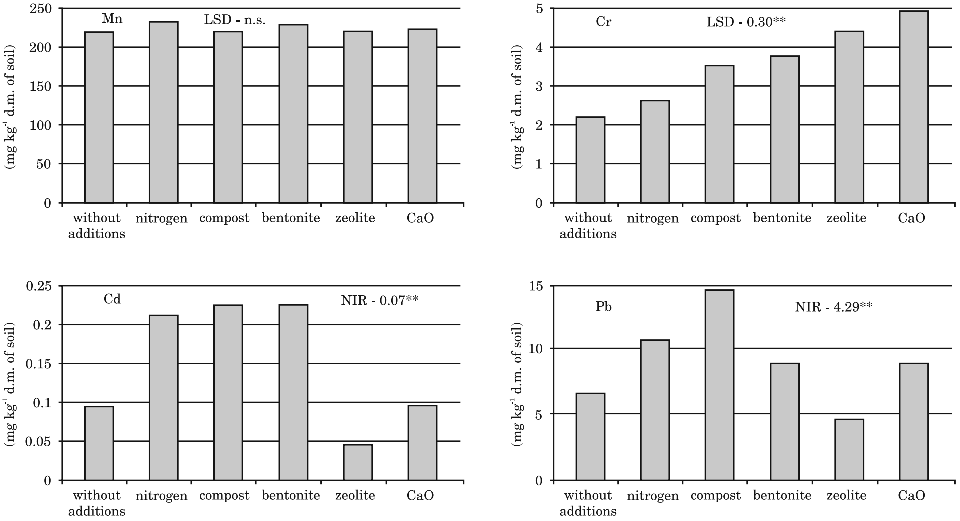

Fig. 1. Content of trace elements in soil after plants harvest (average from series contaminated with 5-20 g heating oil per kg of soil)
differences significant for: ** -P=0.01, * -P=0.05

a severe threat (Baran et al. 2009). Yet, an elevated concentration of heavy metals in topmost layers of soil make it chemically degraded (Terelak et al. 2002). Heavy metals belong to the most persistent and slowly migrating soil contaminants.

Organic fertilizers add large amounts of organic matter to soil, have a positive influence on its pH and improve soil absorbing properties (Buczkowski et al. 2002). Organic matter can form strong bonds with chromium, iron, lead, nickel and cobalt ions, as well as weaker bonds with manganese and zinc ions in soil. Clay minerals are characterized by strong affinity to iron ions, but a weaker one to nickel and cobalt ions, and their oxides form strong bonds with chromium and lead ions (Kabata-Pendias, Pendias 2001).

Heavy metals are the elements which do not undergo biodegradation to simple compounds. Sorption does not cleanse the soil but causes further accumulation of metals. Minerals which have good adsorption properties improve the soil pH or form insoluble compounds. Zeolite is characterized by good affinity to Cd^{2+}, Pb^{2+}, Zn^{2+} and Cu^{2+} ions, but complete desorption of cadmium occurs at low soil pH, which may explain why zeolite introduced to acid soil under maize had no effect on the content of cadmium, chromium and lead in soil. In order to decrease the content of lead from 500 to 5 mg kg^{-1} in sandy soil, 5% of zeolite per soil mass should be added; when the contamination reaches 5000 mg Pb kg^{-1}, the required amount of zeolite equals 35% of the soil mass. In fact, much worse sorption of nickel and cadmium by zeolite has been recorded (Buczkowski et al. 2002). When zeolites are added to soil, the available forms of cadmium are converted into unavailable ones, which diminishes the content of this element in soil (Chen et al. 2000). Usman et al. (2005) achieved positive effects of applying zeolite to soil, as the concentrations of metals in the soil solution fell by 13 to 57%. Usman et al. (2005) suggest that bentonites are more effective than zeolites in immobilization of metals, which may be attributed to the high content of montmorillonite in bentonites; in zeolites, this mineral makes up just 10% of the total content. In the authors' own investigation, only zeolite had a positive, reducing effect on the content of some of the trace elements in soil.

The soil reaction affects the mobility of heavy metals. All trace metals can form insoluble oxides, hydroxides, carbonates and phosphates only in alkaline environment. Below pH=6, even iron and manganese hydroxides become soluble, which leads to the release of metals adsorbed on their surface (Buczkowski et al. 2002). Kabata-Pendias and Sadurski (2003) report that soil liming depressed the mobility of metals by rising the soil pH, which favours formation of metal hydroxides and carbonates. In soil of higher pH, bonds between metals and humic acids become stronger. In soils of neutral or alkaline reaction, as well as in the presence of calcium carbonate, metals are slowly transferred to deeper soil horizons Kabata-Pendias and Pendias (2001).

CONCLUSIONS

1. The effect of incremental doses of heating oil on the content of analyzed trace metals in soil was varied and depended on the application of alleviating substances to soil.

2. The increasing amounts of heating oil contributed to a decrease in the content of cadmium and chromium in soil, but had no such regular influence on the content of lead or manganese.

3. None of the applied alleviating substances had a reducing effect on the content of manganese, cadmium, chromium or lead in the tested soil. Zeolite was an exception as it reduced the content of cadmium and lead in soil.

4. The content of heavy metals in soil, even when contaminated with the highest levels of heating oil, did not exceed the permissible amounts defined in the Regulation of the Ministry of the Environment of 9 September 2002 and therefore allowed us to classify the soil as a zero contamination degree according to the IUNG Puławy classification.

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EFFECT OF SUBSTRATES CONTAINING MUNICIPAL SEWAGE SLUDGE COMPOST ON THE ACCUMULATION OF MACROCOMPONENTS IN *IMPATIENS WALLERIANA* HOOK

Agnieszka Zawadzińska, Piotr Salachna

Department of Ornamental Plants

West Pomeranian University of Technology in Szczecin

Abstract

Municipal sewage sludge is often used in compost production for agriculture. Composting sediments, which include structure-forming materials, results in the formation of matter rich in organic substances and nutrients that can be used as a component of horticultural substrates, as an alternative to peat. The aim of this research paper is to assess the impact of substrates containing municipal sewage sludge compost on $N_{tot.}$, P, K, Ca, Mg and S levels in the leaves and roots of *Impatiens walleriana* Hook. The two-year experiment used four types of compost; in the first year the tests were conducted after a 7-month period of decay and the second year after an 18-month period. The composition of compost material was as follows: compost I – 70% municipal sewage sludge, 30% rye straw; compost II – 70% municipal sewage sludge, 30% coniferous tree sawdust; compost III – 35% municipal sewage sludge, 35% potato pulp, 30% rye straw; compost IV – 35% municipal sewage sludge, 35% potato pulp, 30% coniferous tree sawdust. During the first and second year, five experimental sites were used: four substrate with 50% of the tested compost and 50% sphagnum peat and one sphagnum peat reference substrate with a 2.5 g dm^{-3} dose of Azofoska multipurpose fertiliser.

After 12 weeks of *Impatiens walleriana* cultivation the recorded level of total nitrogen, calcium, magnesium and sulphur in the leaves was lower than the optimal range of the macrocomponent content recommended for this species. The levels of phosphorus and potassium were the exception. Impatiens cultivated in substrates with compost after a 7-month decay period contained 1.7 times more nitrogen and 1.8 times more potassium in the leaves; they had similar phosphorus and sulphur content and lower calcium and magnesium content than the plants cultivated in the compost substrates after an 18-month decay period. The roots of impatiens contained more calcium than the leaves in both years of the study.

The nutrients supplied in the substrates and through top dressing fertilisation were not sufficient to achieve the best cultivation results for *Impatiens walleriana*.

Keywords: composts, sewage sludge, *Impatiens waleriana* Hook., macrocomponents.

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dr inż. Agnieszka Zawadzińska, Department of Ornamental Plants, West Pomeranian University of Technology, Papieża Pawła VI 3, 71-434 Szczecin, Poland, e-mail: kro@zut.edu.pl

WPŁYW PODŁOŻY ZAWIERAJĄCYCH KOMPOSTY Z KOMUNALNEGO OSADU ŚCIEKOWEGO NA AKUMULACJĘ MAKROSKŁADNIKÓW W NIECIERPKU WALLERIANA (*IMPATIENS WALLERIANA* HOOK.)

Abstrakt

Komunalne osady ściekowe są odpadem wykorzystywanym często w rolnictwie do produkcji kompostów. Kompostowanie osadów z materiałami strukturotwórczymi powoduje, że powstaje bogata w substancję organiczną i składniki pokarmowe materia, którą można stosować alternatywnie do torfu, jako składnik podłoży ogrodniczych. Celem pracy była ocena wpływu podłoży zawierających komposty z komunalnych osadów ściekowych na zawartość N_{og}., P, K, Ca, Mg, S w liściach i korzeniach niecierpka Walleriana. W doświadczeniu dwuletnim wykorzystano cztery rodzaje kompostów: w pierwszym roku po 7- miesięcznym okresie rozkładu, a w drugim po 18-miesięcznym okresie rozkładu. Skład rzeczowy kompostów był następujący: kompost I – 70% komunalny osad ściekowy, 30% słoma żytnia; kompost II – 70% komunalny osad ściekowy, 30% trociny z drzew iglastych; kompost III – 35% komunalny osad ściekowy, 35% wycierka ziemniaczana, 30% słoma żytnia; kompost IV – 35% komunalny osad ściekowy, 35% wycierka ziemniaczana, 30% trociny z drzew iglastych. W pierwszym i drugim roku zastosowano pięć obiektów doświadczalnych: cztery podłoża z udziałem 50% danego kompostu i 50% torfu wysokiego oraz jedno podłoże kontrolne z torfu wysokiego, z dodatkiem Azofoski w dawce 2,5g dm^{-3}.

Po 12 tygodniach uprawy niecierpka Walleriana zawartość azotu ogólnego, wapnia, magnezu i siarki w liściach, z wyjątkiem fosforu i potasu, była mniejsza niż optymalny zakres zawartości tych makroskładników zalecany dla tego gatunku. Niecierpki uprawiane w podłożach z udziałem kompostów po 7-miesięcznym okresie rozkładu gromadziły w liściach 1,7 razy więcej azotu ogólnego i 1,8 razy więcej potasu, miały podobną zawartość fosforu i siarki, natomiast mniej wapnia i magnezu niż w podłożach z kompostami po 18-miesięcznym okresie rozkładu. Korzenie niecierpka zawierały więcej wapnia niż liście w obydwóch latach badań. Składniki pokarmowe dostarczone w podłożach i w nawożeniu pogłównym nie zaspokoiły w pełni wymagań niecierpka Walleriana.

Słowa kluczowe: kompost, osad ściekowy, niecierpek Walleriana, makroskładniki.

INTRODUCTION

Impatiens walleriana Hook. is an ornamental plant originating from Africa. In Poland, it is primarily grown on flowerbeds and as a cover plant in parks and gardens. It is characterized by rapid growth, abundant flowering and lasting ornamental value.

Peat is the most common substrate in the cultivation of bedding plants (Rumpel 1998, Ribeiro et al. 2007). Alternative substrates developed from different types of industrial and municipal waste are becoming increasingly popular due to environmental and economic considerations (Grigatti et al. 2007, Krzywy et al. 2007, Zaller 2007). These types of waste are rich in organic substances and contain elements that may provide plants with necessary nutrients. Municipal sewage sludge can also be used for this purpose (Ribeiro et al. 2000, Zubilaga, Lavado 2001) despite a number of limitations as to its use, including an unpleasant odour, greasy texture and, most importantly, excessive concentrations of heavy metals and pathogens

(Dusza et al. 2009). Therefore, before it is used for plant growing, the waste must comply with the requirements set out in the Polish legislation (*Regulation…*2008, *Regulation…*2010). Composting is the most effective and economic method of sewage treatment (Raviv 1998, Ciećko, Harnisz 2002). Composts based on municipal sewage sludge are very rich in macro-and micronutrients; on the other hand, they may present an elevated pH and salinity (Kalembasa, Kuziemska 1999, Baran 2004), which is why they should be mixed with other less rich substrates, e.g. peat (Abad et al. 2001, Moore 2004).

The study was conducted in order to evaluate the effect of substrates containing peat and municipal sewage sludge compost, potato pulp, and straw or sawdust on the macronutrient content of leaves and roots of *Impatiens walleriana*.

MATERIAL AND METHODS

A study on *Impatiens walleriana* was conducted in 2005-2006. It involved analyses of sphagnum peat and compost prepared in 2004from municipal sewage sludge, potato pulp, straw or sawdust. The plant material consisted of *Impatiens walleriana* seedlings of the variety Fiesta Lavendr Orchid, obtained from the Ball FloraPlant nurseries. Four variants of compost were added to the substrate, left to mature for seven months (in 2005) and for eighteen months (in 2006). The four variant composts had the following composition (in percentage of dry weight):

- compost I – 70% municipal sewage sludge, 30% rye straw;
- compost II – 70% municipal sewage sludge, 30% coniferous tree sawdust;
- compost III – 35% municipal sewage sludge, 35% potato pulp, 30% rye straw;
- compost IV – 35% municipal sewage sludge, 35% potato pulp, 30% coniferous tree sawdust.

The origin of the above materials, their chemical composition, descrption of the composting process and detailed chemical characteristics can be found in Krzywy et al. (2007). In early April 2005, four substrate mixtures were prepared from sphagnum peat and one of the four compost variants each (50% of volume). The reference consisted of peat substrate, whose pH was 6.0, with an addition of 2.5 kg m^{-3} of the fertilizer Azofoska (13.6+6.4+19.1). Results of chemical analyses of the substrates and their constituents material are presented in Table 1. The substrates whose pH was assessed as too low were neutralized with chalk and dolomite up to pH 5.8-6.0. Some substrates with the content of ammonium nitrate (V) and potassium below recommended values were supplemented with ammonium sulphate and potassium sulphate to the upper recommended level for impatiens. The plants were seeded in the substrates in the first ten days of June; 0.75 dm^3 capacity pots were placed in a plastic tunnel. Top dressing of all variants was performed after 7 weeks of the experiment in the first year and after 4 weeks in the second year. The plants were supplemented once weekly with 50 ml of Peters Professional (27+15+12) fertilizer per pot, in 0.5% concentration.

In both years, after 12 weeks of cultivation, plant material was sampled for chemical analysis. The slevel of $N_{tot.,}$ P, K, Ca, Mg and S were determined in leaves and roots. The plant material for analysis was obtained by taking pooled samples from all plants in each variant. The tests were made on samples dried at 60°C using the methods generally adopted in agricultural chemistry and pedology (Ostrowska et al. 1991, Nowosielski 1988).

In both years, the experiments were established in complete randomization with 4 replications, each comprising 4 plants. The results of analyses of the chemical composition of plants were verified with the help of the ANALWAR 4.3. software and variance analysis for univariate experiments. The significance of the means was verified with the Tukey's range test at a level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Impatiens walleriana requires lightweight, permeable substrates with high water holding capacity (Kessler 2005). A German company called Benary (www.benary.com), which produces *Impatiens walleriana*, states that these plants respond well to 15-30% of clay and 0-20% of perlite added to the peat base. It is also recommended to use 1-1.5 kg m^{-3} of balanced multi-component fertilizer for supplementation of the levels of nutrients. The substrates prepared from compost containing municipal sewage sludge were rich in nutrients in both years of the study (Table 1). Composts used for preparation of the substrates did not exceed standards for the heavy metal content (Krzywy et al. 2007).

Impatiens walleriana has moderate nutritional requirements but it is sensitive to soil salinity. There are no precise data available on the optimal nutrient content in substrate for this species. Zawadzińska et al. (2009) presents general guidelines for growing *Impatiens* sp. According to her recommendations, the preferred salinity of soil for impatiens crops should be maintained below 2.5 g NaCl dm^{-3} and the pH between 5.5 and 6.5. The range of nutrients is as follows: 180-230 mg $N\text{-}NO_3$ dm^{-3}; < 40 mg $N\text{-}NH_4$ dm^{-3}; 160-210 mg P dm^{-3}; 200-250 mg K dm^{-3}; 500-1500 mg Ca dm^{-3}; 150-200 mg Mg dm^{-3}; 50-100 mg $S\text{-}SO_4$ dm^{-3}.

When analyzing the substrate prepared in 2005 (Table 1), a slightly lower pH than recommended for *Impatiens* was recorded; the salinity, on the

Table 1

Physical properties and chemical composition of substrates used for cultivation of *Impatiens walleriana* in 2005-2006

Substrate*	pH_{H_2O}	Dry matter (%)	Density (g cm^{-3})	Salinity (g NaCl dm^{-3})	Total form content (g kg^{-1} d.m.)						Available form content (mg dm^{-3})				
					N	P	K	Ca	Mg	S	$N-NO_3$	P	K	Ca	Mg
2005															
Peat	3.60	59.8	0.18	0.35	8.90	1.60	1.60	2.10	0.30	0.50	17.0	20.0	6.00	42.0	27.0
I	5.35	36.3	0.39	3.66	17.6	17.3	3.70	7.70	0.63	5.30	785	687	379	2265	500
II	5.80	38.1	0.39	1.69	16.6	13.0	2.20	6.70	0.56	4.70	346	671	224	2690	309
III	5.80	44.8	0.36	1.44	16.1	14.8	5.60	6.50	0.60	5.40	386	518	644	1596	285
IV	5.90	45.4	0.38	0.83	11.0	14.5	1.60	4.40	0.63	3.50	281	348	180	1625	202
2006															
Peat	3,50	58.8	0.20	0,35	9.30	1.60	0.50	2.47	0.14	0.17	16,5	19,0	5,0	40,0	27,0
I	5.40	36.8	0.28	3.36	11.4	6.95	5.05	2.12	1.75	1.90	330	810	845	3843	554
II	5.40	36.6	0.34	3.33	10.3	5.50	2.45	1.82	1.30	1.85	379	620	226	3272	413
III	5.40	22.2	0.30	2.10	9.90	4.10	4.90	1.70	1.20	1.75	223	574	703	2121	306
IV	5.90	24.6	0.38	2.64	9.00	3.90	2.65	1.70	1.30	1.71	228	449	295	3110	384

* Composition of substrates:
I – 50% compost I + 50% peat; II – 50% compost II + 50% peat; III – 50% compost III + 50% peat; IV – 50% compost IV + 50% peat;

other hand, was elevated in compost I. Moreover, the content of available nutrients, especially nitrogen, phosphorus and calcium, in some substrates exceeded the recommended limits. In 2006, elevated salinity was determined in compost I and II substrates, which were composed of 70% municipal sludge and 30% straw or sawdust (Table 1). Despite the added share peat, poor in nutrients, the excessive salinity found in the prepared mixtures was not mitigated. In most substrates, the nutrient content was higher than recommended.

In 2005, a significant impact of the substrate on the content of nitrogen, phosphorus, potassium and sulphur in leaves of *Impatiens* was recorded after 12 weeks of cultivation (Table 2). The highest level of total nitrogen was found in treatment II, where the compost included municipal sludge (70%) and sawdust compost (30%); the lowest level of total nitrogen was found in treatment III, with the compost variant containing sewage sludge (35%), potato pulp (35%) and sawdust (30%). The phosphorus content was the lowest in leaves of plants grown without any compost. The phosphorus content did not differ significantly between any of the pots containing municipal sludge compost. The highest level of potassium was found in treatment III, with compost containing sewage sludge (35%), potato pulp (35%) and straw (30%). This was due to the high content of potassium in the substrate (Table 1). The content of calcium (0.29-0.36%) and magnesium (0.077--0.079%) in leaves of *Impatiens* did not vary in any of the examined variants.

Table 2

Macronutrient composition of *Impatiens walleriana* leaves (% d.m.) at the end of the cultivation, depending on the substrates

Substrate*	2005					
	N_{total}	P	K	Ca	Mg	S
Control - peat	2.37 *bc***	0.69 *b*	1.79 *d*	0.33 *a*	0.078 *a*	0.20 *a*
I – compost I + peat	2.27 *cd*	0.99 *a*	2.17 *c*	0.29 *a*	0.078 *a*	0.18 *ab*
II – compost II + peat	2.58 *a*	0.99 *a*	2.24 *c*	0.36 *a*	0.079 *a*	0.13 *b*
III – compost III + peat	2.17 *d*	0.99 *a*	2.64 *a*	0.36 *a*	0.078 *a*	0.17 *ab*
IV– compost IV + peat	2.48 *ab*	1.01 *a*	2.42 *b*	0.34 *a*	0.077 *a*	0.19 *ab*
Mean	2.37	0.93	2.25	0.33	0.078	0.17
Substrate*	2006					
	N_{total}	P	K	Ca	Mg	S
Control - peat	1.06 *c*	0.79 *b*	0.98 *c*	0.89 *a*	0.086 *a*	0.11 *a*
I – compost I + peat	1.57 *a*	1.10 *a*	1.48 *a*	0.98 *a*	0.092 *a*	0.14 *a*
II – compost II + peat	1.53 *a*	1.14 *a*	1.49 *a*	0.99 *a*	0.093 *a*	0.18 *a*
III – compost III + peat	1.27 *b*	0.86 *b*	1.17 *b*	0.96 *a*	0.088 *a*	0.13 *a*
IV – compost IV + peat	1.31 *b*	0.83 *b*	1.18 *b*	0.96 *a*	0.088 *a*	0.14 *a*
Mean	1.34	0.94	1.26	0.96	0.090	0.14

* key: see Table 1

** figures designated same letters do not differ significantly

The sulphur content in leaves was the highest versus the control (0.20% S d.m.), but no significant differences in its content were recorded between variants I, III and IV.

In 2006 a significant impact of the substrates on the total nitrogen, phosphorus and potassium in leaves of *Impatiens* was recorded. Higher levels of total nitrogen were recorded in leaves of plants grown in substrates containing compost developed from sewage sludge (70%) and straw (30%) or sawdust (30%) than in substrates containing composts with potato pulp (variants II and IV). Higher levels of phosphorus and potassium were also recorded in leaves of plants from pots in the variants I and II than in the other pots. As regards the levels of calcium, magnesium and sulphur in plant leaves, no significant differences between the treatments were recorded.

According to KESSLER (2005), leaves of properly nourished *Impatiens walleriana* plants should contain N_{total} 3.6-5.8% d.m., P 0.8-0.9% d.m., K 1.4-2.4% d.m., Ca 1.8-2.4% d.m., Mg 0.9-3.6% d.m. and S 0.8-0.9% d.m. In both years of the present study, despite the high content of nutrients in the substrates and top dressing with compound fertilizer, the nitrogen content in leaves was lower (2.58-1.06% $N_{tot.}$ d.m.) than an optimal range for *Impatiens walleriana*. In the first year of our experiment, the phosphorus content in leaves of plants cultivated in the pots filled with composts (0.99-1.01% P d.m.) exceeded the recommended range of P 0.8-0.9% d.m. (KESSLER 2005). In the second year, the recommended range of phosphorus content in leaves appeared only in treatments I, II and IV. In both years, the content of potassium in leaves of *Impatiens walleriana* cultivated without any compost and in treatments I and II, were within the optimal range (1.4-2.4% K d.m.); in treatments III and IV (2.64 and 2.42% K d.m., respectively) it exceeded the recommended values. In both years, deficits of calcium, magnesium and sulphur in leaves of *Impatiens* occurred. The magnesium content was more than ten-fold less than recommended.

In 2005, it was only the phosphorus and potassium content in roots of *Impatiens* that varied depending on the substrates (Table 3). The phosphorus root content in treatments I, II, III and IV was significantly higher than in roots of plants grown without any compost (0.65% P d.m.). The highest amount of potassium (1.30% K d.m.) was recorded in roots of *Impatiens* grown in variant IV; there were no significant differences between the variants II and III.

During the second year, the substrates modified the level of total nitrogen, phosphorus, potassium and calcium in roots of *Impatiens*. The highest level of total nitrogen and phosphorus was recorded in roots of plants cultivated in treatments I and II, in which composts contained sewage sludge (70%) and straw (30%) or sawdust (30%). No significant differences in the total nitrogen content were recorded between roots of plants from the control and variant III, containing compost made from sewage sludge (35%), potato pulp (35%) and straw (30%). The highest level of potassium (0.40% K d.m.)

Table 3

Macronutrient composition of *Impatiens walleriana* roots (% d.m.)
at the end of the cultivation, depending on the substrates

Substrate	2005					
	N_{total}	P	K	Ca	Mg	S
Control - peat	1.55 *a*	0.65 *b*	0.57 *c*	0.63 *a*	0.006 *a*	0.03 *a*
I – compost I + peat	1.56 *a*	0.80 *a*	0.79 *b*	0.61 *a*	0.008 *a*	0.05 *a*
II – compost II + peat	1.60 *a*	0.82 *a*	1.19 *a*	0.60 *a*	0.006 *a*	0.10 *a*
III – compost III + peat	1.52 *a*	0.77 *ab*	1.24 *a*	0.60 *a*	0.006 *a*	0.06 *a*
IV – compost IV + peat	1.53 *a*	0.84 *a*	1.30 *a*	0.62 *a*	0.008 *a*	0.08 *a*
Mean	1.55	0.78	1.01	0.61	0.007	0.06
Substrate	2006					
	N_{total}	P	K	Ca	Mg	S
Control - peat	0.95 *ab*	0.35 *b*	0.36 *ab*	0.59 *b*	0.007 *a*	0.04 *a*
I – compost I + peat	1.01 *a*	0.58 *a*	0.38 *ab*	1,16 *a*	0.008 *a*	0.08 *a*
II – compost II + peat	1.08 *a*	0.57 *a*	0.40 *a*	1.17 *a*	0.007 *a*	0.09 *a*
III – compost III + peat	0.90 *ab*	0.37 *b*	0.27 *bc*	1.17 *a*	0.010 *a*	0.05 *a*
IV – compost IV + peat	0.78 *b*	0.38 *b*	0.24 *c*	1.20 *a*	0.008 *a*	0.08 *a*
Mean	0.94	0.45	0.33	1.06	0.008	0.07

key: see Table 1
* figures designated same letters do not differ significantly

was found in roots of plants cultivated on substrate with compost II, which contained municipal sludge (70%) and sawdust (30%). The calcium content in roots of *Impatiens* did not differ significantly between the treatments containing composts, being the lowest in plants from the control pots.

No data can be found on the nutrient composition of roots of ornamental plants grown on substrates containing municipal sewage sludge compost. The information presented by LIS-KRZYŚCIN (2006) proves that the content of total nitrogen, phosphorus, potassium, calcium and magnesium in roots of *Pelargonium × hortorum* L.H. Bailey is the highest in the active growth phase, afterwards decreasing until the end of the growing period. The total nitrogen content level in roots of *Impatiens* during the first year (0.77-0.84% P d.m.) was higher than in roots of *Pelargonium* fertilized with higher doses of nitrogen. Also, the phosphorus content in roots *Impatiens* in the first year of cultivation was higher than that of *Pelargonium* (0.41-0.22% P d.m.). This can be explained by a very high level of phosphorus content in the compost substrates (Table 1). The calcium content in roots of *Impatiens* cultivated on compost substrates was lower in the first year than in *Pelargonium* fertilized with nitrogen, but higher in the second year (1.16-1.20% Ca d.m.) than in roots of *Pelargonium* during the period of rapid growth (1.11% Ca d.m.).

During the first year of the cultivation, leaves of *Impatiens* accumulated 1.7-fold more total nitrogen and 1.8-fold more potassium than during the second year; the levels of phosphorus and sulphur were comparable, and those of calcium and magnesium were slightly lower. This was due to a higher content of available forms of N-NO_3, phosphorus, potassium and sulphur (Table 1) in the substrate during the first year. *Impatiens* showed signs of component deficits in leaves much later; top dressing was started after 7 weeks of cultivation (Zawadzińska, Dobrowolska 2009). In the second year, top dressing was introduced after the 4th week of cultivation. *Impatiens* develops ornamental flowers; it blooms especially profusely in summer months and can produce over 200 flowers during the growing season. In an experiment run by Zawadzińska and Dobrowolska (2009), *Impatiens* grown on substrates containing compost made from sewage sludge (70%) and straw (30%), added at 50% and 70% ratios, produced an average of 234 flowers. Abundant flowering in another experiment (data not included) resulted in a rapid depletion of nutrients from substrates. A similar response occurred in New Guinea Impatiens (*Impatiens hawkerii*) grown on substrates containing compost with the same ingredient composition (Zawadzińska et al. 2009).

The current experiment proves that roots were more capable of accumulating calcium than other macronutrients. Calcium was the only trace element that was determined in higher concentrations in roots than in leaves of *Impatiens* in both years.

The key factor influencing the assimilation of nutrients is the ratio of ions in a substrate. During the nutrient absorption, potassium, calcium and magnesium act antagonistically. It is only when the substrate ensures Ca:K, Ca:Mg and K:Mg ratios optimal for a given species that the absorption of these elements can be efficient (Lis-Krzyścin 2007, Osemwota et al. 2007, Francke 2010). Impaired magnesium absorption may result from a higher soil potassium content than recommended. Also, large doses of available potassium and ammonium nitrogen in the form of NH_4^+ inhibit magnesium absorption (Marschner 1993). On the other hand, shortage of calcium in leaves is due to excess NH_4^+, K^+, Mg^{2+} and Na^{2+} ions in a substrate as well as high salinity (Geraldson 1957). In an experiment conducted by Romero et al. (2007), excessive calcium fertilization decreased wet and dry weight of plants and reduced the potassium content in leaf cells. In our experiment, the substrates were rich in potassium, calcium and magnesium (Table 1), sometimes above the recommended levels. The antagonism between elements may have contributed to the poor absorption of calcium and magnesium from the substrates, leading to a deficit of these compounds in leaves of *Impatiens*.

CONCLUSIONS

1. The content of nitrogen, calcium, magnesium and sulphur in leaves of *Impatiens walleriana* grown on substrates containing municipal sludge compost, potato pulp and rye straw or coniferous tree sawdust was lower than the recommended optimal range for this species.

2. Impatiens cultivated on substrates with compost maturing for 7 months contained 1.70-fold more nitrogen and 1.8-fold more potassium in leaves; they had a similar phosphorus and sulphur content and but less calcium and magnesium than plants cultivated in the compost substrates after an 18-month ripening period.

3. Roots of *Impatiens walleriana* contained more calcium than leaves in both years of the experiment.

4. The nutrients supplied with the substrates and through top dressing fertilization were not sufficient to achieve the best cultivation results for *Impatiens walleriana*.

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REVIEW PAPERS

THE EFFECT OF DIETARY SODIUM CHLORIDE CONCENTRATIONS ON BLOOD ELECTROLYTE CONCENTRATIONS, THE INCIDENCE OF FOOT PAD DERMATITIS AND BONE MINERALIZATION IN BROILER CHICKENS AND TURKEYS

Jan Jankowski[1], **Zenon Zduńczyk**[2]

[1]Chair of Poultry Science
University of Warmia and Mazury in Olsztyn
[2]Institute of Animal Reproduction and Food Research
Polish Academy of Sciences

Abstract

Among numerous changes that have occurred in poultry feeding over the last decades, an important one consists in an increase in the dietary content of strong electrolytes (Na, K and Cl) for slaughter chickens and turkeys. This coincided with a higher incidence of such unfavourable developments as the wet litter syndrome, foot-pad dermatitis (FPD) and deformations of the birds' legs. This paper reviews the results of the latest experiments that have analyzed the effect of different amounts of supplemental NaCl as a source of Na in diets for broiler chickens and turkeys on the concentrations of electrolytes in their blood, the incidence of FPD and leg bone deformations. It has been determined that an increase in Na in chickens' diets within the range of 0-0.25% did not affect FDP symptoms. Turkey diets containing 0.20% of Na intensified the symptoms of FDP compared to a diet without added sodium chloride. In chickens, significant disorders in the mineral balance, including worse physicochemical parameters of the tibia, appeared only when the birds were given feeds with a very low content of sodium (0.02 and 0.07%), made without or with very low sodium supplementation. Increasing the amount

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prof. dr hab. Jan Jankowski prof. zw., Chair of Poultry Sciences, Uniwersity of Warmia and Mazury, Michała Oczapowskiego 5, 10-719 Olsztyn, Poland, e-mail: janj@uwm.edu.pl

of added sodium to over 0.14% of a diet for broiler chickens and to 0.13% for turkeys did not improve the analyzed bone mineralization parameters. The reports reviewed in this paper indicate that the risk of inferior bone mineralization process, mainly due to Na deficiency, as well as a higher litter moisture content and more frequent cases of FPD (as the dietary Na content increases) are more profound among slaughter turkeys than broiler chickens.

Keywords: electrolyte balance, sodium, faeces moisture, FPD, tibia mineralization.

WPŁYW ZAWARTOŚCI CHLORKU SODU W PASZY NA STĘŻENIE ELEKTROLITÓW WE KRWI, CZĘSTOŚĆ WYSTĘPOWANIA ZAPALENIA SKÓRY STÓP I MINERALIZACJĘ KOŚCI U KURCZĄT BROJLERÓW I INDYKÓW

Abstrakt

Wśród wielu zamian, jakie zaszły w żywieniu drobiu w ostatnich dekadach, ważne miejsce zajmuje wzrost zawartości silnych elektrolitów (Na, K i Cl) w dietach kurcząt i indyków rzeźnych. W tym samym bowiem czasie nasiliło się występowanie takich niekorzystnych zjawisk, jak syndrom mokrej ściółki, foot-pad dermatitis (FPD) oraz deformacje kończyn ptaków. W pracy podsumowano wyniki najnowszych doświadczeń, w których oceniano wpływ zawartości elektrolitów w dietach na występowanie zakłóceń w odchowie kurcząt i indycząt, pogarszających dobrostan (głównie wskutek występowania FPD i deformacji kończyn) tych ptaków. Stwierdzono, że zwiększenie zawartości Na w diecie kurcząt w zakresie 0-0,25% nie miało wpływu na występowanie FPD. W żywieniu indyków diety o zawartości do 0,20% Na nasilały objawy FPD, w stosunku do diety bez dodatku chlorku sodu. U kurcząt istotne zakłócenia gospodarki mineralnej, w tym pogorszenie parametrów fizykochemicznych kości piszczelowej, występowały jedynie w przypadku mieszanek o bardzo niskiej zawartości sodu (0,02 i 0,07%), sporządzonych bez dodatku lub z bardzo niewielkim dodatkiem tego pierwiastka. Zwiększenie dodatku sodu u najmłodszych kurcząt ponad 0,14% i u indycząt ponad 0,13% diety nie poprawiało analizowanych parametrów mineralizacji kości. Wyniki doświadczeń wskazują, że ryzyko pogorszenia procesu mineralizacji kości, głównie w przypadku niedoboru Na, oraz możliwość zwiększenia wilgotności ściółki i występowania FPD (wraz ze zwiększeniem zawartości Na w diecie) są znacznie większe u indyków rzeźnych niż u kurcząt broilerów.

Słowa kluczowe: bilans elektrolitów, dodatek paszowy sodu, wilgotność odchodów, mineralizacja kości piszczelowej.

INTRODUCTION

Freedom from discomfort by providing an appropriate environment and freedom from pain, injury or disease by prevention or rapid diagnosis and treatment are important elements of animal welfare (Beaumont et al. 2010). In this context, it is worrying to notice that mass-scale poultry production and more intensive feeding of reared birds create worse indoor environmental conditions, leading to such health problems as foot-pad dermatitis (FPD) as well as deformations of and damage to legs (Oviedo-Rondon et al. 2006, Venäläinen et al. 2006). Leg diseases are frequently initiated by excessive moisture of the litter, which is caused by a variety of factors, not fully recognized yet but associated with intensive feeding of poultry (Francesch, Brufau 2004, Mayne et al. 2007). An excessive level of dietary sodium, administered to birds to accelerate their growth, is indicated among the aforementioned factors. An increased dietary intake of sodium stimulates higher water consumption and consequently raises the moisture content of faeces (Borges et al. 2003, Oviedo-Rondon et al. 2006, Mushtaq et al. 2007).

Deformations of legs and leg damage deteriorate the welfare of birds and slow down their growth rate (Oviedo-Rondon et al. 2006, Nääs et al. 2009). Inadequate bone mineralization in poultry is most often attributed to certain disorders in the mineral balance, especially calcium and phosphorus (Kestin et al. 2001, Rao et al. 2003, Venäläinen et al. 2006, Tatara et al. 2011). Less is known about the effect of strong electrolytes such as sodium, potassium and chlorine, in diets for young chickens and turkeys on the course of mineralization processes and stability of the skeleton which stores 1/3 of the sodium resources found in the whole body.

It has been demonstrated experimentally that disorders in mineralization processes, which manifested themselves as a reduced bone ash content, resulted from an increased amount of sodium in chickens' diet, while a higher dietary content of chlorine counteracted this effect (Murakami et al. 1997*a*,*b*). It has also been observed that sodium interacting with calcium can play an important role in the pathogenesis of skeletal muscles in broiler chickens (Sandercock, Mitchell 2004), which may enhance problems induced by bone deformations. Similar though scanty findings have stimulated the most recent experiments, recapitulated herein, which focused on the effect of sodium and – to some extent – potassium and chlorine on such litter moisture, incidence of FDP as well as mineral composition and deformations of the skeleton of slaughter chickens and turkeys. The purpose of this article has been to summarize the results of such experiments.

CONTENT AND BALANCE OF ELECTROLYTES IN DIETS FOR BROILER CHICKENS AND TURKEYS

According to the most commonly applied nutritional requirements of broiler chickens (NRC 1994), the optimum doses of sodium, potassium and chlorine in starter diets are 0.20, 0.30 and 0.20%, respectively. For grower and finisher diets, it is recommended to ensure the following rates of these elements: 0.15 and 0.12% of sodium, 0.15% and 0.12% of chlorine and 0.30% of potassium, respectively.

Higher doses of Na in diets were tested in numerous experiments conducted in the early 21st century, in the hope that they would contribute to an increased feed consumption and growth rate of chickens. From 1 to 21 day of age, for example, an optimum dose of sodium in a diet was suggested as 0.25% (Murakami et al. 1997*a*) and even higher: 0.28-0.30% (Oviedo-Rondon et al. 2001). In the second nutritional period of broiler chickens (21-35 days of age), 0.15-0.29% of Na in a diet was recommended as an optimal level (MURAKAMI et al. 1997b). Because of the widespread use of NaCl as a source of dietary sodium, diets have also become richer in chlorine.

With respect to turkeys, the percentage of sodium in a diet should be 0.17% in the first and 0.15% and second month of rearing turkey poults; afterwards, until the fattening is terminated, 0.12% of Na in a diet is considered as sufficient (NCR, 1994). The percentage of chlorine in a diet should be on a similar level (a little lower in the early phase), while the recommended percentage of K in a starter diet is 0.70% and should gradually decrease down to 0.40% in finisher feeds (NCR 1994).

In recent years, the content of K in a diet has risen considerably. There are several reasons, e.g. higher potassium fertilization of crops, the EU ban on using animal meals in animal nutrition, as a result of which plant feed mixtures are now richer in plant protein sources, especially soybean meal. Due to a high content of potassium in soybean meal, feed mixtures without any other high-protein ingredients are characterized by a relatively high content of K. This is illustrated by the data set in Table 1 concerning diets for broiler chickens and turkeys made without supplemental feed salt or a different source of sodium.

Table 1

Content of Na, K and Cl in feed mixtures without NaCl addition (JANKOWSKI et al. 2011*a*)

Specification	Sodium	Potassium	Chlorine
Content in a feed mix, g kg^{-1}	0.03	0.94	0.14
Nutritional requirement coverage for chickens, % of the NRC standard	16.5	313	72
Nutritional requirement coverage for turkeys, % of the NRC standard	19.4	134	96

Na, K and Cl have a substantial and mutually correlated impact on the water and electrolyte balance as well as the acid and alkaline balance of a living organism, hence they are known as strong electrolytes. When preparing animal feed mixtures, it is important to consider the content of all these elements as well as their sum, including the valence of a given element. This is known as the dietary electrolyte balance (DEB), which encompasses the sum of milliequivalents of Na^+ and K^+ minus the content of milliequivalents of Cl^- (MONGIN 1980).

It is assumed that under neutral environmental conditions, the DEB value for chickens is approximately 250 mEq kg (MONGIN 1981), being a mean value of quite a wide range of 220-270 mEq kg^{-1} (MUSHTAQ et al. 2007). For young turkeys, the DEB value derived from the recommended content of Na, K and Cl (NRC 1994) is 211 mEq kg^{-1}, thus being much lower than the optimal value in nutrition of broiler chickens.

EFFECT OF THE CONTENT OF NA ON LEVEL OF ELECTROLYTES IN BLOOD OF BROILER CHICKENS AND TURKEYS

Analysis of the level of electrolytes in blood serum, indicating possible consequences of the deficiency of excess of sodium in diets for broiler chickens and turkeys has been the subject of the experiment presented in Table 2, in which six diets were tested: with different amounts of supplemental NaCl and without this compound. As the synthetic presentation of the results of the tests on chickens shows, statistically significant differences were found between the group fed a diet without added NaCl versus the other nutritional regimes. Supply of diet no 1 caused a decrease in Na concentrations, while raising the concentrations of K, Ca and Mg, which suggests that Na deficit disturbed the mineral balance in chickens. Even a low level of supplemental

Table 2

Effect of different amounts of supplemental NaCl on the concentrations of electrolytes in the blood serum of broiler chickens (Tykałowski et al. 2011) and turkeys (Lichtorowicz et al. 2012)

Specification	NaCl addition to diet (%)					
	0	0.13	0.25	0.38	0.51	0.64
Content of electrolytes in diet, %*						
Na	0.02	0.07	0.12	0.17	0.22	0.26
K	0.89	0.90	0.89	0.88	0.89	0.88
Cl	0.11	0.20	0.27	0.34	0.41	0.48
Content of electrolytes in chickens' blood						
Na, mmol l^{-1}	136^{b}	151^{a}	150^{a}	150^{a}	149^{a}	148^{a}
Cl, mmol l^{-1}	103^{b}	115^{a}	116^{a}	114^{a}	113^{a}	112^{a}
K, mmol l^{-1}	8.46^{a}	5.72^{b}	5.32^{b}	5.21^{b}	5.45^{b}	5.54^{b}
Ca, mgl dl^{-1}	12.1^{a}	11.1^{b}	10.5^{b}	11.2^{b}	10.6^{b}	10.5^{b}
P, mgl dl^{-1}	7.04^{a}	7.29^{a}	6.41^{b}	6.78^{b}	6.52^{b}	6.12^{b}
Mg, mgl dl^{-1}	3.09^{a}	2.56^{b}	2.31^{c}	2.41^{b}	2.30^{c}	2.24^{c}
Content of electrolytes in turkeys' blood						
Na, mmol l^{-1}	141	150	148	150	146	148
Cl, mmol l^{-1}	105^{b}	110^{a}	110^{a}	109^{a}	110^{a}	110^{a}
K, mmol l^{-1}	3.56	3.85	3.55	3.28	2.71	3.27
Ca, mgl dl^{-1}	13.7^{a}	13.3^{ab}	12.8^{b}	13.3^{ab}	13.3^{ab}	12.9^{b}
P, mgl dl^{-1}	6.30^{c}	9.21^{a}	7.63^{b}	8.56^{a}	8.89^{a}	7.77^{b}
Mg, mgl dl^{-1}	2.83^{a}	2.34^{b}	2.37^{b}	3.37^{b}	2.41^{b}	2.46^{b}

* Data refer to chickens' diets. In turkeys' diets, with a higher content of soybean meal, the content of K was 0.2% higher while the content of Na and Cl was similar.

NaCl, such as 0.13%, which increased the content of Na in a diet from 0.02 to 0.07% and the content of Cl from 0.11 to 0.20%, ensured the concentrations of most of the analyzed elements at a level similar to that determined in diets with 2-5-fold higher NaCl supplementation. Significantly less Mg was observed in groups 3-6 compared to groups 1 and 2. This indicates that higher NaCl supplementation can decrease the concentrations of Mg in blood, but only with respect to groups receiving diets poor in sodium and chlorine, causing certain changes in the blood concentrations of electrolytes.

In an experiment conducted on young turkeys, diets with different amounts of Na and Cl did not cause changes in the concentrations of sodium and potassium in six-week-old turkeys' blood serum. The lowest level of sodium and chlorine in the diet decreased the concentrations of chlorine while increasing the concentrations of Ca and P in blood serum. With respect to a diet deficient in sodium, the highest NaCl supplementation decreased the concentrations of Ca.

In another experiment (JANKOWSKI et al. 2012a), in which the amounts of Na in diets were 0.07, 0.12, 0.17 and 0.22%, the concentrations of microelements were analyzed in the blood serum of turkeys that completed a full rearing cycle at 18 weeks of age. It was found that the blood serum concentrations of all elements (Y) except Mg tended to increase as the percentage of Na in a diet (X) increased. With respect to sodium, the increase followed the equation: $Y = 149.5 + 14X$ ($R^2 = 0.891$, $p = 0.056$). The same tendency was observed regarding the concentration of K ($Y = 2.14 + 2.36\ X$, $R^2 = 0.904$, $p = 0.049$) and the concentration of phosphorus in serum ($Y = 7.95 + 4.64\ X$, $R^2 = 0.945$, $p = 0.028$), while the dependences determined for Cl, Ca and Mg produced low coefficients of statistical significance.

Some other investigations demonstrated that excess of sodium caused increased blood volume and decreased counts of lymphocytes (EKANAYAKE et al. 2004). Another experiment (TYKAŁOWSKI et al. 2011) showed that a sodium-deficient diet (without supplemental NaCl) reduced the concentrations of the analyzed subpopulations of T-lymphocytes, but the differences versus the other groups were not statistically significant. This was also a result of a relatively high variation of the analyzed parameters, which did not show a straight linear dependence on the content of Na in a feed mixture.

The results of the authors' own experiments show that variations in the concentrations of microelements in blood serum, dependent on the amount of added NaCl, are relatively small, which limits the diagnostic value of this analytical determination.

EFFECT OF THE CONTENT OF ELECTROLYTES IN A DIET ON WATER INTAKE AND MOISTURE OF THE LITTER

It is known that dietary sodium is an important factor in birds' response to stress, especially heat stress, for instance through increased consumption of water (Olanrewaju et al. 2007). As evidenced experimentally (Borges et al. 2003), giving chickens a starter diet with the DEB increasing in the range of 0, 120, 240 and 360 mEq kg^{-1} caused an increase in water and feed intake from 2.31 do 2.76 l kg^{-1} during a six-week trial. Typically, as long as the thermal comfort is maintained, water intake is twice as high as the amount of ingested feed, but this ratio increases when the content of electrolytes in a diet, especially sodium, goes up. Then, the moisture content of bird excreta rises, as well.

An experiment conducted by Enting et al. (2009) suggests that the litter moisture is highly significantly correlated with the moisture content of birds' faeces. In an earlier study of Murakami et al. (2001), it was shown that differences in the content of dietary Na within 0.1 to 0.35% caused a proportional increase in the moisture content of litter. In another trial (Vieira et al. 2003), during the first week of age of chickens, as the content of Na in a feed mixture increased successively from 0.12 to 0.48%, so did the water intake while the dry matter content in excreta decreased from 28% do 18%. In another experiment (Mustaq et al. 2007) with similar percentages of Na in diet, i.e. 0.20, 0.25 and 0.3% Na, respectively, the litter moisture (in %) was 33.2^{b}, 37.5^{a} and 29.3^{c} (significance of differences $p<0.05$).

In one of our experiments (Jankowski et al. 2011*a*), it was noted that the addition of NaCl, increasing the content of both elements in the diet, affected some parameters of the small intestine, for instance decreased the pH of the intestinal contents (Table 3). On the other hand, the amount of Na in the diet had no effect on the percentage of dry matter in the caecal contents.

A similar analysis of varied levels of NaCl added to feeds was completed experimentally (Jankowski et al. 2012*a*) for slaughter turkeys. Analogously to the aforementioned experiment on chickens (Table 3), differences in the amounts of Na added to turkeys' diets caused significant changes in the functions of the small intestine, but led to much smaller alterations in the parameters of the caecum. Thus, the content of dry matter in excreta of these birds was even, despite different quantities of dietary Na, ranging from 0.05 to 0.20%. At the completion of both turkey rearing periods, i.e. at 8 and 18 weeks of age, significant intensification of FPD symptoms was observed (Table 4). In the 8th week, the advancement of FPD rose from 1.7 to 2.0 points, and in the 18th week of turkey rearing it went up from 3.2 to 3.5 points. This score can indicate that turkeys are more sensitive to leg disorders classified as FPD. In the final phase of rearing, a small increase in the content of dietary Na, from 0.10 to 0.15%, intensified the symptoms of FPD.

Table 3

Parameters of digestive tract function in chickens fed diets with different amounts of supplemental NaCl (JANKOWSKI et al. 2011*a*)

Specification	Supplemental Na in feed (%)		
	0	0.10	0.25
Small intestine			
the total mass, g kg^{-1} BW	47.0^{a}	43.7^{ab}	42.5^{b}
DM of contents, %	19.3^{a}	17.2^{b}	17.4^{b}
viscosity, mPas	2.11^{a}	1.65^{b}	2.20^{a}
pH of contents	5.77^{a}	5.22^{b}	5.27^{b}
Caecum			
mass of contents, g kg^{-1} BW	4.94^{a}	4.71^{b}	6.29^{a}
DM of contents, %	18.1	16.7	18.0
pH of contents	6.16	6.22	6.08

Table 4

Effect of the Na content in feeds on moisture of faeces and incidence of FPD in turkeys aged 8 and 18 weeks (JANKOWSKI et al. 2012*a*)

Na added to feed (%)	8 weeks		18 weeks	
	dry matter (%)	FPD	dry matter (%)	FPD
0.05	19.0	1.7^{b}	20.5	3.2^{b}
0.10	19.4	1.8^{ab}	20.0	3.3^{b}
0.15	19.2	1.7^{b}	20.2	3.5^{a}
0.20	19.0	2.0^{a}	19.9	3.5^{a}

EFFECT OF SODIUM ON BONE MINERALIZATION IN BROILER CHICKENS AND TURKEYS

Experiments testing the effect of varied amounts of dietary sodium on bone mineralization in poultry are relatively few. Selected results of one of such experiments are presented in Table 5.

Uniform measurements of the tibia's relative mass suggest that different Na and Cl supplementation levels did not reduce the share of the tibia's mass in the total body mass of chickens. This finding also held true for chicken fed on a mix without supplemental Na. However, the growth rate of chickens in that group was inhibited, so that the final body weight was less than 0.5 kg. Likewise, in the second group, where the percentage of Na added to the feed mixture was small (0.05%), the final body weight was less than 1.5 kg. In the other groups, it was significantly higher (1.64-1.71 kg). Feeding chickens on a diet without added Na decreased the content of ash,

Table 5

Parameters of the mineralization and strength of the tibia in chicken fed diets with different supplemental Na (JANKOWSKI et al. 2011*a*)

Specification	Content of Na in diet* (%)					
	0.02	0.07	0.12	0.17	0.22	0.26
Relative bone mass, % body weight	0.49	0.54	0.53	0.51	0.52	0.50
Bone content of:						
ash, % dry matter	50.7^{b}	53.8^{a}	54.8^{a}	52.1^{ab}	52.5^{ab}	50.7^{b}
Ca, % dry matter	18.0^{b}	18.8^{ab}	19.5^{a}	18.2^{b}	18.5^{ab}	17.9^{b}
P, % dry matter	8.69^{B}	9.36^{A}	9.41^{A}	8.82^{B}	9.06^{AB}	8.78^{B}
Minimum breaking strength, kN	0.23^{C}	0.63^{A}	0.62^{AB}	0.56^{AB}	0.59^{AB}	0.54^{B}

* Content in grower diet, given from 15-35 days of age of chickens;
$^{a,\,b}$ and $^{A,\,B}$ – significance of differences at 0.005 and 0.001

Ca and P in the tibia's dry matter and radically increased its vulnerability to fracture. Even a small amount of Na added to a diet (0.05%) sufficed to offset the above negative consequences of sodium deficiency in chickens' diet. In the group which received the feed mixture with the highest Na content (0.26%), a decrease in the content of ash, Ca and P in the tibia was noted, similarly as in the group fed a sodium-deficient diet, but the tibia did not become more breakable.

In an experiment on young turkeys (to six weeks of age), it was found that a sodium-deficient diet (0.035) decreased the body weight of birds, including the weight of the tibia, but did not reduce the content of bone ash, ca and P (JANKOWSKI et al. 2012*b*). This research problem was analyzed on turkeys reared in a full cycle, to 18th week of age, using diets with four levels of Na, from 0.07 to 0.22% (JANKOWSKI et al. 2012*a*). Different concentrations of Na in a diet did not affect the relative bone mass of the tibia or the content of ash, Ca and P in the bone. Significant differences were noted while analyzing the parameters of tibia breaking strength. An increased content of Na in diets, from 0.07 and 0.12 raised to 0.17 and 0.22%, caused a significant increase in the perimeter and density of the tibia, reflected in higher elastic and breaking strength of the bone.

The results of the experiments discussed above imply that deterioration of the parameters of bone mineralization can be induced by both a deficient and an excessive Na content of a diet. Regarding chickens, worse bone mineralization parameters were achieved when the dietary content of Na was either decreased from 0.07-0.22% to 0.02% or increased to 0.26%. With respect to slaughter turkeys, better bone mineralization parameters were observed when dietary sodium levels were increased from 0.07-0.12 to 0.17-0.22%.

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CALCIUM BIOAVAILABILITY FROM DAIRY PRODUCTS AND ITS RELEASE FROM FOOD BY *IN VITRO* DIGESTION

Jan A. Kłobukowski[1], Krystyna A. Skibniewska[2], Ireneusz M. Kowalski[3]

[1]Chair of Human Nutrition
[2]Chair of Foundations of Safety
[3]Chair and Clinic of Rehabilitation
University of Warmia and Mazury in Olsztyn

Abstract

Food products are an exclusive source of nutrients necessary for the development and proper functioning of human organism. The key issue in nutrition is the bioavailability of nutrients rather than the supply of their adequate amounts in a diet. Calcium is the basic structural element of bones and teeth. It is also an element with a wide range of physiological functions, e.g. it is responsible for the right excitability of muscular and nervous system, normal heart function, hormonal secretion, blood clotting, cell membrane permeability and activation of various enzymes. It is universally acknowledged that the main source of calcium for a human organism is milk and dairy products, owing to their rich content and high availability of calcium. However, certain stages in food processing technologies may depress the high bioavailability of dietary nutrients.

In this paper, long-term research findings about the bioavailability of calcium in milk products have been summarized, in addition to the information about calcium uptake from bread or other popular breakfast meals composed of milk products in combination with cereal component. The popular opinion that milk and other dairy products are the best and most valuable source of highly available calcium has indeed been supported. However, an *in vitro* study demonstrates that an addition of cereal to milk or yoghurt significantly diminishes the quantity of calcium released during digestion, which could contribute to the development of calcium insufficiency or augmentation of its symptoms.

Keywords: milk, dairy products; bread, cereals, calcium deficiency.

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prof. dr hab. Krystyna A. Skibniewska prof. zw., Chair of Foundations of Safety, University of Warmia and Mazury , Heweliusza 10, 10-726 Olsztyn, Poland, e-mail: kas@uwm.edu.pl

BIODOSTĘPNOŚĆ WAPNIA Z PRODUKTÓW MLECZNYCH I JEGO UWALNIANIE Z ŻYWNOŚCI PODCZAS TRAWIENIA IN VITRO

Abstrakt

Pożywienie jest jedynym źródłem składników odżywczych niezbędnych do rozwoju i prawidłowego funkcjonowania organizmu człowieka. Kluczowym zagadnieniem w żywieniu jest dostarczenie odpowiedniej ilości składnika odżywczego, w związku z czym duże znaczenie ma biodostępność z diety. Wapń to podstawowy składnik budulcowy kości i zębów, również pierwiastek o bardzo szerokim znaczeniu fizjologicznym, odpowiada bowiem za prawidłową pobudliwość układu mięśniowo-nerwowego, właściwą pracę serca, sekrecję hormonalną, krzepnięcie krwi, przepuszczalność przez błonę komórkową oraz aktywację wielu enzymów. Powszechnie przyjęte jest, że głównym źródłem wapnia dla organizmu człowieka jest mleko i nabiał zarówno ze względu na dużą zawartość, jak i przyswajalność tego pierwiastka z produktów mleczarskich. Zmiany w technologii przetwórstwa żywności są zagrożeniem dla wysokiej biodostępności składników odżywczych z diety.

W pracy podsumowano wyniki wieloletnich badań autorów nad biodostępnością wapnia z produktów mleczarskich oraz uwalnianiem tego pierwiastka z chleba oraz posiłków śniadaniowych składających się z produktów mlecznych z dodatkiem zbożowych. Potwierdzono powszechną opinię o mleku i nabiale jako najcenniejszych źródłach łatwo przyswajalnego wapnia. Jednak w badaniach in vitro wykazano, że dodatek produktów zbożowych do mleka lub jogurtu znacznie zmniejsza ilość wapnia uwalnianego w procesie trawienia, co może wywoływać lub nasilać objawy niedoboru wapnia u człowieka.

Słowa kluczowe: mleko, nabiał, chleb, produkty zbożowe, niedobór wapnia.

INTRODUCTION

Calcium is the most abundant macroelement in a human body (Peckenpaugh 2011, Miciński et al 2012, 2013*b*). Out of the average 1200 g of the element detected in an adult organism, 99% is stored in bones and teeth, and the remaining 1% can be found in body fluids and soft tissues. Calcium has a very broad range of physiological functions, of which the most important ones are:

- calcium is the basic structural element (as calcium phosphate-hydroxyapatite) of bones and teeth, necessary for their proper development;
- it is responsible for the regulation of the nervous and muscular system (e.g. it participates in the systolic and diastolic contractions of the myocardium);
- calcium is cne of the main factors in a cascade of blood coagulation process (transformation of prothrombin into thrombin);
- it acts as activator of some biocatalyzers (enzymes), e.g. lipase, ATP-ase.
- it diminishes the passage of serum through capillaries and is used to reduce amounts of allergic exudates;
- calcium mediates secretion of insulin and calcitonin from the cells.

Symptoms of calcium deficiency in humans may be discussed on two levels: deficiency resulting from an insufficient dietary supply or as hypocalcaemia manifested by decreased Ca concentration in blood, most often determined by the occurrence of hormonal disorders (e.g. parathormone – PTH deficiency), insufficient supply, synthesis and/or absorption disorders of vitamin D, resulting in a lower level of 1,25-$(OH)_2D_3$ (1,25dihydroxycholecalcypherol). Calcium deficiency may result in the growth disorders in children and improper bones calcification (rachitis), leading to deformations and reduced mechanical resistance of bones. Elderly people, especially women after menopause, are exposed to bone softening (osteomalacia) and loss of bone mass, which is a risk factor in osteoporosis, which may lead to pathological bone fractures. Most prevalent are lumbar spine fractures, femur neck fractures, wrist and forearm fractures.

Calcium homeostasis means an organism is able of maintain a constant concentration of Ca in blood serum within the range of 2.3-2.7 mmol dm^{-3}, accepted as physiological norm. Calcium homeostasis is achieved by increasing or decreasing the intestinal Ca absorption, its excretion with urine and faeces and utilization of reserves in bone tissue. The latter coincides with an initiation of hormonal regulatory mechanisms. A decrease in the blood calcium concentration is accompanied by excretion of parathormon from parathyroids; the hormone is responsible for conversion of vitamin D to its active, di-hydroxylic form. As a result, the rate of calcium absorption from the gastrointestinal tract increases. PTH and 1,25-$(OH)_2D_3$ simultaneously, influence the Ca release from bones and inhibit the excretion of this element in urine through the process of Ca re-absorption in kidneys. Calcitonin, secreted by the thyroid gland, is a PTH antagonist, which means that it acts opposite to PTH and decreases calcium concentrations in blood serum (MICIŃSKI et al 2013*a*).

BIOAVAILABILITY

Minerals, including calcium, occur in food as inorganic and organic compounds, often in water solution, which results in the varied Ca release and absorption. Bioavailability (biological availability, bioassimilation) is understood as a degree to which a nutrient is converted by the human organism into the form ready to be absorbed and utilized in metabolic processes and/or for storage (FAIRWEATHER-TAIT 1995, JACKSON 1996). Bioavailability of nutrients is determined through basic measurements: apparent absorption (A) defined as a difference between amounts ingested and excreted with faeces, equal absorbed amount, and apparent retention (R), understood as a difference between amounts absorbed and excreted with urine, equal amount of nutrient retained within the organism i.e. utilized in metabolism or stored. The bioavailability parameters is expressed in mg(μg) day^{-1} or in percentage units in comparison to ingested amount. The so-called indices of specific bioavailability are used for some bioelements (iron, selenium, zinc); a specific bioavailability index defines the portion of a mineral bound into biologically active compounds (e.g. activity of glutathione peroxidase for selenium or level of ferritin in blood serum as an indicator of iron store in the liver).

Many factors influence bioavailability of minerals in human organisms. Generally, they can be divided into two groups: exogenous, directly connected with food, and endogenous, related to the organism. Among the food-related factors, the most important ones are: properties of a given mineral, type and amount of compounds containing the mineral, oxidation status, solubility, presence of antagonistic (competitive) ions, presence of substances facilitating or hindering absorption of the mineral. Endogenous factors are: genetic conditions, age, sex, physiological status (e.g. pregnancy, breast-feeding), mineral supply status (stock), emotional state, illnesses.

CALCIUM BIOAVAILABILITY FROM MILK AND DAIRY PRODUCTS

Milk and dairy products are an excellent source of calcium (Ca) for a number of reasons. This statement refers to total calcium content in this group of food products, ratio of calcium to protein and of calcium to phosphorous (Table 1). The highest Ca content was found in ripening cheeses (from about 400 to 1400 mg 100 g^{-1} of the product). A lower calcium content was determined in milk fermented beverages (103-170 mg 100 g^{-1}) and fresh cheeses (90-100 mg Ca 100 g^{-1}). However, milk fermented beverages were characterized by a nutritionally good ratio of calcium to phosphorous (1.4:1), but the Ca : P ratio in fresh cheeses was low (0.4), similarly to the calcium to protein ratio, which equalled *ca* 5 mg Ca in 1 g of protein.

It is worth emphasizing that consumption of 1 dm^3 of milk supplies 100% of daily calcium requirement of adult person (1000 - 1200 mg per day). Kłobukowski at al. (1997*ab*) found a high calcium content, comparable to that in ripening cheeses, in Ricotta-type non-ripening cheeses, where it ranged from 750 mg in 100 g (cheese No 1) and 600 mg 100 g^{-1} (cheese No 2). The study revealed a highly favourable Ca : P ratio of 1.85 and 1.55, respectively. A higher ratio was found only for Parmesan (1.70) and Ementaler cheese (2.01) – Table 1.

The calcium to protein proportion (mg Ca in 1 g protein) in a diet appeared to be a very helpful index, ready to be used for determination of the influence of dietary protein content on calcium homeostasis and health status of bones. It has been proved that high protein consumption negatively

Table 1

Content of calcium, protein, phosphorus and ratios between these nutrients in milk and dairy products

Dairy product	Content* (g, mg 100 g^{-1} of product)			Ratio**	
	protein (g)	calcium (mg)	phosphorus (mg)	Ca : protein (mg g^{-1} of protein)	Ca : P
Milk 2% of fat	3.4	120	86	35.3	1.40
Natural yoghurt 2% of fat	4.3	170	122	39.5	1.39
Kefir 2% of fat	3.4	103	74	30.3	1.39
Buttermilk 2% of fat	3.4	110	80	32.4	1.38
Whey	0.8	68	40	85.0	1.70
Full-fat Brie cheese	19.8	600	380	30.3	1.58
Full-fat Camembert cheese	21.4	386	310	18.0	1.25
Full-fat Cheddar cheese	27.1	703	487	25.9	1.44
Full-fat Ementaler cheese	28.8	835	416	29.0	2.01
Parmesan cheese	41.5	1380	810	33.2	1.70
Gouda cheese	27.9	807	516	28.9	1.56
Tylżycki cheese	26.1	815	510	31.2	1.60
Acid-type fresh cheese	19.8	96	240	4.85	0.40
Fromage cheese	10.2	55	123	5.4	0.45
Homogenized cream cheese	17.7	88	216	5.0	0.41
Edamski processed cheese	13.5	367	578	27.2	0.63

* protein, calcium and phosphorous content after the Polish tables of food content (KUNACHOWICZ et al. 2008);

** authors' calculations

influences calcium balance in an organism and increased calcium excretion with urine when the Ca : protein index is below 20 (CASHAM 2002, ZITTERMANN 2002). The value of this index is much higher for milk, milk fermented beverages and ripening cheeses (Table 1), which proves that these dairy products are an excellent Ca source. Low values of this index calculated for fresh cheeses were due to the technological process which involved elimination of whey containing even 80% of the milk calcium. Milk souring causes a release of insoluble calcium phosphate from casein micelles, which then dissolves in milk acid, transforms into an ionic form and is transferred into whey after separation of milk curd.

Bioavailability, determined in balance studies, is a very important criterion measuring how a human organism utilizes minerals, including calcium. The highest values (expressed as percentages) of calcium apparent absorption and retention were noted for fresh cheeses produced by both the classical method (acidic coagulation) and with addition of the probiotic *Lactobacillus plantarum*, prebiotics: 2.5% addition of inuline or maltodextrin and synbiotics: *Lactobacillus plantarum* plus 2.5% addition of inuline or maltodextrin. In almost all cases these measures of bioavailability were much above 80% (Table 2). Lower results (by about 20 percent units) of A and R parameters were obtained for diets containing ripening cheeses (Jeziorański and Tylżycki) produced by immersion in traditional (20% NaCl solution) and in modified brine (1 : 1 20% NaCl and KCl solution).

The lowest (about 50-60%) values of A and R (Table 2) were found for Ricotta-type non-ripening cheeses produced by the method of thermal-acidic coagulation of milk protein and for whey and permeate, byproducts of fresh cheese production. It should be stressed that all the values of apparent absorption and retention presented in table 2 are very high, several-fold in excess of the bioavailability of Ca from cereal products (usually <10%).

The highest calcium availability in milk and dairy products among all foodstuffs results from both nutritional and non-nutritional factors. The nutritional factors are connected with the age (higher intestinal absorption by young people), sex, physiological state (pregnancy, breast-feeding), state of organism, saturation with vitamin D, illnesses or medicine taking (e.g. diuretics). It has been proven that young, growing rats absorbed almost three times more calcium from the intestine than mature ones (McErloy et al. 1991). Higher resorption of calcium from bones was observed in women in early menopause due to a lower estrogen level (Nordin et al. 2004). Deficien-

Table 2

Values of calcium apparent absorption (A) and retention (R) coefficients from diet containing dairy products

Dairy products	Calcium bioavailability coefficients* (%)	
	apparent absorption (A)	apparent retention (R)
Ripened cheeses:		
1. Jeziorański (NaCl)	64.4	59.6
2. Jeziorański (NaCl+KCl)	73.0	58.8
3. Tylżycki (NaCl)	78.9	68.0
4. Tylżycki (NaCl+KCl)	81.3	76.3
Ricotta-type unripened cheeses:		
1. Ricotta-type cheese 1	60.5	52.0
2. Ricotta-type cheese 2	57.4	52.2
1. Acid-type fresh cheese	90.3	84.2
2. Acid-type fresh cheese with L. *plantarum*	90.5	86.3
3. Acid-type fresh cheese with L. *plantarum*+inulin	93.5	90.3
4. Acid-type fresh cheese with L. *plantarum*+maltodextrin	84.8	80.1
5. Acid-type fresh cheese with inulin	87.1	80.2
6. Acid-type fresh cheese with maltodextrin	93.3	90.2
Permeate	51.8	49.5
Whey	54.4	52.2

* Bioavailability indexes have been calculated utilizing published (Kłobukowski et al. 1997*a* and *b*, 2004, 2009, Modzelewska-Kapituła et al. 2008) and unpublished data of Kłobukowski et.al.

cy of vitamin D decreases calcium intestinal absorption and utilization in an organism (Lips 2012). An active person, spending much time outdoors and consuming fish, eggs and fatty dairy products does not suffer from vitamin D deficiency, because it is synthesized in the skin from 7-dehydrocholesterol under the influence of ultra violet rays (Zittermann et al. 1998). The vitamin D synthesis rate decreases in elderly people when the solar exposure is insufficient.

The following nutritional factors affect the bioavailability of calcium from diets containing dairy products: amount and form of calcium, content of lactose, proteins and phosphorous. The beneficial effect of lactose on calcium absorption has been demonstrated in studies on the influence of calcium and milk components on values of Ca apparent absorption indices (Buchowski, Miller 1991). Lactose facilitates Ca absorption by stimulating, independently from vitamin D, the diffusive system from the calcium intestinal transfer. In all probability, lactose cooperates with intestinal villi and enhances the Ca penetrability. The resence of calcium bound to casein in the form of colloidal di- and tripeptide (phosphopeptides) may be a barrier to its absorption (Tsuchita et al. 1995). Soral-Śmietana et al. (2013) proved that acid whey concentrates produced by various membrane processes did not differ in the calcium bioavailability coefficients determined for rat diets. On the other hand, Pantako et al. (1994) proved that whey proteins, especially α-lactoalbumin, may favour calcium absorption by rats.

A diet typical for Poland and many other countries, also highly developed one does not supply enough calcium for a human organism. It has been proved (Kerstetteret al. 2003) that an insufficient supply of standard value protein and dietary calcium (e.g. a diet without or with too little of milk and dairy products) increases the risk of bone injuries and prolongs the rehabilitation time after fractures. Owing to a high content of easily bioavailable calcium, milk and its products can provide staple human food with Ca, preventing its deficiency which leads to osteoporosis. As a consequence, the diet with sufficient supply of milk products promote proper development and health of humans.

CALCIUM AVAILABILITY FROM BREAD AND CEREAL ADDED TO DAIRY MEALS

Bioavailability can be assessed accurately on a model of living organisms. Experiments with humans are very rare because of restrictions and high costs; most common are experiments on rats. It is easier and less time-consuming to determine the rate of nutrient release during *in vitro* simulated digestion in the human gastrointestinal tract. This way it is possible to assess the amount of a nutrient ready to be utilized by the organism (Skibniewska et al. 2002).

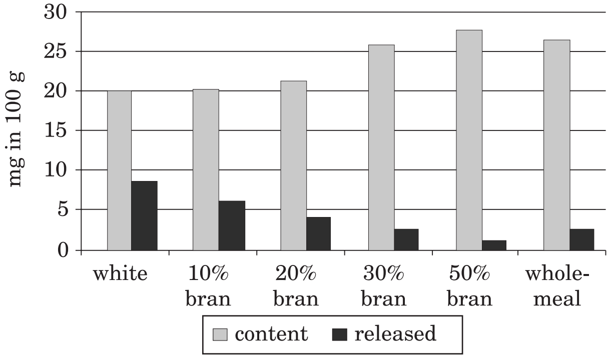

Fig. 1. Content of calcium and its amount released in the process of digestion from bread with various additions of bran

Bread and other cereal products are very popular foodstuffs throughout the world, and are often the main source of energy, nutrients, minerals and vitamins. The content of minerals in bread strongly depends on type of flour: white flour, made mostly from kernel endosperm, contains much less of minerals than darker types of flour, containing some share of germ and bran. Brown bread, especially whole-meal one, contains also phytates and dietary fiber, compounds with anti-nutritional activity which decrease the bioavailability mineral by forming non-digestible, insoluble compounds with minerals (Grases et al. 2001). Figure 1 presents the content of calcium and its amount released in the *in vitro* digestion process from bread baked from white flour and with addition of various amounts of bran. Brown bread contained not much more of the element, but the amount of released Ca dramatically decreased with an increase in the bran proportion.

While searching for a method to raise amounts of minerals released during digestion, bread baked from dough subjected to prolonged fermentation (no less than 5 h, up to 10-11 h) was examined (Skibniewska et al. 2004). The aim was to check whether prolonged fermentation would allow phytase to decompose phytic acid and release minerals. The expected result,

Table 3

Content of calcium in Polish bread (Kunachowicz et al. 2008)

Bread	Ca content (mg 100 g^{-1})
Rye bread (white)	14
Rye bread (brown)	25
Rye bread (Litewski)	61
Rye bread with soya and sunflower seeds	41
Popular bread (wheat and rye)	17
Graham bread	22
Wheat bread	16

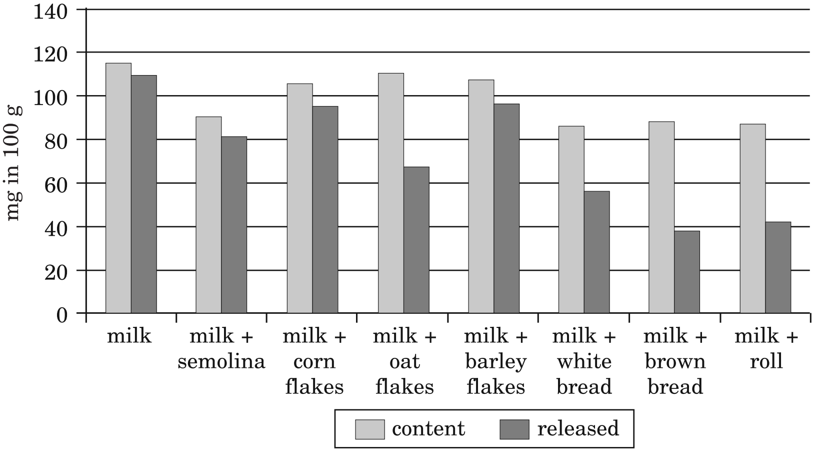

Fig. 2. Content of calcium and its amount released in the process of digestion from breakfast meals

i.e. a high percentage of minerals released from bread produced with this method, was not obtained (63% of Ca was released on average). Another experiment was run to study the influence of various methods of dough preparation on mineral release (SKIBNIEWSKA et al. 2005). Calcium was released in just 5% from whole-meal spelt flour, 16% from whole-meal common wheat flour and 66% (in a range of 34 to 87%) from mixed wheat and rye bread, the most popular type of wheat on the Polish market. SKIBNIEWSKA et al (2002) studied the influence of baking additives (natural leaven, starter cultures and complex dough improver) on the *in vitro* digestibility of minerals from white and whole-meal bread. As in the previous experiments, the share of bran had the strongest influence on the release of minerals: 74-86% of Ca was released from white bread and 16-51% from the whole-meal one (51% was released from bread baked with addition of natural leaven only).

In order to elucidate the influence of intestinal bacteria on mineral release, an experiment with the addition of *Bifidobacterium bifidum* was performed (NALEPA et al. 2012). Amounts of minerals released during enzymatic digestion varied depending on the element, bran content and presence of bacteria. As in all the previous experiments, a higher bran content lowered the release of elements. Addition of bacteria influenced mineral release in a different manner, e.g. the release of calcium (together with iron) was reduced in the presence of *Bifidobacterium bifidum* (bacteria probably utilized some of the elements for their growth).

Bread, even whole-meal one, contains little calcium (Table 3) in comparison to the daily recommended dose (about 1 000 mg depending on age, sex and physiological status), which means that the low availability of calcium from bread is not a problem. But the decreasing Ca availability from food caused by anti-nutrients from cereal products is a cause of worries.

In an experiment carried out in 2005, breakfast meals composed of milk alone or with addition of cereals were digested *in vitro* (Figure 2). Almost all the Ca included in milk was released into the solution. Corn and barley flakes, as well as semolina, decreased only slightly the amount of Ca determined in the solution after digestion, although brown bread and rolls captured about half of the element. Rolls were a surprise because they were made from white flour. They were bought in a shop, hence their exact composition was unknown.

In 2012, a paper on calcium availability from pure yoghurt and yoghurt with an addition of cereal products was published (SKIBNIEWSKA et al. 2010). The yoghurts contained less calcium than reported in the Polish tables of food composition (KUNACHOWICZ et al. 2008). Moreover, a very small portion of the element was released: on average 45%, in a range from 28.5 to 77.9%. At this moment, the results raise a question for discussion: in view of the of general deficiency of calcium in the human diet, should dieticians recommend mixing milk and cereals?

CONCLUSIONS

The above information (high values of apparent absorption and retention indexes) explicitly confirms the opinion that milk and dairy products are the most valuable source of highly available calcium among all the food products is the human diet.

At present, the human bone system is exposed to three problems: a decline in the milk and dairy product consumption causing a dramatic decline in calcium intake, innovations in dairy processing technologies leading to a decreased calcium concentration in food, and dialecticians recommending to increase consumption of whole-meal cereals, which leads to a worse calcium bioavailability. Because osteoporosis has grown to be a global health problem, monitoring calcium bioavailability from food is necessary.

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MERCURY IN MEDICINE AND HEALTH SERVICE

Anna Osinka, Kazimierz Pasternak

Medical University of Lublin

Abstract

People are most often exposed to mercury in water, amalgam dental fillings, vaccines with ethylmercury as a preservative and in methylmercury containing fish. Foetuses and children are most susceptible to toxic mercury, which passes to their organisms through the placenta or with mother's milk.

Distribution of mercury in organs depends on the type of compound absorbed and duration of exposure. Mercury is mainly absorbed through the respiratory system.

There are two types of mercury poisoning: acute and chronic. The critical organs for acute poisoning with mercury vapour are the lungs. Acute poisoning develops when large amounts of mercury are inhaled, which may lead to acute bronchitis, bronchiolitis and pneumonia. Chronic exposure to mercury vapour primarily affects the central nervous system.

Mercury is considered as a contributing factor in aetiology of numerous disorders, including neurologic, renal, immunological, cardiologic, reproductive or even genetic abnormalities. Studies have demonstrated the correlation between mercury toxicity and pathogenesis of Alzheimer's or Parkinson's disease, autism and multiple sclerosis.

Mercury may be involved in four processes which lead to genotoxicity: generation of free radicals and oxidative stress, effects on microtubules and on DNA repair mechanisms as well as direct interaction with DNA molecules.

Keywords: mercury, methylmercury, ethylmercury, thiomersal, toxicity.

RTĘĆ W MEDYCYNIE I LECZNICTWIE

Abstrakt

Człowiek najbardziej narażony jest na kontakt z rtęcią zawartą w wodzie, amalgamatach w plombach dentystycznych emitujących pary rtęci, szczepionkach z etylortęcią stosowaną jako środek konserwujący, metylortęcią w rybach.

Płód i dzieci są bardziej podatne na toksyczną rtęć. Matki spożywające w swojej diecie toksyczną rtęć mogą narazić na jej działanie swoje potomstwo, ponieważ rtęć może przechodzić przez barierę łożyskową lub z mlekiem matki do organizmu dziecka. Rozmieszczenie rtęci w narządach zależy od rodzaju związku wchłoniętego do organizmu i czasu trwania ekspozycji. Główną drogą wchłaniania tego metalu jest układ oddechowy.

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mgr inż. Anna Osińska, Medical University of Lublin

Można wyróżnić dwa rodzaje zatruć rtęcią: ostre i przewlekłe. Narządem krytycznym w zatruciach ostrych parami rtęci są płuca. Gdy do organizmu dostaje się duża ilość rtęci drogą oddechową, może rozwinąć się ostre zapalenie oskrzeli, oskrzelików i zapalenie płuc. Natomiast w przypadku przewlekłego narażenia na pary rtęci układem krytycznym jest ośrodkowy układ nerwowy. Ta postać choroby występuje w przypadku długotrwałego narażenia na niskie stężenia par rtęci.

Rtęć jest uznana za czynnik przyczynowy różnego rodzaju zaburzeń, w tym neurologicznych, nefrologicznych, immunologicznych, kardiologicznych, rozrodczych, a nawet genetycznych. Badania wykazały korelację między toksycznością rtęci a patogenezą choroby Alzheimera lub choroby Parkinsona, autyzmem i stwardnieniem rozsianym.

Rtęć może być zaangażowana w cztery procesy, które prowadzą do genotoksyczności: wytwarzanie wolnych rodników i stresu oksydacyjnego, działanie na mikrotubule, wpływ na mechanizmy naprawy DNA i bezpośrednią interakcję z cząsteczkami DNA.

Słowa kluczowe: rtęć, metyl ortęć, etyl ortęć, tiomersal, toksyczność.

INTRODUCTION

Mercury is a silver-white metal whose Latin name is *hydrargyrum,* meaning liquid silver, hence its symbol Hg. In its liquid form, mercury is poorly absorbed and poses a low health risk. Its vapours, however, are well absorbed into the respiratory system, thus being the major cause of numerous intoxications. Mercury is considered a serious problem as it accumulates in the environment. Organic compounds are more dangerous due to their toxic effects. Compared to inorganic forms, they are more easily soluble in lipids, more mobile in the human body and are highly likely to cause brain damage because they cross the blood-brain barrier (Clarkson et al. 2007, Magos et al., 2006).

Mercury in pharmaceuticals and utility products

Mercury has long been a popular choice for dental amalgams. In individuals with amalgam fillings, chewing gum for example leads to the release of mercury vapours, which are transported with inspired air to the olfactory bulbs, brain and lungs and permeate into the blood vessels (Baird et al. 2004). Thus, the amount of mercury in the brain may be proportional to the number of amalgam fillings. Moreover, individuals with such fillings have 4 to 5-fold higher levels of mercury in urine and blood.

Mercury compounds have antibacterial and antifungal properties, which is why they are used as preservatives or antiseptics in paints, cosmetics, medicines and vaccines. For example, thimerosol is a mercury-containing compound used as a preservative in hepatitis B, diphtheria, pertussis, acellular pertussis and tetanus vaccines. The use of mercury in vaccines has aroused interest due to infant deaths and speculations about its effects (Westphal Hallier 2003). Skin whitening creams and soaps made in some developing countries are a recognized source of chronic mercury poisoning (Harada et al. 2001).

Mercury in the human body

Distribution of mercury in organs depends on the type of compound absorbed and duration of exposure. The major route of mercury absorption is via the respiratory system; 80% of mercury absorbed by breathing is retained in the body, mainly in the blood, brain and foetal tissues.

Metallic mercury vapour enters the bloodstream, causing oxidation of red blood cells; 99% of mercury species circulating in plasma are attached to protein-bound thiol groups. Transport of mercury into organs and subsequent organ distribution is determined by the remaining 1% of mercury bound to "diffusible thiols"-, i.e. low molecular weight thiols that are capable of crossing the cell membranes.

A certain amount of elemental mercury remaining in blood crosses the blood-brain and placental barrier, causing the accumulation of mercury in the brain and foetal tissues. The concentration of mercury in the brain of those occupationally exposed to mercury vapour was found to be several times higher than in other organs (except kidneys). Moreover, in women exposed to mercury during pregnancy, the content of methylmercury in blood was lower compared to foetal blood cells.

In humans, about 95% of alkylmercury compounds are absorbed from the gastrointestinal tract, in contrast to just 7% of inorganic mercury compounds. Methylmercury compounds are also absorbed through the skin. Approximately 90% of inorganic mercury accumulate in kidneys.

The concentration of mercury in urine reflects its level in kidneys. Since kidneys mainly accumulate inorganic fractions of mercury, they may be considered a biomarker of mercury vapours in the entire body (SEŃCZUK 2002).

Much of inorganic mercury is eliminated through the gastrointestinal system, with bile and faeces. High mobility of mercury vapours is associated with its physical properties. It is believed that an atom of mercury crosses the cell membranes by passive diffusion. Mercury passes to the bile as a complex with glutathione. The structure of mercury glutathione complex (Hg-GS-SG) is similar to that of oxidized glutathione (GS-SG) and thus may freely permeate the hepatic cell membranes (CLARKSON et al. 2007, BALLATORI 2005).

Ninety percent of methylmercury compounds are excreted in faeces. Elimination half-life of methylmercury is over 70 days, compared to 40 days for most inorganic salts and 60 days for mercury vapour. Mercury is excreted with urine in the form of metallothionein. In tissues, methylmercury occurs in the form of macromolecular complexes with proteins; small molecular complexes predominate in blood whereas the brain contains mostly methylomercury glutathione.

MERCURY POISONING

Acute poisoning

The critical organs of acute poisoning with mercury vapour are the lungs. Acute poisoning develops when large amounts of mercury are inhaled, which may lead to acute bronchitis, bronchiolitis and pneumonia. Patients die due to respiratory failure. Moreover, inflammatory bowel disease, with dehydration acute heart failure, oral inflammation, drooling and signs of kidney damage have been observed. Ingestion of inorganic salts is followed by salivation, burning sensation in the throat, vomiting, bloody diarrhoea, necrosis of the intestinal mucosa and kidney damage, leading to anuria and uraemia (SEŃCZUK 2002).

Chronic poisoning

In chronic exposure to mercury vapour, the central nervous system is primarily affected. Abnormalities develop with prolonged exposure to low concentrations of mercury vapour. The earliest symptoms include weakness, headache and limb pain. Salivation, inflammation of the mucous membrane and gums, tooth loss, and oral dryness are likely to follow. Moreover, blue-violet plaque on the gums, diarrhoea and symptoms of renal failure are found. Over time, the CNS damage develops, including sleep disorders, impaired concentration or memory, irritability and nervousness, trembling fingers, hands and legs (SEŃCZUK 2002, YILMAZ et al. 2010).

Toxic effects of mercury on organs

Intracellular mercury attaches to thiol residues of proteins, particularly glutathione and cysteine, resulting in inactivation of sulphur and blocking of the related enzymes, cofactors and hormones. Its molecular interactions with sulfhydryl groups in molecules of albumin, metallothionein, glutathione, and cysteine have been implicated in the mechanisms involved in renal and neuronal toxicity (JAMES et al. 2005, FONNUM, LOCK, 2004). Moreover, i blocks the immune function of Mn and Zn leading to deficiency of a principal antioxidant enzyme, superoxide dismutase, involved in various diseases, including Alzheimer’s disease, Parkinson’s disease, cancer, Down’s syndrome, Dengue, etc. (NOOR et al. 2002). Furthermore, in cultured cerebellar granule cells, low concentration of mercury causes a rise in [Ca^{2+}], which may trigger a cascade of events leading to impairment of mitochondrial energy metabolism and generation of reactive oxygen species. By inhibiting the glutamic acid uptake, mercury magnifies the sensitivity of neurones to excitotoxic injury (FONNUM, LOCK 2004). The combination of these mercury - triggered events enhances free radical stress, which is a key factor of disease progression, ageing and degenerative disorders (NAGY 2001).

Central nervous system

The central nervous system starts to develop in the embryonic period and continues through adolescence. During its development, the CNS is sensitive to is exposed to environmental hazards as it is dependent on developmental processes (i.e. proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis).

Comparison of foetuses and adult organisms shows that the former are more susceptible to mercury toxicity. Maternal consumption of methylmercury-contaminated fish or bread during pregnancy may cause psychomotor retardation in the offspring. The common symptoms in children exposed to maternal mercury include impaired motor function, memory, and language, particularly if exposed in the second trimester. Autism is a disorder that can lead to life-long disability. There is a potential link between mercury toxicity and autism in children (Lee et al. 2003).

Low concentrations of some metals, including mercury, can directly induce α-synuclein fibril formation which is the major constituent of intracellular protein inclusions (Lewy bodies and Lewy neurites) in neurons leading to Parkinson's disease (Uversky et al. 2001). Moreover, low concentrations of mercury are able to induce oxidative stress, cytotoxicity and increased secretion of β-amyloid, which may result in neurodegenerative diseases (Olivieri et al. 2002). Mercury binds sulfhydryl groups of proteins and disulfide groups in amino acids, which inactivates the related enzymes, cofactors, hormones. By binding sulfhydryl groups, it also alters the cellular membrane permeability. Blocked or inhibited sulphur oxidation on cellular levels has been observed in many chronic neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, ALS, lupus, rheumatoid arthritis, autism, etc. (Wilkinson, Waring 2002).

Furthermore, mercury can induce decreased manual dexterity, increased muscular fatigue, decreased muscular strength, disrupted attention, impaired motor function and verbal memory. The other concerns include tiredness, memory disturbances, finger tremor, abnormal findings of computerised EEG analysis and impaired performance in neurobehavioral or neuropsychological tests (Farhana et al. 2005).

Urinary system

Kidneys accumulate the highest levels of mercury compared to the brain and liver. Mercuric ion (Hg^{2+}), one of the best-known thiol-binding agents, impairs the NF-KB activation and DNA binding in renal epithelial cells leading to apoptosis (Dieguez-Acuna et al. 2004).

Reproduction

When concentration of methylmercury in mothers is very high they do not conceive; if they do, pregnancy is lost, the foetus aborted or stillborn. In cases of live births, children suffer from serious neurological symptoms. In women exposed to mercury vapour, high incidence rates of menstrual disorders, primary subfecundity and adverse pregnancy outcome are observed. Hg accounts for subfertility of males; both organic and inorganic mercury reduces motility of spermatozoa (Farhana et al. 2005).

Immune system

The immune system plays an important regulatory role in the host-defence mechanisms. Patients with certain autoimmune and allergic diseases, such as systemic lupus, multiple sclerosis, autoimmune thyroiditis, often show increased lymphocyte stimulation by inorganic mercury (Prochazkova et al. 2004).

Cardiovascular system

Cardiovascular diseases, including hypertension, are a leading cause of death in developing and developed countries. Mercury can induce hypertension and atherosclerosis in experimental animals and humans. Therefore assessment of its effects on the development of cardiovascular disturbances in the population is essential. Exposure to methylmercury may affect the development of cardiovascular homoeostasis in children with low birth weight, systolic and diastolic blood pressure (Stern 2005).

Genome

Human exposure to mercury compounds can induce changes in the genetic material; two processes are mainly involved: teratogenesis and carcinogenesis. The former may manifest itself in progeny in the form of congenital abnormalities, while the latter consists in the direct development of tumours in the exposed individuals.

Methylmercury-induced chromosome damage in cells can give rise to abnormal offspring.

The cytogenetic analysis revealed the effects of mercury on mitotic and meiotic chromosomes (Crespo-Lopeza et al. 2009).

MECHANISMS OF MERCURY GENOTOXICITY

Oxidative stress

One of the earliest molecular mechanisms described to explain genotoxic consequences of mercury was oxidative stress (DNA damage caused by action of free radicals generated by the metal). Free radicals are highly reactive chemical species that, in addition to their important physiological role, can also induce DNA damage and consequently, damage to cells leading to carcinogenic processes (Halliwel 2007). Free radicals are responsible for initiating the chain reaction resulting in cell proliferation.

Reactive oxygen species (ROS) constitute the main type of free radicals implicated in pathogenic mechanisms. The most important species are: superoxide radical, hydrogen peroxide, hydroxyl radical, oxygen singlet, alkyl radical, peroxyl radical and nitric oxide.

Mercury compounds are capable of inducing cellular damage by increasing of ROS levels (ERCAL et al. 2001).

Direct action of free radicals on nucleic acids are likely to cause genetic mutations. Moreover, free radicals may induce conformational changes in proteins responsible for the formation and maintenance of DNA, such as repair of enzymes, DNA-polymerases, even tubulin and kinesin motor proteins, responsible for mitotic spindle and chromosomal segregation (CEBULSKA-WASILEWSKA et al. 2005, STOIBER et al. 2004). Glutathione can act as the main cell defence line against mercury compounds. High levels of intracellular glutathione have a neuroprotective effect against intoxication with mercury (HERCULANO et al. 2006).

Moreover, some other antioxidant substances, e.g. L-ascorbic acid, also demonstrate their protective role against mercury genotoxicity by preventing sister chromatid exchanges and abnormal mitosis.

Microtubules

The influence of mercury species on microtubules has been known since the main proteins constituting microtubules, tubulin and kinesin, were described as preferential targets for mercury binding. However, only recently their potential genotoxic effect has been recognized (THIER et al. 2003, STOIBER et al. 2004). Cytoskeletal proteins are involved in cell movement, mitotic spindle formation, chromosomal segregation and nuclear division, implicating that part of inorganic mercury chromosomal genotoxicity could be due to functional impairment of kinesin and/or microtubules, leading to disturbances in chromosome distribution (BONACKER et al. 2004).

DNA repair mechanisms

Another possible mechanism responsible for mercury genotoxicity is associated with its effect on DNA repair mechanisms, which constitute the defence system designated to protect the genome integrity. The deficient defence system may eventually lead to carcinogenic processes (CEBULSKA-WASILEWSKA et al. 2005, HALLIWEL 2007).

Besides the indirect action on the DNA repair system, mercury can also be directly bound to the “zinc fingers” of DNA repair enzymes, affecting their activity. These “zinc fingers” are sequence-specific DNA-binding proteins, which contain an atom of zinc and four atoms of cysteines and/or histidines. Thus, high affinity of mercury to sulfhydryl groups of these cysteines may severely deform the structural integrity and activity of enzymes (CEBULSKA-WASILEWSKA et al. 2005).

Interaction with DNA molecules

All molecular mechanisms described above as potentially responsible for mercury genotoxicity are primarily associated with mercury-protein interactions and mercury species affecting these proteins (DNA repair enzymes, antioxidant enzymes, cytoskeleton proteins, etc.) (Crespo-Lopeza et al. 2009).

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SULPHUR IN THE POLISH FERTILIZATION DIAGNOSITCS

Małgorzata Skwierawska[1], Sławomir Krzebietke[1],
Krzysztof Jankowski[2], Zofia Benedycka[1],
Ewa Mackiewicz-Walec[1]

[1]Chair of Agricultural Chemistry and Environment Protection
[2]Chair of Agrotechnology and Crop Management
University of Warmia and Mazury in Olsztyn

Abstract

Over the years, researchers from Polish research centres have been improving analytical methods as well as plant and soil assays, designed to diagnose demands of crops for sulphur fertilization and to assess their supply with this element. In this article, the authors look back at the last 100 years of the Polish research on sulphur, in the context of analytical methods, soil and plant assays, and their application to assessments of crop fertilization requirements.

Studies on diagnosing crops' demand for sulphur fertilization have a long-standing tradition. Back in 1903, for example, Godlewski and Jentys wrote about nutritional demands of crops and about sulphur nutrition. For over a century since then, the analytical methods have changes, soil and plant assays have been designed and parameters have been established to facilitate assessment of plant nutrient demands. Sulphur-oxidizing autotrophic microorganisms or the fungi *Aspergillus niger* have been used for diagnosis. Another investigated possibility was monitoring the capacity of sulphur for migration, assayed in lisymetric experiments.

The 1960s were a time when modifications of earlier turbimetric methods appeared. In addition, applications of the isotope ^{35}S were checked as a sulphur marker enabling determination of the dynamics of this element in soil and in plants. With the passing of time, new technologies and measuring devices were developed. Some research centres implemented sulphur detection assays on soil and plant material with the following methods: ICP, GC, HPLC or XPF (x-ray fluorescent analysis). With respect to soil and plant tests, which admittedly are a very useful tool for monitoring the sulphur abundance in soil and nutritional demands of plants, it is now the time to state that they need further verification and calibration, in both pot and field experiments.

Key words: sulphur, fertilization demands, diagnostics, analytical methods, soil tests, plant tests.

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dr inż. Małgorzata Skwierawska, Chair of Agricultural Chemistry and Environment Protection, University of Warmia and Mazury in Olsztyn, Oczapowskiego 8, 10-719 Olsztyn, Poland, e-mail: malgorzata.skwierawska@uwm.edu.pl

SIARKA W POLSKIEJ DIAGNOSTYCE NAWOZOWEJ

Abstrakt

Na przestrzeni lat w polskich ośrodkach naukowych badacze udoskonalali metody analityczne oraz testy roślinne i glebowe w celu diagnozowania potrzeb nawożenia siarką i oceny zaopatrzenia roślin w ten pierwiastek. Celem pracy była retrospektywna analiza 100 lat polskich badań nad siarką w kontekście metod analitycznych, testów glebowych i roślinnych oraz wykorzystania ich do oceny potrzeb nawozowych roślin.

Polskie badania dotyczące diagnozowania potrzeb nawożenia siarką mają długą tradycję. Już w 1903 r. Godlewski i Jentys pisali o wymaganiach pokarmowych roślin i nawożeniu siarką. W ciągu 100 lat polskich badań zmieniały się metody analityczne, opracowano testy glebowe i roślinne oraz wskaźniki mające ułatwić ocenę potrzeb nawozowych roślin. Do diagnozy wykorzystywano mikroorganizmy autotroficzne utleniające siarkę elementarną czy grzyby *Aspergillus niger*. Badano możliwości migracji siarki w doświadczeniach lizymetrycznych.

Lata 60. to zarówno modyfikacje metod turbidymetrycznych, jak i poszukiwania zastosowań izotopu ^{35}S do znakowania siarki w celu oznaczania dynamiki siarki w glebie i roślinie. W miarę upływu czasu pojawiły się nowe technologie i aparatury pomiarowe. Niektóre ośrodki naukowe zainicjowały oznaczanie siarki w materiale glebowym i roślinnym za pomocą ICP, GC, HPLC czy XPF – metodą analizy fluorescencyjnej rentgenowskiej. W nawiązaniu do testów glebowych i roślinnych, które są bardzo przydatnym narzędziem w monitorowaniu zasobności gleb w siarkę i wymagań pokarmowych roślin, można na dzień dzisiejszy stwierdzić, że wymagają one dalszych weryfikacji i kalibracji zarówno na poziomie doświadczeń wazonowych, jak i badań polowych.

Słowa kluczowe: siarka, potrzeby nawozowe, diagnostyka, metody analityczne, testy glebowe, testy roślinne.

INTRODUCTION

Sulphur is a life-sustaining element for plants, animals and people. This fact is recognized by giving it the name of a fourth macronutrient, after nitrogen, phosphorus and potassium, which is essential for plant life. At the same time, sulphur is perceived as an element degrading the natural environment, and especially in the 1990s it was seen as a noxious substance. Both excess and deficit of sulphur in nature are an unwanted development. Soil in particular, being the main store of sulphur and a source of this element for plants, should be constantly monitored with various analytical methods and tests (Jakubus 2006).

For years, Polish researchers have looked for more precise ways to determine concentrations of sulphur in soil and in plants. Grzesiuk (1968) wrote that ‘we lack quick and simple methods for determination of sulphates in plants. Such methods should be easy enough to make determinations even in a modestly equipped laboratory, but accurate and quick enough to serve practical diagnostic purposes for sulphur fertilization of crops.’ Koter and Grzesiuk (1967) pointed to the fact that ‘the Polish agricultural literature does not comprise any references dedicated to some simple and accessible method for determination of sulphate sulphur in soil.’

Diagnosing the fertilization demand of soil for sulphur is not an easy task due to the dynamic and seasonal behaviour of the metal as well as its relatively high soil mobility and biochemical transformations. The aim of making a fertilization diagnosis is to estimate the current or to predict the future plant supply with a given element and, based on such information, to estimate possible yield loss or its inferior quality due to some deficit of the analyzed nutrient.

The authors' objective has been to review the last 100 years of the Polish research on sulphur in the context of analytical methods, soil and plant assays used to diagnose sulphur fertilization demand.

IMPROVEMENT OF ANALYTICAL METHODS

For many years, researchers at Polish research stations and centres have been improving analytical methods, soil and plant assays designed to assess the sulphur fertilization demand and to estimate the plant supply with this element.

Detailed methodology regarding the determination of sulphur in soil was published in 1905, in a book authored by Zygmunt Chmielewski, who introduced himself as 'an assistant researcher at the Experimental Chemical and Agricultural Station in Dublany.' In his publication titled A Handbook of Chemical and Agricultural Analysis, Chmielewski (1905) gave a detailed description of particular stages in determination of total and sulphate sulphur with the weight method, in which the value of SO_3 was derived from the weight of $BaSO_4$. The achieved result, he said 'gives us an idea of the general content of sulphur in the earth.'

In the 1920s, microbiological experiments were initiated on sulphur oxidation by soil microorganisms. One of the researchers involved in those investigations was Olszyński (1927), who published a book called Sulphur Oxidation in Soil, in which he analyzed processes of sulphur oxidation in the chemical and biochemical pathway, and discussed the factors which could significantly affect the intensity of sulphur oxidation. One of the conditions which was found to have a strong effect on the rate of sulphur oxidation was the soil pH; another determinant was the type of soil in which oxidation processes occur. The aforementioned microbiological experiments together with the subsequent elaboration of their methodology had a cognitive as well as a practical value (elemental sulphur oxidation to sulphates by soil microorganisms, reduction of soil pH and its consequence such as mobilization of nutrients from soil, i.e. insoluble phosphates). Studies on sulphur oxidising soil-borne microorganisms were also pursued by Król (1983), Król et al. (1986), Król and Kobus (1992), who observed that stimulation of the growth of autotrophic bacteria oxidising elemental sulphur occurred only at the doses of 1.5 and 3.0 t S ha^{-1}; whenever higher amounts were applied, the counts of these bacteria declined. The above results enabled researchers to predict the capacity of microorganisms to oxidise elemental to sulphate sulphur, and to assess the abundance of sulphur in soils.

In the 1930s, physiological studies were commenced, including the uptake of sulphur by oat. An example is the experiment reported by STREBEYKO (1934), in which sulphur in plants was determined according to KÖNIG (1923). Strebeyko analysed the rate of sulphur uptake during the vegetative growth of plants. His studies showed that phosphorus nutrition had a strong influence on the uptake of sulphur, with the sulphur to phosphorus ratio being constant. This implied the parallel uptake of these two elements by plants. The physiological research translated into practical applications in the assessment of crops' nutritive demands for sulphur.

KOZŁOWSKI (1936) reports on 'a painfully felt lack of chemical analysis on mineral salt solutions from individual soil profile horizons. Any changes are closely connected to the agricultural production, therefore their recognition is of utmost importance for investigations on the productivity and fertility of soils.' The author also drew attention to analyses of soil solutions 'with strong acids, which yield „stores of elements" but have no practical value regarding any assessment of the current soil productivity or recommendations for its fertilization.' He suggested extracting fresh soil matter in distilled water, and analyzing macroelements, including sulphur, in such solutions.

In the 1960s, studies began on using the fungus *Aspergillus niger* as an indicator of the soil supplies of available sulphur. This problem was dealt with mainly by NOWOSIELSKI (1961*ab*, 1963*ab*), GÓRSKI et al. (1963). The researchers checked whether it was possible to determine total and available sulphur in soils and plants with the help of fungi called *Aspergillus niger*, comparing that method with the nephelometric one. They discovered a general proportional tendency. Overall, soils which contain much available sulphur diagnosed by nephelometry also contain much sulphur available for *Aspergillus niger*, although a reverse tendency is not repeatable. This can be explained, for instance, by the fact that the fungus is able to absorb sulphur from some organic bonds in addition to sulphate sulphur. The cited authors also emphasized the ease of conducting analyses on the quantitative determination of available forms of elements, and the higher sensitivity of the nephelometric method, which is specially important when soil contains little amounts of sulphur. Based on his studies, NOWOSIELSKI (1961) concluded that the fungus *Aspergillus niger* grows very fast and produced abundant yields of mycelium. It has high nutritional demands for agriculturally important elements. A drawback of the method based on *Aspergillus niger* was its relatively low accuracy and long duration of an analysis, typically lasting from 2.5 to 5 days. As a quantitative method, it could be applied to observations of changes in the concentration of available forms of elements; it could also be used for simultaneous assessment of the abundance of several nutrients in soil. Among the Polish researchers who examined sulphur determinations with the fungus *Aspergillus niger*, Goźliński (1970) is a good example.

Noteworthy is the broad-scale research on nephelometric sulphur determinations, completed in Olsztyn. The nephelometric method for determination of sulphur in plant extracted with 2% acetic acid designed by Grzesiuk (1968) included a simple, quick and fairly accurate technique. Koter et al. (1963) elaborated a simplified version of the Butters-Chenery's method of 1959 for determination of the soil content of sulphates in 2% solution of acetic acid. Koter and Grzesiuk (1967) adapted the method developed by Massoumie and Cornfield (1963) for determination of the soil content of sulphates in different soil solutions. The two researchers verified experimentally that 0.15% $CaCl_2$ was a suitable extraction solution for determination of water soluble sulphates; in turn, an acetate acid and phosphorus mixture could be applied in determinations of the sum of soluble and reversely absorbed sulphates. At present, sulphur determinations in soil are made with the nephelometric and turbidimetric methods, e.g. Kaczor, Brodowska (2009), Skwierawska (2008), Skwierawska (2011). A team of researchers headed Boratyński (Boratyński et al. 1975) must not be left out of our discussion. These scientists will be remembered for their methodological investigations on the analytical determination of sulphate sulphur in soil extracts with the methods by Massoumi and Cornfield (1963) as well as Bardsley and Lancaster (1960) (using a number of different extraction solutions). In conclusion, they suggested several methodological recommendations for an improved use of the two methods. They recommended the Bardsley and Lancaster's method as a simpler one and therefore more useful for series of analytical tests. The methodology of sulphur determinations with turbidimetric techniques was also examined and developed by Skłodowski (1968), who determined the content of organic sulphur with the method designed by Ewans and Rost (1945). Skłodowski modified the turbidimetric method for determination of available sulphates proposed by Ensminger (1954). Indirectly, nephelometry was also employed for determination of concentrations of glucosinolates in plants. A method was developed, which enabled determination of the total content of glucosinolates via quantitative determination of sulphur contained in sulphates generated as a product of the decomposition of glucosinolates (Krzywińska 1978). Krzywińska also worked out a modified Butters-Chenery's method and put forth her own method to determine protein sulphur in plant material (Krzywińska 1977).

In parallel, studies were conducted on the application of the isotope ^{35}S for the sake of evaluating the dynamics of sulphur transformations in soil and plants. The leading researchers in this field were Koter et al. (1965), Koter, Panak (1966), Panak (1966), Raczynskij, Panak (1966), Panak, Szafranek (1967), Panak (1970), Koter et al. (1973). For example, Koter et al. (1965) examined the use of isotope-coded sulphur in experiments on the sorption of sulphur in different types of soil. They compared extraction solutions in terms of their ability to leach sulphates from soil. Afterwards, they put the analyzed solutions in the following order: NaH_2PO_4 > $CaCl_2$ > Na_2SO_4 > H_2O. They arrived at the conclusion that $CaCl_2$ solution was best at extracting sulphates from soil, as it yielded clear and colourless extracts. PANAK (1970) used sodium sulphate tagged with the isotope ^{35}S to determine phytoavailability of sulphates from fermented manure.

Current analytical methods for determination of sulphur in soil, plants and fertilizers are described in detail by KALEMBASA (2004), who underlines that a correct sampling procedure considerably lessens analytical errors. Preparation of samples for chemical analysis can be divided into destructive and non-destructive procedures. Destructive methods involve mineralization of analyzed material, but according to a non-destructive method sulphur is determined in ground material with an elemental analysis or an XRF technique. The latter methods are distinguished by the best precision, provided the instruments are well-calibrated and operate flawlessly. KALEMBASA (2004) distinguished the following sulphur determination methods:

1. The weight method, which should be applied to samples containing large quantities of sulphur. The method consists in precipitating $BaSO_4$ and rinsing the precipitate, which is then dried and weighed.

2. The volumetric method, which takes advantage of changes in the colouration of a solution as it changes its volume. The process of sulphur determination consists of adding a known volume of barium to a solution, and titrating its excess with a titrated solution containing sulphates

3. The spectrophotometric method, which employs absorption characteristics of compounds or complexes with sulphur at an exactly set wavelength.

4. The conductometric method, which relies on the precipitation of sulphates with calcium ions in the presence of acetone. After centrifugation, the precipitate is dissolved in water and concentration of ions is measured on a conductometer.

5. The nephelometric or turbidimetric method, which measures the content of sulphur in the form of sulphates based on the turbidity of barium sulphate suspension. KALEMBASA (2004), while underlining that the method is quick and easy, adds that the final result can be affected by how fast barium chloride is added or how long the suspension is kept; in conclusion, the researcher abandons the above method for the sake of other instrumental techniques.

6. The reduction method, which means that sulphur compounds are reduced to hydrogen sulphide, which in turn is sorbed in a solution of sodium hydroxide containing bismuth. Extinction of the produced collodial bismuth sulphide is measured by spectrophotometry. The method is very sensitive and accurate, but requires corrosive reagents. It is also expensive and labour-consuming.

The instrumental methods, in which determinations can be performed in a solution, according to KALEMBASA (2004):

1. The ICO method, in which argon flows through a specially designed burner and the high temperature of plasma causes evaporation of the sample and creation of free atoms and ions. In addition, through the elimination of chemical interference, it is possible to determine other elements, and the speed and precision makes this method extremely accurate. Disadvantages of the ICP method are the high costs of equipment and occurrence of spectral interferences.

2. Ion-exchange chromatography is used for analysis of soil, plants and fertilizers.

3. The potentiometric method has found applications in the determination of total sulphur. This is a quick method and electrodes are relatively inexpensive, but the major problem is the excess of accompanying ions, sometimes appearing in quantities surpassing the amount of analyzed sulphur.

The instrumental methods in which determinations can be performed on a whole sample, according to KALEMBASA (2004):

1. This method relies on the measured radiation of a sample activated with x-rays. This is a quick method, which is quite suitable for series analyses. Unfortunately, the price of an apparatus is rather high, and series of standards are needed to make analyses on soil and plant samples.

2. A CHNOS analyzer of the elemental composition provides us with a quick and clean determination method. Fully automated, this method does not call for sample preparation other than their fragmentation and drying.

3. The above list shows a wide range of analytical methods to choose from in order to determine sulphur in soil and plant material, but the actual choice depends primarily on the analytical capacity of a given laboratory.

SOIL ASSAYS

Out of a great number of analytical assays, most important are the ones which describe assimilable and available fractions of an element, which are a potential source of plant supply (JAKUBUS 2000).

Chemical soil tests are divided into two primary groups: extracting soluble sulphates, in which H_2O, $CaCl_2$ and LiCl are used, and adsorbed ones, mainly determined in $KHPO_4$, $Ca(H_2PO_4)_2$, NH_4OAc + HOAc extracts. In an assessment of the soil content of sulphur, sulphate sulphur has little significance as a plant available fraction due to its high changeability and dependence on environmental and agronomic factors. The soil assays which are gaining importance are the ones that also account for the content of organic sulphur, which can be transformed via mineralization into an available fraction, thus constituting one of the major sources of phytoavailable sulphur.

Available sulphur is extracted with a 0.25 M KCl solution at the temperature of 40°C for three hours (so-called KCl-40 assay). For better interpretation of results, Jakubus (2000, 2004*a*,*b*) distinguished the sulphate sulphur and organic sulphur fractions in the KCL-40 soil test.

A way to estimate losses of mineral components through leaching is through physicochemical soil assays, of which drainage systems and lysimeters are an intrinsic part. Based on the chemical composition of drainage waters and waters captured in lysimeters, losses of minerals leached into groundwater are assessed and a sulphur balance is made (Ruszkowska 1979, Pondel, Terelak 1981, Motowicka-Terelak, Gądor 1986, Gądor, Motowicka-Terelak 1986, Ruszkowska et al. 1988, 1993, Kiepul 1998*a*, 1999*a*,*b*, 2007). Lysimetric experiments have demonstrated that, given the same sulphur content, plants take up more sulphur from alkaline than from acid soils. Sulphur fertilization of soils with acid reaction raises the concentration of sulphur in plants higher than identical fertilization on alkaline soils. Based on amounts of leached sulphur and potential uptake of sulphur by plants, it is possible to foresee the required sulphur fertilization.

Boreczek (2001*a*) undertook a task to make a sulphur balance on the so called 'field surface'. The author arrived at the conclusion that the balance was negative on plots not fertilized with sulphur, and the size of a sulphur deficit depended on the uptake of this element by plants. Considering a small input of sulphur from the atmosphere, the loss of sulphates through leaching can lead to sulphur deficits on farms with intensive plant production.

PLANT ASSAYS

The visual method was mentioned back in 1903 by Godlewski and Jentys (1903). The authors gave a detailed description of crops grown on soils with controlled deficits of nutrients, suggesting that 'a deficit of just one nutrient in soil causes an inhibition – more or less severe – of the plant's development.' In 1896, Godlewski and Jentys (1903) set up a series of field experiments, including wheat, rye and potatoes, in which they analyzed the chemical composition of crops in order to determine their fertilization demand. It is a notable fact that Godlewski and Jentys (1903) did not refer to any source of methodology for analyses, but specifically named the person who completed these assays. In their article Godlewski and Jentys (1903) explained that 'the analyses were performed by Mr Konstanty Jasiński, then an assistant at the Chair of Agriculture.' They concluded that 'the supply of sulphates to potato tubers is strictly regulated by the plant's needs, because any excessive amounts of these compounds do not accumulate in tubers.' According to these authors, 'the chemical breakdown of yields can ensure more reliable clues as to the shortage or excess of plant nutrients in soil than the chemical decomposition of soil itself,' and give practical information for agriculture such as an evaluation of the soil content of nutrients. Chemical analysis of yields can provide us with valuable clues about the soil nutrient abundance, but as the above researchers stated, 'correct conclusions in this area can be drawn from quantitative ratios between these nutrients rather than from their per cent amounts (...). Thus, for improved theory of fertilization, it is extremely important that each field sampling effort generates as many chemical analyses of harvested plants as possible.'

Later Rejman (1965) noticed that 'there is a general tendency among researchers to conduct observations on the so-called typical plants, the ones which are supposed to respond to sulphur fertilization. (...) the lack of complete studies does not facilitate any synthetic elaboration of crop requirements regarding sulphur.' Analyses of plants suffering from sulphur deficiency demonstrated that they had accumulated nitrates (V) and some non-protein nitrogen bonds. The above author highlighted the fact that plant biological assays do not allow one to draw conclusions about the absolute content of sulphur in soil.

The sulphur nutritional status of crops can be assessed visually or with plant assays. The visual method is applied to detect sulphur deficiency. Most common visual symptoms of sulphur shortage in plants are chlorosis, deformation of new leaves, decolouration of flowers and a smaller number of husks and seeds. Plants are slimmer and their growth rate is slower (Kalembasa, Godlewska 2004).Visual symptoms of sulphur deficiency can be observed on dicotyledonous plants, especially ones from the family of Brassicae (Kalembasa, Godlewska 2004). These signs include the yellowing and drying of leaves, progressing from the edge towards the centre of a leaf blade. Koter and Benedycka (1984) observed evident symptoms of sulphur deficiency in plants, which were characterized by a pale green or yellowish colour of the youngest leaves and advancing necrosis of growth apexes. In cereals, sulphur deficiency manifests itself through the formation of a field with an irregular patter of colours and structure. The gravest problem in the evaluation of sulphur deficiency in plants is the risk of erroneous interpretation. Frequently, sulphur deficiency is demonstrated analytically prior to the manifestation of visual symptoms (Kalembasa, Godlewska 2004).

Plant tests present a highly statistically verified relationship between crop yields and concentration of sulphur in plants. The most popular indicators of sulphur deficiency in plants are concentrations of sulphate sulphur, total sulphur and the value of the N:S ratio. Plant tests can be divided into biological (content of sulphates and glutathione), chemical (content of reducible sulphur) and complex assays (the N:S value and percentage of sulphate sulphur to total sulphur) (Schnug, Haneklaus 1998).

With plant tests, it is possible to determine the volume of present or to predict future deficits of some elements. Plant tests often require frequent sampling at different stages of plant development to keep abreast with dynamic changes of determined parameters. Plant tests will not help to assess the quantitative demand of crops for sulphur; they will just verify whether it is necessary to intervene by applying fast-acting fertilizers. Biological indicators are of little use to the assessment of the plant's supply with sulphur because they depend on a number of factors, for instance biotic and abiotic stresses or applied fertilization. Determination of the range of values of the total sulphur concentration in a plant enables a more precise determination of the plant's supply with this element, and for some researchers it is a better criterion for an assessment of sulphur deficiency in the plant. The N:S value is the most popular diagnostic indicator showing the plant's sulphur nutritional status. It is underlined that this ratio changes far less than other indices during the plant growing season or in response to fertilization. However, researchers argue which of the methods listed above is the best at diagnosing sulphur status (JAKUBUS 2006).

GOŹLIŃSKI (1970, 1974) designed plant tests. He highlighted the potential of complex plant tests (the N:S ratio, percentage of sulphur content) as indicators of sulphur fertilization demand. The percentage of sulphur in plant composition falling down to a certain threshold value as well as an altered N:S ratio can indicate that plants are not sufficiently supplied with this element, and therefore should be nourished with sulphur fertilizers. Similar studies on plant tests were conducted by STARZYŃSKI et al. (1974). BORECZEK (2001*b*) used plant tests to analyze the content of total sulphur and the N:S ratio in oilseed rape, wheat and maize. The plants were harvested at different stages of development according to the BBA scale and the Zadoks scale. Boreczek has concluded that the N:S ratio is a more sensitive indicator of plant nutritional status, but needs more precise calibration.

GRZEBISZ and FOTYMA (1996) evaluated the sulphur nutrition of oilseed rape grown in north-western Poland. The nutritional status of the crop was carried out according to the content of sulphur in dry matter of plants. At a low S content in dry matter (less than 0.35%) visual symptoms of sulphur deficiency appear; when the sulphur content is insufficient (0.36-0.55%), so-called hidden deficit, it cannot be detected visually. An optimal amount of sulphur in plant dry matter is 0.56-0.65%, and above it the sulphur content is high. The studies by Grzebisz and Fotyma proved that over 50% of the analyzed plantations suffered from hidden sulphur deficiency, and on fields in the former Province of Kalisz (województwo kaliskie) up to 75% of oilseed rape plantations had inadequate plant concentrations of sulphur.

The methods which determine total or sulphate sulphur content in soil and plant material are used for diagnostic purposes and for working out reports on plant fertilization needs. The diagnostic and fertilization recommendations, according to KALEMBASA and GODLEWSKA (2004), comprise three stages, of which the first one involves determinations of the content of an analyzed element in soil or in a plant, using extraction reagents for this aim. Determination of the soil content of available sulphur, that is the determination of fertilization demand for sulphur, is rather complicated and requires the use of an adequately selected extraction reagent. An assessment of the achieved results on the content of sulphur in soil or in the plant, and especially the evaluation of the methods chosen for determination of the analyzed element against threshold values, create the second stage of the process. LIPIŃSKI et al. (2003) suggested threshold values to be used by agricultural extension services. These authors analyzed concentrations of sulphate sulphur available to plants in order to estimate the soil content of this element, based on the sulphur content in soil samples. Then, they estimated the fertilization demands of crops for sulphur. An additional research tool was an analysis of linear regression. Such studies help to estimate the soil richness in sulphur and the crops' demands for sulphur fertilization, but the up-to-date results need further verification.

CONCLUSIONS AND RECOMMENDATIONS

Recapitulating, despite an obvious progress in analytical techniques and methods, it is evident that the dominant analytical method applied in the current research is the nephelometric method, originally developed by BUTTERS-CHENERY (1959), BARDSLEY-LANCASTER (1960), and EVENSA-ROSTA (1945), later modified by SKŁODOWSKI (1968), GRZESIUK (1968). Today's science takes advantage of the achievements of predecessors active in the 1960s, when a huge advance was made in terms of sulphur determinations. The sulphur determination with the *Aspergillusem niger* assay has never been implemented in a broader scale. Also, sulphur detection with the isotope ^{35}S-coded sulphur has been practically abandoned. Some research centres are now beginning to implement new sulphur analytical methods in soil or plants, such as the ICP method or the XPF x-ray fluorescent analysis, but these solutions are not popular due to the rather costly equipment they require. Soil and plant assays are a very useful tool for monitoring the soil richness in sulphur and crops' nutritional demands for this element. But these tests still need to be calibrated and verified in pot and field trials, before statistically confirmed results are available.

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