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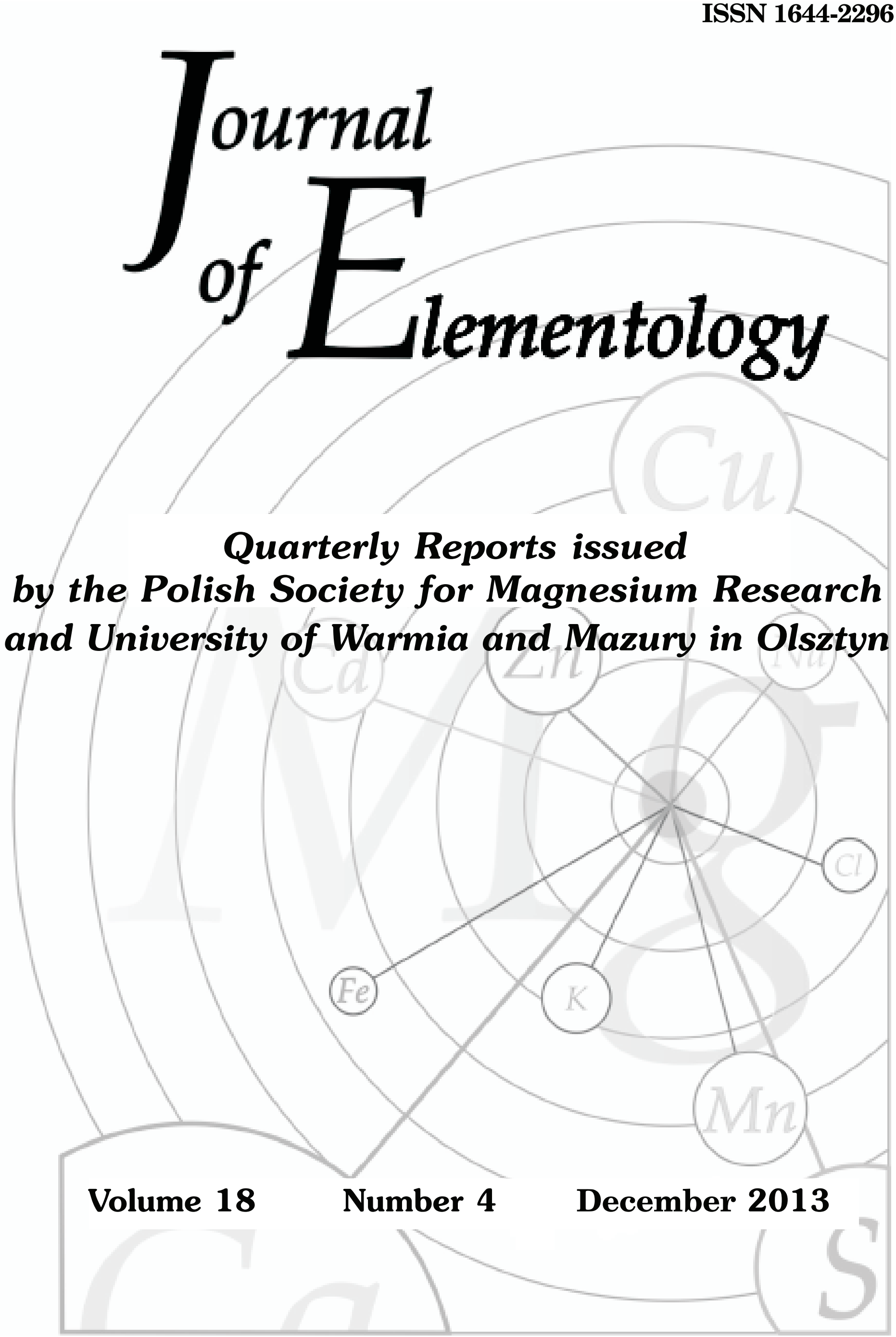

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ORIGINAL PAPERS

EFFECT OF DIFFERENT NITROGEN FERTILIZATION REGIMES ON THE CHEMICAL COMPOSITION OF FIELD-GROWN EGGPLANTS

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Abstract

Eggplant is a vegetable which deserves our interest owing to its high nutritive value and a wide range of possible cooking recipes. The biological value of this vegetable depends on several biotic and abiotic factors. The aim of this research has been to determine the effect of nitrogen fertilization methods on the quality of eggplant fruits.

Application of ammonium sulphate, ammonium nitrate and Entec 26, a slow-release fertilizer containing a nitrification inhibitor, was tested in a three–factorial experiment. The fertilizers were applied once, before planting, making use of the whole dose (100, 150 and 200 kg N ha^{-1}), or twice, divided into a dose given before the growing period and another one supplied as top dressing (75+25, 100+50, 100+100 kg N ha^{-1}). The eggplant fruits contained on average 6.80% – 8.06% of dry matter, 1.85-2.48% of reducing sugars and 2.00-2.56% of total sugars, 10.58 mg 100 g^{-1} f.m. of vitamin C, 4.27-6.56 mg 100 g^{-1} f.m. of anthocyanins, 50.70-71.06 mg 100 g^{-1} f.m. of polyphenols. The mean content of P in dry matter was 0.33%, K – 3.99%, Mg – 0.25% and Ca – 0.25%. Significant differences in the quality of eggplant fruits were demonstrated between particular years of cultivation. The biological value of eggplant fruits did not depend on the method of nitrogen fertilization.

Key words: *Solanum melongena* L., Entec 26, ammonium sulphate, ammonium nitrate, biological value.

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WPŁYW ZRÓŻNICOWANEGO NAWOŻENIA AZOTEM NA SKŁAD CHEMICZNY OWOCÓW OBERŻYNY W UPRAWIE POLOWEJ

Abstrakt

Oberżyna zasługuje na zainteresowanie ze względu na dużą wartość odżywczą oraz szerokie możliwości przygotowania do konsumpcji. Jej wartość biologiczna zależy od wielu czynników biotycznych i abiotycznych. Celem badań było określenie wpływu sposobu nawożenia azotem na jakość owoców tego warzywa.

W trzyczynnikowym doświadczeniu polowym zastosowano siarczan amonu, saletrę amonową i nawóz Entec 26 o przedłużonym działaniu, zawierający inhibitor nitryfikacji. Nawożenie wykonano jednorazowo przed posadzeniem roślin, stosując całą dawkę (100, 150 i 200 kg N ha^{-1}), lub podzielono ją na przedwegetacyjną oraz pogłówną (75+25, 100+50, 100+100 kg N ha^{-1}). Owoce oberżyny zawierały średnio 6.80-8.06% suchej masy, 1.85-2.48% cukrów redukujących oraz 2.00-2.56% cukrów ogółem, 10.58 mg 100g^{-1} ś.m. witaminy C, 4.27-6.56 mg 100 g^{-1} ś.m. antocyjanów, 50.70-71.06 mg 100 g^{-1} ś.m. polifenoli. Zawartość P w suchej masie wynosiła średnio 0.33%, K – 3.99%, Mg – 0.25%, Ca – 0.25%.

Wykazano istotne zróżnicowanie wartości biologicznej owoców oberżyny w poszczególnych latach uprawy. Nie zależała ona od sposobu nawożenia azotem.

Słowa kluczowe: *Solanum melongena* L., Entec 26, siarczan amonu, saletra amonowa, wartość biologiczna.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is a vegetable that is not commonly grown in Poland, although it deserves much more attention owing to its excellent nutritive value and a wide range of culinary uses. Technologies for growing eggplants in plastic tunnels or greenhouses have been improved (Markiewicz et al. 2008, Michałojć, Buczkowska 2008, Buczkowska 2010), but field production under Poland's prevalent climatic conditions remains rather difficult. For eggplant cultivation to be successful, several conditions must be satisfied. Above all, a suitable cultivar must be selected (Raigon et al. 2010). Beside, the weather conditions (Chen, Li 1997, Kashyap et al. 2003), and the type and soil fertility should be adequate. Eggplant plantations are most successful on sandy loam with a high organic matter content and abundance of mineral compounds (Chen, Li 1997). Both biotic and abiotic factors influence the content of different components in vegetables (Biesiada, Tomczak 2012). Among the most important factors is the cultivation regime, mainly nitrogen fertilization, which determines the quality of vegetables. Kaufmann and Vorwerk (1971) reported that eggplants grown in plastic tunnels and greenhouses have a higher demand for nutrients than tomatoes. Rosati and Troisi (2001) determined the maximum dose of nitrogen for field cultivation at 250 kg ha^{-1}. With this dose of N fertilization, the N uptake reached 200 kg ha^{-1}, of which 39% was used up effectively. Aminifard et al. (2010) reported 100 kg N ha^{-1} as an optimum dose. For field cultivation, Rosati and MAGNIFICO (2001) recommend to divide the whole dose of nitrogen into two batches: one applied before the growing period and the other one given in a top dressing treatment early as possible.

Apart from splitting a nitrogen dose, another way to improve the efficiency of nitrogen fertilization to vegetables and to reduce nitrogen leaching from soil is by using slow-release fertilizers., e.g. Entec 26, which ensure good results of cultivation of different vegetables (HÄHNDEL, ZERULLA 2001, PASDA et al. 2001, KOŁOTA, ADAMCZEWSKA-SOWIŃSKA 2006a, b, 2007, 2008). The phytoavailability of slow-release fertilizer is adjusted to particular plant development stages. Entec 26 is a mixture of ammonium sulphate and ammonium nitrate containing 7.5% $N\text{-}NO_3$, 18.5% $N\text{-}NH_4$ and 13% S. In addition, it includes a stabilizer of the ammonium form of nitrogen, DMPP (3,4--dimethyl pirazole phosphate), which inhibits the nitrification process for about 4 to 10 weeks.

Considerable differences between recommended eggplant fertilization regimes appear because both specific conditions and the type of cultivation must be taken account of when making a fertilization plan. The scarcity of information about nutrition of this vegetable species or about the influence of fertilization on the biological quality of eggplant yield encouraged us to conduct a study whose aim was to determine effects of different nitrogen fertilization technologies on the chemical composition of eggplant fruits.

MATERIAL AND METHODS

The study was carried out in 2010-2011, at the Station of Research on Vegetables and Ornamental Plants, which belongs to the Department of Horticulture at the Wroclaw University of Environmental and Life Sciences. A three-factorial field experiment with nitrogen fertilization of eggplants was established in a randomized split-plot design with three replications. The first factor consisted of the following types of fertilizer: ammonium sulphate $(NH_4)_2SO_4$ (20.5% N, 25% S), ammonium nitrate N - NH_4NO_3 (34% N) and the slow-release fertilizer Entec 26 $N\text{-}NH_4NO_3^-$ $(NH_4)_2SO_4$, (26% N, including 7.5% $N\text{-}NO_3$ and 18.5% $N\text{-}NH_4$; 13% S). The second factor was a nitrogen dose (100, 150, 200 kg N ha^{-1}), while the third factor corresponded to how the nitrogen dose was applied. Fertilization was performed once, before planting eggplants, using the whole nitrogen dose, or twice, dividing the N dose into two batches: 75+25 kg N ha^{-1}, 100+50 kg N ha^{-1}, 100+100 kg N ha^{-1}, one applied before the plant growing season and the other one as a top dressing treatment. The experiment was conducted on black degraded soil, developed from medium clay soil of the soil class IIIa, with pH 7.8 and soil salinity equal 105.5 µs cm^{-1}. The soil contained 130 mg P dm^{-3} and 200 mg K dm^{-3}.

Seeds of the Epic F_1 eggplant cultivar were sown in a greenhouse on 26 March. After developing cotyledons, the seedlings were transplanted to pots 12 cm in diameter, filled with peat substrate. While growing the transplants, the plants were fertilized with the liquid fertilizer Florovit, 0.2% in concentration. On 28 May, the transplants were planted on a field, in stripes of 60x50 cm, with 90cm spacing. The size of a plot was 3m^2 (2x1.5m). The transplanting of eggplants was preceded by a nitrogen fertilization treatment with a rototiller, according to an appropriate method. Five weeks afterwards, a nitrogen top dressing treatment was applied. When rainfall was insufficient, the plants were irrigated with a single, 20 mm dose of water. Chemical analyses of fully mature fruits were carried out in mid-August. Eight fruits were collected from each plot. An analytic sample was a quarter slice, 1 cm thick, cut from the middle part of a fruit and composed of the parenchyme and peel. The following parameters were determined: dry matter – by drying at 105°C to the constant weight) (PN-90/A-75101/03), reducing and total sugars - using the method by Lane-Eynon (PN-90/A-7510107), vitamin C – with the Tillmans' method (PN-90/A-75101/11), N-NO_3 – by potentiometry, carotenoids – by the Lichtenthaller and Welburn's colorimetric method (1983), anthocyanins – according to Fuleka and Francis, polyphenols by the Folina-Ciocalteu's method, P and Mg – using the colorimetric method, K and Ca – with the flame photometric method. The results were subjected to statistical analysis using the Tukey's test at the significance level α=0.05.

RESULTS AND DISCUSSION

The results of the chemical analyses showed that the biological value of eggplant fruits varied between years of the experiment. In the second year, during the intensive plant growth after planting, i.e. in June and in the first and second third of July, the mean temperature fluctuated within 18.2--20.5°C (Table 1). Warm August and the first two thirds of September were favourable for the plants' flowering, fruit setting and ripening. In July, there was also much rainfall, which coincided with high air temperature, both stimulating intensive development of the plants. The leading role of ambient temperature as a factor in eggplant cultivation was stressed by Kashyap et al. (2003), while Lawande and Chavan (1998) reported that an optimum temperature for eggplant growth should be 22-30°C in the day and 18-24°C at night.

Higher mean air temperatures during the plant growing season in 2011 contributed to the fruits accumulating nearly twice as much Ca, 1.2-1.3-fold more reducing and total sugars, 3.5-fold more polyphenols and 60% more carotenoids than fruits harvested in 2010 (Table 2). The research proved

Table 1

Mean air temperature and sum of rainfall during the growing period of eggplant in 2010-2011

Months	Temperature (ºC)					Rainfall (mm)				
	decade			mean monthly tempera-ture	deviation from mean monthly temperature for many years	decade			sums of rainfall	deviation from monthly sums of rainfall for many years
	1st	2nd	3rd			1st	2nd	3rd		
2010										
May	13.2	11.6	14.8	13.3	-0.2	29.8	77.2	27.5	134.5	77.5
June	18.6	16.0	17.9	17.5	1.2	13.4	11.4	-	24.8	-54.2
July	20.4	23.3	19.3	21.0	2.9	7.0	34.6	37.5	79.1	-11.9
August	19.8	19.3	17.5	18.8	1.0	16.2	14.0	43.8	74.0	10.0
September	12.6	12.5	12.2	12.5	-1.1	26.0	11.8	51.0	88.8	37.8
2011										
May	10.5	16.0	17.9	14.9	1.4	6.7	13.9	20.8	41.4	-15.6
June	20.4	18.7	18.3	19.1	2.8	3.9	4.5	13.5	21.9	-57.1
July	18.2	20.5	16.3	18.2	0.1	65.5	40.0	47.7	153.2	62.2
August	19.3	19.4	19.5	19.4	1.6	1.6	11.6	9.5	22.7	-41.3
September	17.3	16.1	14.5	15.9	2.3	20.9	0.9	-	21.8	-29.2

Table 2

Biological value of eggplants fruits independently of the method of fertilization, mean from 2010-2011

Components	2010	2011	$LSD_{\alpha=0.05}$
P (%)	0.40	0.25	0.08
K (%)	4.25	3.73	0.11
Mg (%)	0.24	0.25	n.s.
Ca (%)	0.17	0.33	0.02
Dry matter (%)	7.55	6.92	0.36
Reducing sugars (%)	2.02	2.42	0.25
Total sugars (%)	2.04	2.56	0.20
Carotenoids (mg 100 g^{-1} s.m.)	0.80	1.28	n.s.
Vitamin C (mg 100 g^{-1} f.m.)	11.60	9.54	0.76
Anthocyanins (mg 100 g^{-1} f.m.)	5.69	5.03	n.s.
Polyphenols (mg 100 g^{-1} f.m.)	27.19	96.05	5.00
NO_3-N (mg kg^{-1} f.m.)	126	101	12

that the species requires higher temperature, in particular a higher optimal temperature for synthesis of carotenoids (LESTER 2006). The 2010 season, with its high total precipitation and but lower mean temperature, resulted in a distinctly higher content of P and K as well as 21.6% more vitamin C determined in eggplant fruits. These data support the claim that a moderate climate with lower temperatures favours the accumulation of vitamin C in vegetables (LESTER 2006). KOWALSKI et al. (2003) also stressed significant changeability of the biological value of eggplant fruits in the years of their experiments conducted in an unheated foil tunnel.

The statistical analysis of our results did not prove any significant effect of the type of eggplant fertilization on the biological value of its fruits, which on average contained 6.80-8.06% of dry matter, 1.85-2.48% of reducing sugars and 2.00-2.56% of total sugars (Table 3). RAIGON et al. (2010) report the content of dry matter in field-grown eggplants at 8.35%. Investigations carried out in an unheated foil tunnel or in a greenhouse demonstrated that eggplant fruits accumulated higher amounts of reducing sugars (2.44-3.93%) and total sugars (2.65-4.31%), with an approximately same concentration of dry matter (6.63-9.88%) (CEBULA, AMBROSZCZYK 1999, KOWALSKI et al. 2003). In our experiment, the eggplant fruits contained an average 10.58 mg 100 g^{-1} f.m. of vitamin C and 1.04 mg 100 g^{-1} d.m. of carotenoids (Tables 3 and 4). In a cultivation trial under covers, KOWALSKI et al. (2003) recorded the content of vitamin C within the range 9.47-23.18 mg 100 g^{-1} f.m., while CEBULA and AMBROSZCZYK (1999) detected 4.51-6.15 mg 100 g^{-1} f.m. EL-QUDAH (2009) reported that the eggplant had such carotenoids as neoxanthine, violaxanthine, lutein and β-carotene e, which in total amounted to 0.13 mg 100 g^{-1}. KIM et al. (2007) detected that boiled eggplant contained only â-carotene and lutein in the total amount of 0.16 mg 100 g^{-1} f.m., while LAKO et al. (2007) reported that after boiling eggplant did not contain β-carotene.

Table 3

Dry matter, reducing and total sugars, and carotenoids content in eggplant fruits in relation to the fertilization method (mean from 2010-2011)

Type of fertilizer	Rate of N (kg ha^{-1})	Dry matter (%)			Reducing sugars (%)			Total sugars (%)			Carotenoids (mg 100 g^{-1} d.m.)		
		A*	B**	mean	A	B	mean	A	B	mean	A	B	mean
Ammonium nitrate	100	6.90	7.06	6.98	2.11	2.30	2.20	2.19	2.36	2.27	0.74	1.01	0.88
	150	7.44	7.25	7.35	2.30	2.18	2.24	2.40	2.28	2.34	1.03	1.02	1.02
	200	7.05	6.89	6.97	2.00	2.00	2.00	2.30	2.10	2.20	0.90	1.00	0.95
Mean		7.13	7.07	7.10	2.13	2.16	2.14	2.29	2.25	2.27	0.89	1.01	0.95
Ammonium sulphate	100	6.97	7.74	7.35	2.17	2.40	2.28	2.25	2.49	2.37	0.86	1.12	0.99
	150	7.99	7.17	7.58	2.39	2.18	2.28	2.49	2.26	2.37	0.94	0.93	0.93
	200	6.80	6.81	6.80	2.19	2.27	2.23	2.31	2.30	2.30	1.00	1.11	1.05
Mean		7.25	7.24	7.24	2.25	2.28	2.26	2.35	2.35	2.35	0.93	1.05	0.99
Entec 26	100	7.04	6.84	6.94	2.48	2.28	2.38	2.56	2.37	2.47	0.98	2.32	1.65
	150	7.18	8.06	7.62	1.85	2.19	2.02	2.02	2.24	2.13	0.89	0.89	0.89
	200	7.79	7.31	7.55	2.41	2.15	2.28	2.44	2.23	2.34	0.99	1.01	1.00
Mean		7.33	7.40	7.37	2.25	2.21	2.23	2.34	2.28	2.31	0.95	1.41	1.18
Mean for doses	100	6.97	7.21	7.09	2.25	2.33	2.29	2.33	2.41	2.37	0.86	1.49	1.17
	150	7.54	7.50	7.52	2.23	2.18	2.18	2.30	2.26	2.28	0.95	0.94	0.95
	200	7.21	7.00	7.11	2.20	2.14	2.17	2.35	2.21	2.28	0.96	1.04	1.00
Mean		7.24	7.24	–	2.21	2.22	–	2.32	2.29	–	0.92	1.16	–
LSD$_{\alpha=0.05}$ for:													
type of fertilizer (I)				n.s.			n.s.			n.s.			n.s.
rate of nitrogen (II)				n.s.			n.s.			n.s.			n.s.
mode of pplication (III)				n.s.			n.s.			n.s.			n.s

*A – preplanting application,**B – split application

The type of fertilization did not influence the content of anthocyanins or polyphenols in eggplant fruits either (Table 4). The quantity of anthocyanins ranged from 4.27 to 6.56 mg 100 g^{-1} f.m. GAJEWSKI et al. (2006) proved that the content of these compounds in an eggplant peel depended on the maturity stage of the fruit and varied from 3.49 mg g^{-1} f.m. in physiologically immature fruits to 1.12 mg g^{-1} f.m. in fully ripe fruits. LAVANDE and CHAVAN (1998) reported the anthocyanin content in different eggplant cultivars in the range from 6.90 to 752 mg 100 g^{-1} f.m. The data published by LAKO et al. (2007), OKMEN et al. (2009) and RAIGON et al. (2010) also imply a considerable content of polyphenols in an eggplant, i.e. from 41.1 to 138.9 mg 100 g^{-1} f.m. In our experiment, the amount of polyphenols was determined at 50.70 up to 71.06 mg 100 g^{-1} f.m. A decreasing tendency in the content of polyphenols occurred as the dose of nitrogen went up from 100 to 150 and 200 kg N ha^{-1}, by an average of 7.9% and 12.3%, respectively.

The high dietary value of eggplant fruits is attributed to their high concentrations of mineral compounds, especially potassium, magnesium, calcium and iron, present in proportions beneficial for the consumer's health. In our research, the content of P in dry matter was on average 0.33%, K – 3.99%, Mg – 0.25% and Ca – 0.25% (Table 5). RAIGON et al. (2010) determined the following mean amounts of mineral components in field cultivated eggplant: 0.35% P, 3.13% K, 0.10% Mg and 0.11% Ca. According to GOLCZ et al. (2005), eggplant fruits originating from an unheated greenhouse had higher quantities of phosphorus (up to 0.66%) and calcium (up to 0.5%), while the concentrations of K (3.5-4.0%) and Mg (0.25-0.35%) were quite similar. In the experiment by MICHAŁOJĆ and BUCZKOWSKA (2008), conducted in an unheated foil tunnel, eggplant fruits contained an average of 0.26-0.29% P, 2.26-3.02% K, 0.12-0.17% Ca and 0.11-0.15% Mg. MICHAŁOJĆ and BUCZKOWSKA (2008) reported a significant effect of the type of nitrogen fertilizer on the K and Ca content in eggplant fruits. Fertilization with ammonium sulphate stimulated the accumulation of macronutrients. ABDEL-MOUNTY et al. (2011) stressed the high importance of sulphur as a nutrient for eggplants. These authors stated that the quality of eggplant fruit yield improved with an increase in the fertilization dose of this element. Concurrently, the eggplant fruits nourished with more sulphur contained more N, P, K, Fe and proteins. RAIGON et al. (2010) noticed that the content of macronutrients depended on the eggplant cultivation system. In organic cultivation, eggplant fruits contained more K, Mg and Cu in comparison to those from conventional cultivation. In our experiment, the form of applied nitrogen fertilizer did not significantly affect the content of mineral components.

Many authors highlight the positive effect of fertilizers which contain an inhibitor of nitrification on the biological value of vegetables. This is especially true about the diminished content of nitrates in edible parts of plant species prone to the accumulation of this component, e.g. red beet harvested for leaves (KOŁOTA et al. 2007), common cabbage (KOŁOTA, ADAMCZEWSKA-SOWIŃSKA 2008), early leek (KOŁOTA, ADAMCZEWSKA-SOWIŃSKA 2007) as well as

Table 4

Vitamin C, anthocyanins, polyphenols and N-NO_3 content in eggplant fruits in relation to the fertilization method (mean from 2010-2011)

Type of fertilizer	Rate of N (kg ha^{-1})	Vitamin C (mg 100 g^{-1} f.m.)			Anthocyanins (mg 100 g^{-1} f.m.)			Polyphenols (mg 100 g^{-1} f.m.)			N-NO_3 (mg kg^{-1} f.m.)		
		A*	B**	mean	A	B	mean	A	B	mean	A	B	mean
Ammonium nitrate	100	11.22	10.08	10.65	5.50	5.60	5.55	65.89	61.84	63.87	129	88	109
	150	10.86	11.12	10.99	4.27	6.39	5.33	64.42	60.45	62.43	129	108	119
	200	10.50	10.12	10.31	6.56	4.72	5.64	65.20	57.19	61.20	120	126	123
Mean		10.86	10.44	10.65	5.44	5.57	5.51	65.17	59.83	62.50	126	108	117
Ammonium sulphate	100	10.76	9.88	10.32	5.83	4.38	5.11	66.25	71.06	68.66	115	83	99
	150	10.14	10.10	10.12	4.50	5.80	5.15	53.77	65.40	59.58	83	118	100
	200	10.34	11.34	10.84	5.53	5.12	5.32	56.57	50.70	53.64	106	152	129
Mean		10.41	10.44	10.43	5.28	5.10	5.19	58.86	62.39	60.63	101	118	109
Entec 26	100	10.50	11.62	11.06	6.01	5.97	5.99	67.43	63.85	65.64	116	102	109
	150	10.64	9.96	10.30	5.83	4.60	5.21	66.75	54.38	60.57	101	108	105
	200	11.38	9.76	10.57	4.78	5.16	4.97	54.63	63.42	59.02	125	138	131
Mean		10.84	10.45	10.64	5.54	5.24	5.39	62.94	60.55	61.74	114	116	115
Mean for doses	100	10.84	10.44	10.64	5.78	5.32	5.55	66.52	65.58	66.05	120	91	106
	150	10.86	10.44	10.65	4.86	5.60	5.23	61.65	60.08	60.86	104	111	108
	200	10.40	10.44	10.42	5.62	5.00	5.31	58.80	57.10	57.95	117	138	128
Mean		10.70	10.44	10.57	5.42	5.30	–	62.32	60.92	–	114	114	–
$LSD_{\alpha=0.05}$ for:													
type of fertilizer (I)				n.s.			n.s.			n.s.			n.s.
rate of nitrogen (II)				n.s.			n.s.			n.s.			n.s.
mode of pplication (III)				n.s.			n.s.			n.s.			n.s.

*A – preplanting application, **B – split application

Table 5

Macronutrient content in eggplant fruits in relation to the fertilization method (mean from 2010-2011)

Type of fertilizer	Rate of N (kg ha^{-1})	P (%)			K (%)			Mg (%)			Ca (%)		
		A*	B**	mean	A	B	mean	A	B	mean	A	B	mean
Ammonium nitrate	100	0.30	0.32	0.31	3.98	3.95	3.96	0.28	0.27	0.27	0.23	0.24	0.23
	150	0.30	0.32	0.31	4.01	4.03	4.02	0.26	0.30	0.28	0.28	0.22	0.25
	200	0.39	0.29	0.34	4.01	3.91	3.96	0.29	0.24	0.26	0.28	0.27	0.27
Mean		0.33	0.31	0.32	4.00	3.96	3.98	0.27	0.27	0.27	0.26	0.24	0.25
Ammonium sulphate	100	0.33	0.36	0.34	3.94	3.79	3.87	0.23	0.27	0.25	0.25	0.26	0.26
	150	0.31	0.35	0.33	3.91	4.15	4.03	0.25	0.23	0.24	0.28	0.25	0.26
	200	0.32	0.34	0.33	4.05	4.21	4.13	0.22	0.28	0.25	0.26	0.23	0.25
Mean		0.32	0.35	0.33	3.97	3.99	4.01	0.23	0.26	0.25	0.26	0.25	0.26
Entec 26	100	0.30	0.33	0.32	3.97	4.01	3.99	0.21	0.28	0.25	0.27	0.23	0.25
	150	0.36	0.28	0.32	4.05	3.81	3.93	0.22	0.20	0.21	0.23	0.21	0.22
	200	0.35	0.33	0.34	3.86	4.16	4.01	0.19	0.24	0.21	0.28	0.23	0.26
Mean		0.34	0.32	0.33	3.96	3.99	3.98	0.21	0.24	0.22	0.26	0.22	0.24
Mean for doses	100	0.31	0.34	0.32	3.96	3.92	3.94	0.24	0.27	0.26	0.25	0.24	0.25
	150	0.32	0.32	0.32	3.99	4.00	3.99	0.24	0.24	0.24	0.26	0.22	0.24
	200	0.35	0.32	0.34	3.97	4.09	4.03	0.23	0.25	0.24	0.28	0.24	0.26
Mean		0.33	0.33	–	3.98	4.00	–	0.24	0.25	–	0.26	0.24	–
LSD$_{\alpha=0.05}$ for:													
type of fertilizer (I)				n.s.			n.s.			n.s.			n.s.
rate of nitrogen (II)				n.s.			n.s.			n.s.			n.s.
mode of pplication (III)				n.s.			n.s.			n.s.			0.02

*A – preplanting application, **B – split application lettuce, spinach, lamb's lettuce and carrot (Hähndel, Zerulla 2001, Pasda 2001). Eggplant belongs to those vegetables which do not readily accumulate nitrates. In this research, the average level of nitrates was determined at 114 mg in 1 kg f.m. and did not depend on the type of fertilization (Table 4). However, elevated nitrogen doses, from 100 kg ha^{-1} to 150 and 200 kg ha^{-1}, caused an increase in the amount of accumulated nitrates, especially when the fertilization was performed twice: in a split dose applied before the growing period and as a top dressing treatment.

CONCLUSIONS

1. The weather conditions in the two years of the study significantly modified the chemical composition of eggplant fruits.
2. In 2011, when the thermal conditions were more favourable for eggplant, the vegetable accumulated nearly twice as much Ca, 1.2-1.3-fold more reducing and total sugars, 3.5-fold more polyphenols as well as 60% more carotenoids than in 2010.
3. The biological value of eggplant fruits did not depend on the type of nitrogen fertilizer nor on its dose and the way it was applied.

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VERMICOMPOST AFFECTS GROWTH, NITROGEN CONTENT, LEAF GAS EXCHANGE AND PRODUCTIVITY OF PEPPER PLANTS

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Abstract

The purpose of this study was to investigate the effect of the vermicompost Lumbrical, produced by the Californian earthworm *Lumbricus rubellus*, on growth, nitrogen content, leaf gas exchange and productivity of pepper plants (cv. Gorogled 6). The experiments were carried out in 2007-2009, on experimental field of the Agroecological Centre of the Agricultural University of Plovdiv (Bulgaria). Immediately before planting, two levels of the vermicompost were applied: 50 and 100 cm^3 per plant. Thirty days after planting, some of the plants fertilized with 50 cm^3 Lumbrical (the third variant) were additionally fed with a solution of vermicompost (200 cm^3 per plant). Prior to the experiment, chemical and microbiological analyses were performed on both the soil and vermicompost. During plant vegetation, some biometrical indices characterizing the plant growth were measured. The nitrogen concentration and nitrate reductase activity in leaves of pepper plants were determined. The leaf gas exchange was taken into account. The pepper yield and quality parameters of fruits were analyzed. The results of the present study showed that by feeding the pepper with the vermicompost Lumbrical it was possible to increase the plants' vegetative mass improve the development of their generative organs. Incorporation of the vermicompost into the soil increased the content of N in the root area. It also considerably raised the nitrogen content in leaves of pepper plants. A positive correlation between the content N in leaves and the activity of the enzyme called nitrate reductase was observed. The positive effect of the vermicompost was reflected by improved leaf gas exchange parameters and productivity of plants.

Key words: *Lumbricus rubellus*, *Capsicum annuum* L., *nitrate reductase*, photosynthesis, nitrogen, yielding.

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WPŁYW WERMIKOMPOSTU NA WZROST, ZAWARTOŚĆ AZOTU, WYMIANĘ GAZOWĄ LIŚCI I PLONOWANIE PAPRYKI

Abstrakt

Celem badań było określenie wpływu nawożenia wermikompostem Lumbrical, wyprodukowanym z wykorzystaniem dżdżownicy kalifornijskiej (*Lumbricus rubellus*), na wzrost, zawartość azotu, wymianę gazową liści oraz plonowanie papryki odmiany Gorogled 6. Badania przeprowadzono w latach 2007-2009, na polu doświadczalnym Centrum Agroekologicznego Akademii Rolniczej w Płowdiw (Bułgaria). Bezpośrednio przed sadzeniem roślin zastosowano dwa poziomy nawożenia wermikompostem – 50 i 100 cm^3 na roślinę. Trzydzieści dni po sadzeniu część roślin nawożonych 50 cm^3 Lumbricalu (trzeci wariant) dodatkowo zasilono roztworem wermikompostu (200 cm^3 na roślinę). Przed rozpoczęciem badań wykonano analizy chemiczne i mikrobiologiczne gleby i wermikompostu. W okresie wegetacji badano wybrane wskaźniki charakteryzujące wzrost roślin – zawartość azotu i aktywność reduktazy azotanowej w liściach papryki, wymianę gazową liści. Oceniano wielkość plonu oraz parametry charakteryzujące jakość owoców. Wykazano, że nawożenie wermikompostem Lumbrical wpływa na zwiększenie masy wegetatywnej oraz przyspieszenie rozwoju generatywnego roślin. Wprowadzony do gleby wermikompost znacznie wpłynął na zwiększenie zawartości N w strefie korzeni oraz w liściach badanych roślin papryki. Stwierdzono dodatnią korelację między zawartością N w liściach i aktywnością reduktazy azotanowej. Pozytywny wpływ wermikompostu w uprawie papryki wykazano na podstawie wymiany gazowej liści i plonowania roślin.

Słowa kluczowe: *Lumbricus rubellus*, *Capsicum annuum* L., *reduktaza azotanowa*, azot, fotosynteza, plonowanie.

INTRODUCTION

Composting organic waste with various types of earthworms is an intriguing problem from both a scientific and a practical point of view (ROMERO LIMA et al. 2000, MANNA et al. 2003, SALLAKU et al. 2009). Several types of worms have become important in practice: *Eisenia fetida, Lumbricus rubellus, Amyanthes diffrigens and Eudrillus engineac.* They feed on different types of organic manure and other organic waste. While being digested, this raw material undergoes physical and chemical modifications. Around 60% of the food assimilated by worms is released as excrements. The new product (vermicompost) improves soil fertility (HARRIS et al. 1990, KARBAUSKIENE 2000) and has a stimulating effect on the growth and development of plants (ATIYEH et al. 2000 a,b,c, DOMINI et al. 2000, MAKULEC 2002, EDWARDS, ARANCON 2004, ARANCON et al. 2004).

The nutritional value of vermicompost depends on its origin. GOLCHIN et al. (2006) report that vermicompost made from animal manure has a higher nutritional value than that extracted from organic public waste. For example, vermicompost from cattle and pig manure as well as from food waste can improve the germination, growth and flowering rate of a larger number of ornamental and vegetable crops than vermicompost from other sources (ATIYEH et al. 2000 a,b,c). Compared to natural fertilizer, vermicompost contains between 40 and 60% more humic substances and exceeds the universal compost in quality (Dominguez et al. 1997, Makulec 2002).

MATERIAL AND METHODS

The field experiments were carried out in 2007-2009, at the Agroecological Centre of the Agricultural University in Plovdiv, Bulgaria. The experiments were carried out on the Gorogled 6 pepper cultivar, which is grown for ground red pepper. In March, seeds for the production of seedlings were planted at a density of 12-15 g m^{-2}. The seedlings were grown in a polythene greenhouse, on cold seed-beds with tunnel covers above them. The seedlings were transplanted onto a permanent site at the beginning of June, after a lasting increase in the soil temperature to over 16°C. They were planted in long furrows, spaced 60 × 15 cm.

For the purpose of the experiments, vermicompost produced by the Californian earthworm *Lumbricus rubellus* (Lumbrical) was used. The worms were fed with cattle manure. Lumbrical was placed at the bottom of 6-8 cm deep furrows directly before planting the seedlings. During the vegetative season, the peppers, fertilized with 50 cm^3 in the third variant, were treated with Lumbrical extract (30 days after the first nutrition). The extract consisted of aqueous solution of Lumbrical in the 1:10 ratio. The solution was left to mature for at least 24 hours and was periodically stirred. The clear part of the sediment was decanted. The plants were treated with the extract in a dose of 200 cm^3 per plant.

The experiments were carried out under the following scheme: 1) control sample – soil without the vermicompost; 2) soil fertilized with 50 cm^3 of the vermicompost Lumbrical per plant before planting; 3) soil fertilized with 50 cm^3 of the vermicompost per plant before planting and 200 cm^3 of Lumbrical extract per plant after planting; 4) soil fertilized with 100cm^3 of Lumbrical per plant before planting. Each of the tested variants had four replications, each including 24 plants.

The peppers were harvested once in mid-October, when over 80% of the fruits had reached full botanical maturity. The unripe fruits were left to season.

Prior to the experiment, some chemical characteristics and microbiological analyses were performed both on the soil and on the vermicompost. The organic matter was determined using the Tiurin method for determination of organic carbon content. The determination of pH was performed potentiometrically. Solutions used for the determination of pH were prepared with carbon-dioxide-free water R. The total nitrogen was determined titrometrically, after ashing in sulfuric acid (H_2SO_4) and subsequent distillation in a Parnas-Wagner apparatus. The total content of phosphorus was determined colorimetrically with the Egner-Riem. The total quantity of potassium was determined in hydrochloric acid extract (2 M HCl) with the modified method of Milcheva in a flame photometer. The counts of bacteria, spore-forming bacteria, actinomycetes, microscopic fungi and cellulose decomposing bacteria were determined by the dilution agar plate method.

During the growth and development of the pepper, some biometric parameters of the plants were taken (30 and 60 days after the application of the vermicompost). The leaf area was measured with a digital area meter NEO-2 (TU, Sofia, Bulgaria). The nitrate reductase activity (NRA, EC 1.6.6.1) was determined *in vivo* by the method of KAISER and LEWIS (1984) in the first leaf under the first node (30 days) and in the first leaf under the second node (60 days). The concentration of nitrite was calculated by drawing a calibration curve of nitrite. The enzymatic activity was expressed as µg NO_2^- g^{-1} FW h^{-1}. The leaf gas exchange (portable photosynthetic system LCA-4, ADC, England) was reported. Ground red pepper was prepared from the fruit pericarp. The total pigment content was determined with the ASTA-19 method, modified by MANUELYAN (1979), immediately after grinding pepper fruits.

Statistical analyses were carried out by one-way ANOVA using the Tukey's test to validate the significance of differences at $p = 0.05$.

RESULTS AND DISCUSSION

The soil used for the experiment had a low mineral nitrogen content (Table 1). At the beginning of the experiment, the soil contained 22.4 mg NO_3^- kg^{-1} and 5.6 mg NH_4^+ kg^{-1}. The ammonium content of the vermicompost exceeded that of the soil by 8.4 mg kg^{-1} and the nitrate content was 134.4 mg kg^{-1} higher. Furthermore, the amount of mineral nitrogen in vermicompost exceeded that of the soil by 142.8 mg kg^{-1}. The soil was moderately rich in the phosphorus content and had a high quantity of potassium.

The microbiological analysis showed that the count of bacteria mineralizing organic nitrogen compounds and the count of actinomycetes were higher in vermicompost than in the soil. Irrespective of the fact that the number of nitrogen-immobilizing bacteria was reduced in the vermicompost, the balance between mineralization and immobilization was maintained and the bacteria decomposing organic nitrogen compounds dominated. This led to the accumulation of accessible mineral nitrogen compounds in the vermicompost. There were fewer microscopic fungi and cellulose-decomposing bacteria in the vermicompost than in soil. A possible reason was a shortage of oxygen in the vermicompost, limiting counts of the above aerobic microorganisms.

Table 1

Chemical and microbiological analyses of the soil and vermicompost at the beginning of the experiment

Specification	Soil	Vermicompost
Chemical analysis		
pH (H_20)	7.6	7.4
Organic matter (%)	2.3	32.6
NH_4^+ (mg kg^{-1})	5.6	14.0
NO_3^- (mg kg^{-1})	22.4	156.8
Total N (mg kg^{-1})	28.0	28.0
P_2O_5 (mg kg^{-1})	99.0	325.0
K_2O (mg kg^{-1})	430	498
Microbiological analysis		
Bacteria mineral. org. N compounds	0.4×10^6	4.2×10^6
Spore-forming bacteria	2.8×10^4	6.9×10^4
Bacteria transf. mineral. N compounds	7.1×10^5	5.2×10^5
Actinomycetes	2.2×10^5	2.7×10^5
Microscopic fungi	2.7×10^4	$2,5 \times 10^4$
Cellulose decomposing bacteria	0.6×10^4	0.5×10^4

Research shows that vermicompost improves the growth of many agricultural crops (ARANCON et al. 2004, GUTIERREZ-MICELI et al. 2007). A better growth of vegetative organs is accompanied by an increased development of generative organs (HASHEMIMAJD et al. 2004). At the same time, GUTIÉRREZ-MICELI et al. (2007) report that vermicompost in greenhouse production of tomatoes leads to plants growing much taller but has no significant effect on the number of leaves. Positive results have been reported from a turnip growing experiment (*Brassica napa* L.). Plants fed with vermicompost are characterized by a higher number of leaves and larger leaf mass compared with the control (CLASSEN et al. 2007).

The results presented in Table 2 showed that the vermicompost Lumbrical accelerated the vegetative growth of the plants. On the 30th day after feeding with Lumbrical the plants' height and the diameter of their crowns were larger in the fed plants. The number, size and mass of leaves considerably increased in comparison with the control. The formed photosynthesizing surface was by 45.6% larger compared with the control. This was a result of the improved nutrition of plants, which accelerated the growth

Table 2

Effect of Lumbrical vermicompost on some growth parameters of pepper plants 30 days after vermicompost applied

Parameters \ Variants	1*	2	3
Plant height (cm)	33.6 *a***	37.6 *b*	36.6 *b*
Crown diameter (cm)	25.0 *a*	27.3 *a*	27.2 *a*
Number of fully expanded leaves	10.1 *a*	12.6 *ab*	14.8 *b*
Area of mature leaves (dm^2)	1.94 *a*	2.80 *b*	2.86 *b*
Leaf fresh weight (g)	8.9 *a*	13.1 *b*	13.3 *b*
Flower buds number	10.50 *a*	19.10 *b*	20.5 *b*
Flower buds fresh weight (g)	0.31 *a*	0.59 *b*	1.03 *c*

* 1 – control, soil without vermicompost; 2 – soil fertilized with vermicompost, 50 cm^3 per plant before planting; 3 – soil fertilized with vermicompost, 100 cm^3 per plant before planting ;

**Values in columns followed by the same letter do not differ significantly at $p = 0.05$.

and the accumulation of biomass of photosynthesizing organ. The accelerated vegetative growth of the fed plants may have been achieved owing to a considerable quantity of assimilated nitrogen, as well as to the biologically active components of the vermicompost (CANELLAS et al. 2002). Other causes could be the improved structure and mechanical properties of the vermicompost-enriched soil (WANG et al. 2010). The vermicompost also influenced the generative development of the plants. The number and mass of formed leaf buds were much higher in the fed plants. The stimulating effect of the vermicompost on the plants' growth continued until the end of the reported period (Table 3). The additional enrichment of the soil with the vermicompost extract had a positive effect on the growth indices. They improved but were lower than determined for the plants fed once with a higher level of Lumbrical.

The results show (Table 4) that on the 30th day after feeding with Lumbrical, the content of the N-element was within the optimal range. This means that the plants (including the control) were grown under favourable conditions, which could have been expected considering the fact that the preceding crop was wheat. On the 60th day, the nitrogen content fell beyond the optimal range in leaves of both the control and the fed plants. Regardless of the fluctuations in the N balance, a positive effect of Lumbrical was observed throughout the entire reported period. The additional enrichment of the soil with the vermicompost extract increased the nitrogen level in the fed plants. Similar results were achieved by other authors as well. The

Table 3

Effect of Lumbrical vermicompost on some growth parameters of pepper plants 60 days after vermicompost applied

Parameters \ Variants	1*	2	3	4
Plant height (cm)	41.8 *a***	45.5 *b*	45.5 *b*	45.8 *b*
Crown diameter (cm)	28.7 *a*	35.3 *b*	39.1 *c*	39.8 *c*
Leaf number	79.0 *a*	126.0 *b*	137.2 *bc*	154.0 *c*
Leaf area (dm^2)	7.7 *a*	20.0 *b*	20.2 *b*	24.1 *c*
Leaf weight (g)	24.7 *a*	60.7 *b*	61.9 *b*	73.1 *c*
Ovarium number	6.8 *a*	7.8 *a*	12.7 *b*	15.7 *c*
Ovarium weight (g)	1.0 *a*	1.6 *ab*	2.8 *ab*	3.0 *b*
Fruits number	11.8 *a*	13.5 *a*	16.5 *b*	18.3 *b*
Fruit weight (g)	92.0 *a*	270.3 *b*	269.8 *b*	304.9 *c*

*1 – control, soil without vermicompost; 2 – soil fertilized with vermicompost, 50 cm^3 per plant before planting; 3 – soil fertilized with vermicompost, 50 cm^3 per plant before planting and vermicompost extract, 200 cm^3 per plant after planting; 4 – soil fertilized with vermicompost, 100 cm^3 per plant before planting;
** Values in columns followed by the same letter do not differ significantly at $p = 0.05$.

Table 4

Effect of vermicompost on nitrogen (N) concentration ($g\ kg^{-1}$) and nitrate reductase activity ($\mu g\ NO_2^-\ g^{-1}\ h^{-1}$) in the first leaf under the first node (30 days) and 1st leaf under the second node (60 days) of pepper plants

	N ($g\ kg^{-1}$)		NR ($\mu g\ NO_2^-\ g^{-1}\ h^{-1}$)	
Variants	30 days	60 days	30 days	60 days
1*	36.7 *a*	22.4 *a*	82.0 *a*	60.0 *a*
2	38.5 *a*	24.1 *a*	94.0 *b*	61.3 *a*
3	-	26.1 *a*	-	77.5 *ab*
4	42.2 *a*	29.9 *a*	114.0 *b*	81.0 *b*

*Key: see Table 3

liquid vermicompost extract increases the nitrogen content in leaves of tomato plants (TEJADA et al. 2007). SAINZ et al. (1998) indicate that addition of vermicompost to soil leads to increasing the content of the element in substrate and higher N concentrations in cucumber leaves.

Nitrate reductase is the first enzyme which limits nitrogen absorption by plants (CAMPBELL 1999). Its activity is often used as an indicator of plants' ability to utilize NO_3^- from soil (BARFORD, LAJTHA 1992). It was found that in leaves of the fed plants the activity of nitrate reductase increased parallel to the increasing levels of Lumbrical. On the 60th day, this stimulating effect was weaker, probably due to a decrease in the nitrate content in leaves. The additional enrichment of the soil with the vermicompost extract influenced the activity of nitrate reductase. The activity of the enzyme increased by 29.2%. This corresponded to higher nitrogen levels reported in leaves of the additionally fed plants. The higher activity of nitrate reductase suggests that these plants were better at utilizing nitrate ions of the soil. Similar results have been reported in tomatoes by DODDEMA et al. (1986), manioc (CRUZ et al. 2004) and coffee (DAMATTA et al. 1999), fed with organic products.

When comparing the nourishment regime at the beginning of vegetation (Table 1) and 30 days after the soil had been enriched with Lumbrical (Table 5), it appears that the nitrogen content in the plants is slightly reduced (28.0-22.6 mg kg^{-1}). After the incorporation of the vermicompost, the level of N in the soil increased by 102.5% compared with the control. The increased level of the element was mostly achieved by nitrate nitrogen. A higher level of nitrogen than in the control variant continued during the entire reported period.

A considerable increase in the nitrogen level in the soil enriched with Lumbrical was most probably induced by the active influence of nitrogen-fixing bacteria contained in the vermicompost (ANSARI 2008a, b). This confirmed the results obtained by PATTIL (1993), who reports that eight weeks after enrichment of soil with the vermicompost Lumbrical, it contained 75 mg kg^{-1} nitrate nitrogen (67% higher compared with the control).

A comparison of the nitrogen values at the beginning and at the end of the reported period shows certain reduction in N quantities, which can be explained by some specific features of the pepper and its yields. Pepper belongs to annual plants whose vegetative growth continues parallel to the flowering and fruiting. This is why absorption of mineral elements (including nitrogen) continues almost throughout the entire vegetation period. The additional application of the vermicompost had a positive effect on the nitrogen content in the soil. The higher content of N-element in the soil enriched with the vermicompost (Table 5) as well as its higher concentration in leaf tissue of the fed plants (Table 4) allow us to suggest that the improved plant growth may be directly related to the nutritional effect of the vermicompost (TOMATI et al. 1988).

The results on the influence of Lumbrical on the photosynthetic rate (Table 6) showed that the vermicompost had a positive effect on this process. On the 30th day after the enrichment of the soil, the rate of net photosynthesis increased by 17.3-22.2%. An increase in the transpiration rate was not mathematically proven. On the 60th day after the application of the

Table 5

Dynamics of nitrogen in the soil (mg kg^{-1} soil)

Variants	Mineral nitrogen (mg kg^{-1} soil)					
	30 days			60 days		
	NH_4^+	NO_3^-	total	NH_4^+	NO_3^-	total
1*	3.6 *a*	19.0 *a*	22.6 *a*	6.6 *a*	9.9 *a*	16.5 *a*
2	3.9 *a*	39.0 *b*	42.9 *b*	15.0 *bc*	22.8 *b*	37.8 *b*
3	-	-	-	14.1 *b*	26.1 *c*	40.2 *b*
4	4.8 *a*	43.8 *c*	48.6 *c*	17.4 *c*	22.8 *b*	40.2 *b*

*Key: see Table 3

Table 6

Gas exchange in leaves of pepper plants;1st leaf under the first node (30 days)
and 1st leaf under the second node (60 days)

Variants	P_N (µmol $m^{-2}s^{-1}$)		E (mmol $m^{-2}s^{-1}$)		g_s (mol $m^{-2}s^{-1}$)	
	30 days	60 days	30 days	60 days	30 days	60 days
1*	17.56 *a*	14.19 *a*	2.83 *a*	3.01 *a*	0.11 *a*	0.14 *a*
2	20.60 *b*	20.03 *b*	3.29 *a*	3.36 *a*	0.18 *b*	0.25 *a*
3	-	19.58 *b*	-	4.35 *a*	-	0.18 *b*
4	21.45 *b*	20.36 *b*	3.25 *a*	3.65 *a*	0.19 *b*	0.30 *b*

P_N – net photosynthesis; E – transpiration rate; g_s – stomatal conductivity
*Explanations: see Table 3

vermicompost, the same regularities were observed as in the earlier period. The amount of solar energy and CO_2 assimilated by the plants depends on the photosynthetic leaf area and on the rate of CO_2 fixation, i.e. on the photosynthetic rate (JAIN et al. 1999). Our results (Tables 2-3 and Table 6) showed that in this respect the vermicompost Lumbrical had a positive effect on the plants.

Table 7 shows that the yield of the plants treated with the vermicompost Lumbrical was higher than that of the control sample. A tendency for increase in the average fruit mass and the pericarp thickness (the main material for preparation of grinded pepper) was observed. Higher yields under the influence of vermicompost have been recorded in other crops: aubergine and carrots (TOMAR et al. 1998), tomatoes (TRINGOVSKA et al. 2005) and potatoes (MRINAL-SAIKIA et al. 1998). According to some authors (ARANCON et al. 2004), there is no positive correlation between the yield and content of mineral nitrogen in the vermicompost or in the plant. However, the observed increase

Table 7

Pepper yield (raw material for drying) and quality parameters of fruits

Variants	Total yield (kg ha^{-1})	Number of fruits per plant	One fruit weight (g)	Pericarp thickness (mm)	Absolute dry substance in the material (%)	Pigment content (ASTA units)
1*	8898 *a*	11.0 *a*	15.2 *a*	1.9 *a*	9.95 *a*	148 *ab*
2	9619 *b*	13.5 *ab*	15.3 *a*	2.5 *ab*	10.62 *a*	142 *a*
3	10 635 *c*	13.9 *b*	16.8 *ab*	2.4 *ab*	11.13 *a*	165 *ab*
4	11 202 *d*	14.4 *b*	16.9 *ab*	2.7 *ab*	11.27 *a*	170 *b*

*Key: see Table 3

in the yield may be explained by a richer population of useful soil microorganisms and the increased intensity of the processes in which they are involved, which in its turn, preconditions better plant nutrition.

The content of absolute dry substance and the content of pigment in plant material are important indices of the quality of red pepper for grinding (TODOROVA et al. 1999). In the current study, dry substance in raw material increased by 13.3% and the pigment content rose by 14.9% in response to a single treatment of plants with a higher level of the vermicompost.

CONCLUSIONS

1. Feeding pepper plants with the vermicompost Lumbrical increased their vegetative mass and leaf area and leads to enhanced development of their generative organs.
2. Incorporation of the vermicompost Lumbrical in the soil increased the content of N-element in the root area.
3. Feeding with the vermicompost considerably increased nitrogen content in the leaves of pepper plants, and there was a positive correlation between the incorporated dose and the activity of the enzyme *nitrate reductase*.
4. The positive effect of the vermicompost on the functional activity of the photosynthetic apparatus was expressed through an improved leaf gas exchange parameters.
5. The yield was positively influenced by the vermicompost Lumbrical. A clear increasing tendency in the average fruit mass and the pericarp thickness (the main material for preparation of grinded pepper) was observed. The absolute dry substance and pigment content was higher in the variants treated with the vermicompost.

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EFFECT OF NUTRIENT SOLUTION CONCENTRATION ON YIELD AND QUALITY OF GERBERA GROWN IN PERLITE

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Abstract

The aim of the experiments was to achieve a reduction in the concentration of a nutrient solution used for fertigation of miniature gerbera grown in perlite. During the first 4 weeks after the planting of gerberas, the nutrient solution used for fertigation had a concentration of 1.4 mS cm^{-1}. Next, half of the plants were nourished with the standard nutrient (EC 2.0 ± 10% mS cm^{-1}) while the other half received a more dissolved solution (EC 1.6 ± 10% mS cm^{-1}). The overflow was set at 25% of the daily applied nutrient solution. The number of flowers, the diameter of flower heads and the length of peduncle were not affected by a 20% decrease in the concentration of nutrient solution. The nutritional status of gerbera was also satisfactory. The concentrations of most ions in the leakage were slightly higher than in the nutrient solutions applied for fertigation. The results indicate that the application of solutions with a lower EC did not cause any drastic changes in the plant root environment. The study showed that it is possible to use about 20% less of fertilizers without any negative effects on the number and quality of gerbera flowers grown in perlite. Similar studies should be performed to analyze potential savings of fertilizer in the cultivation of other plant species. The results could contribute to lesser environmental pollution.

Key words: fertilization, soil pollution, drainage waters, root environment.

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WPŁYW STĘŻENIA POŻYWKI NA PLON I JAKOŚĆ GERBERY UPRAWIANEJ W PERLICIE

Abstrakt

Celem pracy było zbadanie możliwości zmniejszenia stężenia pożywki stosowanej do fertygacji miniaturowych odmian gerbery uprawianych w perlicie. Przez pierwsze cztery tygodnie po posadzeniu do fertygacji wszystkich roślin stosowano pożywkę o stężeniu 1.4 mS cm^{-1}. Następnie połowę roślin żywiono pożywką standardową (EC 2,0 ± 10% mS cm^{-1}), natomiast pozostałe rośliny pożywką o stężeniu 1,6 ± 10% mS cm^{-1}. Dzienny przelew ustalono na poziomie 25%. Obniżenie EC pożywki o ok. 20% nie wpłynęło na liczbę wytworzonych kwiatów, średnicę kwiatostanów i długość szypuły gerbery. Stan odżywienia roślin mieścił się w zakresie optymalnym. Stężenie większości jonów w pożywce wyciekającej z podłoża było nieznacznie wyższe niż stężenie pożywki dostarczanej roślinom. Wykazano, że zastosowanie roztworów o niższym EC nie spowodowało drastycznych zmian w środowisku korzeniowym roślin, a także jest możliwe ograniczenie zużycia nawozów o ok. 20% bez negatywnego wpływu na liczbę i jakość kwiatów gerbery uprawianej w perlicie. Wyniki wskazują na potrzebę wykonania podobnych badań w celu ograniczenie zużycia nawozów podczas uprawy innych gatunków roślin. Może się to przyczynić do zmniejszenia zanieczyszczenia środowiska glebowego.

Słowa kluczowe: nawożenie, zanieczyszczenie gleby, wycieki, środowisko korzeniowe.

INTRODUCTION

Soilless cultures and fertigation are the base of modern horticultural technologies. Most of them are open system technologies, where any excess of the applied nutrient solution exudes from the substrate to the soil. In order to stabilize the concentration and pH of the solution in the root zone and to adjust the substrate moisture, the volume of nutrient solution must be higher than the nutritional requirements of the plants (De Pascale, Paradiso 2001). Currently, 25-50% of overflow is recommended for soiless culture (Van Os 1995, Kläring 2001, Brajeul 2005). This leads to an uncontrollable leakage of concentrated nutrient solutions to the soil and then to the ground or surface waters. Thus, greenhouse fertigation causes severe environmental pollution, which has been confirmed by studies carried out in the Netherlands (Van Os 1999), in Italy (De Pascale, Paradiso, 2001), in Spain (Thompson et al. 2002) and in Poland (Breś 2009).

In view of the costs of fertilizers and the negative influence of open fertigation systems on the environment, studies have been undertaken aiming at limiting the doses of applied fertilizers. It has been accepted that successful culture must be carried out in a completely inert medium, which does not modify or only slightly modifies the chemical composition of nutrient solution during plant cultivation. This condition is not met by rockwool, especially at the beginning of plant cultivation, as it alkalizes solutions. Perlite is a fully inert medium. Furthermore, solutions percolate through perlite faster than through rockwool. Thus, the fertigation frequency cannot be decreased, but the concentration of nutrient solution could be lowered. The effect of a lower nutrient solution concentration was investigated in an experiment on miniature gerbera cultivated in perlite.

MATERIAL AND METHODS

Two cultivars of the miniature *Gerbera jamesonii* Bolus were grown in the experiment: Cafe® and Kimsey®, both from the Schreurs breeding nursery. On 8 April 2009, single plants were planted in 3.5 dm^3 containers (with holes in the bottom) filled with perlite. The containers were placed on benches (150 containers with one cultivar in each row; 6.5 containers m^{-2}). An automatic fertilizer mixer and a drip irrigation system for fertigation were applied. During the first 4 weeks after planting, the nutrient solution concentration of 1.4 mS cm^{-1} was used for fertigation. Next, half of the plants were nourished with the standard nutrient (EC 2.0 ± 10% mS cm^{-1}) while the other half of plants received a more dissolved solution (EC 1.6 ± 10% mS cm^{-1}). Taking into consideration the age of plants, stage of plant development and weather conditions, the concentration of some nutrients was slightly higher in the second year of gerbera culture than in the first year. This is consistent with the model suggesting that concentration of a nutrient solution is adaptable to the climatic conditions in a greenhouse i.e. e.g. increased solar radiation necessitates a decrease in the EC of a (Kläring, Cierpinski 1998, Kläring 2001). The insolation during the growing seasons in 2009 and 2010 is illustrated in Figure 1. The chemical composition of nutrient solutions supplied though drips to plants is specified in Tables 1 and 2.

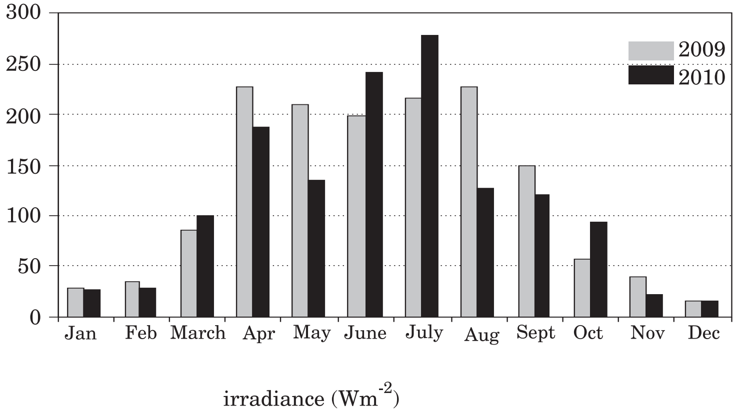

Fig. 1. Insolation during the experiment

Table 1

The nutrient concentration, electrolytic conductivity and pH of the nutrient solution during cultivation of gerberas in 2009 (mean values)

Place of sampling	N- NH_4	N-NO_3	P	K	Ca	Mg	Na	Cl	S-SO_4	Fe	Mn	Zn	Cu	B	EC (mS cm^{-1})	pH
(mg dm^{-3})																
Cafe																
EC 1.6 (mS cm^{-1})																
Drip	1.7	120.5	38.4	194.9	106.4	39.8	27.6	32.4	50.0	1.73	0.71	0.37	0.10	0.19	1.68	6.09
Leakage	1.1	124.9	39.5	196.9	113.3	45.7	37.6	34.8	55.8	1.88	0.57	0.46	0.13	0.20	1.81	6.33
Change (%)*	65	104	103	101	107	115	136	107	112	109	80	124	130	105	108	–
EC 2.0 (mS cm^{-1})																
Drip	1.9	167.7	54.8	297.1	125.9	49.1	35.4	36.2	61.1	2.73	1.04	0.54	0.17	0.24	2.19	5.96
Leakage	1.2	172.7	56.0	299.7	137.6	54.6	48.8	38.6	66.6	2.73	0.96	0.67	0.20	0.24	2.37	6.13
Change (%)*	63	103	102	101	109	111	138	107	109	100	92	124	118	100	108	–
Kimsey																
EC 1.6 (mS cm^{-1})																
Drip	1.7	120.5	38.4	194.9	106.4	39.8	27.6	32.4	50	1.73	0.71	0.37	0.1	0.19	1.68	6.09
Leakage	1.2	123	38.7	173.3	116.8	46.7	36.5	34.3	54.5	1.98	0.45	0.48	0.14	0.20	1.75	6.47
Change (%)*	71	102	101	89	110	117	132	106	109	115	64	130	140	105	104	–
EC 2.0 (mS cm^{-1})																
Drip	1.9	167.7	54.8	297.1	125.9	49.1	35.4	36.2	61.1	2.73	1.04	0.54	0.17	0.24	2.19	5.96
Leakage	1.6	174.1	55.6	268.8	139.6	56.3	46.6	28.2	65.2	2.87	0.9	0.71	0.24	0.25	2.34	6.22
Change (%)*	84	104	102	91	111	115	132	1065	107	105	87	132	141	104	107	–

* modification of nutrient concentrations in leakage in relation to the drip

Table 2

The nutrient concentration, electrolytic conductivity and pH of the nutrient solution during cultivation of gerberas in 2010 (mean values)

Place of sampling	N- NH_4	N-NO_3	P	K	Ca	Mg	Na	Cl	S-SO_4	Fe	Mn	Zn	Cu	B	EC (mS cm^{-1})	pH
(mg dm^{-3})																
Cafe																
EC 1.6 (mS cm^{-1})																
Drip	1.0	146.2	59.1	251.6	103.4	31.2	24.8	27.7	68.1	3.27	0.79	0.27	0.09	0.22	1.79	5.87
Leakage	1.0	168.6	66.8	288.6	110.5	41.5	41.3	30.7	77.5	4.82	0.77	0.37	0.14	0.24	2.07	6.14
Change (%)*	100	116	113	115	107	133	167	112	114	147	98	137	156	109	116	–
EC 2.0 (mS cm^{-1})																
Drip	0.7	177.3	67.4	330.4	109.5	41.0	32.0	30.2	74.3	5.58	0.98	0.37	0.13	0.25	2.23	5.95
Leakage	1.4	207.7	69.4	358.3	120.2	52.4	51.8	33.9	90.2	6.26	0.96	0.51	0.19	0.28	2.54	6.03
Change (%)*	200	117	103	108	110	128	162	112	122	112	98	138	146	112	114	–
Kimsey																
EC 1.6 (mS cm^{-1})																
Drip	1.0	146.2	59.1	251.6	103.4	31.2	24.8	27.7	68.1	3.27	0.79	0.27	0.09	0.22	1.79	5.87
Leakage	1.1	163.3	64.8	258.3	114.5	40.9	38.5	30.3	77.9	4.12	0.82	0.36	0.15	0.24	1.98	6.23
Change (%)*	110	112	110	103	111	131	155	109	114	126	103	133	167	109	111	–
EC 2.0 (mS cm^{-1})																
Drip	0.7	177.3	67.4	330.4	109.5	41.0	32.0	30.2	74.3	5.58	0.98	0.37	0.13	0.25	2.23	5.95
Leakage	0.8	202.3	68.5	322.9	118.6	49.5	45.2	33.0	82.0	5.83	1.06	0.48	0.18	0.27	2.35	6.09
Change (%)*	114	114	102	98	108	121	141	109	112	104	108	130	139	108	105	–

* modification of nutrient concentrations in leakage in relation to the drip

The nutrient solutions were prepared from multi-compound fertilizers (Effect 16-16-8, Effect 5-20-30), calcium saltpeter, magnesium saltpeter and nitric acid. The fertigation frequency was similar for all the plants, ranging from one application per day in winter to seven applications on very hot summer days, each treatment consisting of 100 cm^3. The overflow in the open system was set at 25% of the daily applied nutrient solution. The solution did not remain stagnant in the container as the whole excess leaked through holes in the bottom of containers into the soil.

For biomorphological measurements, 12 plants from each treatment were randomly selected. In 2009, starting with the first harvest (i.e. from 1 June to 31 October), flowers collected from each plant were counted. Also, the flower stems and diameters of flower heads were recorded. Similar measurements were carried out in 2010, from 1 April to 30 June. Samples of fully developed leaves were taken for chemical analyses on 15 June, 15 August and on 15 October in 2009 and on 15 April and 15 June in 2010. The total content of macro- and microelements was determined in dried plant material after its mineralization in strong acids. The total N was determined by the Kjeldahl's procedure. The concentrations of K, Ca, Mg, Fe, Mn, Zn and Cu were determined in an atomic absorption spectrophotometer. Spectrophotometric methods were applied for determination of the P content. Samples of the nutrient solution leaking out from the drips and from the perlite were collected in the middle of each month, during the flower harvest. The concentrations of the following ions were measured: $N\text{-}NH_4$, $N\text{-}NO_3$, P, K, Ca, Mg, Na, Cl, $S\text{-}SO_4$, Fe, Mn, Zn, Cu and B. Furthermore, the pH value and electrolytic conductivity (EC) were determined. The above analytic methods were applied to all elements except nitrogen, sodium, sulphur, chlorides and boron. $N\text{-}NH_4$ and $N\text{-}NO_3$ were determined with the distillation method designed by Bremner and modified by Starck, Na was assayed in an atomic absorption spectrophotometer, while S and Cl were assessed nephelometrically and B was checked by spectrophotometry. The results of biomorphological measurements were evaluated using analysis of variance and the LSD was calculated at $p = 0.05$.

RESULTS AND DISCUSSION

In commercial horticulture, nutrient solution is adapted to specific plant species, the growth and development stage of plants and to the course of climatic conditions. It is a widespread practice to use the same nutrient solution for plant cultivation in an inert medium as a basic solution, ignoring the specific properties of these media. At first, the recommendations elaborated by Sonneveld and Straver (1989) were commonly followed for growing gerbera in inert media, although the nutrient solutions they recommended were designed for plant cultivation in rockwool. The electrolytic conductivity of these solutions was 1.7 mS cm^{-1}. After a few years, some modifications were made, especially for plants grown in a multi-component medium. Paradiso et al. (2003) grew gerbera in a mixture of peat and perlite (1:1) and studied the suitability of nutrient solutions in concentrations of 1.6 and 2.4 mS cm^{-1}. The number of harvested flowers from one plant treated with a higher EC was 10% bigger. However, during the 8-month period, the daily water uptake was also higher in plants fertilized with the more concentrated nutrient solution. Zheng et al. (2004) in an experiment with potted gerbera grown in a mixture of perlite and sphagnum peatmoss (v/v=1/4) studied the use of nutrient solutions in the range of EC 0.2-1.7 mS cm^{-1}. Because of the plant quality, the authors distinguished EC 0.85 mS cm^{-1}, which allowed them to reduce the fertilizer use by 64% (in relation to the nutrient with EC 1.7 mS cm^{-1}). Sirin (2011) investigated the usefulness of five nutrient solutions. However, he did not define the EC value in the cited paper. The solutions differed primarily in the concentration of nitrogen (from 50 to 210 mg N dm^{-3}) and potassium (from 66 to 277 mg K dm^{-3}). The author concluded that the most successful is cultivation of gerbera in perlite and peat mixture (V/V=1/1) is a solution with 150 mg N dm^{-3} and 234 mg K dm^{-3}. In the current horticultural practice, the EC of nutrient solutions for all cultivars of gerbera grown in soilless culture is within the range of 2.0-2.2 mS cm^{-1}.

The above data are contradictory, but our results may help to dispel doubts. For an evaluation of the appropriateness of a fertigation system it is most important to assess yields. The effects of different nutrient solution concentrations on both gerbera cultivars are shown in Table 3. The lowering of nutrient solution concentration by about 20% did not exert any effect on the number of flower heads, diameter of flower heads and length of the stem. Another element of the evaluation of fertigation is an assessment of the nutritional status of plants. The plants showed no symptoms of deficiency or excess of nutrients. The results of the chemical analyses of gerbera leaves in 2010 are shown in Figure 2 as an example. The concentration of macro- and microelements in leaves was compared with the guidelines published by Plank (1988). The one hundred percent of the determined samples were found to be within the recommended value ranged for nitrogen, phosphorus, potassium, calcium, iron, manganese, copper and zinc. According to De Kreij at al. (1990), an optimum concentration of Cu varies from 3.8 to 12.7 mg kg^{-1}. Dole and Wilkins (2005) recommended the range from 2 to 10 mg kg^{-1}. If we agree with these recommendations, the concentration in the nutrient solutions does not need correcting. However, because the Zn concentrations are also very close to the critical values, it would be safer to increase slightly the concentration of these two nutrients (Cu and Zn) in the solutions. The concentration of magnesium was above the recommendation of Plank (1988), but not toxic. According to Valenzuela De Ocampo (2001), the excessive level for magnesium is 1.2%.

Table 3

Effect of nutrient solution concentration on yield and quality of gerbera flowers

Cultivar	Nutrient solution (EC mS cm^{-1})	Number of flower heads produced by the plant	Diameter of flower heads (cm)	Length of the peduncle (cm)
2009				
Cafe	1.6	22.50	8.02	62.09
	2.0	20.75	7.89	60.82
	LSD	ns*	ns	ns
Kimsey	1.6	24.83	7.81	64.84
	2.0	27.83	7.96	64.41
	LSD	ns*	ns	ns
2010				
Cafe	1.6	15.99	8.12	62.90
	2.0	13.83	8.07	62.96
	LSD	ns	ns	ns
Kimsey	1.6	21.00	7.77	65.50
	2.0	23.01	7.97	64.32
	LSD	ns	ns	ns

* non-significant difference

As mentioned earlier, approximately 25% of the daily applied nutrient solution in open systems leaks out from a growing medium directly into the soil. This means that during a greenhouse cultivation of gerbera over an area of 1 ha, the total daily leakage was from 1.6 to 11.4 m^3 of nutrient solution. Interesting results were obtained by comparing the concentration of nutrients in the solutions supplied to the plants and in the drainage waters leaking out to the soil (mean values are shown in Tables 1 and 2). In both years, the concentrations of most ions in the leakage were higher than in the nutrient solutions applied for fertigation. Similarly, increased concentrations of most ions in solutions leaking out from substrates on which roses were grown in systems without nutrient recirculation were found by BLOEMEHARD and MOOLENBROEK (1995). The results of the chemical analyses indicate that the application of solutions with a lower EC did not cause any drastic changes in the plant root environment in comparison with standard treatments (EC 2.0). This result was achieved owing to a very low cation exchange capacity (close to zero), perfect air and water properties of perlite and regular renewal of the pool of nutrients owing to fertigation treatments repeated several times every day. According to ANHTURA (1988), horticultural

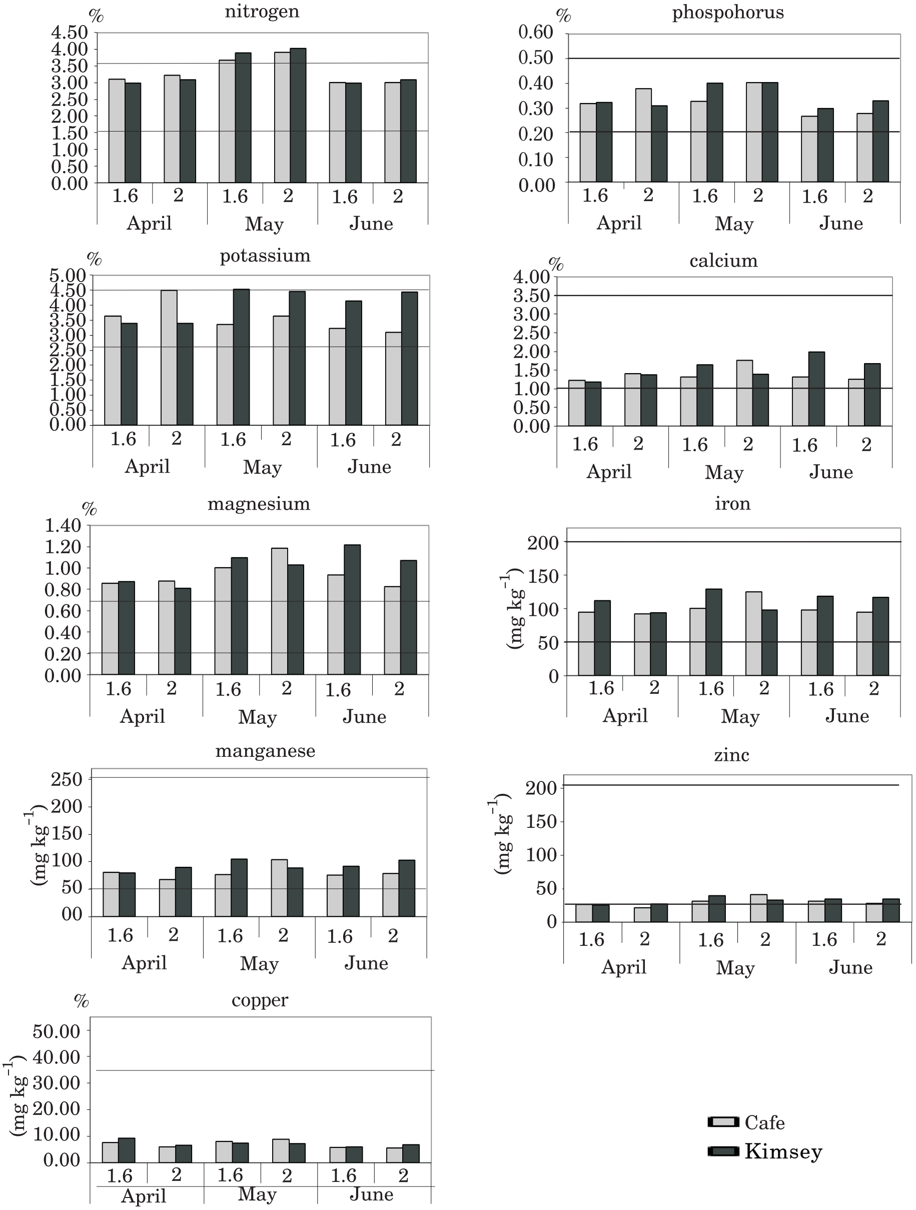

Fig. 2. The concentration of macro – (% d.m.) and microelements (mg kg^{-1}) in gerbera leaves depending on nutrient solution concentrations (mS cm^{-1})

Lines show the optimal range of nutrient concentration recommended by Plank (1988) perlite has the following physical properties: water capacity 21% and air capacity 75%. Perlite retains water around rather than inside granules. Thus, during a fertigation treatment water runs around perlite. Consequently, an increase in the ionic concentrations in drainage water leaking from the medium was negligible. According to BRES (2002), the increase in concentrations of nutrient solutions leaking from the root environment of gerbera grown in rockwool is higher. However, sometimes there is no increase in the nutrient concentration in the root environment. While growing anthurium in expanded clay pellets, a 9.5% decrease of EC was found (KLEIBER, KOMOSA 2006). On the another hand, in an experiment with tomato grown in perlite or expanded clay pellets, the concentration of drainage waters leaking from medium was higher than in solutions applied to the plants (JAROSZ et al. 2011). It seems that modifications in the composition of the rhizosphere and drainage water depend not only on the medium, but also on crop species and even on a cultivation system (DYŚKO, KOWALCZYK 2005).

The open fertigation system applied in horticultural practice is responsible for the chemical degradation of soil environment. During plant cultivation, the chemical composition of the leakages depends on the chemical composition of the nutrient supplied to plants, the plant age, the course of climatic conditions (especially of temperature), the time of the day and on the fertigation frequency (BRES 2010). For example, in Polish horticulture, the biggest pollution of the environment is caused by the fertigation of greenhouse tomato, whose cultivation area covers 2500 ha and the production cycle lasts about 10 months. Much lower pollution is caused by gerbera, which covers only about 90 ha. During commercial gerbera cultivation, the highest losses were shown by potassium (up to 413 kg K month ha^{-1}), nitrates (up to 231 kg NO_3-N month ha^{-1}), calcium (up to 220 kg Ca month ha^{-1}) and sulphur (up to 101 kg SO_4-S month ha^{-1}). The smallest losses were shown by microelements, from 0.01 kg of Mn and Cu to 3.46 kg of Fe month ha^{-1} (BRES 2009). In our experiments, $1m^3$ of leakage flowing from perlite contained 123-208 g N-NO_3, 40-69 g P, 173-358 g K, 111-140 g Ca, 38--52 g Na, 28-39 g Cl and 0.20-0.28 g B (calculations on the basis of chemical analyses of leakage). As mentioned earlier, in a greenhouse with a cultivated 1 ha area, the daily leakage was from 1.6 to 11.4 m^3 of nutrient solution. This input, especially of nitrogen (0.20-2.37 kg N daily ha^{-1}), is dangerous for the environment. Cultivation of gerbera in organic substrates was characterized by a lower run-off. The results of experiments by FERRANTE et al. (2000) showed that only 93 N, 6.8 P, 165 K and 107 Ca kg ha^{-1} per year are released into the environment. In order to restrain the excessive nitrate leaching from agricultural soils, the European Commission has issued the *Council Directive* (1991). The studies presented herein refer to this directive.

CONCLUSIONS

Concentrations of the nutrient solutions typically used for cultivation of miniature gerbera in perlite can be safely reduced to EC 1.6 ± 10% mS cm^{-1}. This will allow us to decrease the use of fertilizers by about 20%. It will also reduce the nutrient leakage to the soil and groundwater. However, a recirculation system is the most ecological solution and its application should be recommended. In the Netherlands, closed systems are compulsory. They largely improve the efficiency of water and nutrient use by cultivated plants and minimize pollution of the environment.

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GEOAVAILABILITY AND PHYTOCONCENTRATION OF ZN: FACING THE CRITICAL VALUE CHALLENGE (POLAND)

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Abstract

The verification of the concept "Facing the *critical value* challenge" has been performed on 9 arable fields of an agricultural farm (52 ha) located in the village Kujawki (Golancz District, Wielkopolska Region, Poland). In total, 99 soil samples were collected at the depth 0-20 cm from 9 fields. Basic properties were determined, i.e., soil particle distribution, organic carbon, pH (in 0.01 moles $CaCl_2$ dm^{-3}), cation exchange capacity (CEC). Moreover, Cu, Zn, Fe and Mn were extracted by 6 moles HCl dm^{-3} (pseudo total forms) and 0.005 moles DTPA, pH 7.3 (active and potentially mobile forms). Plant material (99 samples) was collected from winter triticale, winter wheat, winter barley, grass mixtures, winter oilseed rape and sugar beet at respective growth stages, dried, ground and analysed for Zn. The elaboration of soil ($Zn_{\text{-DTPA}}$) and plant ($Zn_{\text{-Plant}}$) critical values proceeded in two steps, i) tabular interpretation of data through adjustment to critical values in literature, ii) graphical readaptation of the C-Shaped, i.e. Piper-Steenbjerg curve.

The results revealed that the amounts of $Zn_{\text{-DTPA}}$ varied in a wide range, from 0.80 to 4.30 mg kg^{-1}, but its overall share in total Zn fluctuated from 2.0 to 7.9%. This implies that the geoavailability of zinc compounds seems to be relatively high. The relationship established for the pairs $Zn_{\text{-Total}}$ *versus* $Zn_{\text{-DTPA}}$ ($Y_{\text{Zn-DTPA}} = 0.092_{\text{Zn-Total}} - 2.00$; $R^2 = 0.63$) yielded a significantly high coefficient of determination as a proof of the importance of $Zn_{\text{-Total}}$ in controlling the $Zn_{\text{-DTPA}}$ pool. The *critical* $Zn_{\text{-DTPA}}$ *range* varied from 0.80 to 1.43 mg kg^{-1} with a mean value of *ca* 1.08 mg kg^{-1}, reflecting 67% of all investigated sites.

The readapted C-Shaped, i.e. Piper-Steenbjerg curve ($Zn_{\text{-Plant}}$ *versus* $Zn_{\text{-DTPA}}$) allowed establishing a *critical* $Zn_{\text{-Plant}}$ content at 15.3-39.7 mg kg^{-1} for the investigated crop plants. The mean critical value reached 33.3 mg kg^{-1} and divided plants into two groups, i) *experiencing deficiency*: winter wheat, winter oilseed rape, sugar beet and grass mixtures and ii) *not experiencing deficiency*: winter triticale, winter barley and winter wheat. These fin-

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dings give a new insight into the urgent need for elaborating critical values for a wide range of crop plants in use in Poland.

Key words: zinc, geoavailability, phytoconcentration, DTPA, Piper-Steenbjerg curve, critical value, crop plants.

GEODOSTĘPNOŚĆ I FITOKONCENTRACJA ZN: WYZWANIE W STOSUNKU DO WARTOŚCI KRYTYCZNEJ (POLSKA)

Abstrakt

Weryfikację koncepcji „Wyzwanie w stosunku do wartości krytycznej" przeprowadzono na 9 polach uprawnych gospodarstwa rolnego (52 ha) zlokalizowanego w miejscowości Kujawki (gmina Gołańcz, woj. wielkopolskie, Poland). Pobrano 99 próbek gleb z głębokości 0-20 cm i oznaczono następujące właściwości: skład granulometryczny, węgiel organiczny, pH (w 0,01 mola $CaCl_2$ dm^{-3}), kationową pojemność wymienną (CEC). Ponadto Cu, Zn, Fe i Mn ekstrahowano roztworem 6 moli HCl dm^{-3} (formy pseudocałkowite) oraz 0,005 mola DTPA, pH 7.3 (formy aktywne i potencjalnie ruchliwe). Materiał roślinny, tj. pszenżyto ozime, pszenicę ozimą, jęczmień ozimy, mieszankę traw, rzepak ozimy i buraki cukrowe (99 próbek) pobrano w odpowiednich stadiach wzrostu roślin. Po wysuszeniu i zmieleniu oznaczono zawartość Zn. Wartości krytyczne dla gleby ($Zn_{\text{-DTPA}}$) i roślin ($Zn_{\text{-Roślina}}$) opracowano dwustopniowo, stosując a) tabelaryczną interpretację danych przez dopasowanie do literaturowych wartości krytycznych, b) graficzne zaadaptowanie krzywej C-Shaped, tzn. Piper-Steenbjerga.

Wykazano, że ilości $Zn_{\text{-DTPA}}$ wahały się w szerokim zakresie (0,80-4,30 mg kg^{-1}), lecz ogólny procentowy udział w całkowitej zawartości wyniósł od 2,0 do 7,9%, co wskazuje na to, że geodostępność związków cynku jest względnie wysoka. Wysoki współczynnik korelacji dla zależności między $Zn_{\text{-Total}}$ a $Zn_{\text{-DTPA}}$ ($Y_{\text{Zn-DTPA}}$ = $0{,}092_{\text{Zn-Total}} - 2{,}00$; $R^2 = 0{,}63$) był dowodem na ważną rolę $Zn_{\text{-Total}}$ w kontrolowaniu stężenia $Zn_{\text{-DTPA}}$. Krytyczny zakres $Zn_{\text{-DTPA}}$ wynosił 0,80-1,43 mg kg^{-1}, a wartość średnia ok. 1.08 mg kg^{-1}, co odzwierciedla 67% badanych pół uprawnych.

Zaadaptowana krzywa C-Shaped, czyli Piper-Steenbjerga, w formie $Zn_{\text{-Roślina}}$ - $Zn_{\text{-DTPA}}$ umożliwiła opracowanie wartości krytycznych $Zn_{\text{-}Plant}$ mieszczących się w zakresie 15,3-39,7 mg kg^{-1} dla roślin. Średnia wartość krytyczna wynosiła 33,3 mg kg^{-1}, na podstawie której podzielono rośliny na dwie grupy: a) z niedoborem: pszenica ozima, rzepak ozimy, burak cukrowy i mieszanka traw, b) bez niedoboru: pszenżyto ozime, pszenica ozima i jęczmień ozimy. Te wyniki ujawniają nową pilną potrzebę opracowania wartości krytycznych dla wielu roślin uprawianych w Polsce.

Słowa kluczowe: cynk, geodostępność, fitokoncentracja, DTPA, krzywa Piper-Steenbjerg'a, wartość krytyczna, rośliny uprawne.

INTRODUCTION

The sustainable productivity of soil depends mainly on its ability to supply essential nutrients (including Zn) to growing plants (Diatta, Kocialkowski 1998). Physical and chemical changes of Zn in soils are regulated by soil-specific precipitation, complexation and adsorption reactions. Soil Zn occurs in three principal fractions: (i) water-soluble Zn (including Zn^{2+} and soluble organic fractions); (ii) adsorbed and exchangeable Zn in the colloidal fraction (associated with clay particles, humic compounds and Al and Fe hydroxides); and (iii) insoluble Zn complexes and minerals (Lindsay 1979, Barrow, 1993, Alloway 2004).

The mean total Zn content of the lithosphere is estimated to be 80 mg kg^{-1} and a common range for soils is 10-300 mg kg^{-1}, mean 50 mg kg^{-1} (Wedepohl 1972). On the other hand, Kabata-pendias, Pendias (1995) reported that most of surface soils are characterized by Zn levels within the range 17-125 mg kg^{-1}. It should be mentioned that the total Zn content is seldom used as a test for evaluating both its geoavailability and further phytoconcentration. According to Barber (1995), concentrations of water-soluble Zn in the bulk soil solution are generally very low on farmlands and usually fluctuate between $4 \cdot 10^{-10}$ and $4 \cdot 10^{-6}$ moles. However, in calcareous soils, Zn^{2+} may be as low as 10^{-11}-10^{-9} moles, which can severely retard crops' growth (Hacisalihoglu, Kochian 2003). Next, the exchangeable Zn fraction typically ranges from 0.1 to 2 mg kg^{-1}, but insoluble Zn is estimated as > 90% and is practically unavailable for biotic assimilation.

Zinc in the soil solution where pH is below 6.5 may occur as Zn^{2+}, ZnCl, $ZnOH^{+}$, complexed with organic matter or associated with colloidal particles. The extent of zinc speciation depends on stability constants of the species formed, ionic strength, pH and the type and relative concentrations of cations and anions in the solution (Lindsay 1972b). This may be roughly formulated as:

$$K = \frac{(M_a\ L_b)^{ax-by}}{(M^{x+})^a\ (L^{y-})^b} \quad \text{from the equation: } aM^{x+} + bL^{y-} = M_a L_b{}^{ax-by},$$

where:

K – stability constant,
M – Zn ions,
L – ligands,
a – moles of Zn,
b – moles of ligand molecules.

Theoretical approaches on Zn geoavailability have been subjected to experimental measurements, which allowed formulating equilibrium constants (Ma, Lindsay 1990, 1993, Catlett et al. 2002), exhibiting the solubility of Zn as directly proportional to the square of protons' activity as follows: Soil-Zn $+ 2\ H^{+} \Leftrightarrow Zn^{2+}$, then their results generated a log K^{o} for this equation of 5.8. The transformation of this equilibrium reaction gives a log $(Zn^{2+}) \Leftrightarrow 5.8 - 2$ pH, implying that the solubility and therefore Zn geoavailability increase with a decreasing soil pH.

It is well known that an optimum plant growth and crop yield depend not only on the total amount of nutrients present in soil at a particular time but also on their availability (Domańska 2009, Kumar, Babel 2011). Several attempts undertaken over the last century (Mazé 1915, Sommer, Lipman 1926) to evaluate the Zn phytoconcentration as induced or regulated by geochemical processes have faced the challenge of great heterogeneity of soils and unlimited diversity (species, varieties) of plants. The essentiality of Zn forces soil scientists and plant breeders to work out ranges which will be helpful for nutritional remediative interventions. For the purposes of phytoconcentration, the establishment of *critical ranges/values* (or levels) appears to be worth endeavor.

In most crops, *typical* Zn concentration in leaves required for adequate growth approximates 15-20 mg Zn kg^{-1} DW (Marschner 1995). Because plants vary in their requirements for Zn, even among cultivars, it is difficult to set a single critical value. According to Brennan et al. (1993), plants with Zn contents below 20 mg kg^{-1} in dry tissue can be suspected of Zn deficiency, but the *normal ranges* are usually 25 to 150 mg kg^{-1} in dry plant tissues.

For years, the long-term process of standardization of zinc fluxes in the soil–root–shoot continuum has been promoting the application of DTPA (diethylene triamine pentaacetic acid) as a geochemical test for establishing Zn critical values. Next, remarkable achievements in research on the potential of plants to accumulate Zn have been made (Broadley et al. 2007), but linking this process to the DTPA extractable Zn is still scantily highlighted in Polish agricultural conditions.

The aim of the current study was to verify the concept of Zn geoavailability and phytoconcentration under field conditions. The specific purposes concerned the establishment of critical DTPA based Zn values and the respective critical Zn concentrations in winter Triticale, winter wheat, winter barley, grass mixture, winter oilseed rape and sugar beet.

MATERIAL AND METHODS

Location and sampling characteristics

Soil sampling and chemical analyses

Soil samples were collected at the depth 0-20 cm from an agricultural farm (52 ha) located in the village Kujawki (District of Gołańcz; Wielkopolska Region, 17°18»E; 52°57»N, Poland). The soil sampling took place in June and July 2010. The sampling sites consisted of 9 arable fields, where 99 soil samples were gathered under the following cropped fields: 5 soil samples (under winter triticale), 35 (winter wheat), 20 (winter barley), 5 (grass mixture), 23 (winter oilseed rape) and 11 (sugar beet).

The collected soil samples were first air-dried at room temperature, crushed to pass a 2.0 mm screen and stored in plastic bags before chemical analyses. Soil particle distribution was determined by the Casagrande-Proszyński areometer procedure, while organic carbon ($C_{org.}$) by the Tiurin's method (Nelson, Sommers 1986). Next, soil reaction (i.e., pH) was assayed potentiometrically in a 0.010 mole $CaCl_2$ dm^{-3} suspension according to Polish Standard (1994).

The cation exchange capacity (CEC) of the soils was determined with the ammonium acetate test, i.e., 1 mole CH_3COONH_4 dm^{-3} at pH 7.0, followed by the summation of exchangeable alkaline cations (Ca, Mg, K, Na) and exchangeable acidity (1 mole KCl dm^{-3} test), according to Thomas (1982). Zinc was extracted by using 6 moles HCl dm^{-3} and the recovered amounts designated as pseudo-total (Gupta et al. 1996). Briefly, 20 cm^3 of 6 moles HCl dm^{-3} were added to 2 grams of soil (in polyethylene tubes) and the mixture was placed in a low speed shaker (112 rpm) for 1 hour before filtering. Next, the active and mobilisable Zn forms were extracted by the 0.005 moles DTPA dm^{-3}, pH 7.3 (Diethylene triamine pentaacetic acid) test suggested by Lindsay and Norvell (1978) and later Liang and Karamanos (1993). Ten grams of soil were mixed with 20 cm^3 of 0.005 moles DTPA dm^{-3}, pH 7.3 for two hours; the extraction proceeded as described above. Zinc concentrations in filtrates as well as other elements were determined by the FAAS method (Flame Atomic Absorption Spectrometry, Varian Spectra 55B). All chemical analyses were performed in duplications.

Plant sampling and chemical analyses

Plant samples were collected from 6 crop plants, i.e., winter triticale, winter wheat, winter barley, grass mixture, winter oilseed rape and sugar beet, grown on 9 arable fields. The following growth stages were considered:

1) winter triticale (cv. Fredro), winter wheat (cv. Tonacja), winter barley (cv. Matilda) at BBCH29;
2) grass mixture (with 75% of *Lolium multiflorum*) at 25-30 cm height;
3) winter oilseed rape (cv. Cabriolet) at spring regrowth;
4) sugar beet (cv. Raketa) at 6-7 leaves (well developped).

The sampled plant material (in total 99) was dried at 60°C for 3 days in a dryer (SLW 100 ECO). After drying, plant samples were ground in a blender 8010EG; model HGBTWTG4. Adequate portions of the plant mass (0.15 g) were weighted out on an analytical balance (Sartorius A 200S) and digested in 2.5 cm^3 concentrated nitric acid in a MARS 5 apparatus (Microwave Accelerated Reaction System) manufactured by the CEM Corporation. Zinc concentrations in the digests were determined by FAAS (Flame Atomic Absorption Spectrometry, Varian Spectra 55B). All chemical analyses were performed in duplications. Statistical evaluations were performed by using the Statgraphics® software and graphs elaborated with Excel® sheet facilities.

RESULTS AND DISCUSSION

Characteristics of investigated fields (sites)

One of the most common constraints to agricultural plant production is the soil heterogeneity, which implicitly regulates the availability of mineral elements and further shapes spatial biomass distribution (Kiepul, Gediga 2009, Diatta et al. 2012). The same problem appeared on the investigated agricultural fields, where 6 plant species were grown (Table 1). Selected physical and chemical soil parameters revealed markedly high heterogeneity, for example the clay content within the broad range of 317-471 g kg^{-1} and particularly varied levels of organic carbon ($C_{org.}$) fluctuating from 12.7 to 171.1 g kg^{-1}. The analyzed soils may be considered as mostly loamy in nature, which generally manifests in good structural development and relatively high nutrient retention capacity (Dimoyiannis et al. 1998, Schulten, Leinweber 2000).

Table 1

Selected physical and chemical parameters of 9 investigated arable fields, (mean values)

Field/Site (No. of soil samples)	Type of crop plant	Clay (< 0.02 mm)	Organic carbon (Corg)	pH $CaCl_2$	CEC
		(g kg^{-1})			(cmol(+) kg^{-1})
A (*n* = 5)	winter triticale	341	21.3	5.4	4.4
B (*n* = 11)	winter wheat	448	16.2	5.8	4.6
C (*n* = 10)	winter barley	350	13.7	6.1	9.4
D (*n* = 10)	winter barley	318	12.7	6.7	16.7
E (*n* = 5)	grass mixtures	430	171.1	7.6	20.9
F (*n* = 23)	winter oilseed rape	325	33.7	6.0	11.0
G (*n* = 16)	winter wheat	317	12.9	6.5	15.4
H (*n* = 11)	sugar beet	384	14.0	6.2	9.9
I (*n* = 8)	winter wheat	471	17.6	6.1	10.7

The order of soils according to organic carbon suggested by Sequi and De Nobili (2000), that is C_{org} < 5.8 g kg^{-1} (very low); 5.8-10.4 g kg^{-1} (low), 11-14.5 g kg^{-1} (moderate), C_{org} > 14.5 g kg^{-1} (high), translated into three operational groups distinguishable from the data reported in Table 1. These include sites C, D, G, H characterised by C_{org} in the moderate range; A, B, F, I representing the high range and site E with an extremely high C_{org}

level. This means that the investigated fields possess good properties, which should directly help to manage the chemistry of most elements, including Zn, as demonstrated by LINDSAY (1972b) on the basis of soil organic ligands toward zinc ions.

Setting up a good plant stand depends intrinsically on soil reaction (i.e. pH), since pH regulates the solubility as well as the retrogradation of minerals (SKWIERAWSKA et al. 2012). It is most important to keep soil pH on a slightly acid level, which for the 0.01 moles $CaCl_2$ dm^{-3} test is *ca* 6.0-6.5. Most of the investigated sites corresponded to this state, since 6 out of 9 sites had pH between 6.0 and 6.7, except site E characterised by pH of 7.6, (i.e. slightly alkaline). This soil characteristic along with the content of clay and organic carbon are essential factors building and stabilizing buffering capacities, roughly expressed as the cation exchange capacity (CEC).

Ratings for the CEC as suggested METSON (1961) and HAZELTON and MURPHY (2007) – Table 2 show that three prevalent ranges: 6-12 $cmol_{(+)}$ kg^{-1} (sites: C, F, H, T); 12-25 $cmol_{(+)}$ kg^{-1} (sites: D, E, G) and finally 25-40 $cmol_{(+)}$ kg^{-1} (sites: A, B), corresponding to the low, moderate and high CEC, respectively. It should be mentioned that the first two ranges comprised *ca* 78% of all values, which implies that Zn is not strongly retained by soils and should be more easily released (supplied) for biological assimilation. Next, in terms of exchange processes, this may be related to the reduction of the number of negative charges on soil colloids reducing the adsorption of Zn on exchange sites. These geochemical conditions will favor zinc transfer from bulk soils to the *phytosink*, i.e., plants.

Table 2

Rating for the cation exchange capacity (CEC)

Rating	CEC (cmol(+) kg^{-1})
Very low	< 6
Low	6 - 12
Moderate	12 - 25
High	25 - 40
Very high	> 40

Zinc geoavailability versus DTPA critical values

The geoavailability of Zn is controlled by several factors, hence the relative difficulty in elaborating average values (mainly chemical tests), which reasonably express the actual state. Several attempts have been undertaken to outline a “universal” chemical test, but the multitude of soil factors on one hand, and plant heterogeneity (species, varieties) on the other hand substantially dispersed the final target. However, since the 1970s, the use of the DTPA test (Lindsay, Norvell 1978) has been progressing and the achievements, i.e. establishment of some critical values, are more convincing (Sharma et al. 2004, Brennan 2005, Alloway 2008).

The data listed in Table 3 (total and potentially available) show the variation of mean Zn_{-DTPA} values accordingly to the total content and investigated sites. The amounts of Zn_{-DTPA} varied in a wide range, from 0.80 to 4.30 mg kg^{-1}, but the overall share in total Zn fluctuates from 2.0 (site H) to 7.9% (site C), which implies that the geoavailability of zinc compounds seems to be relatively high. The relationship established for the pairs Zn_{-Total} *versus* Zn_{-DTPA} ($Y_{Zn\text{-}DTPA} = 0.092_{Zn\text{-}Total} - 2.00$; $R^2 = 0.63$) yielded a significantly high coefficient of determination as a proof of the importance of the Zn_{-Total} in controlling the Zn_{-DTPA} pool. The values obtained by Ibrahim et al., (2011) fell within a range of 2.62-7.02 mg kg^{-1} (mean = 4.65) slightly narrower than observed in the case of data reported by Kirmani et al. (2001), i.e. 0.47-6.50 (mean = 1.98).

Table 3

Total, potentially available and Zn content in 9 investigated arable fields and plants, respectively, (mean values)

Field/Site (No. of soil samples)	Type of crop plant	Total^a	Potentially available^b	Plant^c
		Zn (mg kg^{-1})		
A (n = 5)	winter triticale	48.6	1.50	43.0
B (n = 11)	winter wheat	39.5	1.15	39.7
C (n = 10)	winter barley	54.2	4.30	39.4
D (n = 10)	winter barley	36.5	1.24	38.7
E (n = 5)	grass mixtures	19.5	1.03	15.3
F (n = 23)	winter oilseed rape	33.1	1.43	22.7
G (n = 16)	winter wheat	54.8	3.20	44.0
H (n = 11)	sugar beet	41.7	0.82	32.6
I (n = 8)	winter wheat	26.0	0.80	24.5

a – 6 moles HCl dm^{-3}, b – 0.005 moles DTPA dm^{-3},
c – concentration in green biomass

One of the challenging aspect in evaluating the potential availability of Zn is the elaboration of the *critical value* for a given site or agricultural field. This is necessary for practical purposes, for instance, predicting possible deficiency. Critical values listed in Table 4 revealed that the reported data vary quite similarly within the suggested ratings, irrespective of the significantly different soil conditions and crop plants (see footnote referen-

Table 4

Critical values[a, b, c, d] (DTPA-based) for interpreting the levels of Zn in agricultural soils (mg kg^{-1})

Parameter/Reference	Rating		
	low	medium	high
Zn[a]; Zn[b]	< 0.8	0.8 - 2.0	> 2.0
Zn[c]; Zn[d]	< 0.6	0.6 - 1.2	1.2 - 2.4
Pakistan[e]	< 0.5	0.5 - 1.0	> 1.0
Brazil[e]	< 0.5	0.6 - 1.2	> 1.2
Canada[e]	< 0.5	0.5 - 1.0	> 1.0
USA[e]	< 0.5	0.6 - 1.0	> 1.0

[a] LINDSAY, NORVELL 1978; [b] IBRAHIM et al. 2011; [c] TAKKAR, MANN 1975; [d] KIRMANI et al. 2011; [e] LHENDUP, DUXBURY 2008

ces). In the current study, an attempt has been made to propose critical Zn_{-DTPA} values as illustrated in the Figure 1. Notably, the optimal critical range varied from 0.80 to 1.43 mg kg^{-1} with the mean value of *ca* 1.08 mg kg^{-1}, reflecting 67% of all investigated sites. The said amount should be rated generally as a medium one, but in overall the investigated sites present a medium-high status. According to BRENNAN et al. (2009), the critical Zn_{-DTPA} for several investigated crop plants fluctuated within the range

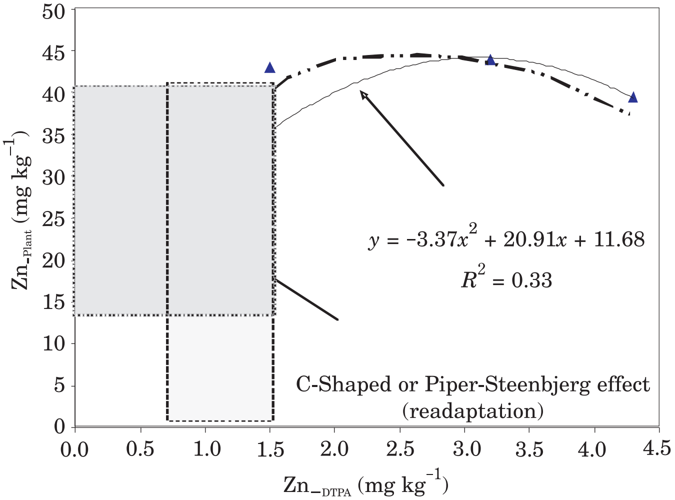

Fig. 1. Graphical readaptation of the C-Shaped or Piper-Steenbjerg curve for elaborating Zn_{-DTPA} and Zn_{-Plan} critical values

0.10-1.00 mg kg^{-1}, but ALLOWAY (2004) has reported a range of 0.45-0.67 mg kg^{-1} as adequate for wheat in India, adding that these values depended on the region and soil characteristics. The same applied to the critical value (0.68 mg kg^{-1}) for dryland in Iran (FEIZIASL et al. 2009). For fields subjected cropped with soybean and wheat in India, BARMAN et al. (1998) suggested a critical operational $Zn_{\text{-DTPA}}$ value of 1.5 mg kg^{-1}, specifically for soils with a high silt and clay content. The latter value is high enough, but significantly lower than the ones elaborated for ginger (*Zingiber officinale* Rosc.) soils, i.e. 2.10-4.53 mg kg^{-1}, mean = 2.95 (SRINIVASAN et al. 2009). It should be mentioned that with such high Zn concentration, most cropped plants suffered biomass loss, probably, due to their response to the toxic effect of the metal.

Most studies dealing with the elaboration of critical $Zn_{\text{-DTPA}}$ values have been performed in geographical zones in which soils are frequently slightly alkaline to alkaline. Under such conditions, zinc geochemistry is mainly controlled by the occurrence of anionic ions, which in turn are not the basic form taken up by plants. The geochemical approach suggested by MA and LINDSAY (1990, 1993) and CATLETT et al. (2002) fully elucidates this state in terms of the direct pH impact over Zn solution activity, i.e. $\log (Zn^{2+}) \Leftrightarrow 5.8 - 2\ pH$. Therefore, the higher the solution pH, the lower the Zn^{2+} activity and *vice-versa*. This may confirm the critical ranges reported by ALLOWAY (2004) or BRENNAN et al. (2009) and also the value equal 0.40 mg kg^{-1} obtained by CAKMAK (2004) for calcareous soils in Anatolia (Turkey). This approach demonstrates that soil pH seems to play a crucial role, but additional factors such as clay and silt levels should not be omitted.

Phytoconcentrations: elaboration of critical Zn values

Modern agricultural practices appear to have increased the extent and severity of zinc deficiency due to i) inadequate soil pH, particularly alkaline conditions, ii) increasingly growing biomass yield, which induces Zn mining from soil reserves. These characteristics have a direct impact on Zn uptake and its subsequent accumulation in plant organs. Zinc concentrations in six crop plants (Table 3), i.e. winter triticale, winter wheat, winter barley, grass mixture, winter oilseed rape and sugar beet, vary within a wide range of 15.3-44.0 mg kg^{-1}, with the lowest and highest levels found for grass mixtures and winter wheat, respectively. The linear relationship obtained from the pairs $Zn_{\text{-Total}}$ *versus* $Zn_{\text{-Plant}}$ ($Y_{\text{Zn-Plant}} = 0.80_{\text{Zn-Total}} + 0.93$; $R^2 = 0.67$) makes it clear that 67% of Zn taken up by tested crop plants should be strictly linked to its total content in soils. This is supported by the relationship reported earlier (i.e., $Y_{\text{Zn-DTPA}} = 0.092_{\text{Zn-Total}} - 2.00$; $R^2 = 0.63$) indicating that the Zn-DTPA pool should potentially reflect Zn accumulated in plants.

As shown in Figure 1, the concentrations below the lower critical level (15.3 mg kg^{-1}) will indicate potential deficiency and the need for remedial action (such as the use of zinc fertilizers or foliar sprays). Values between the lower and upper critical concentrations (15.3-39.7 mg kg^{-1}) will indicate an appropriate zinc status and no need for corrective action, while those above the upper critical value (39.7 mg kg^{-1}) will express a high zinc status reflecting the possibility of toxicity toward susceptible crops. Therefore, the 2nd polynomial relationship characterized by a relatively low coefficient of determination (R^2 = 0.33) visibly displays the *plateau-generated* Zn-DTPA levels higher than 1.5 mg kg^{-1}.

The C-Shaped or Piper-Steenbjerg effect (hand-fitted curve for zinc, readapted), illustrated in Figure 1, deserves due attention. In fact, this curve reflects the relationship between the Zn concentration in plant tissue and the respective yield/biomass build-up (PIPER 1942, STEENBERG 1951, ALLOWAY 2004, BRENNAN 2005). The lower portions of the growth response curve can show an increase in growth with a decline in zinc concentration and can cause problems in the interpretation of plant analyses (IFA 1992). Some authors have suggested that problems with the C-shaped response curve can be minimized by analyzing whole shoot samples (as performed in the current study), but others still consider whole shoots to be unsuitable (ROSELL, ULRICH 1964, GENC et al. 2002). Nevertheless, in many parts of the world, when farmers collect samples for plant analysis, it is often whole shoots of young plants which are sampled (ALLOWAY 2004).

For the purpose of this study, the Piper-Steenbjerg approach has been readapted in order to illustrate the relationship for the pairs $\mathrm{Zn}_{\text{-Plant}}$ *versus* $\mathrm{Zn}_{\text{-DTPA}}$, hence critical values of Zn were obtained for the investigated crop plants. This approach seems advisable, since it links the direct flow of Zn from labile Zn pools and its expected sink (i.e., plants), irrespective of the soil type and plant characteristics (species, varieties). Next, it uncovers a possibility of simultaneously elaborating targeted critical values for physiologically different crop plants.

Elaborated critical plant values versus interpretative standards

A rule of the thumb states that one of the biggest challenges is confronting empirical data with existing standards. In fact, interpretation standards are frequently discrepant as a result of the extensive heterogeneity of plant materials (Table 5). Two levels may be operationally considered for critical values established in this study: a *deficiency* and a *sufficiency* status, which tend to overlap according to ranges suggested by authors (footnote). The calculated mean critical value (data in Figure 1) is equal to 33.3 mg kg^{-1}, a value reflecting generally a sufficient rather than a deficient level. The investigated crop plants may be divided into two basic sites (Figure 2).

Table 5

Critical values[a, b, c, d] for interpreting the levels of Zn in crop plants (mg kg^{-1})

Parameter/Reference	Status		
	deficiency	sufficiency	excess
	(mg kg^{-1})		
Zn[a]	10 - 20	27 - 100	100 - 400
Zn[b]	15	10 - 100	-
Zn[c]	< 15 - 30	15 - 70	> 70
Zn[d]	reference plant - 50		

[a]KABATA-PENDIAS, PENDIAS 1995; [b]PAIS, JONES JR. 1997; [c]CAKMAK, MARSCHNER 1987; [d]MARKERT 1994

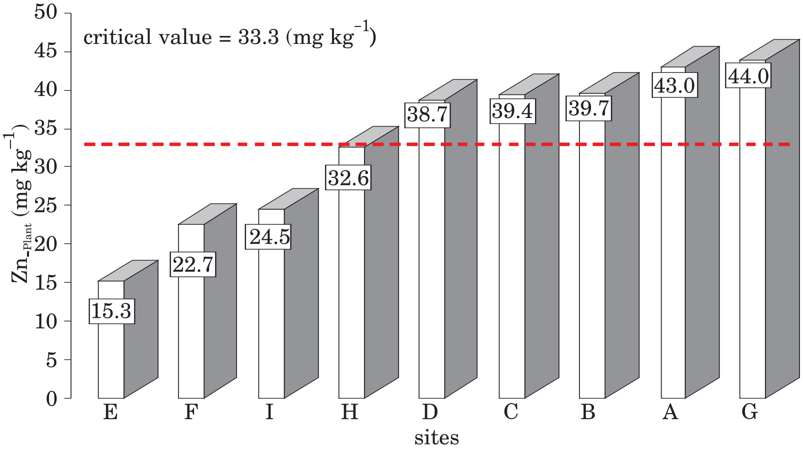

Fig. 2. The Zn$_{\text{-Plant}}$ critical value delimitation of investigated sites into experiencing deficiency (Zn$_{\text{-Plant}}$ < 33.3 mg kg^{-1}) and not experiencing deficiency (Zn$_{\text{-Plant}}$ > 33.3 mg kg^{-1})

Sites where plants may experience deficiency, i.e. Zn$_{\text{-Plant}}$ < 33.3 mg kg^{-1}:

E – winter wheat,
F – winter oilseed rape,
I – sugar beet,
H – grass mixtures;

Sites where plants do not experience deficiency, i.e. Zn$_{\text{-Plant}}$ > 33.3 mg kg^{-1}:

D – winter Triticale,
C – winter wheat,
B – winter barley,

A – winter barley,

G – winter wheat.

These sets are highly interesting for the following reasons: the deficiency group consists of crop plants characterised by high vegetative biomass (except winter wheat), whereas the sufficiency group is made of crops with moderate yields of vegetative biomass. These findings shed new light on the urgency with which we need to elaborate critical values for a wide range of crops to use in Poland and possibly in other countries. The case of zinc reported in this paper seems encouraging.

CONCLUSIONS

1. The nine investigated fields have presented substantially diverse soil properties, but provided growth conditions which seemed favorable to growing crops, except for two sites characterized by $\mathrm{pH}_{\mathrm{CaCl}_2}$ ca 5.5 and CEC ca 4.5 $\mathrm{cmol}_{(+)}$ kg^{-1}.

2. The amounts of $\mathrm{Zn}_{\text{-DTPA}}$ varied from 0.80 to 4.30 mg kg^{-1}, i.e., 2.0 to 7.9% of total Zn. These percentages imply that the geoavailability of zinc compounds was relatively high. The mean critical $\mathrm{Zn}_{\text{-DTPA}}$ value amounted to 1.08 mg kg^{-1} (range: 0.80-1.43 mg kg^{-1}), reflecting 67% of all investigated sites.

3. On the basis of the readapted C-Shaped, i.e. Piper-Steenbjerg curve, the critical $\mathrm{Zn}_{\text{-Plant}}$ content for the investigated crop plants was determined at 15.3-39.7 mg kg^{-1} with the mean value of 33.3 mg kg^{-1}.

4. The mean critical $\mathrm{Zn}_{\text{-Plant}}$ divided plants into two groups: i) experiencing deficiency: winter wheat, winter oilseed rape, sugar beet and grass mixtures and ii) not experiencing deficiency: winter triticale, winter barley and winter wheat. There is a need to elaborate critical values for most crops cultivated in Poland.

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THE INFLUENCE OF DIFFERENT METHODS OF CROPPING AND WEED CONTROL ON THE CONTENT AND UPTAKE OF Fe AND Mn BY DENT MAIZE

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Abstract

Strip cropping is a form of intercropping in which two or more species of plants are grown in adjacent strips. Strips should be wide enough for independent mechanical cultivation, but sufficiently narrow to allow for interaction between species. This may affect not only the size and structure of yield, but also the chemical composition of the plants.

The aim of this study was to assess the impact of strip cropping and different weed control methods on the content of iron and manganese and uptake by dent maize. The study was conducted on a private farm in the village of Frankamionka in the district of Zamość. It was run from 2004 to 2006. It consisted of a field experiment established on soil with an average Fe and Mn content. The experimental factors were two methods of cultivation: sole cropping and strip cropping (common bean, dent maize, and spring wheat in adjacent strips), and three methods of weed control: mechanical (inter-row cultivation applied twice), mechanical-chemical (the herbicide Gesaprim 90 WG 1.5 kg ha^{-1} + single inter-row cultivation), and chemical (the herbicides Gesaprim 90 WG 1.5 kg ha^{-1} + Milagro 040 SC 1.5 L ha^{-1}). Maize was grown for silage and harvested at the milky-wax stage. Iron and manganese in the dry matter of maize were determined by atomic absorption spectrometry (AAS) after digestion in HNO_3 (extra pure) in accordance with PN EN ISO 6869:2002.

On average for the experiment, strip cropping of maize with common beans and spring wheat increased the iron and manganese content in maize crop in comparison with sole cropping. The Fe and Mn content varied depending on the position of a row in strip cropping. Location adjacent to beans was more conducive to iron accumulation in maize, but reduced the manganese content. Strip cropping significantly increased the uptake of both iron and manganese by maize. The iron content was the highest where mechanical weed

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control was applied, while manganese was the highest where herbicides alone were used. The uptake of iron and manganese was the highest under the chemical weed control method. The results confirm the impact of strip cropping on the uptake of these minerals by maize and their content in the maize.

Key words: maize, strip cropping, weed regulation, iron, manganese, content, uptake.

WPŁYW METOD UPRAWY I REGULACJI ZACHWASZCZENIA NA ZAWARTOŚĆ I POBRANIE Fe I Mn PRZEZ KUKURYDZĘ PASTEWNĄ

Abstrakt

Uprawa pasowa jest formą uprawy współrzędnej i polega na uprawie dwóch lub więcej gatunków roślin w sąsiadujących pasach. Szerokość pasów powinna umożliwić zarówno niezależną mechaniczną uprawę roślin, jak i wzajemne oddziaływanie. To z kolei może wpływać nie tylko na wielkość i strukturę plonu, ale również na skład chemiczny roślin.

Celem pracy była ocena wpływu uprawy pasowej i metod regulacji zachwaszczenia na zawartość oraz pobranie żelaza i manganu przez kukurydzę pastewną. Eksperyment polowy przeprowadzono w gospodarstwie indywidualnym położonym we wsi Frankamionka w powiecie zamojskim, w latach 2004-2006, na glebie o średniej zasobności w Fe i Mn. Badanymi czynnikami były dwa sposoby uprawy: siew czysty i uprawa pasowa (w sąsiadujących pasach fasola zwyczajna, kukurydza pastewna, pszenica jara) oraz trzy metody regulacji zachwaszczenia: mechaniczna (2-krotne opielanie międzyrzędzi), mechaniczno-chemiczna (herbicyd Gesaprim 90 WG 1,5 kg ha^{-1} + jednokrotne opielanie międzyrzędzi); chemiczna (herbicydy Gesaprim 90 WG 1,5 kg ha^{-1}+ Milagro 040 SC 1,5 l ha^{-1}). Kukurydzę pastewną uprawiano na zielonkę i zbierano w fazie dojrzałości mleczno-woskowej. Zawartość żelaza i manganu w suchej masie kukurydzy oznaczono metodą absorpcyjnej spektrofotometrii atomowej (ASA) po mineralizacji w HNO_3 (ekstra czystym), zgodnie z normą PN-EN ISO 6869:2002.

Średnio w doświadczeniu, uprawa pasowa kukurydzy z fasolą zwyczajną i pszenicą jarą wpłynęła na zwiększenie zawartości żelaza i manganu w kukurydzy w porównaniu z uprawą w siewie czystym. Zawartość Fe i Mn zmieniała się w zależności od położenia rzędu w uprawie pasowej. Sąsiedztwo z pasem fasoli sprzyjało gromadzeniu większej ilości żelaza w kukurydzy, zmniejszało zaś zawartość manganu. Uprawa pasowa wpłynęła na istotne zwiększenie pobrania zarówno żelaza, jak i manganu z plonem kukurydzy. Zawartość żelaza była największa w warunkach stosowania mechanicznej regulacji zachwaszczenia, zaś manganu tam, gdzie stosowano wyłącznie herbicydy.

Pobranie z plonem żelaza i manganu było największe w warunkach stosowania chemicznej metody regulacji zachwaszczenia. Potwierdzono wpływ uprawy pasowej na zawartość oraz pobranie składników mineralnych z plonem.

Słowa kluczowe: kukurydza, uprawa pasowa, regulacja zachwaszczenia, żelazo, mangan, zawartość, pobranie.

INTRODUCTION

Strip cropping is used in many regions of the world to protect soil against water and wind erosion and to reduce losses due to leaching of minerals (Bucur et al. 2007, Rogobete, Grozav 2011). It involves two or more crop species grown in strips wide enough to allow for independent mechanical cultivation, yet sufficiently narrow so that interaction between species occurs. This system affects not only crop yield but also nutrient content in plants, with the strength and direction of these changes depending on a nutrient and on the neighbouring crops (Li et al. 2001, Głowacka 2010). Maize is often selected for strip cropping because it responds to the edge effect with significant increases in yield. It has the potential to produce large amounts of both silage and grain of high nutritional value. Maize plays an important role as animal feed, food for people and raw material for various industries (Szymańska et al. 2009). Therefore, as with other plants, the nutritional value and composition as well as proportions of nutrients in maize are very important to the health of humans and animals (Graham et al. 2007, White, Broadley 2009).

In the Polish literature, few studies deal with the impact of strip cropping on weed infestation, yield and the content or uptake of macronutrients (Burczyk 2003, Głowacka 2008, Głowacka et al. 2011). There have been no reports on the impact of strip cropping on the iron and manganese content in plants. Therefore, the aim of this study was to evaluate the impact of strip cropping and sole cropping, together with weed control methods, on the content and uptake of iron and manganese in maize.

MATERIAL AND METHODS

Field experiments were carried out in 2004-2006, on a private farm located in the village of Frankamionka in the district of Zamosc. The experiment was set up in a split-plot randomized block design in four replications. It was conducted on clayey silt soil with granulometric composition, slightly acid (pH in 1 mol KCl dm^{-3} 6.5), containing 19 g kg^{-1} organic matter and with an average content of available forms of manganese and iron (219.4--230.6 mg Mn kg^{-1}, 904-912 mg Fe kg^{-1}).

The experimental factors were as follows: 1) method of cultivation: a) sole cropping, in which the size of plots was 23.75 m^2 for sowing and 17 m^2 for harvesting, with 10 rows of maize spaced at 50 cm, and b) strip cropping, in which three crops – common bean, dent maize and spring wheat – were grown side by side in strips of the width of 2.5 m each. Five rows of maize were planted in each strip, spaced at 50 cm. The size of the maize plots was 11.75 m^2 for sowing and 10.5 m^2 for harvesting; 2) method of weed control: a) mechanical – inter-row cultivation twice; b) mechanical--chemical – the herbicide Gesaprim 90 WG 1.5 kg ha^{-1} (a.i. atrazine 135 g ha^{-1}) + inter-row cultivation; c) chemical – the herbicides Gesaprim 90 WG 1.5 kg ha^{-1} (a.i. atrazine 135 g ha^{-1}) + Milagro 040 SC 1.5 L ha^{-1} (a.i. nicosulfuron 60 g ha^{-1}). The cultivar Veritis of dent maize (FAO 230-240) was sown between 25 April and 5 May. Mineral fertilization was applied uniformly in the amount of 160 kg N ha^{-1}, 40 kg P ha^{-1} and 108 kg K ha^{-1}. Maize was harvested at the milky-wax stage (BBCH 79/83), in the second third of September. Spring wheat was harvested in the second third of August (BBCH 89), and common beans in the last third of August or first week of September (BBCH 88/89). A detailed description of the methodology of the experiment can be found in an earlier study (Głowacka 2008). Meteorological conditions during the years of the research are shown in Figure 1.

Each year prior to harvest, two maize plants were randomly collected from middle rows of each plot. Additionally, from each plot with strip cropping two maize plants were collected from the border rows adjacent to the rows of common bean and spring wheat. After the plants were crushed, dried and ground, the iron and manganese content in the samples (following wet mineralization in extra pure HNO_3) was determined by atomic adsorption spectroscopy (AAS) according to PN EN ISO 6869:2002. the results were calculated on the dry weight basis. The analyses were performed at the Agroecological Central Laboratory of the University of Life Sciences in Lublin. The results were analyzed statistically using analysis of variance (Kala 2009). Differences between averages were evaluated by Tukey's test. The significance of differences was determined at a 95% confidence level.

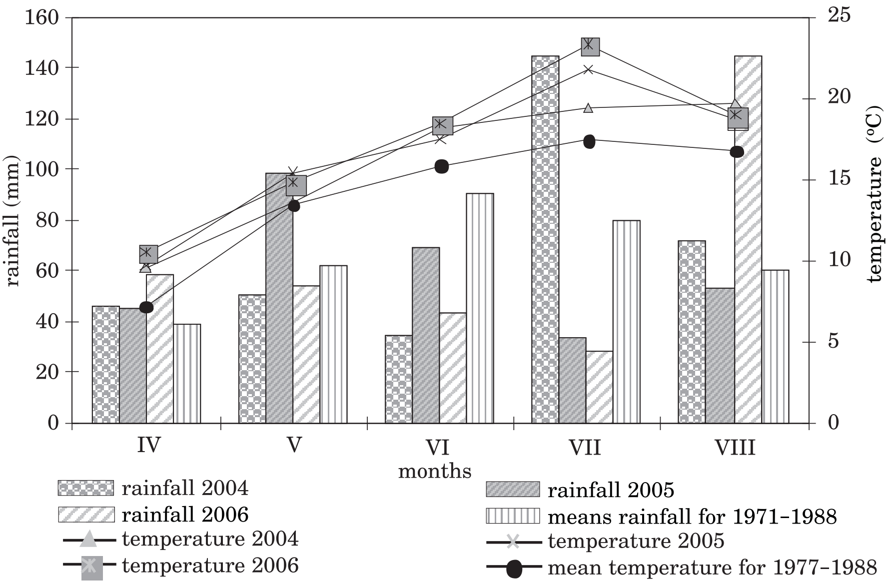

Fig 1. Rainfall and air temperature in months April-August 2004-2006 against the background of long-term means (1971-1988), according to the Meteorological Station in Zamość

RESULTS AND DISCUSSION

In the first year of the study, maize yield in the sole cropping was significantly higher than in the strip cropping (Table 1). On average for the study, however, maize yield was not significantly affected by the cropping methods. Of the three methods of weed control, the mechanical method had the least favourable effect on yield. The low maize biomass in this series was probably due to the inadequate efectiveness of the mechanical weed control treatments (Abdin et al. 2000). The differences between the mechanical-chemical and chemical methods were small and statistically insignificant. Some significant differences in the maize yield in successive years of the study could be due to the weather conditions in the growing season.

Table 1

Yield of maize depending on the method of cropping and weed control (Mg d.m. ha^{-1})

Method of cultivation	Weed control	Years			Average
		2004	2005	2006	
Sole cropping	A*	20.6	14.6	12.3	15.8
	B	22.9	15.9	16.7	18.5
	C	23.2	18.7	16.1	19.3
Strip cropping – mean for plot	A	18.8	14.0	11.6	14.8
	B	19.8	17.9	15.3	17.7
	C	20.9	18.4	15.2	18.2
LSD (α = 0.05)		n.s.	n.s.	n.s.	n.s.
Average for factors					
Sole cropping	–	22.2*b*	16.4	15.4	17.9
Strip cropping – mean for plot	–	19.9*a*	16.8	14.0	16.9
LSD (α = 0.05)		0.94	n.s	n.s.	n.s.
–	A	19.7*a*	14.3*a*	11.9*a*	15.3*a*
–	B	21.3*b*	16.2*b*	16.0*b*	18.1*b*
–	C	22.0*b*	18.5*c*	15.7*b*	18.8*b*
LSD (α = 0.05)		1.45	1.81	3.05	1.59
Years	2004	21.0*c*			
	2005	16.6*b*			
	2006	14.5*a*			
LSD (α = 0.05)		1.65			

* weed control: A – mechanical, B – mechanical-chemical, C – chemical
a, b, c – means in columns marked with the same letter do not differ significantly

There was much less rainfall in 2005 and 2006 than in 2004 or than the long-term average. Moreover in 2006 it was very unevenly distributed.

The iron content in plants is relatively high in comparison with other micronutrients. It also varies markedly depending on the organ and the developmental stage of the plant. In a study by SKOWROŃSKA and FILIPEK (2009), the iron content was 15.5-29.0 mg kg^{-1} d.w. in the grain of maize, 47.5--77.0 mg kg^{-1} d.w. in leaves, and as high as 1,137.0-1,482.0 mg kg^{-1} d.w. in roots. In the present study, the Fe content in the aboveground biomass of maize ranged from 50.8 to 124.4 mg kg^{-1} d.w.; it varied significantly depending on weather conditions in different years of the study, method of cultivation and weed control method (Table 2). The iron content in the maize grown in strip cropping with common bean and spring wheat was substantially higher, by an average of 32%, than in sole cropping. ZANGH and LI

Table 2

Content of iron in maize depending on the method of cropping and weed control (mg Fe kg^{-1} d.w.)

Method of cultivation	Weed control	Years			Average
		2004	2005	2006	
Sole cropping	A*	93.3*c*	53.2*c*	68.7*c*	71.8*b*
	B	88.2*b*	55.3*d*	47.9*a*	63.8*a*
	C	80.0*a*	55.8*d*	58.3*b*	64.7*a*
Strip cropping – mean for plot	A	111.5*d*	50.8*a*	109.3*f*	90.5*d*
	B	124.4*e*	58.2*f*	78.4*d*	87.0*c*
	C	112.1*d*	52.2*b*	92.9*e*	85.7*c*
LSD (α = 0.05)		1.42	1.22	3.32	1.77
Average for factors					
Sole cropping	–	87.2*a*	54.8*a*	58.3*a*	66.7*a*
Strip cropping – mean for plot	–	116.0*b*	53.7*a*	93.5*b*	87.8*b*
LSD (α = 0.05)		1.15	n.s.	2.47	2.27
–	A	102.4*b*	52.0*a*	89.0*c*	81.1*b*
–	B	106.3*c*	56.8*c*	63.2*a*	75.4*a*
–	C	96.0*a*	54.0*b*	75.6*b*	75.2*a*
LSD (α = 0.05)		1.23	1.66	2.72	1.42
Years	2004	101.6*c*			
	2005	54.3*a*			
	2006	75.9*b*			
LSD (α = 0.05)		3.78			

* weed control: A – mechanical, B – mechanical-chemical, C – chemical

a, b, c, d, e, f – means in columns marked with the same letter do not differ significantly

(2003) also indicate that strip cropping affects not only crop yield, but also the uptake and use of nutrients. Research by Głowacka (2011) shows that the neighbouring plants in strip cropping affect the content of macronutrients in maize. Plants in rows adjacent to common beans contain more phosphorus and less potassium than those from the middle rows and from rows adjacent to wheat. Li et al. (2001) reported that wheat is more competitive in the uptake of macronutrients such as N, P, and K than maize and soy accompanying it in strip cropping. In the present study, too, the iron content in maize varied depending on the position of a row in a strip and the adjacent plant. (Figure 2). On average, irrespective of the method of weed control, the iron content in maize growing in rows adjacent to beans was higher than in maize from middle rows or rows growing adjacent to wheat, between which there was no pronounced difference. Changes in the iron content in plants from individual rows were similar, irrespective of the method of weed control (Figure 3).

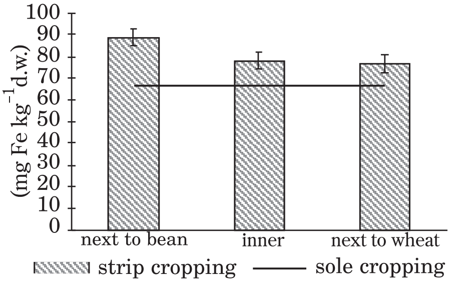

Fig. 2. The influence of row position in a strip on the iron content in maize plants

The manganese content in the aerial parts of a plant to some extent depends on the properties of a species (Szteke et al. 2004, Bowszys et al. 2006, Rogóż, Niemiec 2010, Rachoń et al. 2012) and can range widely from 20 to 500 mg kg^{-1} d.m. In our study, the manganese content in the maize ranged from 8.4 to 28.3 mg kg^{-1} d.w. and was similar to that noted by Rabikowska and Piszcz (2004), but much lower than what is reported to be optimal in animal feed (Gorlach, 1991, Falkowski et al. 2000).

Strip cropping decreased the manganese content in maize only in the first year of the experiment. In the following years and on average for the experiment, the manganese content was significantly higher in strip cropping, by an average of 16.4%, than in sole cropping (Table 3). Differences in the manganese content in maize depending on the crop adjacent to the row of maize in a strip were different than in the case of iron. The lowest manganese content was noted in rows adjacent to common bean, while the highest content was observed in middle rows (Figure 4). Głowacka et al. (2011) reported that maize grown in a row adjacent to wheat contained more calcium than maize grown adjacent to beans. The ability of different plant spe-

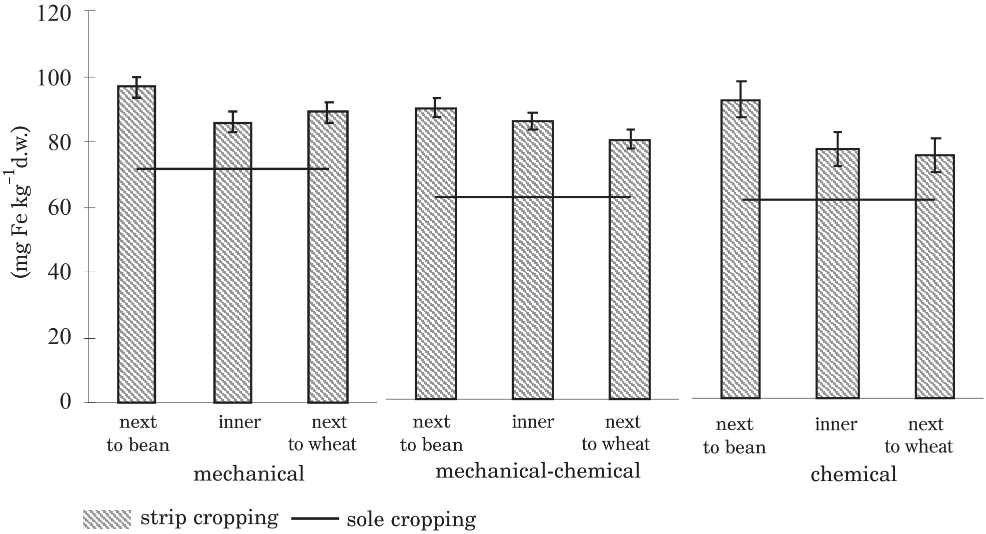

Fig. 3. The influence of a row position in a strip and weed control method on the iron content in maize plants

cies to take up minerals from soil is affected by the unequal valency and cation exchange capacity of their roots (Drake et al. 1951). This exchange capacity in dicotyledonous plants such as beans is twice that of monocots. Moreover, the accumulation of available forms of manganese in soil can be depressed by magnesium and calcium ions (Sienkiewicz et al. 2009). Our analysis of changes in the manganese content in plants from each row under different methods of weed control reveals that wherever the mechanical-chemical and chemical methods were used, the manganese content was the lowest in maize from rows adjacent to common beans. When mechanical treatments alone were applied, the highest Mn content was in maize from the center row, with insignificant differences between the rows bordering with beans and with spring wheat (Figure 5).

High-yielding crops contain smaller quantities of micronutrients per unit weight in relation to their availability in soil than crops that produce less biomass, because theformer are unable to take up from soil sufficiently large amounts of nutrients, which in turn are ‘diluted’ in the high biomass of the plant (Cakmak 2004). This was confirmed in the present study with respect to iron. The highest iron content was noted in the maize from sites where mechanical weed control alone was used. Significantly less iron was accumulated by plants when the mechanical-chemical or chemical method was used. However, the differences between these methods were not significant (Table 2). These methods were conducive to the production of large quantities of biomass by maize (Table 1). The negative correlation between the volume of maize yield and the iron content in biomass is confirmed by the

Table 3

Content of manganese in maize depending on the method of cropping and weed control (mg Mn kg^{-1} d.w.)

Method of cultivation	Weed control	Years			Average
		2004	2005	2006	
Sole cropping	A*	15.3*a*	8.4*a*	13.2*a*	12.3*a*
	B	15.7*ab*	8.9*b*	19.6*b*	14.7*b*
	C	25.3*e*	10.7*d*	19.5*b*	18.4*e*
Strip cropping – mean for plot	A	16.2*b*	9.3*c*	20.2*d*	15.9*c*
	B	17.2*c*	127*e*	25.5*c*	17.8*d*
	C	20.0*d*	9.5*c*	28.3*e*	19.3*f*
LSD (α = 0.05)		0.63	0.04	0.38	0.21
Average for factors					
Sole cropping	–	18.7*b*	9.3*a*	17.4*a*	15.2*a*
Strip cropping – mean for plot	–	17.8*a*	10.5*b*	24.7*b*	17.7*b*
LSD (α = 0.05)		0.22	0.02	0.14	0.07
–	A	15.7*a*	8.9*a*	17.7*a*	13.8*a*
–	B	16.5*b*	10.8*c*	22.6*b*	16.6*b*
–	C	18.3*c*	10.1*b*	23.7*c*	18.9*c*
LSD (α = 0.05)		0.34	0.02	0.21	0.11
Years	2004	18.3*b*			
	2005	9.9*a*			
	2006	21.5*c*			
LSD (α = 0.05)		0.32			

* weed control: A – mechanical, B – mechanical-chemical, C – chemical
a, b, c, d, e, f – means in columns marked with the same letter do not differ significantly

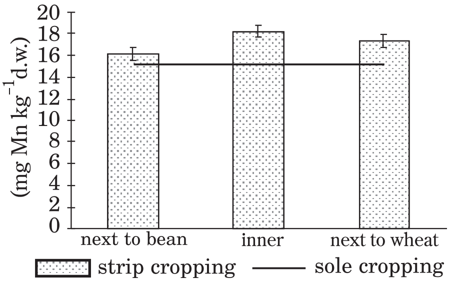

Fig. 4. The influence of a row position in a strip on the manganese content in maize plants

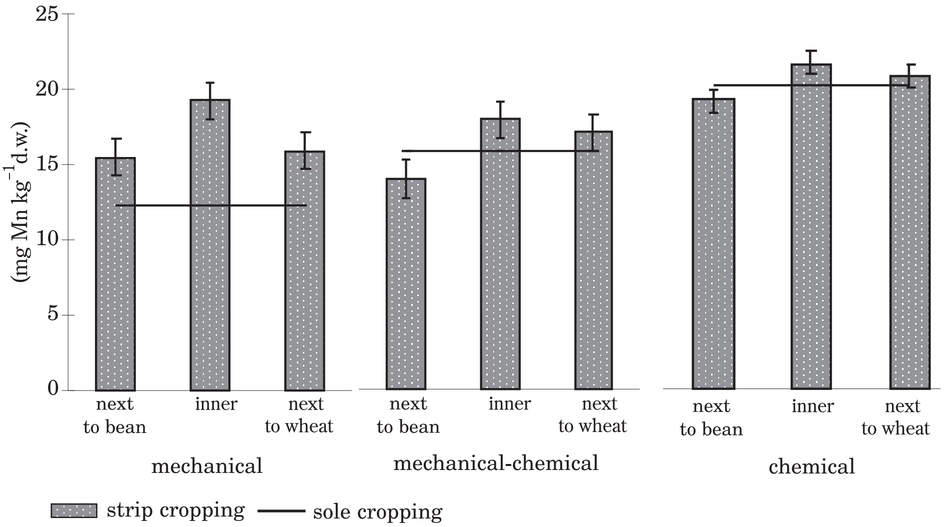

Fig. 5. The influence of a row position in a strip and weed control method on the manganese content in maize plants

Pearson's correlation coefficient (r = -0.471, $p < 0.05$). The manganese content, however, was the lowest in mechanically-weeded maize (Table 3). Inter-row cultivation repeated twice did not completely eliminate weeds from maize rows; the dominant species of weeds, i.e. Echinochloa crus-galli, Chenopodium album, and Galinsoga parviflora, are much more competitive than maize in the uptake of manganese, containing 7-10-fold more of this element, which could have resulted in its limited availability for maize.

The weather conditions substantially affected the iron and manganese content in maize. The smallest amounts of Fe and Mn in the dry matter of the maize were noted in 2005, which was characterized by low rainfall in July and August. The highest iron content was observed in 2004, and the highest manganese content appeared in 2006, the last year of the study (Tables 2 and 3). The impact of weather conditions on the absorption of micronutrients and their higher content in plants in years with more rainfall is also confirmed by the results of other experiments (RAJCAN, SWANTON 2001, SZTEKE et al. 2004, KLIKOCKA 2011).

The uptake of manganese and iron by plants is competitive, with Mn activity greater than that of Fe, especially where pH is acidic (ROGÓŻ 2009). In our study, the manganese content in maize was positively correlated with the iron content – r = 0.410 ($p < 0.05$). According to FALKOWSKI et al. (2000), an optimal Fe/Mn ratio in maize for animal feed should be 1.5-2.5:1. When the value is less than 1.5, symptoms of Mn toxicity and Fe deficiency appear, and when it is above 2.5 there is harmful excess of iron and symptoms of Mn deficiency. Therefore, it is important to maintain an appropriate balance between these two elements. However, the value of this ratio in different species of plants has a much broader range, which appears to be a taxonomic characteristic. In plants of the families Solanaceae, Brassicaceae, and Chenopodiaceae, the ratio is greater than 4, while for the families Fabaceae and Poaceae the optimum Fe/Mn ratio is 2.5-4. In our experiment, the Fe/Mn ratio was 4.9 in strip cropping and 4.6 in sole cropping, which was close to that characteristic of the Poaceae family, but well above the limit proposed for animal feed. The lowest Fe/Mn ratio was found in maize grown with chemical weed control, while the highest was noted in maize weeded mechanically (Table 4).

In a study by Rabikowska and Piszcz (2004), the uptake of manganese by maize ranged from 128 to 375 g from 1 ha. The manganese uptake by maize was similar in the present study: an average of 289 g per 1 ha (123-430.8 g

Table 4

Proportion of the Fe/Mn in maize depending on the method of cropping and weed control

Method of cultivation	Weed control	Years			Average
		2004	2005	2006	
Sole cropping	A*	6.1*c*	6.3*c*	5.2*e*	5.8*e*
	B	5.6*b*	6.2*c*	2.5*a*	4.3*b*
	C	3.2*a*	5.2*b*	3.3*b*	3.5*a*
Strip cropping – mean for plot	A	6.9*d*	5.5*b*	4.0*d*	5.4*d*
	B	7.2*e*	4.6*a*	3.3*c*	4.9*c*
	C	5.6*b*	5.5*b*	3.0*c*	4.4*b*
LSD (α = 0.05)		0.5	0.6	0.6	0.3
Average for factors					
Sole cropping	–	5.0*a*	5.9*b*	3.5*a*	4.6*a*
Strip cropping – mean for plot	–	6.6*b*	5.2*a*	3.6*a*	4.9*b*
LSD (α = 0.05)		0.15	0.16	0.12	0.21
–	A	6.5*b*	5.9*b*	4.8*b*	5.4*c*
–	B	6.4*b*	5.4*a*	2.9*a*	4.6*b*
–	C	4.4*a*	5.4*a*	3.1*a*	4.0*a*
LSD (α = 0.05)		0.18	0.19	0.24	0.31
Years	2004	5.8*b*			
	2005	5.5*b*			
	2006	3.6*a*			
LSD (α = 0.05)		0.36			

* weed control: A – mechanical, B – mechanical-chemical, C – chemical

a, b, c, d, e – means in columns marked with the same letter do not differ significantly from 1 ha). On average for the experiment, significantly more manganese was taken up by maize grown in strip cropping with spring wheat and common beans than in sole cropping (Table 5). The least manganese was taken up by maize from plots weeded mechanically, significantly more from plots where the mechanical-chemical weed control was applied, and the most in the case of chemical weed control. Pearson's correlation coefficients confirm a close relationship between the uptake and micronutrient content, $r = 0.831$ ($p < 0.001$), and between the uptake and yield, $r = 0.819$ ($p < 0.001$). On average in the experiment, strip cropping significantly increased the uptake of iron by maize compared to sole cropping (Table 6). The total uptake of iron was the lowest when the mechanical weed control method was used. A significantly higher uptake of micronutrients was noted for the mechani-

Table 5

Uptake of manganese with maize yield depending on the method of cropping and weed control (g ha^{-1})

Method of cultivation	Weed control	Years			Average
		2004	2005	2006	
Sole cropping	A*	315*a*	123*a*	162	199*a*
	B	359*ab*	142*a*	328	277*b*
	C	585*c*	199*b*	314	366*d*
Strip cropping – mean for plot	A	304*a*	130*a*	292	242*ab*
	B	342*a*	228*b*	360	310*c*
	C	421*b*	175*ab*	431	342*cd*
LSD (α = 0.05)		62.9	53.3	n.s.	46.4
Average for factors					
Sole cropping	–	420*b*	156*a*	268*b*	281*a*
Strip cropping – mean for plot	–	355*a*	178*b*	361*b*	298*b*
LSD (α = 0.05)		22.3	19.0	39.1	16.5
–	A	309*a*	127*a*	227*b*	221*b*
–	B	351*b*	185*b*	344*b*	293*b*
–	C	503*c*	187*b*	372*b*	354*c*
LSD (α = 0.05)		34.3	29.0	60.1	25.3
Years	2004	387*c*			
	2005	166*a*			
	2006	314*b*			
LSD (α = 0.05)		37.8			

* weed control: A – mechanical, B – mechanical-chemical, C – chemical
a, b, c, d – means in columns marked with the same letter do not differ significantly

Table 6

Uptake of iron with maize yield depending on the method of cropping and weed control ($g\ ha^{-1}$)

Method of cultivation	Weed control	Years			Average
		2004	2005	2006	
Sole cropping	A*	1920	779	842	1180
	B	2016	881	800	1233
	C	1851	1041	941	1278
Strip cropping – mean for plot	A	2100	709	1263	1357
	B	2466	1041	1198	1568
	C	2345	963	1415	1574
LSD (α = 0.05)		n.s.	n.s.	n.s.	n.s.
Average for factors					
Sole cropping	–	1929*a*	900*a*	861*a*	1230*a*
Strip cropping – mean for plot	–	2303*b*	905*a*	1292*b*	1500*b*
LSD (α = 0.05)		110.1	n.s.	125.4	58.9
–	A	2010*a*	744*a*	1052*a*	1269*a*
–	B	2241*b*	961*b*	999*a*	1401*b*
–	C	2098*b*	1002	1178*a*	1426*b*
LSD (α = 0.05)		169.1	151.4	n.s.	90.4
Years	2004	2116*c*			
	2005	902*a*			
	2006	1077*b*			
LSD (α = 0.05)		98.7			

* weed control: A – mechanical, B – mechanical-chemical, C – chemical

a, b – means in columns marked with the same letter do not differ significantly

cal-chemical and chemical methods of weed control. However, the differences between these two methods were not significant. The correlation analysis confirmed no significant relationship between the iron uptake and maize yield. Instead, it confirmed the close relationship between uptake of this micronutrient and its content in the biomass of the maize, $r = 0.691$ ($p < 0.001$), which is consistent with results obtained by MAZUR and SIENKIEWICZ (2009).

CONCLUSIONS

1. Strip cropping of maize with common beans and spring wheat significantly increased both the content and uptake of iron and manganese by maize in comparison with sole cropping.

2. The iron content was highest in the maize where the mechanical method of weed control was used, while manganese content and iron and manganese uptake was highest in the case of the chemical weed control.

3. The study confirms the influence of neighbouring plant species in strip cropping on the uptake of different amounts of iron and manganese by maize. Location next to common beans was more conducive to the accumulation of iron in maize, but reduced the manganese content.

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

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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. The residual effect of fertilization with pig manure and compost from biodegradable waste on the spring wheat grain yield was much better than that of 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.

Key words: 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

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prowadzono w warunkach doświadczenia polowego przez 3 lata. Ograniczony dostęp roślin do składników pokarmowych (głównie 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 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 agrotechnical 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 a result of insufficient amounts of natural, organic and also calcium fertilizers introduced to soil. The annual export of biogenic components with plant yields and losses of these components necessitates fertilization, regulating amounts of nutrients required by plants.

Adequate amounts of nutrients supplied to crops, alongside ensuring optimal soil conditions, are the main factors securing the expected yield with an adquate biological and technological value; at the same time they may determine doses of applied manure, compost or sewage sludge.

Cereal grain contains varied quantities 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; among amino acids the limiting role is attributed to lysine and sulfur amino acids. The content of these elements and compounds may be conditioned 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 research.

Table 1

Monthly rainfall and average daily temperature at the site in 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

The experiment was set up with the randomized block 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 liming was conducted according to $^1/_2$ hydrolytic acidity value (962.0 kg CaO ha^{-1}) In the spring of the subsequent year, after basic cultivation meas-

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

ures, manure, compost and sewage sludge were evenly spread over the surface 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 test plant was spring wheat cv. Jagna, sown at the density of 485 plants per 1 m^2. Herbicides and fungicides were applied during the vegetative season to protect the field against weeds and fungal diseases.

The duration of a wheat growing period in each years depended on the weather conditions. 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 under field conditions, the plants were harvested from an area of 4 m^2, separately from each plot.

Manure used in the research came from pigs that prior to its use had been stored on a manure pad for 6 months. The compost was made from plant waste and other biodegradable waste using the Mut-Kyberferm technology, 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 originated from a composting plant located in the city of Krakow. Stabilized sewage sludge (SS) came 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 potentiometer, electrolytic conductivity with a conductometer, total nitrogen content by the Kjeldahl's method. The content of organic matter and ash components was determined in dried and ground material after sample mineralization in a chamber furnace (at 450° C for 5 h). The phosphorus content was determined by colorimetry on a Beckman DU 640 spectrophotometer, and the potassium content was assayed by flame emission spectroscopy (FES) on a Philips PU 9100X apparatus. The concentrations of copper, zinc and manganese were determined by the ICP-AES method on a JY 238 Ultrace apparatus. The analyses were performed according to the methodology described in papers by BARAN and TURSKI (1996), and KRZYWY (1999), and the analytical results are presented in Table 3.

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
C_{utot}	mg kg^{-1} d.m.	156	34	80
Z_{ntot}	mg kg^{-1} d.m.	284	194	950
M_{ntot}	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

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 on a JY 238 Ultrace apparatus.

Concentrations of selected amino acid were assessed in 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 (the Chemical Score) designed by Block and Mitchell (Bęza 1967) was computed, which consisted in determining the ratio of the content of exogenous limiting amino acid in the analyzed protein to the content of the same amino acid in 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. The precision of the Zn, Cu and Mn determinations was determined with the reference material NCS DC733448 (China National Analysis Center for Iron & Steel). The data on the precision and accuracy of the 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 a completely randomized design using f-Fisher test was conducted for weighted mean (from 3 years) content of the microelements, protein and 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 the Statistica PL package (Stanisz 1998).

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.)	Precision	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

RESULTS

Grain yields

On the plots 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, respectively, 0.95 t, 1.23 t, and 0.56 t d.m. ha^{-1} 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.

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 in columns indicate significant differences ($\alpha < 0.05$, Duncan's multiple range test)

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, in comparison to yields from the treatment 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 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 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 from 3.26 mg kg^{-1} d.m. (grain from the treatment where municipal sewage sludge was used) to 3.61 mg kg^{-1} 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 .

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 ($\alpha < 0.05$, Duncan's multiple range test)

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).

Table 7

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

Treatments		Total protein (g kg^{-1} d.m.)	Σ 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	31.3b^{c} ± 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 restrictive amino acid; WE – whole egg protein standards;
MH – mature human
* average of three years ± standard error. Different letters in columns indicate significant differences ($\alpha < 0.05$, Duncan's multiple range test)

The highest weighted mean content of exogenous amino acids (Σ 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 (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 that are highly deficient in fodders. Fertilization with compost from biodegradable waste generally increased the content of the examined exogenous amino acids the highest (except for the methionine content). In comparison to the amino acid composition of mature human protein or hen egg protein, it was concluded that lysine was the amino acid limiting spring wheat grain quality (Table 8).

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	0.71a ± 0.16	2.14a ± 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	0.76ab ± 0.13	2.30b ± 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 ($\alpha < 0.05$, Duncan's multiple range test)

DISCUSSION

There is a wealth of research results indicating that it is possible to use organic waste material in 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 fertilization (STEWART, HASH 1982, BONDE et al. 1988) and the 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 from treatments fertilized with organic materials resulted from the inhibited nutrient utilization by plants, particularly in the case of nitrogen, whose availability from organic materials depends on the weather conditions during the vegetative season, dose of applied fertilizer and the value of the carbon to nitrogen ratio (BARBARIKA et al. 1985, AGEHARA, WARNCKE 2004). Fertilization with organic materials makes it difficult to synchronize the amounts of N released from those materials with plant requirements for this component (MIKKELSEN, HARRTZ 2008). The research results clearly indicate that the depression in wheat grain yield in the first year of the research might have been caused by the spring term of organic materials application.

With time, the residual effect of organic materials becomes distinguishable, resulting in higher yields, the outcome that has been confirmed by our three-year scientific research. A beneficial effect of fertilization with sewage sludge and composted biodegradable waste on wheat biomass yield was also 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 need to balance amounts of nutrients supplied with fertilization.

The content of micronutrients in plant biomass is a product of the content of their assimilable forms in soil, which in turn is strongly modified by soil pH, organic matter content, soil sorption capacity as well as the cultivated plant species and fertilization (KOPEĆ, PRZETACZEK-KACZMARCZYK 2006). An insufficient content of copper, in terms of fodder quality, was found in our research. Apart from a lower fodder value of grain, a copper deficit also leads to limited amount and quality of yield, especially because copper is incorporated in many enzymes and proteins involved in specific metabolic processes (PRASAD 1995, YRUELA 2005). The soil reaction and content of organic matter, for 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 cause low efficiency of soil fertilization with this element. The manure used in the research contained the most copper of the tested organic materials. Despite this, the copper concentration in spring wheat grain from plots fertilized with manure did not increase markedly. The results showed no significant effect of compost from biodegradable waste or municipal sewage sludge on the zinc and manganese concentrations in spring wheat grain in comparison to plants from the treatment fertilized with manure. It might have due to the relatively low content of these elements in the organic materials applied. The zinc content in grain was within the optimal range 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 as well as its biological value. With respect to the chemical composition, which shapes the fodder nutritional value, it may be said that cereal grain is 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 our research, the nitrogen dose in all treatments (except the control) was the same, and the use of a dose of this element corresponding to the wheat's 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 is usually low in lysine. Also in our experiment, lysine in grain was the amino acid that limited the protein quality 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 research results presented herein, however, are not confirmed by the research of JASIEWICZ and BARAN (2011), where it was found out that methionine was the amino acid limiting the protein quality in maize. The differences in the results may be attributable to the applied fertilization (type of organic material), the size of nitrogen dose and to the cultivated plant species. An adequate balance of the amino acid composition in food rations, matching the nutrient supply with animal requirements, is crucial, both because it enables optimal use of one of the most valuable nutrients, such as fodder protein, and because of the environmental aspect, such as a decrease in nitrogen excreted with urine whenever there is excess of each amino acid in relation to the demand.

CONCLUSIONS

1. The limited access of plants to mineral components (mainly nitrogen) introduced with organic waste materials 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. Organic waste materials used in moderate doses based on the plant's requirements for nutrients did not decrease 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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EFFICIENCY OF CHELATE FORMS OF MICRONUTRIENTS IN NUTRITION OF GREENHOUSE TOMATO GROWN IN ROCKWOOL

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Abstract

Cultivation of vegetable crops on rockwool medium under controlled conditions requires the use of completely soluble forms of fertilizers for preparing nutrient solution. In order to prevent immobilization of metal microelements, especially Fe, they are often used in chelated forms. The low biodegradability of currently popular chelating agents may lead to contamination of the environment by these compounds. The aim of the present study was to evaluate whether it is necessary to use chelated forms of Cu and Mn. Another objective was to assess the applicability of two chelating agents of iron in cultivation of tomato on rockwool slabs. Mineral forms of manganese ($MnSO_4$ H_2O) and copper ($CuSO_4$ $5H_2O$) were compared with EDTA+DTPA chelate forms of these nutrients. In the case of Fe, the suitability of EDTA and DTPA as the chelating agents was evaluated, taking into account the crop yield as well as nutritional status of tomatoes in different stages of growth. The results of our greenhouse trials proved that application of chelate forms of Cu and Mn did not alter the total yield of tomato fruits but accelerated fruit maturation, so that early tomato yield was higher than in response to mineral forms of these nutrients. Plants supplied with Cu and Mn chelates contained higher amounts of these nutrients in index parts of tomatoes analysed from May to August in one-month intervals. The use of DTPA and EDTA as chelating agents was just as productive in terms of tomato fruit yield. However, the DTPA chelate contributed to better plant nutrition with Fe, as well as Cu and Mn.

Key words: iron, copper, manganese, chelates, mineral forms, yield, nutrient status.

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SKUTECZNOŚĆ CHELATOWYCH FORM MIKROELEMENTÓW W NAWOŻENIU POMIDORA SZKLARNIOWEGO UPRAWIANEGO W WEŁNIE MINERALNEJ

Abstrakt

Uprawa warzyw pod osłonami w wełnie mineralnej wymaga stosowania pożywek, do których przygotowania stosuje się nawozy całkowicie rozpuszczalne w wodzie. Aby zapobiec uwstecznianiu mikroelementów metalicznych, przede wszystkim żelaza, często stosuje się formy chelatowe tych składników. Niektóre związki chelatowe, zwłaszcza EDTA, z powodu swojej trwałości mogą prowadzić do skażenia środowiska naturalnego. Przedmiotem badań była ocena zasadności stosowania chelatowych form miedzi i manganu oraz porównanie dwóch chelatów stosowanych do kompleksowania żelaza w uprawie pomidora szklarniowego w wełnie mineralnej. Formy mineralne manganu ($MnSO_4$ H_2O) i miedzi ($CuSO_4$ $5H_2O$) porównywano z chelatami EDTA+DTPA tych mikroelementów. W przypadku żelaza porównywano przydatność dwóch chelatów, tj. EDTA i DTPA, do nawożenia pomidora. W obydwu doświadczeniach oceniano wielkość plonu i stan odżywienia roślin mikroelementami w różnych stadiach rozwoju. Wykazano, że chelatowe formy Mn i Cu nie wpływały istotnie na plon ogółem owoców pomidora, przyczyniły się jednak do zwiększenia plonu wczesnego w porównaniu z formami mineralnymi. Rośliny nawożone chelatami Mn i Cu zawierały z reguły większe ilości tych składników w częściach wskaźnikowych, które analizowano w okresie od maja do sierpnia. Zastosowanie żelaza skompleksowanego chelatami DTPA i EDTA skutkowało zbliżonym plonowaniem pomidora. Użycie Fe-DTPA przyczyniło się do lepszego stanu odżywienia pomidora żelazem, miedzią oraz manganem.

Słowa kluczowe: żelazo, miedź, mangan, chelaty, formy mineralne, plon, stan odżywienia.

INTRODUCTION

Highly advanced technologies of horticultural crops production involve the application of fertilizers characterized by high phytoavailability of nutrients. Currently, the widespread hydroponic system of tomato and cucumber production involves the use of completely soluble salts used to prepare nutrient solutions. However, ions of metallic microelements, mainly iron and manganese and less so zinc and copper, may quickly change their valence in the presence of oxygen, thus becoming less available to plants (Höfner 1992). This phenomenon is frequent in inert media, distinguished by high porosity, excellent aeration and the lack of cation exchange holding capacity. Moreover, the immobilization of metallic micronutrients takes place under the conditions of excessive high pH, surplus of phosphates and carbonates in a growing medium (Kołota et al. 2006).

The application of chelated forms of micronutrients, characterized by good solubility in water and low value of dissociated constant, is crucial for the prevention of immobilization. Chelates make metal cations being gradually released to a medium solution or absorbed by plants in complexed forms (Wreesmann 1996). As proven by Komosa et al. (2005), durability and availability of microelements to plants depend on properties of ligands. The most common chelating agents used in fertilizers include EDTA, DTPA and

EDDHA (Lucena 2003), which differ in strength of chemical bonds of the ion-ligand complex with metals as a function of pH. The shortcoming of these chelating agents, especially EDTA, is their low biodegradability (Borowiec et al. 2007, Albano 2012). Until today, most of the greenhouse tomato production has been based on open fertigation systems, in which the surplus of nutrient solution from rockwool slabs sinks to the soil and causes the groundwater contamination. Chelating agents can persist in the environment, retaining the capacity to extract and solubilize heavy metals from sediments (Albano, Merhaut 2012).

There are numerous research data indicating high efficiency of plant nutrition with chelated forms of iron (Ylivainio et al. 2004, 2006). However, in the case of Cu and Mn, the mineral forms of fertilizers assured higher yields of lettuce grown on peat substrate than EDTA + EDTA chelates of these nutrients (Kozik et al. 2008a,b). Some differences in yields of this crop were found between the application of Zn chelated and mineral forms (Kozik et al. 2009). Little information is available on the efficiency of chelate and mineral forms of microelements, especially copper and manganese, in cultivation of tomato on rockwool slabs with nutrient solution continuously supplied to the rhizosphere.

The aim of the present study was to evaluate whether it is necessary to use chelated forms of copper and manganese. Another objective was to assess the suitability of two chelating agents of iron for cultivation of greenhouse tomato on rockwool medium.

MATERIAL AND METHODS

Two greenhouse experiments with tomato cv. Cunero F_1 were conducted in 2002-2005 at the Horticultural Experimental Station of the Wrocław University of Environmental and Life Sciences.

In the first experiment, established in 2002-2003, the following forms of manganese and copper were evaluated:

1) mineral forms of manganese ($MnSO_4$ H_2O) and copper ($CuSO_4$ $5H_2O$),
2) EDTA+DTPA chelate of manganese and mineral copper ($CuSO_4$ $5H_2O$),
3) mineral form of manganese ($MnSO_4$ H_2O) and EDTA+DTPA chelate of copper.

In both years, well-developed transplants were placed on rockwool slabs (100x20x7.5 cm in size; Grodan BV, Master type) in the first third of March. Plants were trained to one stem and tied up with a string as required in the high wire system for a long growing cycle. During the whole growing period, until the end of October, tomato plants were supplied the same nutrient solutions with the following concentration of nutrients (in mg dm^{-3}): $N\text{-}NO_3$–225, P–6, K–394, Ca–160, Mg–90, Fe–1.40, Mn–0.80, B–0.45, Zn–0.48, Cu–0.08, Mo–0.08, pH 5.50, EC-3.20 mmhos cm^{-1} (Breś et al. 2003). The solutions differed only in forms of manganese and copper.

The second experiment was conducted to evaluate the suitability of DTPA (Librel FeDP7 by Tuhuamij BV) and EDTA (Pionier Fe13 by Intermag Ltd.) as chelating agents for the source of iron in nutrition of tomato plants grown on rockwool. The date of planting, plant management and composition of nutrient solution were similar to those in the first experiment.

Both experiments were established in a randomized block design with 4 replications. A single plot contained 8 plants grown on 2 slabs, in the density of 2.7 plants per m^{-2}. Fully ripe tomato fruits were harvested twice a week. The yield was recorded, divided into total and marketable yield of fruits > 3.5 cm in diameter. In addition, early yield, harvested within the first 35 days of yielding, was distinguished. On three occasions during the first experiment, namely in the third decade of June, July and August, and four times during the second experiment, that is at one-month intervals from May to August, samples of index parts of tomato plants were taken for chemical analysis. The index parts of tomato plants were represented by the 8th and 9th leaves from the plant's top. Fifteen leaves from each plot were collected. The plant material was digested with the microwave method and the content of iron, manganese, copper and zinc were determined by the ASA method. The results were subjected to statistical analysis in a one-factorial design, and the least significant differences were calculated by the Tukey's test at significance level α=0.05.

RESULTS

The research results did not prove any significant effect of the examined copper and manganese chelate fertilizers on total yields of tomato fruits. The situation looked different in the case of marketable yields, which appeared to be much lower when copper chelate fertilizer was applied as a source of that nutrient (Table 1). The yields in the remaining fertilization treatments did not significantly differ one from another. The application of Cu and Mn chelate fertilizers had a positive effect on the earliness of tomato fruiting. The early yield from plants fertigated with nutrient solutions containing copper and manganese chelates was considerably higher as compared to that obtained by using mineral forms of these nutrients.

The status of tomato plants' nutrition with iron was not dependent on the application of copper and manganese chelates in the nutrient solution and the mean values were only slightly different, within the range of 112.7--113.4 mg Fe kg^{-1}. In June and in August, the iron content in the index parts of tomato plants was similar, being significantly lower in July.

The results shown in Table 2 indicate that manganese used in the nutrient solution as a chelate caused a large increase in the concentration of this microelement in tomato leaves in each term of analysis compared to its

Table 1

The effect of chelates and mineral forms of manganese and copper on yield of greenhouse tomato cultivar Cunero F_1 (kg plant^{-1})

Fertilizer treatments	Yield		
	total	marketable	early
Mn+Cu mineral forms	9.80	8.50	2.75
Mn-chelate + Cu-mineral form	9.98	8.53	2.95
Cu-chelate + Mn-mineral form	9.66	8.11	3.00
Mean	9.81	8.83	2.90
LSD$_{\alpha\text{-}0.05}$	n.s.*	0.32	0.11

*n.s. – differences not significant

Table 2

The effect of chelates and mineral forms of manganese and copper on the content of manganese and iron in index parts of greenhouse tomato cultivar Cunero F_1 (mg kg^{-1})

Fertilizer treatments	Iron				Manganese			
	terms of analysis				terms of analysis			
	June	July	August	mean	June	July	August	mean
Mn+Cu mineral forms	116.9	107.0	116.1	113.3	209.7	198.2	193.2	200.3
Mn-chelate + Cu-mineral form	124.7	97.1	116.2	112.7	235.6	215.9	204.8	218.7
Cu-chelate + Mn-mineral form	114.8	104.4	121.1	113.4	203.1	195.3	196.9	198.4
Mean	118.8	102.8	117.8	113.1	216.1	203.1	198.3	205.8
LSD$_{\alpha-0.05}$ for: term of analysis				1.0				1.7
forms of microelement				n.s.				1.8
interaction				2.9				3.5

n.s. – non-significant differences

mineral form. It is worth noticing that in the subsequent terms of analysis, the Mn content in leaves tended to decrease, from 216.1 to 198.3 mg Mn kg^{-1}.

On each date of analysis and also as an average value for the whole period of analysis, the copper concentration in the index parts of tomato supplied with Cu-chelate was significantly lower than in the treatment where the mineral form of this nutrient was applied (Table 3). However, the copper chelate improved the uptake of zinc by plants. This effect became distinctly high on the last two dates of sampling, that is in July and August. The manganese chelate did not cause any significant differences in the uptake of zinc compared to its mineral form.

The data from experiment II did not prove any significant influence of the examined fertilizers containing iron complexed by different chelators

Table 3

The effect of chelates and mineral forms of manganese and copper on the content of copper and zinc in index part of greenhouse tomato cultivar Cunero F_1 (mg kg^{-1})

Fertilizer treatments	Iron				Manganese			
	terms of analysis				terms of analysis			
	June	July	August	mean	June	July	August	mean
Mn+Cu mineral forms	9.85	9.65	11.95	10.48	28.95	25.90	24.15	26.33
Mn-chelate + Cu-mineral form	9.40	9.40	11.30	10.03	29.55	26.95	22.80	26.43
Cu-chelate + Mn-mineral form	9.40	8.35	10.05	9.27	29.15	35.45	32.05	32.22
Mean	9.55	9.13	11.10	9.93	29.22	29.43	26.33	28.33
LSD$_{\alpha-0.05}$ for: term of analysis forms of microelement interaction				0.15 0.20 0.30				0.55 0.60 1.15

n.s. – non-significant differences

(EDTA and DTPA) on tomato total, marketable and early yield of fruits (Table 4).

However, the investigated Fe chelates significantly affected the tomato's nutritional status regarding iron (Table 5). The highest mean iron content in leaves, calculated for the two years of research and four terms of analyses, was determined in plants fertilized with the DTPA chelate, where it ranged around 149.9 mg Fe kg^{-1} d.m. of leaves. A markedly lower mean value of the iron content was found when the element was supplied by the EDTA chelate (141.7 mg Fe kg^{-1}). The dynamics of iron uptake and its consequent content in the index parts of tomato were characterized by a high diversity depending on the type of chelate and term of analysis. In the first term of analysis, in May, the mean iron concentration was 152.7 mg Fe kg^{-1},

Table 4

The effect of iron chelates on yield of greenhouse tomato cultivar Cunero F_1 (kg plant^{-1})

Type of chelate	Yield		
	total	marketable	early
DTPA-Fe	9.06	8.67	3.17
EDTA-Fe	9.13	8.73	3.46
Mean	9.10	8.70	3.32
LSD$_{\alpha\text{-}0.05}$	n.s.	n.s.	n.s.

Table 5

Effect of iron chelates on iron content in the index parts of greenhouse tomato (mg Fe kg^{-1} d.m.)

Type of chelate	Terms of analysis				Mean
	May	June	July	August	
DTPA-Fe	118.1	140.1	205.3	136.1	149.9
EDTA-Fe	187.2	121.5	118.9	139.3	141.7
Mean	152.7	130.8	162.1	137.7	145.8
LSD$_{\alpha\text{-}0.05}$ for: term of analysis					2.0
kind of chelates					2.5
interaction					4.3

decreasing to 130.8 mg Fe kg^{-1} d.m. on the following term, but peaking in July to its highest level 162.1 mg Fe kg^{-1} d.m. In the last term, in August, a significant decrease in the mean value of the iron content was recorded, down to 137.7 mg Fe kg^{-1} d.m.

The iron chelates evaluated in the study considerably modified the tomato's nutrition with manganese (Table 6). A higher mean content of manganese (249.9 mg Mn kg^{-1}) d.m. was detected in leaves of plants cultivated with the use of DTPA chelating agent, while a significantly lower mean value of this nutrient was determined in plants fertilized with EDTA (237.7 mg Mn kg^{-1} d.m.). While analyzing the dynamics of the manganese uptake through the plant growing period, it can be noticed that from May to June its content in leaves rapidly increased, on average from 135.6 to 285.9 mg Mn kg^{-1} d.m. and maintained on the level of 242.0 and 311.8 mg Mn kg^{-1} d.m. in July and August, respectively.

Table 6

Effect of iron chelates on the manganese content in the index parts of greenhouse tomato (mg Mn kg^{-1} d.m.)

Type of chelate	Terms of analysis				Mean
	May	June	July	August	
DTPA-Fe	146.1	270.2	244.5	338.9	249.9
EDTA-Fe	125.1	301.5	239.4	284.6	237.7
Mean	135.6	285.9	242.0	311.8	243.8
LSD$_{\alpha\text{-}0.05}$ for: term of analysis					2.8
kind of chelates					3.2
interaction					5.6

The nutritional status of tomatoes with copper was less dependent on the type of iron chelate than in the case of manganese and zinc (Table 7). Plants fertilized with the nutrient solution combined with the EDTA chelate contained significantly less copper (9.16 mg Cu kg^{-1} d.m.) than those fertilized with the nutrient solution with the DTPA chelate (9.36 mg Cu kg^{-1} d.m). The mean value of the copper content in May equaled 9.48 mg Cu kg^{-1}, and decreased to 8.35 mg Cu kg^{-1} d.m in June and 8.67 mg Cu kg^{-1} d.m in July, while increased up to 10.72 mg Cu kg^{-1} in August. The mean value of the copper content calculated for both years and all terms of analyses reached 9.30 mg Cu kg^{-1} d.m, which is similar to the data reported by other researchers.

Table 7

Effect of iron chelates on the copper content in the index parts of greenhouse tomato (mg Cu kg^{-1} d.m.)

Type of chelate	Terms of analysis				Mean
	May	June	July	August	
DTPA-Fe	10.07	8.45	8.48	10.78	9.45
EDTA-Fe	8.88	8.25	8.85	10.65	9.16
Mean	9.48	8.35	8.67	10.72	9.30
$LSD_{\alpha\text{-}0.05}$ for: term of analysis					0.10
kind of chelates					0.12
interaction					0.21

The highest mean content of zinc in leaves (46.3 mg Zn kg^{-1}) was detected in plants fertilized with iron supplied by the EDTA chelate, while distinctly less zinc appeared in leaves of plants nourished with DTPA (36.4 mg Zn kg^{-1} d.m.). In the course of the growing period, the content of zinc became significantly diversified in subsequent terms of analysis. The lowest mean content of zinc (35.0 mg Zn kg^{-1} d.m.) was observed in May (Table 8), while in the other terms of growing period varied between 46.2 mg Zn kg^{-1} d.m in June and 41.9-42.5 mg Zn kg^{-1} d.m. in July and August. During all terms of analysis, except May, plants supplied with nutrient solution with EDTA-Fe chelate, contained substantially higher quantities of zinc in the index part of leaves.

Table 8

Effect of iron chelates on the zinc content in the index parts of greenhouse tomato (mg Zn kg^{-1} d.m.)

Type of chelate	Terms of analysis				Mean
	May	June	July	August	
DTPA-Fe	36.6	38.7	35.4	35.0	36.4
EDTA-Fe	33.3	53.7	48.3	49.9	46.3
Mean	35.0	46.2	41.9	42.5	41.4
LSD$_{\alpha\text{-}0.05}$ for: term of analysis					0.5
kind of chelates					0.6
interaction					1.1

DISCUSSION

Yields of tomato Cunero F$_1$ cultivar obtained in our greenhouse experiments were quite similar to the ones reported from other trials (Nurzyński 2004, 2006, Pawlińska, Komosa 2004, Jarosz, Dzida 2011). The application of mineral forms of Mn and Cu appeared to be as efficient as the use of DTPA chelates of these nutrients with respect to the total yield of fruits. However, the Cu chelate was less effective in stimulating marketable yield, while both tested chelates caused accelerated maturation of fruits and consequently higher early yields of tomatoes. In a trial conducted by Kozik et al. (2012), the yield of greenhouse lettuce grown on peat substrate was unaffected by the source of micronutrients applied in lower doses, but mineral forms of Mn, Cu and Zn produced better effects than their EDTA and DTPA chelates when supplied in higher amounts to a growing medium. The good results achieved by mineral forms of micronutrients in a nutrient solution may be attributed to creating favorable conditions for their uptake and preventing immobilization. Likewise, in an experiment conducted by Komosa et al. (2002), the source of Fe in a nutrient solution did not affect the yield of tomato fruits. No differences in the growth and final weight of marigold biomass supplied with Fe-EDTA and Fe-DTPA sources of this nutrient were observed by Albano and Merhaut (2012).

The iron content in plants is generally between 50 and 200 Fe kg^{-1} d.m. (Sonneveld, Voogt 1985), and the recommended level for tomatoes should exceed 60.0 mg Fe kg^{-1} d.m. of leaves. The amounts of Fe in our study, which varied from 97.1 to the maximum 205.3 mg Fe kg^{-1} d.m., justify the conclusion that irrespective of the type of iron chelate and source of Mn and Cu in a nutrient solution all tomato plants were properly fed with this nutrient. Similar levels of iron in the tomato's index part were obtained by Chohura and Komosa (2003) Komosa et al. (2002), and Dyśko et al. (2009).

Numerous research data have proven that both deficiency and excess of manganese can be harmful to tomatoes (Shenker et al. 2004). Seresinhe (1996) reported that an optimum manganese content in fully developed tomato leaves ranges from 50 to 500 mg Mn kg^{-1} d.m. Even a wider range of its content, within 25-1000 mg Mn kg^{-1} d.m., was proposed by Horst (1988). Shenker et al. (2004) determined that at a concentration of 16.8 mg Mn kg^{-1} d.m, the chlorophyll content in tomato leaves was not markedly lower than at 207.4 mg Mn kg^{-1} d.m. which appeared to be phytotoxic. Le Bot et al. (1990b) reported that 250 mg Mn kg^{-1} d.m is the upper safe limit for the manganese content in tomato leaves. Other researchers have pointed out that the toxic effect of this component can be reduced by maintaining an appropriate manganese: magnesium ratio (Horst 1988, Le Bot et al. 1990a). In our trials, the amounts of this element were within 193.2-235.6 mg Mn kg^{-1} d.m. in experiment I and 125.1 - 338.9 mg Mn kg^{-1}d.m. in experiment II. The data indicate a good supply of the tomato plants with Mn, irrespective of the source of this nutrient and type of Fe chelating agent. A similar level of Mn was also determined by Chohura and Komosa (2003). High quantities of manganese usually induce a reduced uptake of iron, because these two ions compete for proteins transporting them through the plasma membrane (Korshunova et al. 1999, Gunes et al. 1998). Such relationships have become apparent in our research, namely an increase in the manganese content in tomato leaves was usually accompanied by a decrease in the iron concentration.

The copper content in the tomato's index parts was less varied than that of the remaining microelements. In the first experiment, the content of this nutrients did not exceed the range of 8.35-11.95 mg Cu kg^{-1} d.m., with the mean value for all analyses determined at 9.93 mg Cu kg^{-1} d.m. In the second experiment, the mean concentration of copper in tomato leaves equaled 9.30 mg Cu kg^{-1} d.m., and the results of analyses in particular terms were between 8.25 and 10.78 mg Cu kg^{-1} d.m. Such small variation could be have been caused by the retention of copper ions in root tissues. Chaignon et al. (2002) concluded that when this is the case, less copper is transported through the xylem so that the variation in the copper content in leaves is much smaller. The mean copper content determined in our research was similar to the data presented by Chohura and Komosa (2003), while being higher than reported by Ginocchio et al. (2002), who assumed that an optimum level of this nutrient is above 4.0 Cu kg^{-1} d.m. of tomato leaves.

The mean content of zinc, ranging around 28.3 mg Zn kg^{-1} d.m in the first experiment and 41.4 mg Zn kg^{-1} d.m in the second one, was on a level of optimal tomato nutrition determined by Gunes et al. (1998). The same authors claimed that the zinc content in tomato leaves may be found within a wide range from 25 to 250 Zn kg^{-1} d.m. KAYA and HIGS (2001) report that a minimum content of this component for tomato equals 25 Zn kg^{-1} d.m. KAYA and HIGGS (2002) recorded visible symptoms of zinc deficiency such as curled leaves and shortened internodes at the concentration of 23.0 Zn kg^{-1} d.m. In our first experiment, the Zn content was in the range of 22.8-35.45 Zn kg^{-1} d.m. Those values were close to the lower limit, but no symptoms of ill health of plant could be observed. In the second experiment, the range of zinc content in leaves was considerably wider: from 33.3 to 53.7 Zn kg^{-1} d.m. A similar content of zinc in index parts was determined by CHOHURA and KOMOSA (2003). Higher quantities of this element in leaves of tomato cultivated in rockwool were reported by PAWLIŃSKA and KOMOSA (2002, 2004).

Recapitulating, with the output from our experiments, we can conclude that the use of Mn chelate brought about better results while Cu-chelate had an adverse effect on the uptake of these nutrients by greenhouse tomato in rockwool cultivation compared to mineral forms of manganese and copper. The only beneficial impact of these forms of Mn and Cu was a bigger early yield of fruits. In an assessment of different aspects of using Mn and Cu chelates in practice it is also necessary to consider that they are more expensive then mineral forms.

CONCLUSIONS

1. The application of chelate forms of copper and manganese did not influence the total yield of tomato fruits, but it contributed to considerable increase in a very early yield if compared to mineral forms of these nutrients.
2. Manganese used as a chelate and copper in the mineral form significantly improved the nutritional status of plants by Mn and Cu, respectively.
3. The application of iron complexed with different chelating agents did not cause any significant variation in tomato marketable as well as early yield of fruits.
4. The use of DTPA as an Fe source contributed to a better status of tomato nutrition with iron, manganese and copper relative to the use of EDTA Fe.

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ANALYSIS OF CHOSEN MACRONUTRIENTS AND VITAMIN D IN DAILY FOOD RATIONS OF ELDERLY MEN AND WOMEN IN THE CONTEXT OF CALCIUM-PHOSPHORUS HOMEOSTASIS

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Abstract

The loss of bone mineral components is an obvious and progressing process in elderly men and women. The rate of the bone metabolic turnover is associated with the nutrition and environmental conditions. Vitamins and minerals in a diet are indispensable for both prevention and treatment of diseases. The aim of the present study was to evaluate vitamin D, calcium (Ca) and phosphorus (P) content and to compare Ca-P homeostasis in daily dietary intake among the elderly members of a population-based study in Kołobrzeg.

The examined population comprised a group of 479 randomly chosen people aged > 51 years, including 316 women and 163 men. The eating habits of the respondents were evaluated in 2009. The information on individual food consumption was obtained by dietary history interviews (3 x 24-hour dietary recall).

The research proved that the vitamin D and Ca supply with a daily diet was extremely low, in contrast to the P content, which significantly exceeded the recommended daily intake for this age group. Daily food rations of both women and men were not properly balanced in terms of Ca and P and vitamin D. It was revealed that the vitamin D supply in the daily dietary intake of the elderly persons was very low, covering from 22 to 40% of the Adequate Intake (AI). Vitamin D is necessary for maintaining an appropriate calcium and phosphorus homeostasis in the body and ensuring that many tissues, organs and cells function properly. A diet rich in vitamin D and Ca has been identified as playing a potential role in the prevention of bone diseases.

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A change in dietary habits can be an important factor in reducing the incidence of chronic diseases, particularly an elevated fracture risk in osteoporosis.

Key words: calcium-phosphorus homeostasis, vitamin D, daily food rations.

ANALIZA WYBRANYCH SKŁADNIKÓW MINERALNYCH I WITAMINY D W CAŁODZIENNYCH RACJACH POKARMOWYCH OSÓB STARSZYCH W ASPEKCIE GOSPODARKI WAPNIOWO-FOSFORANOWEJ

Abstrakt

Utrata masy kostnej u ludzi starszych jest zjawiskiem naturalnym i postępującym zarówno u kobiet, jak i mężczyzn. Tempo obrotu metabolicznego tkanki kostnej zależne jest od sposobu odżywiania i uwarunkowań środowiskowych. Odpowiednia zawartość witamin i składników mineralnych w racjach pokarmowych oraz proporcje między nimi są istotne w prewencji i terapii żywieniowej. Celem pracy była analiza podaży witaminy D, wapnia i fosforu w całodziennych racjach pokarmowych osób starszych oraz ocena homeostazy wapniowo-fosforanowej.

Badania przeprowadzono w 2009 r., w Kołobrzegu, metodą sondażu diagnostycznego, stosując kwestionariusz wywiadu (na podstawie trzech wywiadów 24-godzinnych) wśród losowo wybranych 479 osób (316 kobiet i 163 mężczyzn) powyżej 51. roku życia.

W analizie ilościowej badanych racji pokarmowych stwierdzeno, iż posiłki badanych kobiet i mężczyzn były nieprawidłowo skomponowane; wykazano niedobory witaminy D i wapnia, a jednocześnie nadmierną zawartość fosforu w stosunku do rekomendacji żywieniowych oraz niewłaściwe proporcje między analizowanymi składnikami diety. Zawartość witaminy D w analizowanych racjach pokarmowych kobiet i mężczyzn była bardzo niska, pokrywała zapotrzebowanie badanych od 22 do 40% określonego zapotrzebowania na poziomie wystarczającego spożycia (AI). Witamina D jest niezbędnym elementem do utrzymania homeostazy wapniowo-fosforanowej w organizmie i zapewnia właściwe funkcjonowanie tkanek, narządów i komórek.

Zmiana zwyczajów żywieniowych badanych osób starszych może obniżyć ryzyko rozwoju przewlekłych chorób niezakaźnych, w tym złamań kości w osteoporozie.

Słowa kluczowe: homeostaza wapniowo-fosforanowa, witamina D, dzienne racje pokarmowe.

INTRODUCTION

Nutritional deficiencies of the ageing population are attributed to chronic diseases, inadequate nutrition, reduced absorbability as well as inefficient use of nutrients. The loss of bone mineral constituents is an obvious and progressing process in elderly men and women. It is understood that the bone mass accumulates in the first two decades of human life, afterwards remaining stable until approximately 40 years of age. The rate of the bone metabolic turnover is associated with the nutrition and environmental conditions. Nutrition should be among bone disease prevention strategies, especially as the human population is ageing and a diet produces an important effect on bone health. Bone is the major store of both phosphorus and calcium (ANDERSEN et al. 2005, BONJOUR et al. 2009, PEACOCK 2010). There are some nutrients that are essential for attaining good bone tissue formation and maintaining its optimal metabolism, e.g. Ca, P, Mg, Zn, F as well as vitamins D, A, K, C and protein (TANG et al. 2007, ZHU, PRINCE 2012).

Calcium is an essential element that is available to the body only through dietary sources. An adequate Ca supply in a diet appears to be the main condition for keeping the right level of bone mass and slowing down its decrease (ZHU, PRINCE 2012). Possible reasons for Ca deficiency in an organism may be its insufficient supply, reduced absorption from the alimentary tract and excessive excretion with urine. Calcium is deposited in bones in the form of calcium-phosphate salt. Phosphorus is essential for the normal bone formation and therefore plays a very important role during the skeletal development. However, there are much larger stores of phosphorus than calcium in soft tissues, reflecting the central role of phosphorus in energy metabolism, intracellular signaling and cell structure (PEACOCK 2010). Phosphorus is ubiquitous in almost all foods. Although P deficiency was never a real problem in human nutrition, a surplus of phosphorus in the diet was more of the concern.

Vitamin D, parathormone (PTH), calcitonin and sex hormones play a principal role in maintaining calcium and phosphorus homeostasis and the balance between bone creation and bone resorption. Vitamin D is necessary to keep the right calcium and phosphorus homeostasis in the body and also to ensure that many tissues, organs and cells, unrelated to mineral economy, function properly (HOLIK 2006, 2007, HINES et al. 2010, CHRISTAKOS et al. 2011).

The aim of the study was to evaluate the content of vitamin D as well as Ca and P and to compare the status and calcium-phosphorus homeostasis in daily dietary intake for the elderly by a population-based study in Kołobrzeg.

MATERIALS AND METHODS

The study was conducted in 2009, in the Rehabilitation and Recreation Center in Kołobrzeg, Poland. The participants were healthy adults aged >51 year. The examined group comprised 479 persons (316 women and 163 men) who were selected randomly from 1200 people. Three different age groups were distinguished for men and women: age 51-65; 66-75 and > 75 years. Each of the participants was individually inquired about the diet from 3 days, according to recommendation by the Food and Nutrition Institute in Warsaw.

The serving size was established based on products and portions reported by the respondents. The energy value and contents of nutrients in daily food intake were appraised with the aid of a computer program based on the current tables of values of nutritional products worked out at the Food and Nutrition Institute in Warsaw. The computer program takes into account the loss of nutritional value during food processing technological processes versus raw products. The assessment of the participants' eating habits was carried out using Polish recommendations (BUŁHAK-JACHYMCZYK 2008, WOJTASIK, BUŁHAK-JACHYMCZYK 2008).

An Excel 2010 calculation sheet was used to process the results, while the Polish version of Statistica 10.0 for Windows (StastSoft® Krakow, Polska) was applied to submit them to statistical evaluation. Statistical significance was determined by the Mann-Whitney U test for comparison of quantitative data between two groups. The Kruskal-Wallis test was applied to comparative analysis of more than two groups. The level of statistical significance was set at $p < 0.05$ and the Spearman's correlation coefficient for nonparametric variables distribution was used.

RESULTS AND DISCUSSION

The examined population of elderly subjects, who did not take any dietary supplements, was divided into different age groups. It was revealed that the levels of the basic nutrients in the tested cohort's rations had been not well-balanced (Table 1). Differences in the energy delivered with food within the tested groups were noticed. The results indicate that the group of men <75 y received less energy with daily dietary intake than required, while seniors >75 y were provided with more energy with food than needed. Irrespectively of the age range, energy values found for elderly women always exceeded the required levels. The protein content exceeded the recommended quantity limits for all the age groups of both men and women. Statistically significant ($p = 0.0004$) differences appeared in the consumed proteins within the examined age groups.

It was established that the energy intake from eaten fat was too high, exceeding 35% among all the respondents, while the percentage contribution of carbohydrates to energy intake was on the lowest recommended level. Actually, the average level of carbohydrates consumed in g d^{-1} was found to be on an acceptable level among the participants of the survey. However, the ratio between digestible carbohydrates and fats was inappropriate compared to the energy supply.

Statistically significant differences in the calcium and phosphorus intake within all examined population divided by gender were established by the Man Whitney U test, $p = 0.002$ for Ca and $p = 0.02$ for P (Figure 1).

Table 1

Distributions of dietary energy and macronutrients intake per day (means ± SD and range) by different age and gender groups (women-W, men-M)

Macronutrient intake per day	W 51-65 y (*n* = 155)	M 51-65 y (*n* = 51)	W 66 -75 y (*n* = 133)	M 66 -75 y (*n* = 85)	W >75 y (*n* = 28(	M >75 y (*n* = 27)
Total energy (kcal)	2104 ± 449 (831-3150	2341 ± 394 (1505-2859)	2201 ± 562 (870-4174)	2033 ± 455 (1254-3006)	2252 ± 736 (928-3980)	2382 ± 410 (1666-2751)
Total protein (g)	92.1 ± 23.4 (37.5-172.4)	89.2 ± 21.9 (52.2-115.0)	86.6 ± 20.4 (42.2-136.1)	85.3 ± 16.4 (54.5-112.8)	91.3 ± 2 3.2 (42.0-132.3)	96.3 ± 20.5 (53.0-120.9)
Animal protein (g)	66.2 ± 22.8 (23.3-144.2)	59.0 ± 19.3 (21.7-81.9)	58.8 ± 16.8 (22.2-95.4)	58.9 ± 13.8 (34.3-87.3)	62.8 ± 19.1 (29.7-110.1)	67.3 ± 17.9 (35.5-89.3)
Fat (g)	88.4 ± 24.1 (35.5-158.1)	94.0 ± 19.0 (56.3-117.6)	87.2 ± 29.3 (27.3-177.4)	77.6 ± 15.8 (38.1-112.2)	87.9 ± 28.4 (41.5-139.9)	89.6 ± 16.5 (69.2-116.1)
Carbohydrates (g)	253.3 ± 75.1 (32.4-454.1)	305.1 ± 69.2 (164.4-411.7)	286.9 ± 77.2 (118.5-551.1)	270.5 ± 82.1 (152.6-469.4)	298.1 ± 121.3 (99.1-603.5)	314.2 ± 63.2 (168.8-372.2)
Energy from protein (%)	12.6 ± 3.5 (5.0-21.8)	10.1 ± 2.9 (3.9-12.7)	10.9 ± 2.8 (5.4-23.2)	11.9 ± 2.9 (7.2-19.0)	11.7 ± 3.0 (6.1-17.6)	11.3 ± 2.5 (6.9-14.5)
Energy from fat (%)	37.9 ± 6.0 (23.7-52.6)	36.2 ± 4.9 (30.7-47.1)	35.8 ± 6.1 (19.8-53.4)	34.8 ± 4.8 (26.5-45.1)	35.5 ± 5.7 (22.4-44.0)	34.3 ± 5.4 (26.2-44.5)
Energy from carbohydrates (%)	49.5 ± 8.1 (28.2-66.1)	53.7 ± 4.7 (45.9-62.1)	53.3 ± 7.0 (37.9-74.8)	53.3 ± 6.2 (42.6-63.9)	52.9 ± 7.8 (40.8-68.6)	54.3 ± 4.0 (45.8-60.8)

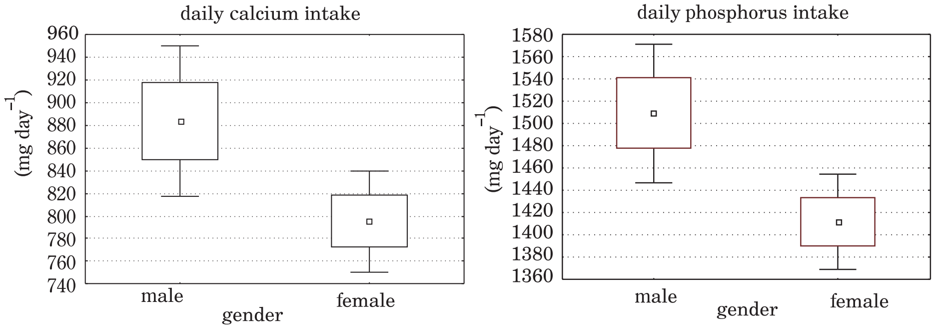

Fig. 1. Comparison of daily calcium and phosphorus intake by the examined males and females (Man-Whitney U test, $p = 0.002$ for Ca and $p = 0.02$ for P)

The average Ca, P and vitamin D concentrations in the daily food portions of the interviewees are presented in Tables 2 and 3. The RDIs for Ca, P and vitamin D are different in different countries and regions. There is no consensus in Europe, e.g. in Poland RDIs are different for adults, post-menopausal women and the elderly (Who/Fao 2004). It is claimed that while quite a number of people between the age of 60 up to 75 are still active physically, socially and professionally, most of those over 75 years of age have worse present worse life quality indicators.

The updated standards for calcium intake have been agreed on a level of sufficient consumption; for adults over 50 years old, 1300 mg of calcium per day is recommended (Wojtasik, Bułhak-Jachymczyk 2008). The average content of calcium has been determined from 786±350 to 809±488 mg in women's daily dietary intake, irrespective of their age, which enables them to achieve a sufficient calcium intake level from 60 to 62% of the standard (Table 2). Markiewicz and co-workers have obtained similar outcome when analyzing the calcium content in daily food rations of elderly people from Podlasie, revealing calcium deficiency of the diets (Markiewicz et al. 2009). It appears that men's daily food rations contained from 853±225 to 883±213 mg of calcium, which corresponds to 66- 68% of the level considered as a sufficient calcium level intake. The insufficient calcium intake in daily food rations among the examined elderly population is a disturbing finding (Andersen et al. 2005, Tang et al. 2007, Bronkowska et al. 2010). A much lower calcium intake among women than men has been ascertained in our study; moreover, the most severe calcium deficiency (786±350 mg d^{-1}) has been found within the 66-75 y age group. We have shown that calcium deficiency in a daily food ration of the subjects does not depend on the gender. Ca deficiency is a common problem, especially among elderly women. There has been an ongoing process of decreasing the estrogen level among the tested women. This causes a growing risk of a bone mass decrease within aging and the risk of osteoporosis can only be reduced by sufficient Ca (Tang

Table 2

Intake of the analyzed nutrients per day (means ± SD and range) and percent of the RDI and AI realization in women's age groups

Women (years)	Ca (mg)	AI (%)	P (mg)	RDI (%)	Ca:P	Vitamin D (µg)	AI (%)	*r* Ca - P	*r* Ca - Vit. D
51 -65	809 ± 488 (977-4559) *M*- 733	1300 mg 62%	1421 ± 488 (761-3128) *M*- 1467	700 mg 245%	1.8	4.0 ± 2.8 (1.1-11.3) *M*- 2.9	10 µg 40%	0.823	-0.045
66 -75	786 ± 350 (172-2105) *M*- 764	1300 mg 60%	1419 ± 343 (732-2263) *M*- 1365	700 mg 245%	1.8	4.4 ± 4.0 (1.1- 22.1) *M*- 2.5	15 µg 29%	0.775	0.278
>75	804 ± 357 (236-1747) *M*-802	1300 mg 62%	1617 ± 445 (954-2680) *M*- 1564	700 mg 279%	2.0	4.0 ± 3.1 (1.7 - 11.0) *M*- 2.5	15 µg 27%	0.909	0.490

r – Spearman correlation coefficient for nonparametric variables distribution, *M* – median

Table 3

Intake of the analyzed nutrients per day (means ± SD and range) and percent of the RDI and AI realization in men's age groups

Men (years)	Ca (mg)	AI (%)	P (mg)	RDI (%)	Ca:P	Vitamin D (µg)	AI (%)	*r* Ca - P	*r* Ca - Vit. D
51 -65	859 ± 269 (481-1206) *M*- 756	1300 mg 66%	1426 ± 328 (817-1877) *M*- 1643	700 mg 245%	1.7	2.7 ± 1.2 (1.0 - 4.8) *M*- 2.8	10 µg 27%	0.705	0.199
66 -75	883 ± 213 (579-1504) *M*- 867	1300 mg 68%	1354 ± 237 (929-1815) *M*- 1470	700 mg 234%	1.5	3.6 ± 3.0 (1.0 - 11.2) *M*- 2.4	15 µg 24%	0.731	0.259
>75	853 ± 225 (401-1223) *M*- 822	1300 mg 66%	1363 ± 206 (909-1536) *M*- 1491	700 mg 235%	1.6	3.3 ± 2.3 (1.7 - 9.0) *M*- 2.5	15 µg 22%	-0.024	-0.190

r – Spearman correlation coefficient for nonparametric variables distribution, M – median et al. 2007, Stransky, Rysava 2009, Peacoc 2010). There are several vital roles that Ca plays in the human body apart from building the skeletal system. For example, calcium is essential in sustaining proper cardiac functions. It also participates in metabolic processes, nerve impulse conduction, muscle contraction, etc. (Velicer 2008, Peacoc 2010). Despite positive changes in the eating habits that have recently been occurring in Poland, Poles often eat diets which do not satisfy the nutritional recommendations. Apparently, the level of calcium intake in Poland is low, reaching 598 mg d^{-1} (Wojtasik, Bułhak-Jachymczyk 2008).

The results of the daily dietary intake analysis carried out among all the men's and women's age groups showed higher than required phosphorus content, i.e. 245-273% for women and 234-273% for men. The systemic phosphoric metabolism together with the calcium metabolism are the main factors responsible for the maintenance of calcium/phosphate homeostasis. An optimum Ca:P ratio in a daily dietary intake is 1:1, but after converting into the gravimetric unit index it equals 1.3 (Bonjour et al. 2009). The average Ca:P ratio (presented in grams) within the analyzed diets of different women's age groups was 1.8 within the age group of 51 to 65 y, 1.8 from 66 to 75 y of age and 2.0 among the most senior group. The respective results for men were: 1.7, 1.5 and 1.6. Comparing the content of phosphorus to calcium in the respondents' food rations, we obserevd simultaneous growth trends in all diets except the diets of men aged > 75 y.

Presence of phosphorus in such food products as cheese, poultry, fish and cereal is responsible for an unstable balance between calcium and phosphorus in a human organism. This is due to the fact that phosphates and polyphosphates are added to food products. Chronic hyperphosphatemia might lead to stimulation of bone tissue resorption (Bonjour et al 2009, Stransky, Rysava 2009, Zhu, Prince 2012). The reason for hyperparathyroidism, which is manifested by higher than normal PTH secretion. might be the calcium/ phosphorus low ratio in a diet. Moreover, vitamin D plays an essential role in calcium and phosphorus metabolism. Without vitamin D, only 10-15% dietary calcium and about 60% of phosphorus are absorbed (Holik 2006). The relationship between Ca and vitamin D in daily intake rations was investigated. Within most of the examined groups, the correlation between Ca and vitamin D is very weak or nonexistent.

The standards proposed in Poland are adapted to the ones by FAO/WHO and expressed as an Adequate Intake (AI) of vitamin D. The organism's requirement for vitamin D can vary and a daily dose ranges from 10 to 15 μg of vitamin D per se according to the age (Bułhak-Jachymczyk). The vitamin D supply including daily dietary intake among the examined elderly persons was very low. The lowest and the highest levels in the women's daily dietary intake were 4.0±2.8 and 4.4±4.0 μg d^{-1}, respectively. Among the men, they ranged from 2.7±1.2 to 3.6±3.0 μg d^{-1}. In all of the men's and women's diets, the required supply of vitamin D was covered from 22 to

40% of the AI levels. Vitamin D deficiency in elderly population may be a cause of bone mineralization disorders and muscle tone decrease, which raise the risk of falls. The reduced dermal synthesis and insufficient quantity of vitamin D delivered in a diet are presumably the reason for a vitamin D shortage in elderly population (Andersen et al. 2005, Holik 2007).

The results of studies have shown relatively low vitamin D intake levels in Poland and in other European countries, like Denmark and Ireland, reaching no more 3.8 µg day^{-1} (Andersen et al. 2005, Bonjour et al. 2009).

The research carried out among the elderly population proved that the vitamin D and calcium supply including daily dietary intake was extremely low. In contrast, the dietary phosphorus content exceeded the recommended daily intake for this age group by 234 to 279%. Results of studies across the world (Andersen et al. 2005, Holik 2007, Tang et al. 2007, Velicer 2008, Kaviani et al. 2012) show insufficient calcium and vitamin D levels among elderly people.

CONCLUSIONS

Particular attention must be drawn to educational programs for elderly people and the need of calcium and vitamin D supplementation in a diet in order to prevent negative effects of malnutrition. In conclusion to the above data, in order to restore the lost metabolic stability in senior people it will be necessary to correct their lifestyles, including changes in the nutritional habits, which should be accompanied by intensive educational efforts regarding nutrition. Low calcium and vitamin D levels along with an excessive phosphorus content in the analyzed diets might be the reason for developing health disorders related to the lack of calcium/phosphorus homeostasis.

Regular monitoring of the elderly people's nutrition seems to be a good starting point for improving their diets, which may help to slow down the biological ageing process and to deter physiological and pathological changes.

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CHANGES IN THE SOIL NITROGEN CONTENT CAUSED BY DIRECT AND RESIDUAL EFFECT OF MEAT AND BONE MEAL

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Abstract

Animal meat and bone meals need to be used for crop production since they have been eliminated from feeds in animal nutrition following the breakout of BSE (Bovine Spongiform Encephalopathy) in cattle. The Decision of the Council of the European Union of 4 December 2000 prohibited adding processed animal protein to feeds for cattle, pigs and poultry. In Poland, this ban was imposed by the decision of the government of 1 November 2003. Meat and bone meals can be an alternative source of nutrients to mineral nitrogen and phosphorus fertilizers because they are rich in these two elements; in addition, they contain calcium, micronutrients and organic substance. The objective of this study has been to evaluate the direct and residual effects of meat and bone meal (MBM) applied in different doses on the content of total nitrogen and its mineral forms in soil, which can indicate a potential threat of environmental contamination and demonstdose the uptake of nitrogen by plants. In 2006-2010, field tests were carried out at the Experimental Station in Bałdy, owned by the University of Warmia and Mazury in Olsztyn. The field trials were set up on typical brown soil. The first order experimental factor was the MBM dose (1.0, 1.5, 2.0 and 2.5 t ha^{-1} year^{-1} and 2.0, 3.0, 4.0 and 5.0 t ha^{-1} every two years); the second order factor was the year of the experiment (4 years). The four-year experiment shows that changes in the soil content of mineral nitrogen forms depended on the dose of MBM, species of crops and weather conditions. Meat and bone meal introduced to soil in large doses, irrespective of the frequency of application, caused a significant increase in the soil's richness in total and mineral nitrogen against the mineral fertilization. The content of N-NO$_3^-$ in soil increased significantly due to the residual effect of large doses of MBM (4.0 and 5.0 t ha^{-1}) and the annual application of 2.5 t MBM ha^{-1}. All the determined concentrations of N-NO$_3^-$ in soil were within the range of very low levels. The lowest content of N$_{min.}$ was detected in soil after winter wheat in the third year of the investigations, which was characterized by an uneven distribution of precipitations, and

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the amount of N-NO_3^- was the lowest in the first year, after winter triticale. The crops took up more nitrogen from soil enriched with MBM (except for the smallest dose) than from fields given mineral fertilization.

Key words: total and mineral nitrogen, soil, meat and bone meal (MBM), N uptake.

ZMIANY ZAWARTOŚCI AZOTU W GLEBIE JAKO EFEKT BEZPOŚREDNIEGO I NASTĘPCZEGO DZIAŁANIA MĄCZKI MIĘSNO-KOSTNEJ

Abstrakt

Konieczność rolniczego zagospodarowania mączek zwierzęcych wynika z eliminacji ich jako komponentów paszowych w żywieniu zwierząt z powodu wykrycia choroby BSE (Bovine Spongiform Encephalopathy) u bydła. Decyzją Rady UE od 4 grudnia 2000 roku obowiązuje zakaz stosowania przetworzonego białka zwierzęcego do pasz dla bydła, trzody chlewnej i drobiu. W Polsce zakaz ten wprowadzono decyzją rządową 1 listopada 2003 roku. Mączka mięsno-kostna może być alternatywą dla mineralnych nawozów azotowo-fosforowych, ponieważ jest bogata w azot i fosfor, a ponadto zawiera wapń, mikroelementy oraz substancję organiczną. Celem pracy była ocena działania bezpośredniego i następczego mączki mięsno-kostnej (MMK) stosowanej w różnych dawkach na zawartość azotu ogólnego i jego mineralnych form w glebie, będących wskaźnikami potencjalnego zagrożenia skażeniem środowiska, oraz na pobranie azotu przez rośliny. Badania polowe przeprowadzono w latach 2006–2010 w Ośrodku Dydaktyczno-Doświadczalnym w Bałdach, należącym do Uniwersytetu Warmińsko-Mazurskiego w Olsztynie, na glebie brunatnej właściwej. Czynnikiem I rzędu była dawka MMK (1,0; 1,5; 2,0 i 2,5 t ha^{-1} rok^{-1} oraz 2,0; 3,0; 4,0 i 5,0 t ha^{-1} co dwa lata), II rzędu – rok doświadczenia (4 lata). Z czteroletnich badań wynika, że zmiany w zawartościach mineralnych form azotu w glebie zależały od dawki MMK, gatunku uprawianej rośliny i warunków pogodowych. MMK wprowadzana do gleby w dużych dawkach, niezależnie od częstotliwości stosowania, wpłynęła na istotne zwiększenie jej zasobności w azot ogólny i mineralny, w odniesieniu do nawożenia mineralnego. Zawartość N-NO_3^- w glebie zwiększyła się istotnie w wyniku następczego działania dużych dawek mączki (4,0 i 5,0 t ha^{-1}) oraz po corocznym zastosowaniu 2,5 t MMK ha^{-1}. Wszystkie oznaczone zawartości N-NO_3^- w glebie mieściły się w przedziale bardzo niskiej zawartości. Najmniejszą zawartość $N_{min.}$ oznaczono w glebie po pszenicy ozimej w trzecim roku badań, charakteryzującym się nierównomiernym rozkładem opadów, a N-NO_3^- w pierwszym roku po pszenżycie ozimym. Rośliny pobrały więcej azotu z gleby wzbogacanej MMK (oprócz najmniejszej dawki) niż nawożonej mineralnie.

Słowa kluczowe: azot ogólny i mineralny, gleba, mączka mięsno-kostna (MMK), pobranie N.

INTRODUCTION

Field use of organic waste is an economically and ecologically viable solution. First of all, nutrients enclosed in MBM can be used for crop production. Secondly, MBM improves soil sorption quality. Because less solid or liquid manure is produced and mineral fertilizers are increasingly more expensive, using animal meals as substances rich in nitrogen, phosphorus and organic matter could help to maintain soils' fertility and fecundity (SPYCHAJ-

-Fabisiak et al. 2007). Meat and bone meals contain nitrogen in the form of protein compounds, so that through mineralization processes the element is slowly released to soil and typically becomes plant available already in the first year of its application (Chaves et al. 2005, Jeng et al. 2004, 2006, Jeng, Vagstad 2009, Nogalska et al. 2012). The dynamics of this process depends on several habitat-related factors and may vary between years and seasons of the year. For over twenty years now, the content of mineral nitrogen in soil has been a recognized test of crops' nutritional demands for this element in many European countries.

The experiment presented in this article involved an analysis of the direct and residual effect of different doses of meat and bone meal on the soil abundance of total and mineral nitrogen and on the uptake of this element by plants in a four-year field rotation. In order to evaluate the environmental effects of soil amendment with large doses of MBM, the content of $N\text{-}NO_3^-$ in soil was determined in autumn, after harvest.

MATERIAL AND METHODS

Field trials were carried out at the UWM Experimental Station in Bałdy in 2006–2010. A two-factor experiment was set up with four replications according to the random blocks method. It was established on typical brown soil developed from loamy sand, class IVa, good rye complex in the Polish soil valuation system. The soil was slightly acid (pH in 1 mol KCl dm^{-3} = 6.3). Its mineral composition was as follows: $C_{org.}$ 7.65 g kg^{-1}, $N_{og.}$ 0.94 g kg^{-1}, $N_{min.}$ 10.25 mg kg^{-1}, and plant available elements (in mg kg^{-1}): 49.0 phosphorus, 96.4 potassium and 31.0 magnesium. The surface area of a plot for harvest was 20 m^2. The trials were run in a four-year rotation cycle composed of winter triticale, winter oilseed rape, winter wheat and maize grown for silage. The first order factor was the dose of MBM (1.0, 1.5, 2.0 and 2.5 t ha^{-1} $year^{-1}$ and 2.0, 3.0, 4.0 and 5.0 t ha^{-1} every two years); the second order factor was the year of the experiment (4 years). This design of the experiment enabled us to compare the immediate and residual effects of the same dose of meal. The effect of MBM was compared to the result achieved on plots fertilized with mineral NPK fertilizer (mineral fertilization according to the agronomic recommendations for the four test crops). In the mineral fertilization treatment, nitrogen was introduced to soil as urea and ammonium nitrate, while phosphorus was applied in the form of granular triple superphosphate. The meal had a small content of potassium (3.4 kg K per ton), which is why it was always applied together with additional potassium as 50% potassium salt in an amount corresponding to the dose applied in the NPK treatment. The doses of nutrients introduced to soil with the mineral fertilizers and MBM are presented in Table 1. The Regulation of the Ministry of Agriculture and Rural Development of 7 December 2004 on

Table 1

Doses of nutrients with meal and bone meal and mineral fertilizer in four-years rotation (kg ha^{-1})

Treatment		Winter triticale (2006/2007)			Winter rape (2007/2008)			Winter wheat (2008/2009)			Maize (2010)		
		N	P	K	N	P	K	N	P	K	N	P	K
NPK		110.0	30.0	100.0	190.0	40.0	150.0	140.0	35.0	120.0	150.0	40.0	140.0
Yearly	1.0 t MBM+K*	78.8	46.7	103.4	78.8	46.7	153.4	78.8	46.7	123.4	78.8	46.7	143.4
	1.5 t MBM+K	118.2	70.1	105.1	118.2	70.1	155.1	118.2	70.0	125.1	118.2	70.1	145.1
	2.0 t MBM+K	157.6	93.4	106.8	157.6	93.4	156.8	157.6	93.4	126.8	157.6	93.4	146.8
	2.5 t MBM+K	197.0	116.7	108.5	197.0	116.7	158.5	197.0	116.7	128.5	197.0	116.7	148.5
Every two years	2.0 t MBM+K	157.6	93.4	105.1	0	0	150.0	157.6	93.4	126.8	0	0	140.0
	3.0 t MBM+K	236.4	140.1	110.2	0	0	150.0	236.4	140.1	130.2	0	0	140.0
	4.0 t MBM+K	315.2	186.8	113.6	0	0	150.0	315.2	186.8	133.6	0	0	140.0
	5.0 t MBM+K	394.0	233.5	117.0	0	0	150.0	394.0	233.5	137.0	0	0	140.0

*MBM+K - meat and bone meal + potassium mineral fertilization as in NPK veterinary requirements concerning soil enrichment substances state that a dose of meat and bone meal cannot exceed 5.0 t ha^{-1} in two years. The animal meal used in our tests was the third category product, i.e. low risk one, and originated from the Animal Waste Utilization Plant owned by Saria Polska in Długi Borek near Szczytno. It contained on average: 965 g dry matter, 714 g organic substance, 276 g crude ash, 137 g crude fat, 78.8 g N, 46.7 g P, 3.42 g K, 100.3 g, Ca, 6.8 g Na and 2.0 g Mg kg^{-1} d.m.

After the first year of the experiment (2007) and after the four-year rotation (in 2010), soil samples were taken from the arable horizon (0-30 cm) for determination of total nitrogen. Soil tests performed in late autumn are increasingly more useful in the assessment of the environmental impact of nitrogen fertilization, because excessive amounts of mineral nitrogen in soil, especially $N\text{-}NO_3^-$, left in soil after harvest may pose a threat to soil and ground waters (Fotyma et al. 2004). In our experiment, the content of ammonia and nitrate nitrogen was tested after harvest each year. Total nitrogen was determined by the Kjeldahl's method, mineral forms of nitrogen were assayed by colorimetry in fresh samples after extraction with 1% K_2SO_4 solution ($N\text{-}NH_4^+$ with the Nessler's reagent and $N\text{-}NO_3^-$ with fenoldisulfonic acid). Mineral nitrogen ($N\text{-}NH_4^+$ + $N\text{-}NO_3^-$) was converted into kg $N_{min.}$ ha^{-1} (Fotyma et al. 2004). The results were subjected to an ANOVA analysis of variance in the Statistica 10 software programme, and the significance of differences between arithmetic means was tested with the the Tukey's test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The analyzed period of time (2006-2010) was characterized by very high variability of the weather conditions (Table 2). The mean temperature from September 2006 to August 2007 was 2.3°C higher than the means from the long-term period of 1961-2000. In January 2007, the air temperature was 5.5°C higher and the precipitations were four-fold larger than the multi-year means. The deficit of rainfall in April and its excess in July (2.4-fold more than in the multi-year period) were unfavourable for the plant growth and processes of the mineralization of meat and bone meal. The weather conditions in the winter 2008 were much different from the multi-year averages: in January and February the temperature was 3.3 and 4.9°C higher, respectively, and the snowfall in January was nearly twice as high as the average. From April to July, the amount of rainfall corresponded to 64.6% of the multi-year mean for this season of the year. The most severe water deficit appeared in May and June (47% of the multi-year averages). The distribution of temperatures in 2008-2009 was similar to the multi-year means, but the precipitations were distributed unevenly, being about 30%

Table 2

Weather conditions in 2006-2010 (data from the Meteorological Station in Tomaszkowo)

Month	Mean monthly temperature (°C)						Precipitation total (mm)					
	2006	2007	2008	2009	2010	1961-2000	2006	2007	2008	2009	2010	1961-2000
January	-8.5	2.6	0.4	-3.2	-9.0	-2.9	19.8	115.4	66.2	24.7	19.4	28.8
February	-3.3	-2.5	2.5	-2.0	-3.0	-2.4	27.6	23.5	24.7	31.7	22.5	20.4
March	-2.5	5.6	2.8	1.3	2.1	1.2	6.0	27.8	52.4	57.9	36.7	26.8
April	7.3	7.5	7.7	9.4	8.1	6.9	25.6	24.7	31.4	4.8	18.2	36.1
May	12.5	13.8	12.3	12.4	12.0	12.7	89.2	93.5	27.0	52.9	131.9	51.9
June	16.0	17.7	16.9	14.9	16.4	15.9	79.2	88.1	32.7	136.9	84.8	79.3
Julay	20.9	17.7	18.5	20.4	21.0	17.7	29.3	173.7	57.7	48.3	80.4	73.8
August	17.2	18.3	18.4	17.6	19.3	17.2	165.0	68.0	102.1	19.3	95.3	67.1
September	14.8	12.6	15.1	14.2	12.0	12.5	51.0	57.9	22.9	25.7	40.5	59.0
October	9.9	7.5	8.6	5.8	5.0	7.8	38.3	30.2	82.8	55.7	24.1	43.4
November	5.1	1.3	4.0	5.0	4.4	2.7	79.3	33.8	29.4	43.8	121.4	47.7
December	4.3	0.5	0.1	-1.9	-6.8	-1.3	45.4	27.8	35.1	30.6	57.2	36.2

lower than the multi-year average, which may have been responsible for the lower content of mineral nitrogen in soil assayed in that season of the experiment (Table 3). More intensive rainfalls occurred in March (nearly twice as much as on average), but they were followed by a severe water deficit in April (13% of the multi-year average rainfall). Apart from a cooler (0.7°C lower temperature than the multi-year mean) and rainy (2.5-fold more rains than on average) May, the year 2010 did not diverge from the multi-year means.

Table 3

Direct and residual effect of MBM doses on the content of mineral nitrogen ($N_{min.}$) in soil (kg ha^{-1})

Treatment		Years of research				Mean (*a*)
		I (2007)	II (2008)	III (2009)	IV (2010)	
NPK		40.29	84.36	33.21	73.77	57.91
Yearly	1.0 t MBM+K*	46.80	85.27	32.30	78.00	60.59
	1.5 t MBM+K	43.95	82.98	34.49	79.15	60.15
	2.0 t MBM+K	48.87	82.90	35.37	77.44	61.15
	2.5 t MBM+K	50.64	84.67	42.59	76.98	63.72
Every two years	2.0 t MBM+K	44.66	78.49	29.78	71.93	56.21
	3.0 t MBM+K	51.73	76.04	35.48	71.30	58.64
	4.0 t MBM+K	52.48	76.23	44.28	89.02	65.51
	5.0 t MBM+K	54.72	76.89	44.68	91.50	66.95
Mean (*b*)		48.24	80.87	36.91	78.79	-
$LSD_{\alpha \leq 0.05}$		(*a*) = 3.92; (*b*) = 2.61; (*a*×*b*) = 7.84				

Legend: *a* – MBM dose, *b* – years of research, *a*×*b* – interaction
*MBM+K – meat and bone meal + potassium mineral fertilizatio as in NPK

Recapitulating, the meteorological conditions during the four-year period of our experiment were changeable, especially in terms of the unevenly distributed precipitations. The growth and development of the plants took place at temperatures about 1°C higher than the mean temperatures from the years 1961-2000. The high temperatures during our experiment should have had positive influence on the mineralization and activation of nutrients from meat and bone meal, but the variable amounts of rain, especially in the first and third year did not aid the process. KLIMEK (2006) and BASSIRIRAD (2000) claim that the soil temperature together with its moisture content significantly affect the rate of metabolic processes in soil organisms which participate in mineralization of organic matter and in microorganisms which have an indirect influence on the rate of organic matter decomposition. During the degradation of organic matter from natural and organic fertilizers, only a certain share of the nutrients is released immediately, while the remaining amounts, especially of nitrogen, are transiently bound (the immobilization process) by soil microorganisms, an effect which to a large extent prevents losses of nutrients.

Over 90% of the nitrogen found in the surface layer of soil is present in organic compounds, which is why there is a close dependence between the content of these compounds and total nitrogen (SPYCHAJ-FABISIAK et al. 2007). In our study, the mean content of nitrogen before the experiment equalled 0.94 g kg^{-1} d.m. About 79 kg N ha^{-1} was introduced to soil with one ton of MBM. After the four-year rotation cycle, the soil enriched with mineral fertilizers contained slightly less nitrogen: 0.90 g $N_{og.}$ kg^{-1}, while the soil nourished with meat and bone meal had about 17% more nitrogen than initially (Table 4). Compared with the NPK treatment, MBM added in the highest doses (2.0 and 2.5 t ha^{-1}) every year and in the doses of 3.0, 4.0 and 5.0 t ha^{-1} every other year caused a significant increase in the content of total nitrogen in soil. The smallest dose (1.0 t ha^{-1}) of MBM stabilized the con-

Table 4

Direct and residual effect of MBM doses on the content of total nitrogen ($N_{tot.}$) in soil (g kg^{-1} d.m.)

Treatment		Years of research		Mean (*a*)
		I (2007)	IV (2010)	
NPK		0.952	0.902	0.927
Yearly	1.0 t MBM+K*	0.920	0.945	0.932
	1.5 t MBM+K	0.995	0.960	0.977
	2.0 t MBM+K	0.983	1.105	1.044
	2.5 t MBM+K	1.007	1.250	1.129
Every two years	2.0 t MBM+K	1.065	0.870	0.966
	3.0 t MBM+K	1.205	1.132	1.169
	4.0 t MBM+K	1.235	1.265	1.250
	5.0 t MBM+K	1.327	1.307	1.317
Mean (*b*)		1.077	1.082	-
$LSD_{\alpha \leq 0.05}$		(*a*) = 0.109; (*b*) = n.s.; (*a*×*b*) = 0.154		

Explanations as in Table 3

*MBM+K – meat and bone meal + potassium mineral fertilization as in NPK

n.s. – non-significant

tent of total nitrogen, while the application of 2.0 tons of MBM every other year resulted in a significant decrease in this element in soil in the final year. The highest dose of MBM (2.5 t ha^{-1}) applied annually differentiated the content of total N in soil to a higher degree than the dose of 5.0 tons of meal introduced to soil every other year. An increase in the content of total nitrogen under the influence of various types of waste from the animal processing industry has also been observed by other researchers (BOHACZ, KORNIŁŁOWICZ-KOWALSKA 2005, JENG et al. 2006, SPYCHAJ-FABISIAK et al. 2007). In contrast, STĘPIEŃ and MERCIK (2002) completed a pot experiment, in which they determined similar concentrations of total nitrogen in soil fertilized with animal meals or fat waste and in soil treated with farmyard manure.

As the dose of MBM increased, irrespective of the frequency of application, a gradual increase in the content of mineral nitrogen in soil was reported (Table 3). Compared to the NPK treatment (110 kg N ha^{-1}), the content of $N_{min.}$ rose significantly in soil which received the highest dose of meal 2.5 t ha^{-1} (197 kg N ha^{-1}) every year or the doses of 4.0 t ha^{-1} (315 kg N ha^{-1}) and 5.0 t ha^{-1} (394 kg N ha^{-1}) applied every other year. Less mineral nitrogen than in the control was found only in the treatment with the smallest dose of meal (2.0 t ha^{-1}), which contributed to soil 157.6 kg N ha^{-1} once every other year. Noteworthy is the fact that although fertilization with the higher doses of MBM led to a statistically confirmed increase in the quantity of easily available forms of nitrogen compared to the NPK fertilization, the differences were small, ranging between 6 and 9 kg N ha^{-1}. The results obtained by BOHACZ and KORNIŁŁOWICZ-KOWALSKA (2005) prove that composts made from feathers, bark and straw caused an increase in the content of mineral nitrogen, which occurred with the passage of time since application. In our experiment, we were able to demonstrate evident differences in the concentrations of mineral nitrogen in soil between the years of the experiment. A significantly higher (nearly twice as much) content of mineral nitrogen forms in soil in the second and fourth year of the experiment was proven, regardless of the frequency of MBM application, compared with the other two years. This can be explained by a relatively long process of mineralization of the organic compounds with nitrogen found in meal, which take two years to decompose, as implied by the results reported by SPYCHAJ-FABISIAK et al. (2007) or BOHACZ and KORNIŁŁOWICZ-KOWALSKA (2005). In turn, JENG et al. (2004, 2006) believe that as much as 80% of the nitrogen in MBM is released and becomes available to plants in the first year. Mineralization of organic nitrogen compounds in soil results in the release of mineral nitrogen, whose assimilability depends on its form, weather conditions and the species of crops (BASSIRIRAD 2000). In our trials, winter oilseed rape grown in the second year did not use up the excessive supply of nitrogen. Amounts of nitrogen taken up by oilseed rape were significantly lower (on average 131.09 kg N ha^{-1}) than amounts absorbed by the other crops (Table 5). A relatively high concentration of $N_{min.}$ was determined in

Table 5

Direct and residual effect of MBM doses on the uptake nitrogen of plants (kg N ha^{-1})

Treatment		Winter triticale (2006/ /2007)	Winter rape (2007/ /2008)	Winter wheat (2008/ /2009)	Maize (2010)	Mean (*a*)
NPK		165.31	104.61	164.72	205.73	160.09
Yearly	1.0 t MBM+K*	124.59	128.40	135.30	187.77	144.01
	1.5 t MBM+K	164.37	149.90	166.13	194.76	168.79
	2.0 t MBM+K	153.67	123.80	162.73	242.04	170.56
	2.5 t MBM+K	157.35	133.67	192.73	242.20	181.49
Every two years	2.0 t MBM+K	126.38	137.49	185.60	180.22	157.42
	3.0 t MBM+K	147.50	148.80	216.49	228.14	185.23
	4.0 t MBM+K	143.31	118.16	205.12	191.07	164.42
	5.0 t MBM+K	151.51	134.99	235.02	198.46	179.99
Mean (*b*)		148.22	131.09	184.87	207.82	-
$LSD_{\alpha \leq 0.05}$		(*a*) = 15.85; (*b*) = 10.57; (*a*×*b*) = 31.70				

Explanations as in Table 3
*MBM+K – meat and bone meal + potassium mineral fertilization as in NPK

soil in the last year of the experiment, after harvesting maize, a plant with the highest nutritional demand (an average uptake of 207.82 kg N ha^{-1}) among the cultivated crops. Also, higher concentrations of $N_{min.}$ were determined in the control treatment after the harvest of winter oilseed rape and maize, whose post-harvest residues could have become an additional source of nitrogen (Table 6). This result cannot be explained by the accumulation of nitrogen gradually released from meal because the supply of nitrogen in the third year was the smallest, clearly due to the unfavourable distribution of precipitations and relatively large amounts of nitrogen absorbed by winter wheat (on average 184.87 kg N ha^{-1}) – Table 5. Under controlled, laboratory conditions corresponding to the natural temperatures in autumn in spring, DELIN and ENGSTROM (2010) showed that 65% of the nitrogen embedded in organic compounds found in meat and bone meal becomes phytoavailable after 35-50 days of mineralization. Also, CHAVES et al. (2005) report that 43-64% of the nitrogen in organic compounds in five types of animal meals was mineralized during 20-day-long incubation of soil.

Nitrogen is considered to be the most important yield stimulating element. But it can also turn into the most severe threat to the natural environment. Particularly dangerous are mineral forms of nitrogen, especially

Table 6

Direct and residual effect of MBM doses on the content of nitrate nitrogen (N-NO_3^-) in soil (kg ha^{-1})

Treatment		Years of research				Mean (*a*)
		I (2007)	II (2008)	III (2009)	IV (2010)	
NPK		13.55	31.87	22.86	27.39	23.92
Yearly	1.0 t MBM+K*	15.87	32.88	21.23	27.12	24.28
	1.5 t MBM+K	17.68	28.99	22.02	26.98	23.92
	2.0 t MBM+K	17.08	25.83	27.32	31.15	25.34
	2.5 t MBM+K	19.62	31.67	31.23	30.49	28.25
Every two years	2.0 t MBM+K	14.68	24.12	18.58	26.70	21.02
	3.0 t MBM+K	20.32	22.17	24.60	30.69	24.45
	4.0 t MBM+K	23.17	25.57	32.31	28.50	27.39
	5.0 t MBM+K	23.77	24.01	32.50	28.38	27.17
Mean (*b*)		18.42	27.46	25.85	28.60	-
$LSD_{\alpha \leq 0.05}$		(*a*) = 2.45; (*b*) = 1.63; (*a*×*b*) = 4.90				

Explantations as in Table 3
*MBM+K – meat and bone meal + potassium mineral fertilization as in NPK

nitrates, whose large amounts are transferred deep into the soil profile (GRABIŃSKA et al. 2005). The unwanted effect of nitrogen can appear when the applied doses of mineral or natural nitrogen fertilizers are in excess of the agronomic recommendations. The introduction of meat and bone meal to soil in our experiment resulted in a higher content of ammonia than nitrate nitrogen (Tables 3, 6). It should be underlined that this result was achieved in soil sampled after the vegetative season (September), when the process of N-NO_3^- leaching in the autumn and winter season is typically more intensive. It can be therefore assumed that the application of MBM has positive influence on the soil environment, because ammonia ions are rather strongly adsorbed by soil. Contrary results were reported by BOHACZ and KORNIŁŁOWICZ-KOWALSKA (2005). In our experiment, significantly higher quantities of nitrates in soil appeared in response to the doses of 4.0 and 5.0 t MBM ha^{-1} introduced every other year and the highest annual dose (2.5 t MMK ha^{-1}) than in soil which received mineral fertilization (Table 6). Nonetheless, even the highest determined amounts of N-NO_3^- in soil were within the threshold levels deemed as a very low content of nitrogen by the Regional Agricultural and Chemical Stations, based on the results of monitoring observations of arable soils from 5.000 checkpoints across Poland

(Fotyma et al. 2004). It was also verified that the year of the experiment had a significant influence on the content of ammonia nitrogen, which occurred in the lowest amounts in soil after the harvest of triticale (first year). This was most probably due to the uneven distribution of precipitations and a very short time period of the mineralization of nitrogen bound in organic compounds in meat and bone meal. Better stability of nitrates in soil, especially in the second year of the tests, was demonstrated after an annual application of animal meal.

The factors which influenced the abundance of mineral nitrogen compounds in soil were the high doses of MBM, species of the grown crop and weather conditions. Relatively small quantities of $N\text{-}NO_3^-$ in soil suggest a low risk of environmental contamination with this element. They can also indirectly imply that there is some deficit of plant available nitrogen in soil. However, compared to mineral fertilization, it was only the smallest dose of MBM (1.0 t ha^{-1} a year 2.0 t ha^{-1} every other year) that resulted in a lower uptake of nitrogen by the grown crops (Table 6). Besides, meal introduced to soil in the highest dose every year or in the doses of 3.0 and 5.0 t ha^{-1} every two years caused a significantly higher uptake of nitrogen by plants against the NPK treatment. Jeng and Vagstadt (2009) do not recommend application of animal meals in early spring or late autumn due to possible leaching of nitrates, even though in a lysimetric experiment they conducted the amounts of nitrates leached from soil fertilized with animal meals were half the quantities leached from soil fertilized with mineral preparations.

CONCLUSIONS

1. The content of mineral nitrogen forms in soil depended on the dose of meat and bone meal, species of the grown crop and the weather conditions.

2. Meat and bone meal applied in high doses, irrespective of the frequency of application, significantly raised the soil abundance of total and mineral nitrogen versus mineral fertilization. All the determined concentrations of $N\text{-}NO_3^-$ in soil were within the levels corresponding to a very low content.

3. The smallest concentration of $N_{min.}$ was determined in soil after winter wheat cultivation, in the third year of the experiment, which was characterized by an uneven distribution of precipitations. The smallest amount of $N\text{-}NO_3^-$ was detected in soil after winter triticale, in the first year of the trials. Meal introduced to soil in very high doses raised the uptake of nitrogen by plants compared the treatment with mineral fertilization.

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DISTRIBUTION OF HEAVY METALS IN BOTTOM SEDIMENTS OF FLOODPLAIN LAKES AND THEIR PARENT RIVER – A CASE STUDY OF THE SŁUPIA*

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Abstract

Concentration of trace elements in bottom sediments can be a result of natural accumulation or caused by anthropogenic factors. The content of heavy metals (Cd, Cr, Cu, Ni, Mn, Pb and Zn) in bottom sediments of oxbow lakes and river-bed of the Słupia River at Słupsk was determined. Sediment samples were taken from two oxbow lakes: naturally disconnected and artificially re-connected with the river. The research showed variation in the content of the elements in sediments in relation to the accumulative and erosive activity of the river. At the water inflow into the re-connected oxbow lake, the transporting strength of the river decreased, which favoured the accumulation of heavy metals. The content of heavy metals in bottom sediments taken from the re-connected floodplain lake at the outlet was several-fold lower than at the inlet, for example there was 11-fold less Mn, 9-fold less Cu 9-fold, 3-fold less As and half the amount of Cd, Cr and Ni. The content of heavy metals at the outlet was similar to the one found in sediments of the oxbow lake separated from the river.

Key words: floodplain lake, Słupia river, bottom sediments, heavy metals.

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ROZMIESZCZENIE METALI CIĘŻKICH W OSADACH DENNYCH STARORZECZY I ICH MACIERZYSTEJ RZEKI (NA PRZYKŁADZIE DOLINY SŁUPI)

Abstrakt

Koncentracja pierwiastków śladowych w osadach dennych może być wynikiem naturalnej akumulacji lub jest zdeterminowana czynnikami antropogenicznymi. Badania terenowe wykonano w dolinie rzeki Słupi, na przedmieściach miasta Słupska. Próbki osadów pobrano na 4 stanowiskach zlokalizowanych w dwóch starorzeczach (w ich ramionach) oraz w rzece Slupi między zbiornikami. Zawartość Cd, Cr, Cu, Ni, Mn, Pb i Zn oznaczono po mineralizacji próbek na mokro w stężonym kwasie azotowym metodą ASA. Wykazano różnice w zawartości pierwiastków w osadach w zależności od poziomu akumulacji i erozyjnej działalności rzeki. W wyniku napływu wody do udrożnionego starorzecza i spadku siły transportowej rzeki zaobserwowano spadek poziomu akumulacji metali ciężkich. Ich zawartość w osadach dennych pobranych na wyjściu (ramię dolne) z OLS-4 była kilkakrotnie niższa niż na wlocie (ramię górne). Zawartość Mn zmalała 11-krotnie, Cu 9-krotnie, As 3-krotnie, Cd, Cr i Ni 2-krotnie. Zawartość metali ciężkich w miejscu wypływu (ramię dolne) była podobna do ich zawartości w osadach starorzecza oddzielonego od rzeki (OLS-5).

Słowa kluczowe: tereny zalewowe, rzeka Słupia, osady denne, metale ciężkie.

INTRODUCTION

Bottom sediments are an important element of a river valley, which are conducive to the absorption of chemical particles, whose content in the riverbed exceeds by several-fold the respective concentrations in the overlying water column (Linnik, Zubenko 2000). In relation to the riverbed morphology and hydrological conditions, dissolved or suspended matter may undergo accumulation along the watercourse, often many kilometers below the source of pollution (Choi et al. 2006, Anicic et al. 2009). The source of heavy metals in bottom sediments, excluding natural processes of parent rock leaching, are municipal and industrial wastes, aerial runoff supplied by dry and wet atmospheric depositions, fertilizers and crop protection products (pesticides) (Zhao et al. 1999, Skorbiłowicz, Skorbiłowicz 2010, Benchea et al. 2011, Glina, Bogacz 2013).

Some meandering river sections are more likely to be exposed to the deposition of the matter transported by the river. In the middle course, the transportation strength of the river weakens and bigger fractions are deposited as sandbanks. The accumulative and erosive activity of the Słupia River appears more profoundly along the meandering section of the river channel, where numerous oxbow lakes mark a former watercourse. Topographic depressions forming single loops along both sides of the river channel are permanently filled with water. Artificial oxbow lakes were created in the 20th century during hydro-engineering works on the river floodplain. The river regulation works composed of the reinforcement of the channel and the cutting-off of some river curves aimed at accelerating the water outflow by raising the slope of the riverbed. Beside some obvious changes in the habitat conditions for phyto- and zoocenoses, the water potential for self-purification decreased significantly (GALLARDO et al. 2008, LIGĘZA, WILK-WOŹNIAK 2011). A solution was found by redirecting the river water flow through oxbow lakes previously separated from the river, which would therefore be re-connected (OBOLEWSKI, GLIŃSKA-LEWCZUK 2011). The re-connection of oxbow lakes to the river may enhance the resuspension of bottom sediments and thus release previously accumulated heavy metals into the water. These are the underlying consideration for an attempt that has been made towards determination of effects of the unblocking of ox-bow lakes and the water inflow of the Słupia on the content of heavy metals in bottom sediments. The problem was discussed against the background of sediments in an ox-bow lake disconnected from the river as well as the river itself.

MATERIAL AND METHODS

Study area

The studied area covered the middle part of the Słupia River, rich in numerous floodplain lakes (Figure 1). This river is one of the biggest watercourses on the East European platform in Poland, which flows into the Baltic Sea (water catchment basin covers 1,620 km^2; the river is 138.6 km long, the average annual flow equals 15.5 m^3 s^{-1}, floods and a flow rate higher than 100 m^3 s^{-1} usually appear between October and March). Most wetlands are agriculturally used and constitute 5.6% of the total studied area. In the early 20th century, a number of river regulation works were performed, mainly cutting off the river meanders. As a result, almost 50 oxbow lakes appeared. The oxbow lakes are located on the right-hand side of the river. The one re-connected to the river (OLS-4) is a water body covering 0.13 ha and its maximal depth reaching 2 m. The re-connection was performed in 2000 using two PCV pipes (inlet and outlet ones) 0.3 m in diameter (OBOLEWSKI, GLIŃSKA-LEWCZUK 2011). The other lake (OLS-5) is separated from the river. Its area is to 0.7 ha and the maximal depth reaches 1.65 m (Table 1).

Samples

The investigation on the content of heavy metals in bottom sediments was conducted on the floodplain of the Słupia River above the town of Słupsk. In the autumn of 2007-2008, low stages of the river water facilitated the collection of sampling material. Sediment samples were taken from the river bank zone and 2 arms of both oxbow lakes. In the case of the re-connected oxbow lake (OLS-4) samples were taken from the zones of water inlet and outlet (Figure 1).

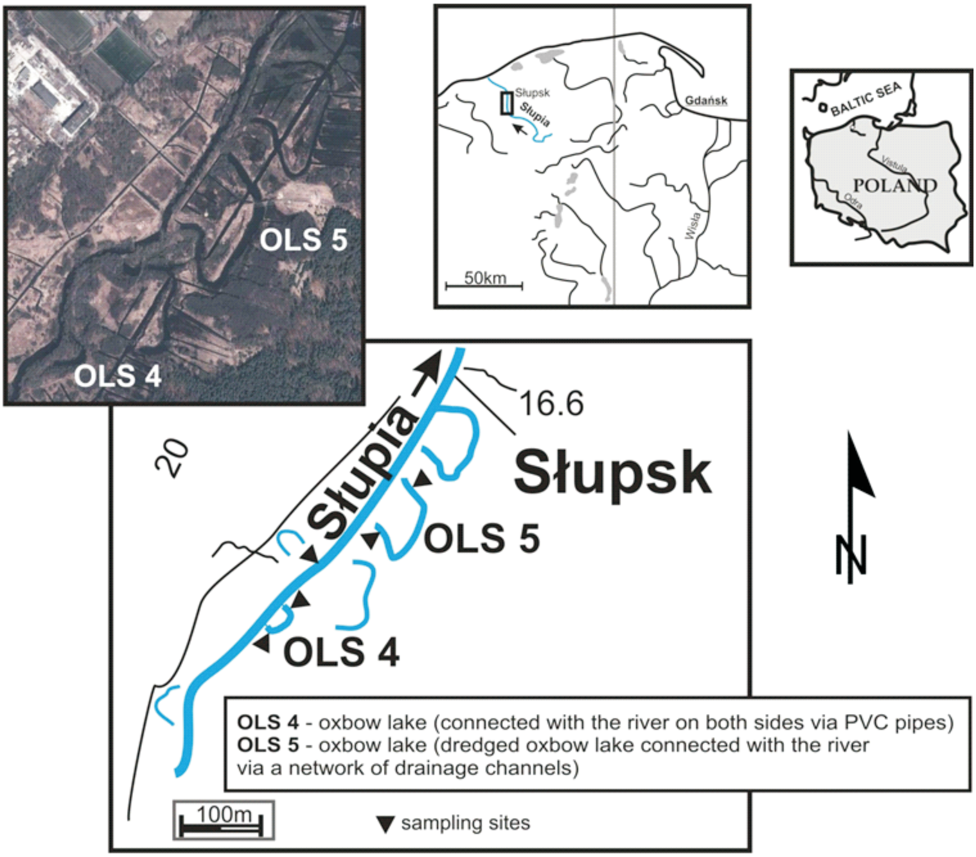

Fig.1. Location of sampling sites along the Słupia floodplain near the city of Słupsk, N Poland

At each sampling site, soil samples were taken at depths of 0 to 10, 10-20 and 20 to 30 cm. Representative samples (*ca* 1000 g) consisting of mixed sediment material were transported in linen bags to a laboratory and air-died. In the laboratory, before chemical analyses, the soil samples were dried at 40°C for 48 h and then passed through a 0.2-mm nylon sieve to obtain an optimal fraction for the assessment of pollution. A sediment fraction with the grain size below 0.125 mm was used for determination of the total content of contaminants. Portions of about 0.5 g from mixed samples were weighed out accurately and placed in 100 ml Teflon digestion tubes, where they were mixed with 8 ml of concentrated HNO_3 and 2 ml of 30% H_2O_2 (SALMINEN et al. 2005). Afterwards, the samples were digested for two hours in a microwave digester CEM MARS 5 (Microwave Accelerated Reaction System, USA).

Table 1

Morphological characteristics of the studied oxbow-lakes

Oxbow-lake			OLS-4		OLS-5	
Geographical coordinates			N 54°26'45.21"	E 17°01'38.70"	N 54°26'52.99"	E 17°01'58.41"
Type of connection with river			LOTIC (pipe PVC)		LOTIC (melioration ditches)	
Length	D (m)		116		468	
Width	S (m)		13.7		20.0	
Area	A (ha)		0.13		0.71	
Sinuosity	S=D/a-c (-)		1.5		2.5	
Distance between arms	a-c (m)		75		188	
Distance from river	upper arm (m)		23		37	
	lower arm (m)		21		22	
	max.(m)		46		160	
Width	river	S_{rz}(m)	15.2		14.5	
	river valley WWW	S_{dol} (m)	374		308	
Depth	$h_{śr}$ (m)		0.88		0.79	
	h_{max}(m)		1.82		1.65	
Volume	$V_{śr}$ (thou m^3)		1.14		5.51	

The following trace metals: Cd, Cr, Cu, Ni, Mn, Pb, Zn and As were determined in bottom sediments in an atomic absorption spectrometer Spectra AA100 (Varian, Australia). The accuracy of determinations of the total content of heavy metals was verified against certified reference material, which was analyzed at the beginning and the end of the sampling series. The observed error was less than 5% of the certified value.

The non-parametric Kruskal-Wallis and Dunns ($p<0.05$) tests were applied in order to indicate statistical significance of differences in environmental conditions between the sampling sites.

RESULTS AND DISCUSSION

The Słupia River belongs to young glacial watercourses, which are characterized by numerous channel bends, meanders and oxbow lakes (OBOLEWSKI, GLIŃSKA-LEWCZUK 2011). The character of the river are conducive to the deposition of material transported by the river. Near the town of Słupsk, the slope of the riverbed diminishes, which results in more intensive formation of sandbanks. These are potential sites for the accumulation of many chemical elements. Bottom sediments in the re-connected oxbow lake are also the result of intensive accumulation of matter originating from the river. The decreased velocity of water inflowing into the oxbow lake enables the deposition of substances just behind the inlet pipe.

The quality of bottom sediments depends on amounts of pollutants introduced to the river above the study site, the chemical composition of water as well as the parent rock on which the river valley lies (GLIŃSKA-LEWCZUK 2005). Heavy metals are mainly derived from agriculture in the catchment basin of the Słupia River and from the wastewater, whose handling and treatment remain unregulated. Another source of pollution is municipal sludge discharged into the river or its tributaries.

Despite the low pollution of the sediments with heavy metals, the results demonstrated significant differences between the sampling sites (Table 2). The river, which acts as a direct recipient of pollutants generated by people in the catchment area, showed the highest content of such heavy metals as Cd, Cr, Cu, Ni and Zn in bottom sediments relative to the other sampling sites, except Mn, Pb and As, which were found in higher concentrations in samples from OLS-4, a lake permanently connected with the Słupia River. The highest concentrations of the above metals were determined in sediments at the in-flow zone of the river water to the oxbow lake.

The analyses showed that bottom sediments in the river channel and the site of the oxbow lake connected to the river contain significantly higher amounts of heavy metals than the geochemical background defined by BOJAKOWSKA and SOKOŁOWSKA (1998) or LIS and PASIECZNA (2001). Comparing the lead contents in bottom sediments in the Słupia riverbed to the background value computed for flowing water sediments *i.e.* 10-15 mg Pb kg^{-1} d.m., it needs to be pointed out that the content of Pb (54.80 mg kg^{-1}) was four-fold higher than the natural level. Similarly, the Cd content (0.79 mg kg^{-1}) was found to exceed the background value (0.5 mg Cd kg^{-1}). The content of chromium in the investigated bottom sediments ranged from 3.9 to 33.7 mg Cr kg^{-1}, being also higher than the geochemical background for aquatic sediments in Poland: 5 mg Cr kg^{-1} according to BOJAKOWSKA and SOKOŁOWSKA (1998), or 10 mg Cr kg^{-1} according to LIS and PASIECZNA (2001). Sediments in the river and in the re-connected oxbow lake contained copper at amounts five-fold higher than the background, *i.e.* 6 mg Cu kg^{-1} (Table 2). Nickel varied from 11 to 32 mg kg^{-1}, and was 2- to 6-times as much as the natural

Table 2

Content of heavy metals (mg kg^{-1}±s.d.) in bottom sediments at the study sites in the Słupia River valley

	Oxbow lake				the Słupia River (N=4)	Average
	OLS-4 N=4		OLS-5 N=4			
	in-flow	out-flow	upper arm	lower arm		
	1	2	3	4	5	
Cd	0.44±0.32	0.23±0.14	0.10±0.08	0.12±0.06	0.79±0.29	0.34±0.21
Cr	7.9±0.2	3.9±2.2	6.2±1.9	6.1±2.0	33.7±11.5	11.6±3.2
Cu*	30.9±13.72	3.4±0.71	3.7±1.1	2.8±0.9	34.9±14.7	15.1±6.8
Ni	10.6±3.7	4.8±1.0	6.5±1.6	4.7±1.6	32.2±10.6	11.8±4.8
Mn	1190±112	111±33	193±76	130±54	645±289	454±89
Pb*	164.9±78.92	12.3±2.71	8.4±1.85	8.0±2.25	54.8±23.63,4	49.7±8.9
Zn*	67.4±28.92,5	18.8±4.11,5	22.0±11.05	19.4±5.75	142.6±35.21,2,3,4	54.0±15.9
As	2.66±0.88	0.96±0.12	0.50±0.20	0.60±0.33	2.03±0.90	1.4±0.53

*statistically significant differences between the studied sites; non parametric Kruskal-Wallis test, $P<0.05$
1-5 statistically significant differences between the studied sites; non parametric Dunns test, $P<0.05$
N – number of samples

level, *i.e.* 5 mg Ni kg^{-1}. The cadmium content in sediments was similar to the geochemical background level (0.5 mg Cd kg^{-1}). The geochemical background level was not exceeded by arsenium versus the normal level in aquatic sediments in Poland (5 mg As kg^{-1}).

The results showed that the direct connection of OLS-4 with the river channel did not influence the quality of all bottom sediments in the water body. The comparison of heavy metals in its sediments showed significant differences between the inlet (OLS4- in-flow) and outlet (OLS-4) sites. At the outlet site, the Pb concentration was 13-fold lower than at the inlet (P < 0.05). The highest decrease was observed for manganese, copper, zinc and arsenium (Table 2). The smallest differences in the content of metals between the inlet and outlet zones in the sediments of OLS-4 were found for cadmium, chromium and nickel.

The sediments from OLS-5, an oxbow lake separated from the river, were characterized by the content of the heavy metals similar to that at the outlet from the re-connected oxbow lake. In comparison to the amounts of heavy metals found in bottom sediments of the river channel, the content of the elements was several-fold lower: Cu 12-fold, Cd, Ni, Pb and Zn by 7-fold, Cr by 6-fold, Mn by 5-fold and As by 3-fold. Samples taken from both arms of OLS-5 (upper and lower arm) showed similarly low levels of all the investigated heavy metals.

Any motion of river water in the re-connected oxbow lake (in-flow) may cause the resuspension of bottom sediments and secondary water pollution. Although oxbow lakes are thought to serve as a biogeochemical filter (water - bottom sediments - plants - animals) in the riverine landscape, it is worth noticing that most natural aquatic sediments commonly have a living and active biological component and inorganic and non-living bio-logical particles (Walling, Owens 2003, Schroeder et al. 2005). Presumably, elements which have not been leached out with the river water are partly accumulated in plants overgrowing intensely the oxbow lake and absorbed into a trophic network. This is confirmed by non-significant amounts of heavy metals in the surface water of the re-connected lake (Obolewski, Glińska-Lewczuk 2011).

In any evaluation of the mobility of heavy metals in sediments, the composition of the uppermost layer appears to be very important. Following the river regulation works, the newly formed oxbow lakes are characterized by an amorphic layer composed mostly of organic matter, which corresponds to the sediments stored for about 30 years, and demonstrates the up-to-date environmental pollution in the region. This layer holds the maximal (direct) accumulation of pollutants that normally reside within the upper 30 cm of a profile (Zhao et al. 1999, Ibragimow et al. 2010). Beneath surface deposits, the parent material is built mostly of the clay, which – owing to its absorptive properties – is supposed to accumulate particles of heavy metals. Thus, the parent material should indicate the background (uncontaminated) levels of heavy metals in aquatic deposits. It can be useful in further studies on sediment pollution by trace elements.

CONCLUSIONS

1. Bottom sediments of the Słupia River and oxbow lakes are characterized by a low level of heavy metal contamination, close to the natural content determined for sediments of aquatic systems in Poland.

2. The maximal content, exceeding the background levels, of the metals under investigation was determined in the sediments taken both from the river channel and the inlet of river water to the re-connected oxbow lake.

3. The results showed that concentrations of heavy metals in sediments of oxbow lakes generally decrease after they have been reconnected to a river. Unblocked oxbow lakes may play a role of filters for heavy metal pollution transported down the river. Although significant accumulation of heavy metals was found at the inlet to the oxbow lake, the content of heavy metals found at the outlet was several-fold lower. The manganese content at the outlet site decreased by 11-fold, copper by 9-fold, zinc by 4-fold, arsenic by 3-fold and cadmium, chromium and nickel by two-fold.

4. Most of the investigated heavy metals in sediments of the oxbow lake separated from the river represented values similar to those found in sediments at the outlet from the re-connected oxbow lake. The lack of direct connection to the river allowed maintaining relatively low contents of heavy metals in the oxbow lake sediments in comparison to river sediments: Cu by 12-fold, Cd, Ni, Pb and Zn by 7-fold, Cr by 6-fold, Mn by 5-fold, whereas As by 3-fold lower than in the riverbed sediments.

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METAL CONCENTRATIONS IN SELECTED ORGANS OF CRAYFISH – *ORCONECTES LIMOSUS* AND *PACIFASTACUS LENIUSCULUS* FROM MAZURIAN LAKES*

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Abstract

Crayfish accumulate trace metals, which is why they often serve as environmental indicators. Among heavy metals, Hg, Cd and Pb are considered toxic substances, while Fe, Zn, Cu, Cr, Ni, Mn, V, Al and Li are involved in vital functions, which makes them essential elements in animal organisms.

The study objective was to determine concentrations of the above metals in the abdominal muscles, hepatopancreas and exoskeleton of spinycheek crayfish (*Orconectes limosus* Raff.) and signal crayfish (*Pacifastacus leniusculus* Dana) from Dgał Wielki Lake and Pobłędzie Lake in the Mazurian Lake District, Poland.

The analyses were carried out with the use of the following methods: CV AAS (Hg), GF AAS (Cd, Pb) and ICP-AES (Al, Cr, Cu, Fe, Li, Mn, Ni, V, Zn) after wet digestion in HNO_3 and $HClO_4$ concentrated acid mixture.

Regarding mean concentrations (mg kg^{-1} wet weight) in crayfish, metals formed the following order: Al (14.8-123.4) > Mn (0.26-91.3) > Fe (0.54-81.1) > Zn (6.80-51.91) > Cu (1.21-4.34) > Ni (0-0.782) > Cr (0.032-0.606) > V (0-0.245) > Li (0.011-0.215) > Pb (0.018-0.079) > Hg (0.004-0.045) > Cd (0.001-0.017).

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Metal concentrations in crayfish organs were varied, and for different metals they decreased in different order: Al, Mn, Cu, Ni, Cr, V and Li – exoskeleton > hepatopancreas > muscles; Zn and Hg – hepatopancreas > muscles > exoskeleton; Fe – hepatopancreas > exoskeleton > muscles, while for lead and cadmium no overall correlation was observed. The comparison of metal concentrations in the organs of spinycheek crayfish from Dgał Wielki Lake and Pobłędzie Lake did not prove any statistically significant discrepancies. Also, some minor interspecies differences in the metal content proved insignificant.

According to the European Union regulations, concentrations of toxic heavy metals (Hg, Cd, Pb) in the muscles of both species of the tested crayfish were significantly below the maximum levels.

Key words: metals, crayfish, muscles, hepatopancreas, exoskeleton.

STĘŻENIE METALI W WYBRANYCH NARZĄDACH RAKÓW – *ORCONECTES LIMOSUS* I *PACIFASTACUS LENIUSCULUS* Z JEZIOR MAZURSKICH

Abstrakt

Raki kumulują w swoim organizmie metale, i dlatego są często traktowane jako wskaźnik stanu środowiska. Wśród metali ciężkich za toksyczne uznaje się Hg, Cd i Pb, natomiast Fe, Zn, Cu, Cr, Ni, Mn, V, Al i Li mają udział w funkcjach życiowych, co czyni je niezbędnymi dla organizmu. Celem pracy było określenie stężeń ww. metali w mięśniach odwłokowych, wątrobotrzustce i pancerzu raków pręgowatego (*Orconectes limosus* Raff.) i sygnałowego (*Pacifastacus leniusculus* Dana) z jezior Dgał Wielki i Pobłędzie (Pojezierze Mazurskie).

Analizy przeprowadzono z użyciem następujących metod: CV AAS (Hg), GF AAS (Cd, Pb) i ICP – AES (Al, Cr, Cu, Fe, Li, Mn, Ni, V, Zn) po zmineralizowaniu próbek w mieszaninie stężonych kwasów azotowego i nadchlorowego.

Pod względem średniego stężenia (mg kg^{-1} mokrej masy) metale były uszeregowane w następującej kolejności: Al (14,8-123,4) > Mn (0,26-91,3) > Fe (0,4-81,1) > Zn (6,80-51,91) > Cu (1,21-4,34) > Ni (0-0,782) > Cr (0,032-0,606) > V (0-0,245) > Li (0,011-0,215) > Pb (0,018-0,079) > Hg (0,004-0,045) > Cd (0,001-0,017).

Stężenie metali w narządach raków było zróżnicowane; dla różnych metali zmniejszało się następująco: Al, Mn, Cu, Ni, Cr, V i Li – pancerz > wątrobotrzustka > mięśnie; Zn i Hg –wątrobotrzustka > mięśnie > pancerz; Fe – wątrobotrzustka > pancerz > mięśnie. Nie obserwowano takich zależności dla ołowiu i kadmu.

Porównanie stężeń metali w narządach raka pręgowatego z jeziora Dgał i jeziora Pobłędzie nie wykazało istotnych statystycznie różnic. Podobnie nieistotne były międzygatunkowe małe różnice w stężeniach metali.

Stężenia toksycznych metali ciężkich (Hg, Cd, Pb) w mięśniach obu badanych gatunków raków plasowały się poniżej najwyższego dopuszczalnego poziomu ustalonego dla środków spożywczych przez Rozporządzenie Komisji WE.

Słowa kluczowe: metale, raki, mięśnie, wątrobotrzustka, pancerz.

INTRODUCTION

Among the native fauna species in Poland, crayfish belong to the largest invertebrates. Until the end of the 19th century, the noble crayfish (*Astacus astacus*) and Danube crayfish (*Astacus leptodactylus*) had been widespread in the European water bodies. But due to their considerable vulnerability to crayfish plague and sensitivity to pollution, at present they are very rare in Poland and under strict protection. These native species were supplanted by the spinycheek crayfish (*Orconectes limosus*), also called American crayfish, imported from the United States of America in 1892, and signal crayfish (*Pacifastacus leniusculus*) introduced to Polish waters in the early 1970s (Kossakowski et al. 1978, Śmietana, Strużyński 1999). Having been introduced to Polish waters, these foreign species were unintentionally spread by humans. For example, it is believed that implementation of nylon fishing nets accelerated the phenomenon, because such nets – unlike cotton ones – do not need drying so crayfish caught in nylon nets can be transferred in good condition from one water body to another, even at considerable distances (Śmietana, Strużyński 1999). Besides, foreign species are less susceptible to crayfish plague and they cope better with unfavourable environmental conditions.

Moreover, female signal crayfish lay eggs under the abdomen as early as spring, while female noble crayfish and Danube crayfish do so in autumn, which additionally contributes to quicker expansion of signal crayfish in Europe. The incubation period lasts 6-7 months in native crayfish and just 5-6 weeks in alien ones. Owing to such short incubation, eggs carried under the abdomen of a female are less likely to be lost (Ulikowski, Borkowska 1999). However, signal crayfish do not grow big and an individual 12-centimetre in length is considered very large (Śmietana, Strużyński 1999).

Signal crayfish are equipped with many practical adaptations and reach sizes similar to noble crayfish. Representatives of the species mature one year earlier than the native crayfish and are more productive of offspring; also, their body length increases faster compared to the native crayfish (Krzywosz et al. 1995, Krzywosz, Krzywosz 2001, Śmietana, Krzywosz 2006). However, their introduction is not always successful, especially where spinycheek crayfish are abundant. It should also be remembered that signal crayfish and spinycheek crayfish are a serious threat to noble crayfish, which have become very rare in Poland (Gydemor 1996, Krzywosz 2006, Kozubíková et al. 2008).

Regarding the above, attempts should be made to eliminate the American species from Polish waters. Attaining this goal can be facilitated by the development of fish farming, as farms can preserve native species in their natural environment (Bernad et al. 2001). It is also supported by Polish legal regulations, stipulating that caught spinycheek crayfish (*Orconectes limosus*

Raff.) and signal crayfish (*Pacifastacus leniusculus* Dana.) individuals should not be released to the same fishing ground, nor to any other water bodies (Dz. U. 2001, 2003).

Crayfish may be used as an environmental indicator because their tissues tend to accumulate metals, including heavy ones (ANDERSON et al. 1997b). Among heavy metals, mercury, cadmium and lead are toxic and not essential to organisms. Other metals from the same group, namely iron, zinc, copper, chromium, nickel, manganese and vanadium, are essential to organisms because of positive roles they play in many vital functions. Also aluminium and lithium, which belong to heavy metals, are essential to animals. An extensive review of the distribution and role of trace elements in the environment has already been provided by KABATA-PENDIAS, PENDIAS (1999). Situations when toxic metal concentrations exceed the tolerable level or when the essential metals exceed concentrations physiologically needed to maintain a biochemical balance pose a threat to animal health and life.

Bioaccumulation of metals in crayfish bodies may impair the immune system, reproduction, heart rhythm, breathing processes, regeneration and moulting processes; it may also cause changes in pigmentation, increase glucose concentration, change pH of digestive juices and induce histopathological changes in the hepatopancreas (MEYER et al. 1991, REDDY et al. 1994, STYRISHAVE, DEPLEDGE 1996, ANDERSON et al. 1997b, AHERN, MORRIS 1999, RODRIGUEZ et al. 2003, WARD et al. 2006, WOODBURN et al. 2011). The hepatopancreas, an organ involved in the processes of nutrient absorption and storage as well as detoxification, is particularly prone to bioaccumulation of metals. They are stored in metal-containing vacuoles, as demonstrated by a study on lead accumulation carried out in laboratory conditions (ANDERSON et al. 1997a).

The objective of this study was to test concentrations of metals, mainly heavy ones, in tissues (exoskeleton, hepatopancreas and muscles) of spinycheek crayfish (*Orconectes limosus* Raff.) and signal crayfish (*Pacifastacus leniusculus* Dana). A hypothesis was formulated that metal concentrations in particular organs of crayfish depended on which lake given crayfish originated from (Dgał Wielki or Pobłędzie Lake) and on the species.

MATERIAL AND METHODS

Study area

The lakes where the crayfish were caught are located in the Mazurian Lake District, Poland. Dgał Wielki Lake is a flow-through lake with an area of 94.5 ha, maximum depth 17.6 m, average depth 5.3 m, maximum length 1,300 m, maximum width 1,120 m and the shoreline length of 5,200 m. The lake has a developed shoreline, hard sandy bottom with muddy sediments in its bays. The lake's direct catchment area comprises 35% of poor arable land, 30% of pastures and fallows, 25% of coniferous forest and almost 10% of farm buildings. This mesotrophic lake is inhabited by such fish as roach, pike, bream, perch, sheatfish, eel, sturgeon and bighead carp.

Pobłędzie Lake is half the size of Dgał Wielki. It has an area of 57.6 ha, maximum depth 15.4 m and an average depth of 5.9 m. Pobłędzie is a flow-through lake located in a protection zone of the Puszcza Romnicka Landscape Park. Perch and pike are dominant species in its ichthyofauna, but eels are absent. The lake is distinguished by relatively low trophy, although it cannot be classified as an oligotrophic water body (KRZYWOSZ, KRZYWOSZ 2002).

Material

The tested material consisted of 40 individuals, including 30 individuals of spinycheek crayfish (*Orconectes limosus* Raff.) and 10 individuals of signal crayfish (*Pacifastacus leniusculus* Dana). Spinycheek crayfish were caught in two lakes: Dgał Wielki (*n*=15, 12 males and 3 females) and Pobłędzie (*n*=15, 9 males and 6 females); signal crayfish (*n*=10, 9 males and one female) were caught only in Pobłędzie Lake. The crayfish were caught on 28th September 2003, then transported to a laboratory, weighed and measured (Table 1). The following organs were dissected: abdomen muscles, hepatopancreas and exoskeleton. Because of the small weight of spinycheek crayfish, the material from three individuals of the same gender was aggregated for analysis. The samples were tightly packed, frozen and stored for analyses at –30°C.

Table 1

Body weight and length (from the rostrum to the end of telson) of spinycheek crayfish (*Orconectes limosus* Raff.) and signal crayfish (*Pacifastacus leniusculus* Dana.) f rom Dgał Wielki Lake and Pobłędzie Lake, Poland

Species	Lake	Gender*	*n*	Weight (g)		Length (cm)	
				min-max	mean ±SD	min-max	mean ±SD
Spinycheek crayfish	Dgał Wielki	*m*	12	20.36-32.94	25.68 ±3.69	8.5-10.6	9.2 ±0.6
		f	3	10.56-25.26	17.24 ±7.44	7.3-9.9	8.6 ±1.3
	Pobłędzie	*m*	9	16.17-34.87	25.24 ±5.66	7.8-10.2	8.7 ±0.8
		f	6	21.64-31.37	25.85 ±3.31	8.7-10.5	9.6 ±0.6
Signal crayfish	Pobłędzie	*m*	9	67.80-114.90	90.79 ±16.85	12.0-13.5	12.7 ±0.5
		f	1	-	47.63	-	11.3

* *m* – males, *f* – females

Analytical methods

Mercury determination. The samples of the muscles and hepatopancreas were digested in mixture of concentrated nitric acid and perchloric acid (7:3), as in ADRIAN (1971), while the samples of the exoskeleton were mineralized for 12 hours in concentrated nitric acid in an incubator at 70°C. After dissolution with deionized water, the samples were filtered to a volumetric flask and made up to 100 ml. The content of mercury was determined by cold vapour atomic absorption spectrometry (CV AAS) in a Bacharach Coleman MAS 50D apparatus, after reducing mercury ions with 5 ml 10% $SnCl_2$ solution.

Determination of other metals. Tissue samples were placed in test tubes and mineralized in a mixture of concentrated nitric acid and perchloric acid (4:1) in a heating block. The block temperature was gradually increased from 50°C to 200°C in order to avoid untimely evaporation of the acids and carbonization of the residue. The dry residue was diluted in 2 ml of 15% HNO_3, transferred to a volumetric flask and made up to 20 ml with deionized water.

Cadmium and lead were determined using graphite furnace atomic absorption spectrometry (GF AAS) in a Perkin Elmer ZL 4110 apparatus, while the other chemical elements (Al, Cr, Cu, Fe, Li, Mn, Ni, V, Zn) were determined using inductively coupled plasma atomic emission spectroscopy (ICP AES) in a Jobin Yvon JY-24 apparatus.

RESULTS AND DISCUSSION

It is common knowledge that the content of elements in aquatic organisms depends on their environmental concentrations and their involvement in metabolism (ANDERSON et al. 1997b). Average concentrations (mg kg^{-1} wet weight) of the analysed metals in the three selected organs of crayfish were as follows: Hg – 0.004-0.045; Cd – 0.001-0.017; Pb – 0.018-0.079; Cu – 1.21-4.34; Zn – 6.80-51.91; Cr – 0.032-0.606; Ni – 0-0.782; Fe – 0.54-81.1; Mn – 0.26-91.31; V – 0-0.245; Li – 0.011-0.215 and Al – 14.8-123.4 (Table 2). With regard to the concentrations, the metals formed the following order: Al > Mn > Fe > Zn > Cu > Ni > Cr > V > Li > Pb > Hg > Cd.

The observed order of increasing metal concentrations in crayfish organs is consistent with their content in the lithosphere and hydrosphere provided by KABATA-PENDIAS, PENDIAS (1999); it is also consistent with the results obtained by MACKEVIČIENĖ (2002).

Mercury, cadmium and lead are explicitly toxic substances, although their low concentrations are tolerated by organism. The other metals belong to the group of elements essential for life; they have to be supplied to a body but their excess is harmful.

Mercury, cadmium and lead are found in animal tissues even when there is no environmental pollution. In increased concentrations, the metals inhibit enzymes, bind to amino acids, hemoglobin, RNA and DNA, cause histopathological changes in the hepatopancreas and exhibit mutagenic and carcinogenic activity (ANDERSON et al. 1997b, KABATA-PENDIAS, PENDIAS 1999, KOUBA et al. 2010).

KOUBA et al. (2010) discussed the content of these metals in the abdominal muscles and hepatopancreas of some species of crayfish caught in various regions of the world, based on a publication covering the period from the early 1970s until 2007. Metal concentrations (mg kg^{-1} wet weight) in the organs of crayfish caught in unpolluted areas were varied: 0.010-0.274 mercury in the muscles and 0.048-0.420 in the hepatopancreas; cadmium <0.004-0.116 and 0.02-0.72 respectively, and lead 0.012-2.14 and 0.008-1.66, respectively. This indicates that accumulation of mercury, cadmium and lead in the crayfish from the Mazurian Lakes (Table 2) lied within the lower range of the results obtained by the above authors.

Essential elements (Cu, Zn, Cr, Ni, Fe, Mn, V, Li, Al) are present in crayfish bodies as a result of their physiological functions. Besides the data on three toxic metals, KOUBA et al. (2010) also provided results of studies on the content of essential elements, namely: Cu, Zn, Cr and Ni. Their content (mg kg^{-1} wet weight) in the abdominal muscles and hepatopancreas of crayfish caught in unpolluted water bodies ranged within: copper 0.594-1.44 and 2.07-117.0, zinc 1.06-25.5 and 5.02-62.0, chromium <0.02-0.62 and 0.03-0.18, and nickel <0.08-0.97 and 0.25-0.93, respectively. The results of our study (Table 2) seem to be in agreement with the results provided by KOUBA et al. (2010). Similar data were also reported in publications of other authors (FINERTY et al. 1990, ANDERSON et al. 1997b, MOELLER et al. 2003), and some of them additionally provided data on metal content in exoskeleton (MACKEVIČIENĖ 2002, POURANG, DENNIS 2005).

There are fewer publications on iron, manganese, vanadium, lithium, and aluminium in crustaceans. The content of these metals in crayfish organs varied depending on where they were caught and on the species. Concentrations detected in this study (Table 2) did not differ significantly from those reported by other authors for crayfish caught in unpolluted areas. According to KURUN et al. (2010), iron concentrations (mg kg^{-1} wet weight) in crayfish ranged within 67.77-73.41 in muscles and 68.07-75.34 in hepatopancreas. Taking into account the proportion of dry weight in the tested organs, these concentrations did not differ from iron content determined in our study (Table 2). Similar concentrations of iron level were found for whole crayfish (MOELLER et al. 2003).

MACKEVIČIENĖ (2002) reported the manganese content (mg kg^{-1} wet weight) in crayfish at the level of 5.56±3.87 in muscles, 118±11 in exoskeleton, and 56.00±8.81 in hepatopancreas. Similar concentrations of the element in crayfish muscles and hepatopancreas were also determined by KURUN et al. (2010).

Table 2

Metal concentrations in the hepatopancreas, exoskeleton and abdominal muscles of spinycheek crayfish *(Orconectes limosus* Raff.) and signal crayfish *(Pacifastacus leniusculus* Dana.) from Lake Dgał Wielki and Lake Pobłędzie, Poland

Species	Lake	Organ*	Content ** (mg kg^{-1} wet weight)											
			Hg	Cd	Pb	Cu	Zn	Cr	Ni	Fe	Mn	V	Li	Al
Spinycheek crayfish	Dgał Wielki *n* = 5	*h*	0.035 ±0.011	0.002 ±0.002	0.025 ±0.018	1.70 ±0.29	21.28 ±8.92	0.032 ±0.045	0.156 ±0.147	20.4 ±15.1	4.16 ±1.70	0.000	0.011- ±0.009	52.3- ±20.1
		e	0.007 ±0.007	0.006 ±0.006	0.057 ±0.023	2.92 ±0.33	6.80 ±1.07	0.606 ±0.198	0.782 ±0.092	6.8 ±4.5	35.76 ±8.81	0.245 ±0.033	0.215 ±0.021	123.4 ±8.1
		m	0.028 ±0.034	0.002 ±0.001	0.022 ±0.015	1.21 ±0.41	10.66 ±4.21	0.040 ±0.034	0.000	0.54 ±0.78	0.260 ±0.134	0.000	0.013 ±0.003	26.6 ±14.1
	Pobłędzie *n* = 5	*h*	0.030 ±0.011	0.002 ±0.001	0.018 ±0.018	11.86 ±11.34	20.71 ±10.78	0.072 ±0.053	0.084 ±0.081	32.9 ±17.7	9.12 ±4.76	0.005 ±0.012	0.020 ±0.010	47.2 ±29.0
		e	0.004 ±0.001	0.004 ±0.002	0.079 ±0.058	3.98 ±0.92	7.51 ±3.04	0.446 ±0.087	0.749 ±0.246	7.9 ±9.2	70.78 ±20.08	0.242 ±0.013	0.189 ±0.031	106.3 ±11.4
		m	0.009 ±0.007	0.003 ±0.002	0.033 ±0.025	2.77 ±1.04	14.96 ±1.02	0.092 ±0.037	0.000	2.38 ±0.84	0.76 ±0.30	0.000	0.018 ±0.014	14.8 ±9.1
Signal crayfish	Pobłędzie *n* = 10	*h*	0.045 ±0.017	0.017 ±0.019	0.046 ±0.021	3.18 ±1.12	51.91 ±28.47	0.120 ±0.061	0.379 ±0.298	81.1 ±45.4	6.54 ±5.50	0.015 ±0.013	0.030 ±0.012	47.2 ±25.3
		e	0.020 ±0.026	0.012 ±0.009	0.025 ±0.063	4.34 ±0.53	8.85 ±3.33	0.374 ±0.215	0.518 ±0.083	5.2 ±5.8	91.31 ±60.38	0.182 ±0.038	0.149 ±0.021	99.4 ±10.3
		m	0.026 ±0.045	0.001 ±0.001	0.023 ±0.010	2.02 ±0.68	9.97 ±5.07	0.060 ±0.030	0.016 ±0.032	1.29 ±1.58	0.430 ±0.306	0.000	0.025 ±0.006	34.0 ±11.2

**h* – hepatopancreas; *e* – exoskeleton; *m* – abdominal muscle; ** mean ±standard deviation

The study of Finerty et al. (1990) showed that the vanadium content (mg kg^{-1} wet weight) in organs of crayfish caught in unpolluted areas was 0.000-0.946 in abdominal muscles and 0.000-0.554 in the hepatopancreas. The same authors observed several-fold higher concentrations in muscles (2.190--5.134 mg kg^{-1}) and the hepatopancreas (3.577-7.271 mg kg^{-1}) of crayfish caught from polluted areas.

Lithium occurs in all waters as hydroxide usually combined with colloidal suspension but rarely as an ion. The element also appears in all animal tissues, and is particularly abundant in parenchymatous organs (Kabata-Pendias, Pendias 1999). The available literature lacks publications on the lithium content in crustaceans.

Recent studies have proven that aluminium impairs functions of the hepatopancreas of crayfish (Woodburn et al. 2011) and may also have an adverse effect on the immune system (Ward et al. 2006). The growing interest in this chemical element is stimulated by the fact that when environmental acidification increases, the bioavailability of aluminium increases as well. According to the reports published so far, the aluminium content (mg kg^{-1} wet weight) in crayfish muscles and the hepatopancreas ranged within 0.00-44.59 and 0.00-74.34, respectively (Finerty et al. 1990, Kurun et al. 2010).

The crayfish organs tested in our study presented various degrees of metal bioaccumulation. According to the decreasing metal concentrations, the following order of organs was observed: Al, Mn, Cu, Ni, Cr, V and Li – exoskeleton > hepatopancreas > muscles; Zn and Hg – hepatopancreas > muscles > exoskeleton; Fe – hepatopancreas > exoskeleton > muscles. For lead and cadmium, no regular pattern of their concentrations in the particular organs was observed. The content of these metals depended on the species and on where they were captured (Table 2). Thus, among the three tested organs the highest metal concentration was observed in the exoskeleton or the hepatopancreas, being usually the lowest in muscles, an observation confirmed in publications by other authors (Mackevičienė 2002, Pourang, Dennis 2005, Naghshbandi et al. 2007, Kouba et al. 2010). Lead, cadmium, zinc and mercury were exceptions. The lead and cadmium content was dependent more on the catching location and the species than on the organ. The zinc and mercury concentrations in muscles were higher than in the exoskeleton, but lower than in the hepatopancreas. A similar relationship for zinc was reported by Pourang and Dennis (2005).

A high content of metals in the hepatopancreas results from its function. It is a well-known fact that this organ is involved in absorption and storage of nutrients, and in detoxification processes, which makes it prone to bioaccumulating metals (Anderson et al. 1997b, Pourang, Dennis 2005). High levels of metals in the exoskeleton may be due to the absorption of metals from water (Anderson et al. 1997a, b) or the fact that it is the route for both absorption and excretion of metals from the organism (Mackevičienė 2002). Some of the detected differences in metal concentrations in crayfish organs between the species and the catching areas proved to be statistically insignificant (Table 2). However, significant differences were demonstrated in some earlier research (Kouba et al. 2010). Our results allow us to conclude that metal concentrations in the lakes where the crayfish had lived were within the range of natural background.

Abdominal muscles of crayfish are treated as food, therefore it is essential to compare the determined concentrations of mercury, cadmium and lead with the legally set maximum limits. In Poland, which is a member state of the European Union, concentrations of the three toxic heavy metals are regulated by Commission Regulation (EC) No 1881/2006, which sets threshold levels for certain contaminants in foodstuffs (Dz.U. 2006). This Regulation stipulates that the content of mercury, cadmium and lead in meat of crustaceans shall not exceed 0.50 mg kg^{-1} wet weight. Concentrations of these metals in the meat of the tested crayfish were several-fold lower, hence it should be safe for the health of potential consumers.

CONCLUSIONS

1. With regard to the concentration in crayfish organs, the following order of metals was observed: Al > Mn > Fe > Zn > Cu > Ni > Cr > V > Li > Pb > Hg > Cd.

2. The tested crayfish organs presented different degrees of metal bioaccumulation; regarding concentrations of metals, the following order of organs was observed: the exoskeleton > hepatopancreas > muscles for Al, Mn, Cu, Ni, Cr, V and Li; hepatopancreas > muscles > exoskeleton for Zn and Hg; hepatopancreas > exoskeleton > muscles for Fe. No such explicit correlations were found for Pb and Cd.

3. Some differences in the metal content in crayfish organs between the species and catching areas proved to be statistically insignificant.

4. The concentrations of toxic metals in the abdominal muscles of the tested crayfish were several-fold lower than the maximum permissible levels, which means that the meat was safe for the health of potential consumers.

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EFFECTS OF SOIL PROPERTIES ON COPPER SPECIATION IN SOIL SOLUTION

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Abstract

The numerical speciation analysis relies on quantitative assessment of concentrations of different forms of an element in soil solution (free ions, complex ions, neutral complexes), which have different abilities to react (ion activity). The reactivity affects the element's mobility and bioavailability. This method can be employed to estimate potential bioavailability and toxicity of a given element. This study was undertaken to evaluaate effects of selected soil properties on changes in the total concentration of copper (Cu) and percentages of particular forms of this element in soil solution.

The study was based on a microplot experiment. The investigated factors were the soil texture, pH, organic carbon content and degree of soil copper contamination. Soil solutions were obtained with the vacuum displacement method. The concentration of copper in soil solution was determined with the ICP method, and the percentages of particular copper forms in the total copper concentration were calculated with the MINTEQA 2 software. It was found that copper in the analysed soil solutions occurred mainly in the form of metalorganic complexes. The increasing soil acidity was correlated with an increased percentage of free copper ions and copper complexes with organic matter. Simultaneously, the share of bonds with carbonates, sulfates and hydroxyl groups decreased. A decrease in the percentage of Cu^{2+} free ions in the soil solution was observed in response to an increasing organic carbon content, while the percentage of copper complexes with organic matter rose. The degree of soil copper contamination and soil texture had no influence on the percentages of different copper forms in the soil solution.

Key words: soil solution, copper, soil properties, numerical speciation, MINTEQA.

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WPŁYW WŁAŚCIWOŚCI GLEBY NA SPECJACJĘ MIEDZI W ROZTWORZE GLEBOWYM

Abstrakt

Numeryczna analiza specjacyjna polega na ilościowej ocenie stężenia różnych form badanego pierwiastka w roztworze glebowym (wolne jony, jony kompleksowe, obojętne kompleksy), które charakteryzują się różną zdolnością do reagowania (aktywnością jonów), co wpływa na ich ruchliwość i biodostępność. Metoda ta może więc być narzędziem umożliwiającym prognozowanie ich potencjalnej biodostępności lub toksyczności dla organizmów. W związku z tym podjęto badania dotyczące oceny wpływu wybranych właściwości gleby na zmiany całkowitego stężenia Cu oraz udziału poszczególnych form tego pierwiastka w roztworze glebowym.

W doświadczeniu mikropoletkowym badanymi czynnikami były skład granulometryczny, pH, zawartość Corg. w glebie oraz stopień zanieczyszczenia gleby miedzią. Roztwór glebowy pozyskiwano metodą podciśnieniową. Stężenie miedzi w roztworze glebowym oznaczono metodą ICP, a udział poszczególnych form miedzi w całkowitym stężeniu tego pierwiastka w roztworze glebowym obliczono za pomocą programu komputerowego MINTEQA 2. Stwierdzono, że w analizowanych roztworach glebowych miedź występuje głównie w formie kompleksów metaloorganicznych. Wraz ze wzrostem zakwaszenia zwiększał się w roztworze glebowym udział wolnych jonów i kompleksów miedzi z materią organiczną, a zmniejszał udział połączeń z węglanami, siarczanami i grupami hydroksylowymi. Wraz ze wzrostem zawartości C-org. w glebie, zmniejszał się w roztworze glebowym udział wolnych jonów Cu^{2+}, a zwiększał udział kompleksów miedzi z materią organiczną. Stopień zanieczyszczenia gleby metalami ciężkimi i skład granulometryczny nie wpływały na procentowy udział różnych form miedzi w roztworze glebowym.

Słowa kluczowe: roztwór glebowy, miedź, właściwości gleby, specjacja numeryczna, MINTEQA.

INTRODUCTION

The chemical composition of soil solution can be exploited to predict both bioavailability of trace elements to plants as well as potential contamination of plants grown for feed or food (Hirsh, Banin 1990, Khoshgoftar et al. 2004). Considering the number and diversity of chemical forms of trace elements in soil solution, special attention should be paid to the method of numerical speciation analysis. The method is based on quantifying concentrations of various forms of a given element in soil solution in an attempt to calculate its bioavailability or toxicity to living organisms (Parker, Pedler 1986, Checkai et al. 1987, Minnich et al. 1987, Cancés et al. 2003).

The aim of the study was to determine the total concentration of copper (Cu) an percentages of individual chemical forms of this element in soil solution depending on such soil properties as the soil grain size composition, soil reaction, organic carbon content and degree of soil copper contamination.

MATERIAL AND METHODS

The study was based on a microplot experiment run at the Experimental Station of Agriculture, the Faculty of Biology at Warsaw University of Life Sciences in Skierniewice (central Poland). The microplots were represented by stoneware pots (1.2 m deep and 40 cm wide), which were filled with soil and placed outdoors.

The experimental factors were:

- three levels of soil pH: 4, 5 and 6;
- two soils: loamy sand (7% clay, 6% silt, 87% sand), sandy loam (13% clay, 5% silt, 82% sand);
- three levels of the content of soil organic carbon: 6, 9 and 12g C kg^{-1};
- four degrees of soil contamination with Cu: natural content – 0 (10 mg kg^{-1}), I (30 mg kg^{-1}), II (50 mg kg^{-1}) and III (80 mg kg^{-1}) (Kabata-Pendias et al. 1993).

The experiment consisted of 216 microplots, which represented 72 combinations of the investigated factors. All the factors were observed in three replications arranged in a split-plot experimental design. The experimental samples were collected from each microplot's topmost soil layer (0-30 cm down). The soil samples were dried up to the level of air humidity. Afterwards, they were ground in a china mortar and put through a sieve with the 1 mm diameter mesh net. The samples thus prepared were used to obtain soil solutions according to the vacuum displacement method with a vacuum pump (Dynavac OP4). The total concentration of copper was determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES method) (apparatus: IRYS Advantage ThermoElementar). The MINTEQA2 for Windows software was used for calculations of shares of particular forms of Cu ions in soil solution. The results were statistically analyzed with ANOVA and simple linear regression. The differences between means were analysed with the Tukey's test at p=0.05. Statistical analyses were conducted with Statgraphics 5.1. software.

RESEARCH RESULTS

The total concentration of Cu in the analysed soil solutions ranged from 1.23 to 2.25 μmol dm^{-3} and differed depending on soil properties.

The results of ANOVA showed that the total concentration of copper in soil solutions increased significantly as the soil reaction became more acidic and the degree of copper soil contamination increased. The concentration of Cu was higher in soil solution of loamy sand than of sandy loam. The content of organic carbon did not significantly affect Cu concentrations in soil solution (Table 1).

Table 1

Total Cu concentration (μmol dm^{-3}) versus soil properties

Item	Soil texture		Soil reaction (pH_{KCl})			Content of organic carbon (g kg^{-1})			Degree of Cu soil contamination			
	loamy sand	sandy loam	4	5	6	6.0	9.0	12.0	0	I	II	III
Cu	1.76	1.26	2.05	1.46	1.38	1.52	1.52	1.49	1.23	1.31	1.75	2.25
$LSD_{0.05}$	0.18		0.21			0.27			0.25			

The results of speciation analysis of copper in soil solutions indicated the highest percentage of Cu complexes with organic matter and carbonates. The prevalent forms of copper were Cu-org. complexes, whose average share ranged from 28.2 to 93.2% of the total concentration of copper in soil solution. The second most abundant form of copper observed in soil solutions were Cu-CO_3 complexes, whose contribution to total copper ranged from 12.7 to 62.3%, depending on soil properties (Tables 2–5).

Table 2

Percentages of different copper forms in soil solution according to soil pH value (pH_{KCl})

Form of Cu	Soil pH		
	4.0	5.0	6.0
Cu^{2+}	6.5	5.9	4.4
Cu-org.	93.2	56.2	28.2
$CuOH^+$	tc*	3.0	3.4
Cu-CO_3	tc	34.6	62.3
$Cu(CO_3)_2^{2-}$	tc	tc	1.4

*trace content

The highest percentage (93.2%) of Cu-org. complexes in the total concentration of copper was observed in the soil solutions of most acid soils (Table 2). As the soil pH was increasing, amounts of metalorganic complexes of copper were steadily decreasing, while the percentage of Cu-CO_3 complexes was growing. Copper complexes with carbonates reached 34.6% in the solutions of soils with pH 5.0 and 62.3% in the solutions of soils with pH 6.0. Other forms of copper ($CuOH^+$, Cu-CO_3 and $Cu(CO_3)_2^{2-}$) were not observed in the solutions of soils with the lowest pH value. At the same time, their share in the total Cu concentration in solutions of soils with higher pH was relatively low (Table 2).

Regardless of the degree of soil Cu contamination, the dominant forms of copper in soil solution were Cu-org. complexes, which approximately made up 59% of this element's total soil solution concentration. At the same time, a relatively high percentage (32%) of Cu-CO_3 complexes was observed. In both Cu unpolluted and contaminated soils, an average share of free ions Cu^{2+} was just 5.5% of the total concentration of copper in soil solution (Table 3).

Table 3

Percentages of different copper forms in soil solutions according to soil copper contamination

Form of Cu	Degree of Cu soil contamination			
	0	I	II	III
Cu^{2+}	5.4	5.4	5.7	5.8
Cu-org.	59.4	58.6	59.0	59.8
$CuOH^+$	2.1	2.1	2.2	2.1
Cu-CO_3	33.0	33.0	32.3	31.6
$Cu(CO_3)_2^{2-}$	0.5	0.5	0.5	0.5

Table 4

Percentages of different copper forms in soil solution according to content of soil organic carbon

Form of Cu	Content of soil organic carbon (g kg^{-1})		
	6.0	9.0	12.0
Cu^{2+}	6.5	5.3	5.0
Cu-org.	37.1	61.7	78.8
$CuOH^+$	2.6	2.1	1.8
Cu-CO_3	52.6	30.1	14.2
$Cu(CO_3)_2^{2-}$	0.9	0.5	-

The effect of the content of soil organic carbon on copper speciation in soil solutions was considerable. A two-fold increase of soil organic carbon content nearly doubled the share of Cu-org. in the total concentration of copper in soil solution (from 37.1% to 78.8%). Simultaneously, the percentage of Cu-CO_3 complexes decreased three-fold (from 52.6% to 14.2%). Free ions Cu^{2+} made up 5.0% of all Cu forms in soil solutions of the soils characterized by the highest content of organic carbon, and 6.5% in soil solution of soils with the lowest content of organic carbon. Other forms of copper represented low percentages in the total content of copper in the soil solution (Table 4).

Soil texture had a weak effect on the share of copper forms in the distribution of this element in soil solution. The Cu-org. form was prevalent in the solutions of all the analysed soils: an average share of this form in the total copper concentration in soil solutions was 56.7% in sandy loam soil and 60.8% in loamy sand. The Cu-CO_3 complexes had a large share in the overall distribution of copper forms in soil solutions. Unlike Cu-org., they were more abundant in sandy loam soil solutions (on average 34.5%). In loamy sand soils, complexes of copper with carbonates constituted 30.0% of the total amount of copper in the soil solution (Table 5).

Table 5

Percentages of different copper forms according to soil texture

Form of Cu	Soil texture	
	loamy sand	sandy loam
Cu^{2+}	5.8	5.6
Cu-org.	60.8	56.7
$CuOH^+$	2.9	2.3
Cu-CO_3	30.0	34.5
$Cu(CO_3)_2^{2-}$	0.5	0.5

DISCUSSION

The results of the present study suggest a significant effect of soil properties on the total concentration of copper in soil solution. In solutions of the analysed soils, total copper concentrations increased due to soil Cu contamination and in response to soil acidification. At the same time, the copper total concentration was higher in solution of loamy sand soil. On the other hand, no effect of the soil organic carbon content on the total concentration of Cu in soil solution was observed (Table 1). Such influence of soil properties on copper concentration in soil solution has been confirmed by other authors (Jeffery, Uren 1983, McGrath et al. 1988, Sauvé et al. 1997). The observed effects are attributed to the high affinity of copper for organic matter (Norvell 1991).

The observed high ratio of Cu-org. complexes in the total concentration of copper in soil solution is confirmed in literature (Sanders 1982, 1983, McBride, Bouldin 1984, Fotovat, Naidu 1997). The share of metalorganic complexes estimated in this experiment at 28.2 to 93.2% corresponds well with the results of Smal (1999), which were obtained with the use of SOILCHEM software. The latter showed that copper organic complexes amounted to 75.4% of the total concentration of this element in soil solution of a humus layer of forest soils and 48.4% in agricultural soils. The results of the numerical analysis of organic soil solutions carried out by Rutkowska (2008) with the MINTEQA2 software indicated that the percentage share of Cu-org. complexes in the total concentration of copper in soil solution ranged from 74 to 86%. Even a higher percentage of copper organic complexes, reaching 99.5%, was found by McBride and Bouldin (1984).

In the analysed solutions, copper was also found in the form of complexes with carbonates, whose share ranged from 12.7 to 62.3% of the total concentration of copper in soil solution, depending on soil reaction and soil richness in organic carbon (Tables 2-5). The share of Cu-CO_3 complexes in soil solution decreased with an increase of soil organic carbon content and a change of soil reaction into acid one. Smal (1999), Saeki et al. (2002) and Rutkowska (2008) confirmed presence of copper in the form of Cu-CO_3 complexes in soil solution. However, none of these authors obtained a percentage of this form in soil solution as high as observed in the present study. Rutkowska (2008) showed that solutions of agricultural soils contain complexes of copper with carbonates on a level ranging from 5.0 to 6.8% of the total concentration of Cu in soil solution.

Regardless of the soil properties investigated, a small percentage of free Cu^{2+} ions was observed in the total concentration of copper in soil solution. Similar observations were reported by Nolan et al. (2003) who showed a 5% share of free Cu^{2+} ions in the soil solutions analysed in their research. A small share of free Cu^{2+} in soil solution, no more than slightly over 12%, was also observed by Sanders (1983), McGrath et al. (1988) and Fotovat, Naidu (1997). According to Yuan (2009), Wójcikowska-Kapusta, Niemczuk (2009) and Jaworska, Dąbkowska-Naskręt (2012) this result is due to the strong binding of copper by the soil solid phase. As claimed by Cancés et al. (2003), speciation of copper in soil solution is conditioned by soluble forms of organic substance. Salam, Helmke (1998) suggest that complexing is a reaction that substantially conditions the course of copper speciation in soil solution.

According to Smal (1999), Saeki et al. (2002), Artiola (2005) and Rutkowska (2008) copper in soil solution can also form bonds with other nonorganic ligands (e.g. SO_4^{2-}, NH_3, NO_3^-, $H_2PO_4^{2-}$, Cl^-, OH^-). In the numerical speciation analysis carried out in this study, a small proportion of $CuOH^+$ bonds was detected in the analysed soil solutions, but other forms were not observed (Tables 2-5).

CONCLUSIONS

Based on the numerical speciation analysis of copper in the solutions of soils with different physical and chemical properties it was concluded that:
- the estimates derived by running a MINTEQA2 software package of the percentages of ion forms in soil solutions showed that copper occurred in soil solution in the form of complexes with humus acids;
- among the soil properties studied, soil reaction had the strongest influence on the per cent shares of copper forms in soil solution; in a more acid soil, the ratio of copper free ions and copper complexes with organic matter, bonds with carbonates as well as sulfates and hydroxyl groups decreased;
- the degree of soil Cu contamination and soil texture had no effect on percentages of different forms of copper in soil solution;
- with a higher organic carbon content in soil, soil solution contained a lower share of free copper ions, whereas the share of copper complexes with organic matter increased.

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CHANGES IN THE CONTENT OF SOME MACROELEMENTS IN MAIZE (*ZEA MAYS* L.) AFTER APPLICATION OF FUEL OIL AND DIFFERENT NEUTRALIZING SUBSTANCES TO SOIL[*]

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Abstract

The aim of the present study was to determine the effect of incremental doses of heating oil (0-20 g kg of soil) and neutralizing substances – mineral and organic ones – on the content of some macronutrients in maize (*Zea mays* L.). The effect of heating oil and neutralizing substances on concentrations of the analyzed macronutrients in maize was varied. The content of phosphorus in maize was positively correlated to incremental doses of heating oil; the accumulation of sodium, calcium and magnesium in maize assumed a parabolic function, while changes in the content of potassium were irregular. All the substances applied to soil had a limiting effect on the content of phosphorus but stimulated (except nitrogen) the accumulation of potassium in maize grown on soil contaminated with heating oil. Out of the tested soil-amending substances, bentonite was the most stimulating one with respect to sodium and the most limiting to calcium in maize. Zeolite and calcium oxide showed a stimulating effect and increased the content of calcium and magnesium in maize. Compost had a similar effect on the content of calcium, whereas nitrogen stimulated the accumulation of magnesium in maize.

Key words: fuel oil contamination, nitrogen, compost, bentonite, zeolite, calcium oxide, maize, macroelements.

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ZMIANY ZAWARTOŚCI WYBRANYCH MAKROSKŁADNIKÓW W KUKURYDZY (*ZEA MAYS* L.) PO APLIKACJI OLEJU OPAŁOWEGO I RÓŻNYCH SUBSTANCJI NEUTRALIZUJĄCYCH DO GLEBY

Abstrakt

Celem badań było określenie wpływu wzrastających dawek oleju opałowego (0-20 $g \cdot kg^{-1}$ gleby) i substancji neutralizujących – mineralnych i organicznych na zawartość wybranych makroskładników w kukurydzy (*Zea mays* L.). Wpływ oleju opałowego i substancji łagodzących na zawartość badanych makropierwiastków w kukurydzy był zróżnicowany. Zawartość fosforu w kukurydzy była dodatnio skorelowana ze wzrastającymi dawkami oleju opałowego, nagromadzenie sodu, wapnia i magnezu przybierało funkcję paraboliczną, a zmiany zawartości potasu nie były jednoznacznie ukierunkowane. Wszystkie zaaplikowane do gleby substancje działały ograniczająco na zawartość fosforu, a także stymulująco (oprócz azotu) na akumulację potasu w kukurydzy uprawianej na glebie zanieczyszczonej olejem opałowym. Bentonit był dodatkiem najbardziej stymulującym zawartość sodu i najbardziej ograniczającym nagromadzenie wapnia w kukurydzy. Wykazano, że zeolit i tlenek wapnia działały stymulująco i wpłynęły na zwiększenie zawartości wapnia i magnezu w kukurydzy. Podobny wpływ na zawartość wapnia miał kompost, a na nagromadzanie magnezu w kukurydzy – azot.

Słowa kluczowe: zanieczyszczenie olejem opałowym, azot, kompost, bentonit, zeolit, tlenek wapnia, kukurydza, makroskładniki.

INTRODUCTION

Petroleum products become a problem when they turn into soil and water environment pollutants. In Poland, former military grounds, petroleum refinery facilities, petrol stations, intermediate pumping stations or pipelines can be contaminated with petroleum substances. Contamination at such locations exceeds permissible values and can be found as deep as 30 meters underground, for example on airfields formerly occupied by the Soviet Army. These contaminants cause modifications in soil properties (WYSZKOWSKA, WYSZKOWSKI 2006, 2010, ZIÓŁKOWSKA, WYSZKOWSKI 2010, WYSZKOWSKI, SIVITSKAYA 2012, 2013) and threaten surface and ground waters (NOWAK 2008). Regardless of the source of contamination, petroleum substances which permeate into soil enter the soil-plant-animal-human chain (PRZYBULEWSKA 2005, WYSZKOWSKI, ZIÓŁKOWSKA 2013). Hydrocarbons from petroleum products form a fatty film on underground and aerial parts of plants, which depresses the plant's transpiration and respiration rates, impairs the permeability of plant membranes and causes disorders in metabolic processes, leading to changes in the chemical composition of plants (WYSZKOWSKA, WYSZKOWSKI 2006, WYSZKOWSKI et al. 2004, WYSZKOWSKI, ZIÓŁKOWSKA 2009, 2011, ZIÓŁKOWSKA, WYSZKOWSKI 2010). Petroleum compounds are responsible for disorders in the growth of plants, weaker tolerance to pests, disruptions of the growth rate (ZIÓŁKOWSKA, WYSZKOWSKI 2010). They block enzymatic systems, which causes physiological changes and necrosis of tissues and cells (PRZYBULEWSKA 2005). It is therefore important to restrain the effect of petroleum on plants.

The aim of present study was to determine the effect of incremental doses of heating oil and alleviating substances – mineral and organic ones – on the content of magnesium and other macronutrients in maize (*Zea mays* L.).

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 of the grain-size distribution of 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%). The soil properties were as follows: 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. The soil was contaminated with heating oils in the amounts of 0, 5, 10, 15 and 20 g kg^{-1}. In order to reduce the effect of fuel oil on soil, the following neutralizing substances were applied: nitrogen as urea (200 mg N kg^{-1} of soil), compost (3%), bentonite and zeolite (2% relative to the mass of soil) and 50% calcium oxide in a dose corresponding to one full hydrolytic acidity (10.93 g Ca kg^{-1} of soil). Compost was prepared from leaves (44%), manure (33%) and peat (23%) composted for six months. The concentration of macroelements in these substances (in g kg^{-1}) was as follows: compost: P – 2.32, K – 1.33, Mg – 1.47, Ca – 15.86, Na – 0.12; bentonite: P – 0.47, K – 2.43, Mg – 5.03, Ca – 26.72, Na – 12.11; calcium oxide: P – 0.10, K – 0.77, Mg – 2.65, Ca – 347.99, Na – 0.07. Apart from heating oil and the alleviating substances, the soil (9 kg in each pot) was enriched with macro- and micronutrients, added in the following doses: 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. Once the soil was prepared as described above, cv. Reduta maize (*Zea mays* L.) was sown. The trials were conducted on 8 plants per pot. Maize plants were harvested during the intensive stem elongation phase and plant samples were taken for laboratory analyses.

The plant material was dried at 60°C, ground and mineralized in concentrated sulphuric (VI) acid with hydrogen peroxide added as a catalyst. The solution thus prepared underwent the following determinations: content of phosphorus (P^{5+}) by colorimetry with the vanadium-molybdenum method; content of potassium (K^+), sodium (Na^+) and calcium (Ca^{2+}) – by the atomic emission spectrometry method – ESA and the content of magnesium (Mg^{2+}) – with atomic absorption spectrometry ASA (OSTROWSKA et al.

1991). The grain-size distribution in the soil was determined with the laser method using a Mastersizer 2000 meter. Prior to the establishment of the trials, the soil was also tested for its reaction (pH) with the potentiometric method in KCl aqueous solution of the concentration of 1 mol dm^{-3}, hydrolytic acidity (Hh) and sum of the exchange capacity of cations (S) with Kappen method, content of organic carbon (C_{org}) with Tiurin method, content of available phosphorus and potassium with Egner-Riehm method, available magnesium with Schachtschabel method (Lityński et al. 1976), and trace elements according to the US-EPA3051 methodology (1994). The results were processed statistically by means of two-factor analysis of variance ANOVA from a Statistica software package (StatSoft, Inc. 2010).

RESULTS AND DISCUSSION

The content of macronutrients in the maize plants depended on both the dose of heating oil and soil amending substances (Tables 1-2). In the non-amended treatments, the content of phosphorus in maize tended to increase (r=0.885), especially in response to high doses of heating oil. No such tendency existed for potassium, although the treatment contaminated with 20 g of heating oil per kg of soil contained 33% less potassium than the control (no heating oil). Under the influence of increasing doses of heating oil, the sodium concentration increment (+19%) in maize in the objects with non-amended soil was parabolic, with the peak content at the dose of 15 g of heating oil per kg of soil. Analogously, the content of calcium and magnesium increased up to the dose of 10 g of heating oil per 1 kg of soil, but fell in the aerial parts of maize in response to the highest dose of the contaminant (20 g kg^{-1} of soil).

Petroleum substances modify the accumulation of most of macronutrients in plants. Dimitrov and Mitova (1998) found that concentrations of phosphorus and calcium in plants do not change significantly under the effect of diesel oil. Wyszkowski and Ziółkowska (2009) observed a stimulating effect of petrol in the amount of 10 g kg^{-1} of soil on the content of magnesium and calcium in maize, a finding which is confirmed by the present study. An observation that is contradictory to the results reported by the above authors was made for calcium and magnesium, as in the current research the dose of 10 g of heating oil per kg of soil stimulated the accumulation of these elements in maize. In another study, conducted by Krzebietke and Sienkiewicz (2010), soil contamination with anthracene and pyrene caused an increase in the content of calcium and sodium in butterhead lettuce. The experiment completed by Wieczorek et al. (2006) showed that anthracene resulted in an increased content of magnesium in yellow lupine. These relationships were partly confirmed in the authors' own research. The effect of

Table 1

Content of phosphorus, potassium and sodium in maize *Zea mays* L. (g kg^{-1} d.m.)

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
			Phosphorus				
0	1.07	1.09	0.78	0.90	0.79	0.73	0.89
5	0.88	0.96	0.85	0.83	0.77	0.70	0.83
10	1.19	0.99	0.92	1.05	1.00	0.73	0.98
15	1.77	0.93	1.32	1.41	1.74	1.35	1.42
20	1.80	0.91	1.66	1.39	1.27	1.91	1.49
Average	1.34	0.98	1.11	1.12	1.11	1.08	1.12
r	0.885**	-0.873**	0.943**	0.908**	0.754**	0.887**	0.913**
LSD	a – 0.12**, b – 0.13**, $a \cdot b$ – 0.30**						
			Potassium				
0	34.95	29.55	34.70	39.13	33.23	38.14	34.95
5	28.32	20.70	35.69	33.72	30.53	33.23	30.36
10	37.16	22.42	36.42	38.39	37.90	33.23	34.25
15	31.27	24.63	37.16	30.28	33.23	34.95	31.92
20	23.65	31.76	36.67	29.30	27.58	26.35	29.22
Average	31.07	25.81	36.13	34.16	32.49	33.18	31.07
r	-0.580	0.281	0.892**	-0.809**	-0.356	-0.802**	-0.638*
LSD	a – 1.40**, b – 1.54**, $a \cdot b$ – 3.44**						
			Sodium				
0	1.06	1.12	1.09	4.01	1.26	1.23	1.06
5	1.14	1.12	1.29	2.17	1.14	1.26	1.14
10	1.17	1.17	1.12	1.74	1.06	1.14	1.17
15	1.26	1.17	1.29	2.36	1.09	1.17	1.26
20	1.14	1.31	1.23	3.30	1.23	1.09	1.14
Average	1.16	1.18	1.20	2.72	1.16	1.18	1.16
r	0.630*	0.883**	0.477	-0.209	-0.207	-0.861**	-0.114
LSD	a – 0.08**, b – 0.09**, $a \cdot b$ – 0.20**						

LSD for: a – heating oil dose, b – type of neutralizing substance, $a \cdot b$ – interaction;
significant for: *P=0.05, **P=0.01, r – correlation coefficient

Table 2

Content of magnesium and calcium in maize *Zea mays* L (g kg^{-1} d.m)

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
Calcium							
0	5.73	9.40	9.16	6.71	8.18	11.11	8.38
5	6.22	7.44	7.93	3.78	7.20	9.16	6.96
10	6.71	4.76	6.22	3.53	8.42	6.96	6.10
15	6.71	5.24	5.98	3.78	5.98	6.71	5.73
20	5.24	5.73	6.47	3.78	6.71	5.00	5.49
Average	6.12	6.52	7.15	4.32	7.30	7.79	6.53
r	-0.121	-0.791**	-0.856**	-0.691*	-0.646*	-0.977**	-0.944**
LSD	a – 0.45**, b – 0.50**, $a \cdot b$ – 1.11**						
Magnesium							
0	4.34	5.10	4.37	4.97	5.00	5.41	4.87
5	4.80	5.94	4.75	5.05	6.41	5.95	5.48
10	4.93	6.25	4.19	4.71	6.21	5.51	5.30
15	4.85	6.03	4.47	4.32	5.25	5.36	5.05
20	4.55	5.65	4.69	4.75	4.81	4.18	4.77
Average	4.69	5.79	4.50	4.76	5.54	5.28	5.09
r	0.314	0.422	0.242	-0.644*	-0.337	-0.730*	-0.333
LSD	a – 0.12**, b – 0.13**, $a \cdot b$ – 0.29*						

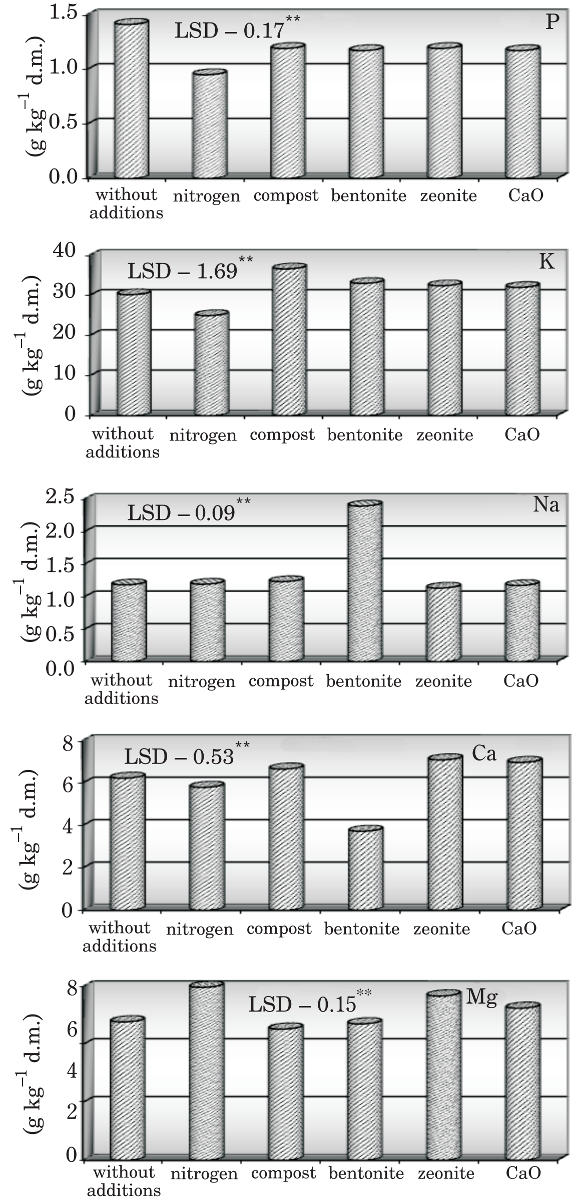
LSD for: a – heating oil dose, b – type of neutralizing substance, $a \cdot b$ – interaction;
significant for: *P=0.05, **P=0.01,, r – correlation coefficient

rising doses of diesel oil in the content of macronutrients has also been investigated by Wyszkowski and Wyszkowska (2005), who concluded that diesel oil stimulated the accumulation of sodium, potassium and calcium in aerial parts of maize. The results of the present study confirm this dependence only for calcium and sodium in soil contaminated with the lower doses of heating oil.

The soil amending substances applied to soil not contaminated with heating oil had little effect on the content of phosphorus in maize (Tables 1-2). Their reducing effect was observed in the treatments enriched with calcium oxide, zeolite and compost. The lowest concentrations of phosphorus in maize were found in the treatment with calcium oxide. Most of the soil-amending substances also depressed the content of potassium in maize. The only substances which caused an increase in the content of potassium were bentonite and calcium oxide. Compared with the objects without soil amending substances, the content of sodium in maize rose by a few to less than twenty per cent following the application of nitrogen, zeolite or calcium oxide, but was four-fold higher when the soil had been enriched with bentonite. In maize grown on soil not polluted with heating oil, all the alleviating substances stimulated an increase in the content of calcium and magnesium. The most effective proved to be calcium oxide, which contributed to a double rise in the content of calcium and a 25% increase in content of magnesium.

All the soil-amending substances were shown to have a negative effect on the content of phosphorus in maize grown on soil contaminated with heating oil (Figure 1), whose content fell by 33% in the objects with nitrogen, 17% in the treatments with bentonite and calcium oxide, and 16% when compost or zeolite had been added, all compared to the objects with no soil alleviating substances. All the substances except nitrogen (-17%) caused an increase in the content of potassium in maize. Its highest rise was observed in the objects with compost (+21%) compared to the non-amending objects. The other soil amending substances caused a non-significant increase in potassium (less than 10%). Bentonite was the only substance which caused an increase (double) in the content of sodium in maize. Compost, zeolite and calcium oxide contributed to an increase in the content of calcium (7, 14 and 12%, respectively) and a decrease in the content of these element in maize (-40%) versus the objects without soil amending substances. The substances which had a beneficial effect on the content of magnesium in maize were nitrogen (+25%), zeolite (+19%) and calcium oxide (+10% versus the objects without alleviating substances).

Filipek-Mazur and Gondek (2006) observed a stimulating effect of mineral fertilizers on the content of potassium, as well as organic fertilizers on the content of phosphorus in oat straw. The negative effect of nitrogen on the content of phosphorus and potassium in crops was recorded by Wyszkowski and Wyszkowska (2005). These authors confirmed the stimulating effect of nitrogen on the accumulation of calcium and magnesium in aerial parts of plants. The present results show an increase in the content of magnesium in maize caused by nitrogen, zeolite and CaO. Rabikowska (1999) found more phosphorus and potassium in maize fertilized with farmyard manure than in the control plants. She also reported that nitrogen fertilization had no effect on the content of potassium in plants. These observations have been verified in the present study in the objects with compost. The effect of FYM fertilization on the content of macronutrients decreased in the following order: P > K > N > Ca > Mg, while that of nitrogen was as follows: N > Ca > Mg > K > P (Rabikowska 1999). Wyszkowski and Ziółkowska (2009) observed a stimulating influence of bentonite (7-, 9- and 11-fold increase) on the content of sodium and noticed a positive effect of calcium oxide on the content of calcium in plants, as well as a negative effect of both substances on the content of magnesium (Wyszkowski, Ziółkowska 2009), observations which



differences significant for: $^{*}P$=0.05, $^{**}P$=0.01

Fig. 1. Content of macroelements in maize *Zea mays* L. (average from objects contaminated with 5-20 g heating oil per kg of soil)

have been partly confirmed by the present research. According to Wyszkowski and Radziemska (2009), among all the tested neutralizing substances, calcium oxide had the strongest effect on the content of macronutrients, mainly calcium and sodium, in plants. Ciećko et al. (2004, 2005) observed a stimulating influence of compost, lime and bentonite on the content of magnesium (Ciećko et al. 2005), and reverse effect on the content of potassium (Ciećko et al. 2004) in the majority of the parts of the examined plants. In another study conducted by Wyszkowski (2007), application of CaO depressed concentrations of macronutrients (except calcium) in aerial parts of plants, including the content of magnesium, which was most strongly reduced.

CONCLUSIONS

1. The effect of heating oil and alleviating substances on concentrations of the analyzed macronutrients in maize was varied.
2. The content of phosphorus in maize was positively correlated to incremental doses of heating oil; the accumulation of sodium, calcium and magnesium in maize assumed a parabolic function while changes in the content of potassium were irregular.
3. All the substances applied to soil had a limiting effect on the content of phosphorus but stimulated (except nitrogen) the accumulation of potassium in maize grown on soil contaminated with heating oil.
4. Out of the tested soil-amending substances, bentonite was the most stimulating one with respect to sodium and the most limiting to calcium in maize.
5. Zeolite and calcium oxide showed a stimulating effect and increased the content of calcium and magnesium in maize. Compost had a similar effect on the content of calcium, whereas nitrogen stimulated the accumulation of magnesium in maize.

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THE INFLUENCE OF ALTERED HOMEOSTASIS ON MAMMARY GLAND RUBIDIUM CONCENTRATIONS IN DOGS

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Abstract

Rubidium is an alkaline metal with exceptional chemical activity. Together with lithium, sodium, potassium and cesium, it belongs to group I of the periodic table of elements.

The aim of this study was to provide data on the rubidium content in normal mammary gland tissue and adenocarcinomas and to elucidate the effects of altered homeostasis on this element in the course of mammary gland neoplastic disease in dogs. The investigation was performed to confirm the hypothesis put forward in human research that this metal is cumulated in neoplastic tumours of the mammary gland.

The research material included *post mortem* collected healthy mammary glands of bitches and mammary gland neoplastic tumours obtained during routine surgical treatments as well as during necropsies. The content of rubidium was determined by the inductively coupled plasma mass spectrometry (ICP-MS) method.

The highest rubidium content was observed in the tissues with neoplastic lesions, where it averaged 10.72 mg kg^{-1} wet mass. In the healthy tissues, the content of this metal was on a much lower level. The mean rubidium concentration in mammary glands without pathological changes was 1.91 m kg^{-1} wet mass. The statistical analysis showed a significant difference (P=0.01) in the rubidium content between the investigated groups. Higher rubidium concentrations were observed in the tissues with neoplastic lesions. This phenomenon is similar to disturbances of the ionic balance observed in the case of breast cancer in women.

Key words: rubidium, dogs, mammary glands, health, tumours.

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WPŁYW ZABURZONEJ HOMEOSTAZY NA ZAWARTOŚĆ RUBIDU W GRUCZOLE SUTKOWYM SUK

Abstrakt

Rubid jest metalem alkalicznym o wyjątkowej aktywności chemicznej. Podobnie jak lit, sód, potas i cez, należy do I grupy układu okresowego pierwiastków.

Celem badań było dostarczenie danych na temat zawartości rubidu w zdrowych gruczołach sutkowych samic psów oraz w gruczolakorakach w celu zrozumienia wpływu zaburzeń homeostazy na kumulację tego pierwiastka w przebiegu nowotworów. Badania prowadzono w celu potwierdzenia hipotezy wysuniętej na podstawie badań dotyczących wzrostu zawartości tego metalu w guzach nowotworowych gruczołu sutkowego kobiet. Materiał badawczy obejmował pośmiertnie zebrane zdrowe gruczoły sutkowe suk oraz wycinki guzów nowotworowych gruczołu sutkowego uzyskane podczas rutynowych zabiegów chirurgicznych. Zawartość rubidu określono za pomocą metody ICP-MS. Najwyższą jego koncentrację stwierdzono w tkankach ze zmianami nowotworowymi, w których średnia zawartość tego pierwiastka wynosiła 10,72 mg kg^{-1} świeżej masy. W zdrowych tkankach wykazano znacznie mniejszą zawartość rubidu, średnio 1,91 mg kg^{-1} świeżej masy. W analizie statystycznej stwierdzono istotną różnicę (p = 0,01) między badanymi grupami. Wyższe zawartości rubidu zaobserwowano w tkankach dotkniętych procesem nowotworzenia. Zjawisko to ma podobny charakter do zaburzeń równowagi jonowej występującej w przebiegu nowotworów sutka u kobiet.

Słowa kluczowe: rubid, psy, gruczoły sutkowe, zdrowie, guzy.

INTRODUCTION

Rubidium is an alkaline metal with exceptional chemical activity. Together with lithium, sodium, potassium and cesium, it belongs to group I of the periodic table of elements; regarding its occurrence in the earth's crust, rubidium is the 16th most frequent element (Selin, Teeyasoontranont 1991). It is quite common in the environment and is able to replace potassium in metabolic reactions, which may cause disturb their course. The role of rubidium in to animals has not been finally determined. The studies conducted to date indicate that it may be essential; however, there is a small margin between its possible required concentration and its toxic effect on an organism (Angelov 1994, Anke, Angelov 1995, Kośla et al. 2002, Milman et al. 2006, Skibniewska et al. 2012). This element is assimilated very quickly and effectively, but excreted very slowly (Schäfer, Forth 1983). The tissues in which the process of carcinogenesis takes place have a special predilection for the accumulation of rubidium (Rizk, Sky-Peck 1983, Drake, Sky-Peck 1989, Tamano et al. 2002). In studies conducted on mice suffering from experimentally induced cancer, a 10-fold increase of the level of rubidium in the tumour tissue as compared to healthy tissues was found (Feldstein et al. 1998). An analogously high content of this element was recorded in malignant breast tumours in women (Majewska et al. 1998).

Only few publications dedicated to the rubidium content in malignant tumours in animals have been published until now. The aim of the current research was to expand our knowledge on the content of rubidium in the tissue of mammary glands of dogs and the changes in its concentration, depending on ongoing carcinogenesis processes. Also, an attempt was made to verify the hypothesis which assumes that during carcinogenesis in dogs, phenomena similar to those described in people could appear, e.g. disturbance of homeostasis causing an increase of concentrations of certain elements in tumour tissues.

MATERIAL AND METHODS

The research material was divided into two groups (Table 1). The first one consisted of *post mortem* collected bitch mammary glands (n=10) without tumour lesions. The second group included neoplastic tumours (n=30) collected during surgical operations, tumours derived from bitch subjected to euthanasia due to advanced cancer disease and from animals dead because of mammary gland cancer. Because of the character of the study which required collection of *post mortem* tissues and tissues obtained from routine mastectomy procedures performed in veterinary clinics, no consent of ethical commission was necessary according to the Polish law. The tissues which revealed dysplasia and hyperplasia were excluded from the study. The specific nature of the research meant that the analysis covered only tumours with dimensions allowing collection of a representative sample for determination of the elemental content as well as histopathological examination. Due to a high morphological diversity of tumours of mammary glands in bitches, the study was conducted on a group of neoplasms of the epithelial origin which were isolated on the grounds of histopathological analysis. This group consisted of adenocarcinomas. The effect of animal age on the tumours tissue distribution of rubidium was also determined in the investigated group. Dogs were divided into the following age categories: 5-9 years of age, 10-13 years and 14-16 years.

The control group consisted of mammary glands collected from animals with documented origin which died during various incidents. The animals from that group were not in the lactation stage. The mean age of the examined healthy animals was 7 years, lower than in the group of dogs with neoplastic changes, where it reached 11 years. In each case, *ca* 5 g of fresh tissues were obtained and the collected material was dissected appropriately with a plastic blade. After preservation in 10% formaldehyde, fragments of tissues were subjected to pathomorphologic examination; the remaining part of each sample was kept frozen in plastic bags until determinations of the content of the elements.

Table 1

Characteristics of the dogs studied

Item	Control group	Neoplastic group
Number of females	10	30
Mean age (years)	7	11
Range of age females (years)	4-10	5-16

Before commencing the chemical analyses, the tissues were homogenized and then 0.5 g of every sample was placed in a Teflon vessel and mineralized in nitric acid under pressure in a microwave. The content of rubidium was determined by the inductively coupled plasma mass spectrometry (ICP-MS) method, with relation to reference material. Statistical calculations of the data was performed using Statistica Pl, version 10 (Statsoft Inc.). Because variables were not normally distributed, nonparametric tests were used for statistical analyses. Dependences of the rubidium concentration associated with the impact of the health state and age were examined using the Kruskal-Wallis *W* test. Differences were considered as significant at the levels of $P = 0.05$ and $P = 0.01$. All data were expressed as an arithmetic mean ± standard deviation SD and median. All statistical analyses were performed on wet mass concentration.

RESULTS

The lowest value of rubidium was found in mammary glands with no visible pathologic changes, i.e. 1.91 mg kg^{-1} wet mass, constituting simultaneously the control group, whereas the highest value was recorded in tumours of the epithelial origin (10.72 mg kg^{-1} wet mass). The presence of a highly significant difference ($P = 0.01$) between the group of epithelial originated tumours and control group was recorded (Table 2).

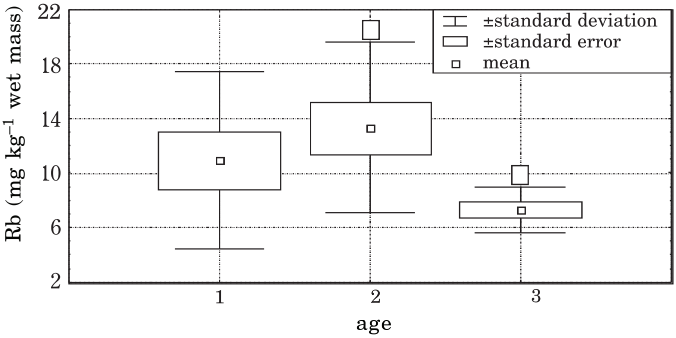
Detailed analysis of the interaction between age and rubidium content in adenocarcinomas (Figure 1.) showed that there was a statistically significant difference between animals from group 10-13 years and 14-16 years. The highest average content of 13.3 mg kg^{-1} of rubidium was observed in individuals from the group 10-13-year-old. It was almost 2-fold higher compared to content of this metal in the oldest females aged 14-16 years. In general, rubidium concentrations increased from the age of 5 to 13 years, and then decreased to >14 years.

Table 2

Statistical distribution of parameters for rubidium concentration ($mg\ kg^{-1}$ wet mass) in the mammary glands tissues without neoplastic lesions and with adenocarcinomas

Statistical parameters	Healthy mammary glands	Adenocarcinomas
Group size (*n*)	10	30
Arithmetic mean	1.91*A*	10.72*A*
Standard deviation	1.97	5.79
Lower quartile (25%)	1.06	6.07
Median	1.46	8.73
Upper quartile (75%)	1.65	14.86

A – statistically significant differences between the groups at $P \leq 0.01$



n=10 (5-9 years), *n*=11 (10-13 years), *n*=9 (14-16 years)

*statistically significant differences between the groups at $P = 0.05$

Fig. 1. Effect of age on rubidium concentration in adenocarcinomas; data are expressed as arithmetic means, standard deviation and standard error

DISCUSSION

The chance to understand the role of mineral elements in human and animal health is created by the technological progress, which has helped to improve the quality of analysis of bioelements in tissues. Analytical techniques demonstrate many types of activity in organisms of humans as well as animals. They include, *inter alia*: structural i.e. inorganic function, electrochemical, and catalytic activities and other phenomena, unknown before, as well as mixed functions (Bowen 1988).

Many observations indicate that various metals play a catalytic role in oxidative damage of biological macromolecules (Ebrahim et al. 2007, Naga-Raju et al. 2006). Cancer is a complex, multi-factor disease. Despite the primary role of such environmental carcinogens such as radiation, viruses and chemical compounds, the formation and development of tumours is induced by many secondary factors. Breast cancer is a hormone-dependent cancer; mineral elements act here by mediation of various mechanisms on the cellular and sub-cellular level (Naga-Raju et al. 2006, Schazer 1980, Kanias et al. 1994). They may be described as interactions between elements and hormones which regulate metabolism of biochemical substrates. Hence, we may and should expect the relationship between the content of mineral elements in tissue and cancer of mammary gland (Ebrahim et al. 2007). Exposure to various carcinogens, including certain mineral elements, induces formation of free radicals, which play a significant role in development of various types of neoplasm. The discussed activity is mainly manifested in mutations and damages of DNA, leading to development of tumour (Naga-Raju et al. 2006, Hartwig 2000).

At already mentioned, the problem of possible indispensability of rubidium for humans and animals still remains unclear. Rubidium is found in the first group of the periodic table of elements and is present in all biological systems (Fieve, Jamison 1982). Alkali metals trespass biological barriers according to the following pattern: lithium substitutes sodium, whereas rubidium and cesium substitute potassium. In physiological processes, rubidium behaves like potassium and it is considered that the both discussed elements supplant each other to a great extent (Angelov 1994, Milman et al. 2006). Until now, few studies concerning the rubidium content in human and animal tissues have been carried out. Their results suggest that its level for example in muscles of dairy cows varies within the limits of 15-26 mg kg^{-1} dry mass whereas in the liver, it is equal to 25-38 mg kg^{-1} dry mass (Kośla et al. 2002). In the respective studies concerning the content of rubidium in human liver it was found in the concentration of 7 mg kg^{-1} fresh mass of the organ, on average, which corresponds to *ca* 24 mg kg^{-1} dry mass. Other results suggest that it is equal to 17 mg kg^{-1}, on average. Therefore, these values are similar to the data concerning animals. Also, no statistically significant differences in the rubidium level depending on climatic zones inhabited by different human populations were found: e.g., the content of rubidium in the liver of native Danish people was similar as that one found in the Inuit who live in Greenland (Milman et al. 2006). In the studies concerning the presence of mineral elements in mammary glands of humans, it was found that the content of rubidium increased in the tissues subject to the process of carcinogenesis; however, values reported by different authors are divergent. Naga-Raju et al. (2006) observed that the mean rubidium content in breast cancer was 22.27 mg kg^{-1} dry mass of tissue and in healthy tissues, it amounted to 12.45 mg kg^{-1}; therefore, the level of rubidium in cancerous tumours was almost twice as high as in the healthy tissues. Both discussed groups differed significantly statistically. Similar relationships were also reported by Hoong et al. (1993). Somewhat different results were obtained by Ebrachim et al. (2007) and Garg et al. (1990). The mentioned authors found an increased level of rubidium in breast cancers as compared to the tissues without those lesions, but it was not as spectacular as in the studies of other research teams. On the other hand, there is lack of respective data concerning the content of rubidium in mammary gland tumours of carnivorous animals. The elaborations concerning the daily intake of the discussed element by the representatives of the mentioned animal species are unavailable. It was revealed that the daily rubidium intake in humans varied within the limits of 1-5 mg (Angelov 1994). In own studies on the tissue of mammary gland which was not subject to carcinogenesis, the average rubidium content was equal to 1.91 mg kg^{-1} wet mass of the organ, which approximately corresponds to 6.7 mg kg^{-1} dry mass. The highest rubidium level was recorded in adenocarcinomas. It equalled 14.14 mg kg^{-1} wet mass of the organ, which translates to about 50 mg kg^{-1} dry mass i.e. the level which already determines the border between micro- and macroelements in tissues because – according to the universally adopted criteria – the elements which occur physiologically in a concentration above 0.01 wt%, that is above 50 mg kg^{-1} dry mass of tissue. are recognized as macroelements (Andrews et al. 2004, Lewińska-Preiss 2011). The very high concentration which was observed herein may be the evidence of extreme insufficiency of homeostatic processes in the examined tissue.

CONCLUSIONS

To sum up, the observed considerable accumulation of rubidium results from advancement of neoplastic changes in the mammary gland of the studied dogs. This phenomenon is similar to the disturbances of ionic balance observed in cases of breast cancer in women. Differences in the level of rubidium in the tissues of adenocarcinomas as compared to pathologically unchanged tissues may suggest a potential applicability of determinations of the discussed element as a discriminating factor.

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EFFECT OF FERTILIZATION ON THE CONTENT OF MACRONUTRIENTS IN FRUITS OF MILK THISTLE (*SILYBUM MARIANUM* L. GAERTN.)

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Abstract

Milk thistle was grown on a substrate with the texture of light soil, slightly acid in reaction and moderately abundant in available phosphorus, potassium and nitrogen. The effect of incremental nitrogen fertilization (0.0, 1.0, 2.0, 3.0 g N per pot) against the background of constant PK fertilization (P – 1.0, K – 1.5 g per pot) on the content of macronutrients in fruits of two forms of milk thistle, a cultivar called Silma and a population line bred in Poland, was tested in the first trial; the influence of boron fertilization was assessed in the second series and the impact of forms of magnesium fertilizers ($MgCl_2$ and $MgSO_4$) was analyzed in the third variant of the experiment. The variety-specific traits had a significant effect on the content of nitrogen, phosphorus and potassium, but did not affect the concentrations of calcium, magnesium and sodium. The content of nitrogen in achenes of the population plant was 26.38 g N kg^{-1} d.m., compared to 25.16 g N kg^{-1} d.m. determined for cv. Silma. Fruits of the population line also contained significantly more phosphorus and potassium (by app. 17%), whereas the levels of sodium, calcium and magnesium did not differ in a statistically significant way between the two types of the plant. The highest nitrogen (27.62 g kg^{-1} d.m.) and phosphorus (8.78 g kg^{-1} d.m.) concentrations were obtained by applying 2 g N per pot. Sulphur introduced to soil in the form of magnesium sulphate raised the content of nitrogen in achenes of both forms of milk thistle.

Key words: milk thistle, N doses, boron, magnesium fertilizers, macroelements.

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WPŁYW NAWOŻENIA NA ZAWARTOŚĆ MAKROELEMENTÓW W OWOCACH OSTROPESTU PLAMISTEGO (*SILYBUM MARIANUM* L. GAERTN.)

Abstrakt

Ostropest plamisty uprawiano na podłożu o składzie granulometrycznym gleby lekkiej, o odczynie lekko kwaśnym i średniej zasobności w przyswajalny fosfor, potas i magnez. W doświadczeniu pierwszym badano wpływ wzrastającego poziomu nawożenia azotem (0,0; 1,0; 2,0; 3,0 g N na wazon) stosowanego na tle stałego nawożenia PK (P – 1,0; K – 1,5 g na wazon), w drugim – boru, a w trzecim – formy nawozów magnezowych ($MgCl_2$ i $MgSO_4$) na zawartość makroelementów w owocach dwóch form ostropestu – odmiany Silma i populacji krajowej. Cechy odmianowe istotnie wpływały na zawartość azotu, fosforu i potasu, natomiast nie miały wpływu na koncentrację wapnia magnezu i sodu. Zawartość azotu w niełupkach roślin populacyjnych wynosiła 26,38 g N kg^{-1} s.m., a u odmiany Silma -25,16 g N kg^{-1} s.m. Owoce roślin populacyjnych zawierały również istotnie więcej fosforu i potasu (o ok. 17%), natomiast zawartości sodu, wapnia i magnezu nie różniły się statystycznie. Największą zawartość azotu (27,62 g N kg^{-1} s.m.) oraz fosforu (8,78 g P kg^{-1} s.m.) uzyskano stosując 2 g N na wazon. Siarka wprowadzona w postaci siarczanu magnezu wpłynęła na zwiększenie zawartości azotu w niełupkach obu form ostropestu.

Słowa kluczowe: ostropest plamisty, dawka azotu, bor, nawozy magnezowe, zawartość makroelementów.

INTRODUCTION

Milk thistle is a popular herbal plant. The medicinal substance silymarin is produced from seed shells, while defatted seeds become a by-product. In some countries, milt thistle sprouts or even whole new plants are consumed as a rich source of anti-oxidants (VAKNIN et al. 2008, HUSSAIN et al. 2010). A high content of protein (about 25%) indicates that defatted milk thistle seeds could be used for animal nutrition (POTKAŃSKI et al. 1991, ŁANGOWSKA et al. 2002, STOPYRA et al. 2006) or for production of functional food (BARANYK et al. 1995, SZCZUCIŃSKA et al. 2003).

Ever since animal meat and bone meals were banned from animal nutrition, new sources of fodder protein have been looked for. The use of genetically modified soybean and maize for production of animal feeds also raises many concerns. Defatted milk thistle seeds seem to be as good a good source of protein as canola meal. A small quantity of flavonolignands found in defatted seeds of milk thistle may – to some extent – replace antibiotics or growth hormones, whose use in animal feed is increasingly often prohibited (CYBULSKI, RADKO 2006, PASCHMA et al. 2010, URBAŃCZYK et al. 2002, SUCHY et al. 2008). Moreover, oil and some seed protein fractions possess the properties that resemble preservative agents used in the pharmaceutical and cosmetics industries (SZCZUCIŃSKA et al. 2003, 2006, 2007).

The total acreage of farmland dedicated to canola grown for diesel oil is limited to about 1 million ha because of certain ecological, organizational and economic considerations. It might be possible to fulfill the requirements on use of energy from renewable resources set by the Directive 2009/28/EC if we raised canola yields or promoted cultivation of other oil plants, for example milk thistle (Baranyk et al. 1995, Kozera, Nowak 2004, Andrzejewska, Sadowska 2007, Andrzejewska, Mielcarek 2011, Sadowska et al. 2011, Wierzbowska et al. 2012b), which has a short vegetative period and can be grown all over Poland. This plant does not require good farmland and can be cultivated on light soils, which are unsuitable for canola (Sadowska, Andrzejewska 2010).

The objective of this study has been to evaluate the effect of mineral fertilization on the content of macronutrients in milk thistle achenes.

METHODS

The research objective was achieved in three, two-factorial pot experiments, set up in a completely random design with four replications. Pots were filled with 10 kg of light soil, which was moderately rich in available forms of phosphorus (58 mg P kg^{-1}), potassium (97 mg K kg^{-1}) and magnesium (32 mg Mg kg^{-1}). The soil reaction was slightly acid (pH_{KCl} = 5.8).

The first experiment was established to investigate the effect of increasing doses of nitrogen (N_0–0.0, N_1–1.0, N_2–2.0, N_3–3.0 g N per pot in the form of urea), applied against the background of constant PK fertilization (1.0 g P as $CaHPO_4$ and 1.5 g K as KCl), on the content of macronutrients in milk thistle achenes. The second experiment focused on the influence of boron (5 mg B per pot in the form of H_3BO_3). The third series tested the effect of magnesium fertilizers (0.3 g Mg per pot in the form of $MgCl_2$ or $MgSO_4 \cdot 7H_2O$) applied together with the increasing nitrogen fertilization and constant PK nourishment. The PKMg fertilization as well as boron and 1 g of nitrogen (treatments N_1, N_2, N_3) were applied before sowing and the remaining part of nitrogen (treatments N_2, N_3) was supplied in a top-dressing treatment during the leaf rosette phase. Two forms of milk thistle were used in the experiments: a cultivar called Silma and a population line grown in Poland.

The plant material harvested during the technological maturity phase was ground and mineralized in concentrated sulphuric acid (IV) with added hydrogen dioxide as an oxidant. Total nitrogen was determined colorimetrically with the hypochlorite method (Baethen, Alley 1989); phosphorus was assayed using the vanadium molybdenum method, while potassium, calcium and sodium were determined with atomic emission spectrophotometry (AES) and the determination of magnesium was performed by atomic absorption spectrophotometry (AAS).

The results of chemical determinations were submitted to statistical analysis aided by a Statistica 10 software package. All statistical calculations were performed at the level of significance p=0.01. The Fisher's test was applied to verify the significance of differences.

RESULTS AND DISCUSSION

The content of mineral constituents in fruits of milk thistle depended on the variety-specific traits and fertilization (Tables 1-5). The characteristics of the two types of milk thistle significantly determined the content of nitrogen, phosphorus and potassium but had no effect on the concentration of the other elements (Tables 1 and 4). The average content of nitrogen in achenes of the population plant was 26.38 g N kg^{-1} d.m., thus being significantly higher than in achenes of cv. Silma (25.16 g N kg^{-1} d.m.). Fruits of the population plant also contained significantly more phosphorus and potassium (by about 17%) than cv. Silma, but there were no differences between the two types of milk thistle in the content of sodium, calcium and magnesium.

Table 1

Content of mineral components in milk thistle fruits depending on the variety

Variety	Components (g kg^{-1} d.m.)					
	N	P	K	Na	Ca	Mg
Silma	25.16±0.68^{a}	8.41±0.15^{a}	6.42±0.15^{a}	0.33±0.01^{a}	9.69±0.39^{a}	3.68±0.06^{a}
Population	26.38±0.70^{b}	8.70±0.13^{b}	7.50±0.15^{b}	0.35±0.01^{a}	9.31±0.29^{a}	3.86±0.07^{a}

On average for the tested nitrogen fertilization doses, the highest content of nitrogen (27.62 g N kg^{-1} d.m.) and phosphorus (8.78 g P kg^{-1} d.m.) in milk thistle fruits was achieved when 2 g N per pot had been applied (Tables 2 and 4). Any further increase in the nitrogen level fertilization did not have significant influence on the content of nitrogen but decreased phosphorus in achenes of milk thistle. The different nitrogen fertilization levels did not have any significant effect on the content of potassium and magnesium in milk thistle fruits, whilst the content of calcium in achenes produced by plants fertilized with 3 g N per pot was over 21% less than in plants not fertilized with nitrogen. The analogous decline in the content of sodium was 14%.

The highest nitrogen concentration of nitrogen in achenes collected from the population line of milk thistle (28.11 g N kg^{-1} d.m.) was obtained after an application of 3 g N per pot; in the case of cv. Silma, the highest content of nitrogen in the fruits (28.13 g N kg^{-1} d.m.) was found in pots fertilized with a dose of 2 g (Tables 3 and 4). The highest dose of nitrogen depressed

Table 2

Content of mineral components in milk thistle fruits depending on nitrogen fertilization

N doses	Components (g kg^{-1} d.m.)					
	N	P	K	Na	Ca	Mg
N0	22.99±0.90^{b}	8.50±0.21^{a}	7.04±0.35^{a}	0.37±0.01^{c}	10.73±0.35^{c}	3.83±0.09^{a}
N1	25.51±0.79^{c}	8.61±0.15^{ab}	6.86±0.30^{a}	0.33±0.02^{ab}	10.18±0.38^{b}	3.78±0.10^{a}
N2	27.62±0.48^{a}	8.78±0.27^{b}	6.92±0.31^{a}	0.35±0.02^{bc}	8.68±0.22^{a}	3.76±0.11^{a}
N3	26.98±0.61^{a}	8.33±0.19^{a}	7.02±0.33^{a}	0.32±0.02^{a}	8.42±0.17*a*	3.73±0.10^{a}

Table 3

Content of mineral components in milk thistle fruits depending on the variety and level of nitrogen fertilization

N doses	Components (g kg^{-1} d.m.)					
	N	P	K	Na	Ca	Mg
Silma						
N0	22.66±1.04^{c}	8.40±0.29^{ab}	6.43±0.29^{a}	0.34± 0.01^{b}	11.25±0.29^{c}	3.89±0.10^{ab}
N1	24.02±0.48^{d}	8.44±0.20^{ab}	6.59±0.42^{a}	0.35±0.03^{bc}	10.36±0.71*b*	3.60±0.10^{c}
N2	28.13±0.65^{b}	8.65±0.46^{ac}	6.38±0.32^{a}	0.31±0.01^{a}	8.76±0.22^{a}	3.66±0.11^{cd}
N3	25.85±0.45^{e}	8.18±0.32^{b}	6.35±0.20^{a}	0.30±0.03^{a}	8.38±0.22^{a}	3.59±0.15^{c}
Population						
N0	23.32±1.63^{cd}	8.60±0.33^{ac}	7.65±0.33^{b}	0.38±0.02^{cd}	10.20±0.50^{b}	3.77±0.17^{ad}
N1	26.99a±0.88^{a}	8.78±0.21^{ac}	7.20±0.37^{c}	0.31±0.02^{a}	10.00±0.37^{b}	3.96±0.11^{b}
N2	27.11±0.66^{ab}	8.95±0.32^{c}	7.46±0.30^{bc}	0.37±0.01^{d}	8.60±0.41^{a}	3.86±0.18^{ab}
N3	28.11±0.67^{ab}	8.49±0.20^{ab}	7.69±0.30^{b}	0.34±0.02^{b}	8.45±0.31^{a}	3.86±0.11^{ab}

the content of phosphorus in achenes of both forms of milk thistle. The level of nitrogen fertilization did not have any larger effect on the content of potassium and magnesium in fruits of both forms. The intermediate and high doses of nitrogen (N_2 and N_3) significantly decreased the content of calcium in both forms of milk thistle (by 22.1 and 25.5%, respectively, in cv. Silma and by 11.6 and 17.1% in the population line compared to the control).

The results of the analysis of variance, shown in Table 4, confirm that the variety-specific traits significantly differentiated the content of all analyzed macronutrients in achenes of milk thistle. The fertilization level affected the content of nitrogen, phosphorus, sodium and calcium but did not have any effect on the concentration of potassium and magnesium.

Table 4

Analysis of variance for the content of mineral components depending on the variety and nitrogen fertilization level

Components	Variety	N doses	Interaction
Nitrogen (N)	**	**	**
Phosphorus (P)	**	*	NS
Potasium(K)	**	NS	NS
Sodium (Na)	**	**	**
Calcium Ca)	**	**	*
Magnesium (Mg)	**	NS	**

* significant for $p \leq 0.05$; **significant for $p \leq 0.01$

High correlation coefficients prove that the content of nitrogen in achenes from both forms of milk thistle was significantly positively correlated with the dose of nitrogen (Table 5). Higher doses of nitrogen introduced as fertilizer significantly depressed the concentration of sodium, calcium and magnesium in cv. Silma fruits; they had the same effect on the content of calcium in achenes from the population line.

Table 5

Coefficient of correlation between the content of mineral components in milk thistle achenes versus the level of nitrogen fertilization

Components	Variety	
	Silma	population
Nitrogen (N)	0.664**	0.675**
Phosphorus (P)	-0.110	-0.040
Potasium(K)	-0.090	0.077
Sodium (Na)	-0.570**	-0.110
Calcium Ca)	-0.860**	-0.740**
Magnesium (Mg)	-0.440*	

* significant for $p \leq 0.05$; **significant for $p \leq 0.01$

WIERZBOWSKA et al. (2012a) obtained a higher yield of achenes and green mass when growing a Polish population line rather than the cultivar Silma. Population plants responded better to higher doses of nitrogen fertilization than cv. Silma. They produced more fruits, which were also more robust. Fertilization composed of 2 g N per pot resulted in the highest contribution of achenes to the total biomass yield. Other experiments conducted by the same authors (WIERZBOWSKA et al. 2012b) indicate that higher nitrogen fertilization levels result in a lower fat content in fruits, especially ones produced by cv. Silma. ANDRZEJEWSKA and SKINDER (2007) demonstrated that the content of potassium in the green matter of milk thistle increased proportionally to the dose of this element, but was on a constant level of about 6 g K kg^{-1} d.m. in achenes irrespective of potassium fertilization doses. The size of a potassium dose did not have any effect on the yield of achenes and their content of silymarin. In turn, Kozera and Nowak (2004) found that the level of NPK fertilization had no effect on the content of nitrogen in milk thistle achenes, but depressed their concentration of potassium. On the other hand, micronutrients applied in the form of Basofoliar 36 Ex raised the content of phosphorus and potassium in achenes compared to plants fertilized with NPK alone.

Overall, boron fertilization did not affect the content of macronutrients in fruits of milk thistle (Table 6), except for the content of magnesium in achenes of cv. Silma, which was higher in the boron-fertilized treatments. Cwalina-Ambroziak et al. (2012) found that the yield of milk thistle achenes grown without nitrogen fertilization or fertilized with a dose of 40 kg N ha^{-1} was higher when boron had been applied. However, boron fertilization caused a decline in yield from plants nourished with higher doses of nitrogen. Moreover, boron applied together with nitrogen changed the structure of pathogenic fungi colonizing the stem base, decreasing the share of *Fusarium* spp. but increasing the number of *Alternaria alternate*. Experiments conducted by Škarpa (2013) showed that foliar application of boron increased the biomass production of sunflower plants. Early application of boron (stage of 4 developed leaves) also increased the uptake of macronutrients (particularly N). The achene yield increased significantly (by 8.3%) after the application of 300 g B ha^{-1} at the beginning of vegetation. Similarly in a study conducted by Wróbel (2009) boron fertilization increased the performance and content of this component in cereal grains.

Table 6

Effect of boron on the content of macronutrients in fruits of milk thistle

Fertilization	Components (g kg^{-1} d.m.)					
	N	P	K	Na	Ca	Mg
Silma						
Without B	25.25±0.73^{a}	8.47±0.13^{a}	6.61±0.34^{a}	0.33±0.04^{a}	9.40±0.62^{a}	3.63±0.14^{b}
B	25.72±0.70^{a}	8.59±0.20^{a}	6.46±0.34^{a}	0.34±0.03^{a}	9.82±0.51^{a}	3.82±0.16*b*
Population						
Without B	27.61±1.31^{b}	8.65±0.21^{a}	7.53±0.16^{b}	0.35±0.02^{a}	9.47±0.81^{a}	3.94±0.11^{b}
B	26.39±1.04^{ab}	8.45±0.13^{a}	7.63±0.33^{b}	0.34±0.03^{a}	9.19±0.56^{a}	3.88±0.13^{b}

Sulphur introduced together with magnesium raised the content of nitrogen in achenes of the population line of milk thistle (Table 7). The form of magnesium fertilizer did not have any significant effect on the content of other macronutrients. In another study by Wierzbowska et al. (2012b), an

Table 7

Effect of the form of magnesium fertilizer on the content of macronutrients in fruits of milk thistle

Fertilization	Components (g kg^{-1} d.m.)					
	N	P	K	Na	Ca	Mg
Silma						
$MgCl_2$	24.69 ± 1.62^{a}	8.30 ± 0.14^{b}	6.18 ± 0.23^{a}	0.33 ± 0.04^{a}	9.98 ± 0.96^{a}	3.63 ± 0.14^{a}
$MgSO_4$	25.25 ± 0.73^{a}	8.47 ± 0.13^{b}	6.61 ± 0.34^{a}	0.33 ± 0.04^{a}	9.40 ± 0.62^{a}	3.63 ± 0.14^{a}
Population						
$MgCl_2$	24.97 ± 1.66^{a}	8.56 ± 0.14^{a}	7.27 ± 0.44^{a}	0.35 ± 0.04^{a}	9.43 ± 0.79^{a}	3.84 ± 0.16^{a}
$MgSO_4$	27.61 ± 1.31^{b}	8.65 ± 0.21^{a}	7.53 ± 0.16^{b}	0.35 ± 0.02^{a}	9.47 ± 0.81^{a}	3.94 ± 0.11^{a}

addition of sulphur contributed to a decrease in the content of crude fat in fruits of cv. Silma. Also, some modifications appeared in the structure of fatty acids, namely there was more linoleic acid ($C_{18:2}$), which is the dominant fatty acid in oil from milk thistle fruits, but less oleic acid ($C_{18:1}$).

According to Sadowska et al. (2011), the weather conditions during the plant growing season had a stronger effect on the chemical composition of milk thistle fruits than agronomic factors. The content of total protein changed very little, whereas the percentages of crude fibre and macronutrients, especially calcium and magnesium, were modified most profoundly. Andrzejewska et al. (2011) claimed that delayed sowing led to an increased content of protein in achenes of this plant. Other experimental data suggest that the content of protein and ash components was independent from the date of sowing (Andrzejewska, Sadowska 2007).

The content of nitrogen in the storage material of achenes was 36-38 g N kg^{-1} (Andrzejewska, Sadowska 2008). Experiments conducted by Sadowska et al. (2011) showed that fruits of milk thistle contained about 160 g kg^{-1} d.m. of total protein and the sum of exogenous amino acids was about 22 g 100 g^{-1} of total protein. Glutamic acid was the dominant amino acid while tryptophan was a limiting one. Potkański et al. (1991) reported that the content of total protein in hulled milk thistle seeds, a by-product of silymarin production, was 215 g kg^{-1} d.m. Other authors (e.g. Baranowska et al. 2003) suggest that such by-product can contain up to 310-360 g kg^{-1} of total protein while the content of crude ash in milk thistle fruits could reach 50-60 g kg^{-1} d.m. (Potkański et al. 1991, Baranowska et al. 2003). Andrzejewska and Sadowska (2007) report that whole achenes compared to hulled seeds had about 80 g kg^{-1} d.m. less total protein and about 4 g kg^{-1} d.m. less phosphorus, but the content of potassium, calcium and magnesium was similar in both types of seeds. Differences in the content of protein and phosphorus were caused by a large share of the pericarp (48.5–55.8%) in the total mass of achenes (Andrzejewska, Sadowska 2008). Finally, the content of silymarin was negatively correlated with the content of nitrogen in achenes.

CONCLUSIONS

1. Fruits of the population plants contained more nitrogen, phosphorus and potassium than achenes of the cultivar Silma.
2. The highest concentration of nitrogen was determined in achenes of the milk thistle cultivar Silma plants fertilized with 2 g N per pot (28.13 g kg^{-1} d.m.); among the population plants, most nitrogen was found in fruits growing on plants nourished with 3 g N per pot (28.11 g kg^{-1} d.m.).
3. The highest content of phosphorus in achenes from both types of milk thistle plants was achieved when the fertilization treatment consisted of 2 g N per pot.
4. Boron fertilization did not affect the content of macronutrients in fruits of either form of milk thistle.
5. Achenes from population plants fertilized with magnesium sulphate contained more nitrogen than from the ones treated with $MgCl_2$.

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APPLICABILITY OF BIOCHEMICAL INDICES TO QUALITY ASSESSMNET OF SOIL PULLUTED WITH HEAVY METALS*

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Abstract

The objective of this study has been to test a series of soil quality biochemical indices so as to select the one(s) that would mainly rely on enzymes participating in the processes of carbon and nitrogen transformations and reflect objectively the quality of soil. Moreover, an ideal index should be comparable to yields of crops.

A vegetative experiment with 5 replications was set up on two soils: loamy sand and sandy loam, which had been polluted with cadmium, copper and zinc. Once the soil moisture content was raised to the level of 60% capillary water retention capacity, the following plants were sown: oat, spring oilseed rape and yellow lupine. Twice during the growing season, the activity of soil enzymes such as dehydrogenases, catalase, urease, ß-glucosidase, acid phosphatase, alkaline phosphatase and arylsulphatase was determined. Next, based on the soil enzymatic activity as well as the content of clay and organic carbon, 21 indices of the biochemical soil activity (BA_1 to BA_{21}) were proposed, which were divided into two groups: simple and complex ones. In addition, coefficients of correlation between yields of plants and biochemical soil quality indices were calculated.

The experiment has demonstrated that the activity of enzymes should be expressed in units of the product of a catalyzed reaction in 1 h time calculated per 1 kg d.m. of soil, i.e. the activity of dehydrogenases in ěmol TFF, catalase – mol O_2, alkaline phosphatase and arylsulphatase – mmol PNP, while that of urease in mmol $N\text{-}NH_4^+$. Introduction of uniform units facilitates comparison of the results and quality assessment of different soils, irrespective of the author or research centre they originate from. It has also been shown that among the 21 tested biochemical indices of soil quality assessment, the best ones are BA_{20} = %C × (Deh + Pal + Pac + Ure), derived from the content of organic

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carbon and activity of four enzymes: dehydrogenase, alkaline phosphatase, acid phosphatase and urease, and BA_{21} = Deh + Kat + Pal + Pac + Ure + Glu + Aryl, calculated from the activity of seven enzymes: dehydrogenases (Deh), catalase (Cat), acid phosphatase (Pac), alkaline phosphatase (Pal), urease (Ure), β-glucosidase (glu) and arylsulphatase (Aryl).

Key words: cadmium, copper, zinc, soil enzymes, biochemical index of soil quality.

ZASTOSOWANIE WSKAŹNIKÓW BIOCHEMICZNYCH DO OCENY JAKOŚCIOWEJ GLEBY ZANIECZYSZCZONEJ METALAMI CIĘŻKIMI

Abstrakt

Celem pracy było przetestowanie wskaźników biochemicznej jakości gleby i zaproponowanie takiego, który byłby oparty głównie na enzymach uczestniczących w procesach transformacji węgla i azotu oraz odzwierciedlał obiektywnie stan gleby. Ponadto byłby porównywany z plonowaniem roślin.

Doświadczenie wegetacyjne wykonano w 5 powtórzeniach na dwóch glebach: piasku gliniastym i glinie piaszczystej, które zanieczyszczono kadmem, miedzią i cynkiem. Po doprowadzeniu gleby do wilgotności równej 60% kapilarnej pojemności wodnej wysiano następujące rośliny: owies, rzepak jary oraz łubin żółty. Dwukrotnie w czasie wegetacji roślin określono aktywność enzymów glebowych: dehydrogenaz, katalazy, ureazy, β-glukozydazy, fosfatazy kwaśnej, fosfatazy alkalicznej i arylosulfatazy. Następnie wykorzystując aktywność enzymów glebowych, zawartość iłu i węgla organicznego zaproponowano 21 wskaźników biochemicznej aktywności gleby (BA_1 – BA_{21}), które podzielono na wskaźniki proste i złożone. Obliczono również współczynniki korelacji między plonem roślin a biochemicznymi wskaźnikami jakości gleby.

Stwierdzono, że aktywność enzymów powinna być wyrażona w jednostkach produktu katalizowanej reakcji w czasie 1 h w przeliczeniu na 1 kg s.m. gleby, tj.: dehydrogenaz – µmol TFF, katalazy – mol O_2, fosfatazy alkalicznej, fosfatazy kwaśnej i arylosulfatazy – mmol PNP, natomiast ureazy – mmol $N\text{-}NH_4^+$. Używanie zunifikowanych jednostek umożliwia porównywanie wyników i ocenę jakości różnych gleb, niezależnie od autora i ośrodka badań. Wykazano również, że spośród 21 przetestowanych biochemicznych wskaźników jakości gleb najlepszymi wskaźnikami są: BA_{20} = %C × (Deh + Pal + Pac + Ure), obliczany na podstawie zawartości węgla organicznego i aktywności czterech enzymów: dehydrogenaz, fosfatazy alkalicznej, fosfatazy kwaśnej i ureazy, oraz BA_{21} = Deh + Kat + Pal + Pac + Ure + Glu + Aryl, obliczany na podstawie aktywności siedmiu enzymów: dehydrogenaz (Deh), katalazy (Kat), fosfatazy kwaśnej (Pac), fosfatazy alklalicznej (Pal), ureazy (Ure), β-glukozydazy (Glu) i arylosulfatazy (Aryl).

Słowa kluczowe: kadm, miedź, cynk, enzymy glebowe, biochemiczny wskaźnik jakości gleby.

INTRODUCTION

Work on constructing biochemical indices has continued for over 100 years as the earliest measurements of soil enzymatic activity were taken in 1905-1910 and involved determination of the activity of catalase and peroxidase (Zahir et al. 2001). The first soil quality index which included the activity of soil enzymes was developed by Hofmann and Seegener in 1950 (Nannipieri et al. 2002). In Poland, back in the early 1980s, Myśków (1981) proposed two soil quality indices (M). One was derived from counts of bacteria, actinomyces and fungi, and another one, which was based on the content of organic carbon and activity of dehydrogenases. Several years later, the same author and his co-workers (Myśków et al. 1996) suggested another biological index of soil fertility, which additionally included soil adsorption capacity. Stefanic et al. (1984) proposed a fertility index (BIF) calculated from the activities of dehydrogenases and the activity of catalase. In the same year, Beck (1984) elaborated a soil quality index (EAN) based on the activities of dehydrogenases, catalase, alkaline phosphatase, protease and amylase.

Garcia and Hernandez (1997) took the content of microbiological carbon as well as the activities of β-glucosidase and arylsulphatase to suggest a biological and biochemical soil quality index (SOM), which made it possible to determine changes in organic matter contained in degraded soils. In the same year, Kucharski (1997) presented a biochemical soil fertility index (M_W), which he had derived from the content of organic carbon (%C) and the activities of dehydrogenases, urease, acid phosphatase and alkaline phosphatase.

Trasar-Cepeda et al. (1998) demonstrated that the content of total nitrogen (N_c) in soil can be described with a linear function which comprised 5 parameters: microbiological biomass, mineralized nitrogen, activity of phosphatase, activity of β-glucosidase and urease. Trinchera et al. (1999) proved that the quality of soil can be evaluated according to the degree of its humification and the amount of humified matter. The same authors also suggest that the ratio of biomass carbon to total carbon content, expressed in %, could be another good indicator of soil quality. They found out that the content of microbiological biomass carbon (C_m) in the 0 to 20 cm deep layer of arable soil corresponded to 1.33% of the total organic carbon (TOC) content, and to 2.09% of TOC in fallow land. In the deeper layer (20-40 cm), these proportions changed, so that they contained 2.06% of C_m relative to TOC in arable soil and just 0.16% in fallow land. Other authors, such as Scharenbroch et al. (2005), also recommended to compare the C_m to TOC ratio. They claim that the said ratio is a good indicator of changes in ecosystems. For example, it identifies the pool of carbon which is mineralizable.

Dick et al. (2000) suggest that the status of mineral soils should be assessed according to the determined activities of soil enzymes. Chemical determinations, in their opinion, are less helpful. They suggest using the activity of alkaline phosphatase (Pal) and acid phosphatase (Pac) to determine whether liming is needed. They claim that if the Pal to Pac ratio is 0.5 or more, soils have proper reaction, but if it falls below 0.5, soils should be limed.

De la Paz Jimenez et al. (2002) worked out a formula that facilitated interpretation of changes in the content of carbon according to the index of microbiological activity (the activity of dehydrogenases and the activity of 3 enzymes responsible for transformations of sulphur, phosphorus and carbon). Koper and Piotrowska (2003) believe that an index derived from the content of organic carbon, total nitrogen, activity of dehydrogenases, alkaline phosphatase, protease and amylase would be suitable for assessment of soil fertility.

Puglisi et al. (2005) worked out a soil alteration index (SAI) based on 15 determinations of fatty acids found in soil phospholipids (PLFA). Their studies demonstrated that SAI was useful in classification of soils depending on their degree of transformation.

A year later, Puglisi et al. (2006) presented three other indices of soil quality based on the activity of seven enzymes (AI 1): arylsulphatase, β-glucosidase, acid phosphatase, urease, invertase, dehydrogenases and phenyloxidase, four enzymes (AI 2): β-glucosidase, acid phosphatase, urease and invertase, three enzymes (AI 3): β-glucosidase, acid phosphatase and urease. In the same year, Bastida et al. (2006) proposed a microbiological degradation index (MDI).

This wide range of microbiological and biochemical indices reported in the literature encouraged us to undertake the present study with an aim of finding out which soil fertility index would assess the condition of soil most objectively. The research was based on the assumption that a good soil quality index should rely on enzymes engaged in the transformation of carbon and nitrogen and should be comparable to yields of crops. Therefore, it was decided to test a series of biochemical indices of soil fertility and find out which one was optimal.

MATERIAL AND METHODS

In order to achieve the above objective, a vegetative (pot) experiment was set up on two soils: loamy sand and sandy loam, which had been polluted with heavy metals. A more detailed description of the characteristics of the soils can be found in Table 1.

The trials were run in a glasshouse at the University of Warmia and Mazury in Olsztyn. Polyethylene pots of the capacity of 3.5 dm^3 were used. The trials were replicated five times. Prior to putting into the pots, soil (3 kg per pot) was mixed in a polyethylene container with macronutrients and heavy metals according to the experimental design. Once portions of the soil were transferred to pots, water was added to bring the soil moisture content to 60% of capillary water holding capacity. Next, the following plants were sown: cv. Kasztan oat, cv. Huzar spring oilseed rape and cv.

Table 1

Physical and chemical properties of soils

Type of soil	pH_{KCl}	Hh	S	T	V (%)	Content					
						C_{org}	N_c	K_w	Na_w	Ca_w	Mg_w
		(mmol(+)g kg^{-1})				(g kg^{-1})		(mg kg^{-1})			
Loamy sand (pg)	6.7	7.8	98.7	106.5	92.7	11.0	0.97	180	28	1429	80
Sandy loam (gp)	6.8	5.2	131.4	136.6	96.2	9.9	1.14	168	57	2214	50

Hh – hydrolytic acidity, S – sum of exchangeable cations, T – total soil adsorption capacity, V – base saturation, C_{org} – organic carbon, Nc – total nitrogen, w – appropriate exchangeable cations

Mister yellow lupine. After emergence, the plants were thinned leaving 12 oat, 8 oilseed rape and 5 yellow lupine plants per pot.

The experimental variables were:

1) type of soil: loamy sand and sandy loam;
2) type of heavy metal: Cd^{2+}, Cu^{2+}, Zn^{2+};
3) quantity of the heavy metal in mg kg^{-1} d.m. of soil:
   Cd^{2}: 0, 50, 150;
   Cu^{2+} and Zn^{2+}: 0, 150, 450;
4) species of the crop: oat, spring oilseed rape and yellow lupine.

Aqueous solutions of $CdCl_2$, $CuCl_2$ $2H_2O$, $ZnCl_2$ were added to batches of soil previously weighed out. They were thoroughly mixed with the soil, which was then placed in pots. When the soil moisture content reached 60% of capillary water holding capacity, the following plants were sown: oat, spring oilseed rape and yellow lupine.

The same fertilization doses were applied to all the treatments (in mg kg^{-1} of soil): 100 (except yellow lupine, which was not fertilized with nitrogen), P – 35, K – 100, Mg – 20. Nitrogen was applied as $CO(NH_2)_2$, phosphorus as KH_2PO_4, potassium as KH_2PO_4 + KCl and magnesium as $MgSO_4 \cdot 7H_2O$.

The plants were grown for 50 days. Then, they were harvested and both green and dry matter yields were determined. On harvest (day 50) and while the plants were growing (day 25), soil samples were taken to determine the activity of soil enzymes: dehydrogenases (EC 1.1) using TTC as substrate (Öhlinger 1996), catalase (EC 1.11.1.6), urease (EC 3.5.1.5), β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1) and arylsulphatase (EC 3.1.6.1) according to Alef and Nannpieri (1998). All the enzymatic determinations, except the activity of catalase, were performed on a Perkin-Elmer Lambda 25 spectrophotometer.

Finally, indices of the biochemical soil activity (BA_1 – BA_{21}) were proposed based on the determined activities of soil enzymes, content of clay and content of organic carbon. The following formulas were used for this purpose:

$BA_1 = \log_{10}C\ \mathrm{Deh}$,

$BA_2 = \log_{10}C\sqrt{\mathrm{Deh}}$,

$BA_3 = \log_{10}\mathrm{clay}\sqrt{\mathrm{Deh}}$,

$BA_4 = \mathrm{Deh} + \mathrm{Cat}$,

$BA_5 = \mathrm{Deh} + \mathrm{Ure}$,

$BA_6 = \mathrm{Deh} + \mathrm{Glu}$,

$BA_7 = \mathrm{Deh} + \mathrm{Pac}$,

$BA_8 = \mathrm{Deh} + \mathrm{Pal}$,

$BA_9 = \mathrm{Deh} + \mathrm{Aryl}$

$BA_{10} = \log_{10}C\sqrt{\mathrm{Deh} + \mathrm{Cat} + \mathrm{Pal}}$,

$BA_{11} = \log_{10}\mathrm{clay}\sqrt{\mathrm{Deh} + \mathrm{Cat} + \mathrm{Pal}}$,

$BA_{12} = \log_{10}C\sqrt{\mathrm{Deh} + \mathrm{Cat} + \mathrm{Pal} + \mathrm{Pac}}$,

$BA_{13} = \log_{10}\mathrm{clay}\sqrt{\mathrm{Deh} + \mathrm{Cat} + \mathrm{Pal} + \mathrm{Pac}}$,

$BA_{14} = \log_{10}C\sqrt{\mathrm{Deh} + \mathrm{Cat} + \mathrm{Pal} + \mathrm{Pac} + \mathrm{Ure}}$,

$BA_{15} = \log_{10}\mathrm{clay}\sqrt{\mathrm{Deh} + \mathrm{Cat} + \mathrm{Pal} + \mathrm{Pac} + \mathrm{Ure}}$,

$BA_{16} = \log_{10}C\sqrt{\mathrm{Deh} + \mathrm{Cat} + \mathrm{Pal} + \mathrm{Pac} + \mathrm{Ure} + \mathrm{Glu}}$,

$BA_{17} = \log_{10}\mathrm{clay}\sqrt{\mathrm{Deh} + \mathrm{Cat} + \mathrm{Pal} + \mathrm{Pac} + \mathrm{Ure} + \mathrm{Glu}}$,

$BA_{18} = \log_{10}C\sqrt{\mathrm{Deh} + \mathrm{Cat} + \mathrm{Pal} + \mathrm{Pac} + \mathrm{Ure} + \mathrm{Glu} + \mathrm{Aryl}}$,

$BA_{19} = \log_{10}\mathrm{clay}\sqrt{\mathrm{Deh} + \mathrm{Cat} + \mathrm{Pal} + \mathrm{Pac} + \mathrm{Ure} + \mathrm{Glu} + \mathrm{Aryl}}$,

$BA_{20} = \%C\ (\mathrm{Deh} + \mathrm{Pal} + \mathrm{Pac} + \mathrm{Ure})$,

$BA_{21} = \mathrm{Deh} + \mathrm{Cat} + \mathrm{Pal} + \mathrm{Pac} + \mathrm{Ure} + \mathrm{Glu} + \mathrm{Aryl}$,

where:

BA – biochemical activity,
Deh – activity of dehydrogenases (μmol TFF kg^{-1} d.m. h^{-1}),
Cat – activity of catalase (mol O_2 kg^{-1} d.m. h^{-1}),
Ure – activity of urease (mmol $N\text{-}NH_4^+$ kg^{-1} d.m. h^{-1}),

Glu – activity β-glucosidase (mmol PNP kg^{-1} d.m. h^{-1}),
Pac – activity of acid phosphatase (mmol PNP kg^{-1} d.m. h^{-1}),
Pal – activity of alkaline phosphatase (mmol PNP kg^{-1} d.m. h^{-1}),
Aryl – activity of arylosulphatase (mmol PNP kg^{-1} d.m. h^{-1}),
C – content of C_{org} in g kg^{-1} d.m. of soil,
%C – content of C_{org} in d.m. of soil in %,
clay – content of clay fraction in %.

Additionally, coefficients of correlations between the crop yields and biochemical indices of soil quality were calculated.

RESULTS AND DISCUSSION

The adamant search for an optimal index of soil quality undertaken by researchers from different parts of the world (Myśków 1981, Beck 1984, Stefanic et al. 1984, Myśków et al. 1996, Garcia, Hernandez 1997, Kucharski 1997, Trasar-Cepeda et al. 1998, Garcia-Gil et al. 2000, De la Paz Jimenez et al. 2002, Koper, Piotrowska 2003, Puglisi et al. 2005, Winding et al. 2005, Puglisi et al. 2006, Fu et al. 2009, Li et al. 2009, Kucharski 2010, Wyszkowska et al. 2010, Kucharski et al. 2011, Grzebisz et al. 2012, Medyńska-Juraszek, Kabała 2012, Wyszkowski, Sivitskaya 2012, Giacometti et al. 2013) proves how important it is to find a suitable model that would reflect objectively soil condition but also take into account all the factors which influence its fertility. Constructing microbiological and biochemical indices of soil quality should rely mainly on enzymes engaged in process of carbon and nitrogen transformations.

The biochemical indices of soil quality presented in this paper were divided into two categories: simple ones derived from two parameters (Tables 2–4) and complex ones, calculated from a higher number of parameters (Tables 5–7). To make the results obtained by different authors comparable, it was decided to express the activity of each enzyme in micromoles or millimoles of the reaction product in kg of d.m. of soil in a specific time unit.

Despite different values of these indices, the authors looked at the sensitivity to heavy metal pollution and correlation with crop yields instead of the absolute value of each analyzed index so as to indicate the best one. When oat was grown (Table 2), the biggest differences between unpolluted soils and the ones polluted with a dose of 150 mg kg^{-1} soil of heavy metals were observable for the indices BA_1 and BA_9. Both of these indices were on average 70% lower for the polluted soils than for the control ones. The smallest differences in this context were demonstrated by the indices BA_2 (49%) and BA_3 (50%).

Table 2

Simple biochemical indices of the quality of soil contaminated with heavy metals under oat

Type of soil	Dose heavy metal (mg kg^{-1})	Simple biochemical indices of the quality of soil*								
		BA_1	BA_2	BA_3	BA_4	BA_5	BA_6	BA_7	BA_8	BA_9
Loamy sand	Cadmium									
	0	17.10	4.16	0.75	17.16	18.27	17.57	19.01	20.14	17.22
	50	11.21	3.39	0.60	11.11	12.15	11.59	12.62	12.65	11.17
	150	5.08	2.30	0.40	5.04	5.45	5.56	6.47	5.89	5.07
	Copper									
	0	17.10	4.16	0,75	17.16	18,27	17.57	19.01	20.14	17.22
	50	2.62	1.64	0.29	2.78	3.37	3.06	4.27	4.75	2.73
	150	0.35	0.60	0.11	0.48	0.72	0.82	1.32	1.28	0.45
	Zinc									
	0	17.10	4.16	0.75	17.16	18.27	17.57	19.01	20.14	17.22
	50	13.82	3.76	0.67	13.73	14.37	14.03	15.29	16.47	13.72
	150	8.23	2.92	0.51	8.17	8.56	8.45	9.54	10.55	8.15

cont. Table 2

Sandy loam	Cadmium									
	0	18.10	4.45	2.15	17.00	19.66	17.48	19.10	21.99	17.05
	50	12.61	3.74	1.78	11.77	14.12	12.22	13.60	15.18	11.72
	150	6.10	2.57	1.25	6.03	7.50	6.48	7.58	8.71	5.90
	Copper									
	0	18.10	4.45	2.15	17.00	19.66	17.48	19.10	21.99	17.05
	50	7.07	2.77	1.35	6.91	7.71	7.24	8.56	11.17	6.90
	150	2.09	1.51	0.73	2.23	2.65	2.50	3.65	5.27	2.17
	Zinc									
	0	18.10	4.45	2.15	17.00	19.66	17.48	19.10	21.99	17.05
	50	13.82	3.85	1.89	13.29	15.46	13.59	15.17	17.95	13.27
	150	9.53	3.18	1.58	9.35	11.04	9.64	11.22	13.34	9.21

*$BA_1 = \log_{10} C\ Deh$, $BA_2 = \log_{10} C\sqrt{Deh}$, $BA_3 = \log_{10} clay\sqrt{Deh}$, $BA_4 = Deh + Cat$, $BA_5 = Deh, Ure$, $BA_6 = Deh + Glu$, $BA_7 = Deh + Pac$, $BA_8 = Deh + Pal$, $BA_9 = Deh + Aryl$

BA – biochemical index of soil quality, Deh – dehydrogenases, Cat – catalase, Ure – urease, Glu – β-glucosidase, Pac – acid phosphatase, Pal – alkaline phosphatase, Aryl – arylsulphatase, C – organic carbon on g kg^{-1} of soil, clay – content of clay fraction in %.

Table 3

Simple biochemical indices of the quality of soil contaminated with heavy metals under spring oilseed rape

Type of soil	Dose heavy metal (mg kg^{-1})	Simple biochemical indices of the quality of soil*								
		BA_1	BA_2	BA_3	BA_4	BA_5	BA_6	BA_7	BA_8	BA_9
Loamy sand	Cadmium									
	0	13.81	3.84	0.65	13.19	13.71	13.65	15.37	16.42	13.21
	50	9.89	3.21	0.56	9.71	10.21	10.16	11.32	11.34	9.73
	150	6.66	2.61	0.46	6.72	7.22	7.16	8.29	7.80	6.77
	Copper									
	0	13.81	3.84	0.65	13.19	13.71	13.65	15.37	16.42	13.21
	50	3.72	1.96	0.34	3.81	4.11	4.18	4.99	5.88	3.81
	150	0.34	0.59	0.10	0.51	0.70	0.84	1.49	1.40	0.52
	Zinc									
	0	13.81	3.84	0.65	13.19	13.71	13.65	15.37	16.42	13.21
	50	8.98	3.06	0.53	8.88	9.31	9.21	10.68	11.83	8.83
	150	4.67	2.22	0.38	4.61	4.88	4.94	5.91	6.42	4.57

cont. Table 3

Sandy loam	Cadmium									
	0	13.13	3.82	1.82	12.22	14.60	12.62	14.45	18.11	12.38
	50	12.40	3.70	1.77	11.62	14.10	12.01	13.49	15.66	11.66
	150	7.67	2.90	1.40	7.35	8.95	7.49	8.78	10.18	7.39
	Copper									
	0	13.13	3.82	1.82	12.22	14.60	12.62	14.45	18.11	12.38
	50	5.49	2.41	1.20	5.55	6.64	5.78	7.23	9.86	5.49
	150	2.23	1.54	0.76	2.41	2.73	2.60	3.82	5.47	2.28
	Zinc									
	0	13.13	3.82	1.82	12.22	14.60	12.62	14.45	18.11	12.38
	50	10.41	3.40	1.62	9.81	12.14	10.41	11.91	13.75	9.75
	150	8.26	2.99	1.45	8.00	9.91	8.28	9.95	11.22	7.94

*explanations see Table 2

Table 4

Simple biochemical indices of the quality of soil contaminated with heavy metals under yellow lupine

Type of soil	Dose heavy metal (mg kg^{-1})	Simple biochemical indices of the quality of soil*								
		BA_1	BA_2	BA_3	BA_4	BA_5	BA_6	BA_7	BA_8	BA_9
Loamy sand	Cadmium									
	0	13.14	3.65	0.65	13.17	14.20	13.62	15.70	16.27	13.49
	50	5.65	2.40	0.43	5.76	6.18	6.18	7.40	7.19	5.74
	150	4.20	2.10	0.36	4.21	4.49	4.53	5.74	5.04	4.19
	Copper									
	0	13.14	3.65	0.65	13.17	14.20	13.62	15.70	16.27	13.49
	50	2.27	1.53	0.27	2.40	2.77	2.76	3.76	4.23	2.41
	150	0.33	0.58	0.10	0.49	0.70	0.82	1.49	1.28	0.43
	Zinc									
	0	13.14	3.65	0.65	13.17	14.20	13.62	15.70	16.27	13.49
	50	7.90	2.88	0.50	7.71	8.29	8.19	9.62	10.43	7.69
	150	3.71	1.98	0.34	3.68	3.94	4.29	4.75	5.22	3.66

cont. Table 4

Sandy loam	Cadmium									
	0	13.65	3.88	1.86	12.76	15.43	13.08	14.78	18.01	12.76
	50	9.12	3.16	1.52	8.69	11.34	9.14	10.52	12.66	8.67
	150	5.71	2.51	1.20	5.50	6.87	5.69	7.22	8.32	5.49
	Copper									
	0	13.65	3.88	1.86	12.76	15.43	13.08	14.78	18.01	12.76
	50	4.18	2.15	1.03	4.16	5.05	4.46	5.94	8.59	4.13
	150	2.11	1.51	0.73	2.28	2.75	2.57	3.84	5.76	2.15
	Zinc									
	0	13.65	3.88	1.86	12.76	15.43	13.08	14.78	18.01	12.76
	50	8.91	3.12	1.51	8.58	11.09	8.92	10.57	12.39	8.50
	150	8.35	3.02	1.46	8.04	10.18	8.30	9.80	11.38	7.91

*explanations see Table 2

Table 5

Complex biochemical indices of soil quality contaminated with heavy metals under oat

Type of soil	Dose heavy metal (mg kg^{-1})	Complex biochemical indices of soil quality*											
		BA_{10}	BA_{11}	BA_{12}	BA_{13}	BA_{14}	BA_{15}	BA_{16}	BA_{17}	BA_{18}	BA_{19}	BA_{20}	BA_{21}
Loamy sand	Cadmium												
	0	4.56	0.82	4.79	0.86	4.93	0.89	5.00	0.90	5.99	0.91	24.17	24.77
	50	3.68	0.65	3.92	0.69	4.08	0.72	4.17	0.74	5.12	0.74	16.61	16.74
	150	2.58	0.45	2.90	0.50	3.01	0.52	3.13	0.54	4.08	0.55	8.98	9.18
	Copper												
	0	4.56	0.82	4.79	0.86	4.93	0.89	5.00	0.90	5.99	0.91	24.17	24.77
	50	2.29	0.41	2.65	0.47	2.81	0.50	2.90	0.51	3.88	0.52	7.71	8.16
	150	1.23	0.22	1.60	0.28	1.73	0.30	1.87	0.33	2.87	0.33	2.86	3.37
	Zinc												
	0	4.56	0.82	4.79	0.86	4.93	0.89	5.00	0.90	5.99	0.91	24.17	24.77
	50	4.19	0.74	4.41	0.78	4.52	0.80	4.58	0.81	5.52	0.82	20.32	20.21
	150	3.40	0.60	3.64	0.64	3.73	0.65	3.80	0.67	4.73	0.67	13.83	13.67

cont. Table 5

Sandy loam	Cadmium											
	0	5.19	2.50	5.48	2.64	5.81	2.80	5.91	2.85	6.54	2.87	34.47	29.63
	50	4.39	2.08	4.69	2.23	5.04	2.40	5.15	2.45	5.79	2.47	26.06	21.86
	150	3.27	1.59	3.60	1.75	3.89	1.90	4.02	1.96	4.83	1.98	15.16	14.05
	Copper												
	0	5.19	2.50	5.48	2.64	5.81	2.80	5.91	2.85	6.54	2.87	34.47	29.63
	50	3.69	1.79	4.00	1.95	4.17	2.03	4.27	2.08	5.07	2.10	17.50	15.89
	150	2.57	1.25	2.95	1.43	3.09	1.50	3.20	1.55	4.07	1.57	9.51	8.87
	Zinc												
	0	5.19	2.50	5.48	2.64	5.81	2.80	5.91	2.85	6.54	2.87	34.47	29.63
	50	4.60	2.26	4.88	2.40	5.18	2.54	5.26	2.58	6.01	2.61	27.07	24.38
	150	3.95	1.96	4.26	2.11	4.53	2.24	4.61	2.29	5.43	2.30	20.53	19.05

$$BA_{10} = \log_{10}C\sqrt{Deh+Cat+Pal},\quad BA_{11} = \log_{10}clay\sqrt{Deh+Cat+Pal},\quad BA_{12} = \log_{10}C\sqrt{Deh+Cat+Pal+Pac},$$

$$BA_{13} = \log_{10}clay\sqrt{Deh+Cat+Pal+Pac},\quad BA_{14} = \log_{10}C\sqrt{Deh+Cat+Pal+Pac+Ure},\quad BA_{15} = \log_{10}clay\sqrt{Deh+Cat+Pal+Pac+Ure},$$

$$BA_{16} = \log_{10}C\sqrt{Deh+Cat+Pal+Pac+Ure+Glu},\quad BA_{17} = \log_{10}clay\sqrt{Deh+Cat+Pal+Pac+Ure+Glu},$$

$$BA_{18} = \log_{10}C\sqrt{Deh+Cat+Pal+Pac+Ure+Glu+Aryl},\quad BA_{19} = \log_{10}clay\sqrt{Deh+Cat+Pal+Pac+Ure+Glu+Aryl},$$

$$BA_{20} = \%C\ (Deh + Pal + Pac + Ure),\quad BA_{21} = Deh + Cat + Pal + Pac + Ure + Glu + Aryl)$$

Table 6

Complex biochemical indices of soil quality contaminated with heavy metals under spring oilseed rape

Type of soil	Dose heavy metal (mg kg^{-1})	Complex biochemical indices of soil quality*											
		BA_{10}	BA_{11}	BA_{12}	BA_{13}	BA_{14}	BA_{15}	BA_{16}	BA_{17}	BA_{18}	BA_{19}	BA_{20}	BA_{21}
Loamy sand	Cadmium												
	0	4.35	0.74	4.66	0.79	4.75	0.81	4.83	0.82	5.63	0.83	22.83	20.80
	50	3.55	0.62	3.82	0.67	3.92	0.68	4.02	0.70	4.93	0.71	15.37	15.07
	150	2.89	0.51	3.19	0.57	3.30	0.59	3.40	0.61	4.39	0.61	10.78	11.36
	Copper												
	0	4.35	0.74	4.66	0.79	4.75	0.81	4.83	0.82	5.63	0.83	22.83	20.80
	50	2.56	0.45	2.84	0.50	2.93	0.51	3.04	0.53	4.01	0.54	8.46	8.83
	150	1.30	0.23	1.71	0.30	1.82	0.32	1.97	0.35	2.99	0.35	3.16	3.81
	Zinc												
	0	4.35	0.74	4.66	0.79	4.75	0.81	4.83	0.82	5.63	0.83	22.83	20.80
	50	3.61	0.63	3.90	0.68	4.00	0.70	4.07	0.71	4.98	0.72	15.92	15.54
	150	2.73	0.47	3.03	0.52	3.11	0.53	3.21	0.55	4.12	0.56	9.67	9.33

cont. Table 6

Sandy loam	0	4.77	2.27	5.09	2.42	5.42	2.58	5.50	2.62	6.13	2.65	30.17	25.18
	50	4.42	2.11	4.72	2.26	5.08	2.43	5.17	2.47	5.84	2.50	26.40	22.39
	150	3.55	1.71	3.84	1.85	4.14	1.99	4.21	2.03	4.99	2.05	17.32	15.14
	Copper												
	0	4.77	2.27	5.09	2.42	5.42	2.58	5.50	2.62	6.13	2.65	30.17	25.18
	50	3.39	1.69	3.72	1.85	3.93	1.96	4.02	2.00	4.89	2.02	15.38	14.65
	150	2.57	1.27	2.92	1.45	3.05	1.51	3.14	1.55	4.04	1.57	9.13	8.86
	Zinc												
	0	4.77	2.27	5.09	2.42	5.42	2.58	5.50	2.62	6.13	2.65	30.17	25.18
	50	4.17	1.99	4.53	2.16	4.89	2.33	5.02	2.39	5.67	2.41	24.44	20.82
	150	3.70	1.80	4.06	1.97	4.38	2.13	4.47	2.17	5.25	2.19	19.37	17.30

*explanations see Table 5

Table 7

Complex biochemical indices of soil quality contaminated with heavy metals under yellow lupine

Type of soil	Dose heavy metal (mg kg^{-1})	Complex biochemical indices of soil quality*											
		BA_{10}	BA_{11}	BA_{12}	BA_{13}	BA_{14}	BA_{15}	BA_{16}	BA_{17}	BA_{18}	BA_{19}	BA_{20}	BA_{21}
Loamy sand	Cadmium												
	0	4.12	0.74	4.45	0.80	4.59	0.82	4.67	0.84	5.67	0.85	20.98	21.70
	50	2.78	0.50	3.11	0.55	3.22	0.57	3.32	0.59	4.31	0.60	10.20	10.80
	150	2.40	0.42	2.77	0.48	2.87	0.50	2.97	0.51	3.91	0.52	8.14	8.20
	Copper												
	0	4.12	0.74	4.45	0.80	4.59	0.82	4.67	0.84	5.67	0.85	20.98	21.70
	50	2.16	0.38	2.51	0.44	2.63	0.46	2.73	0.48	3.73	0.49	6.75	7.28
	150	1.21	0.22	1.63	0.29	1.74	0.31	1.88	0.34	2.90	0.34	2.88	3.56
	Zinc												
	0	4.12	0.74	4.45	0.80	4.59	0.82	4.67	0.84	5.67	0.85	20.98	21.70
	50	3.44	0.59	3.76	0.65	3.88	0.67	3.97	0.69	4.85	0.69	15.07	14.43
	150	2.46	0.42	2.74	0.47	2.82	0.48	2.98	0.51	3.90	0.52	7.95	8.04

cont. Table 7

Sandy loam	Cadmium												
	0	4.73	2.26	5.04	2.41	5.39	2.58	5.47	2.62	6.10	2.64	29.82	24.97
	50	3.96	1.91	4.28	2.06	4.69	2.26	4.80	2.31	5.51	2.33	22.38	19.47
	150	3.25	1.55	3.61	1.73	3.89	1.86	3.97	1.90	4.74	1.92	15.33	13.24
	Copper												
	0	4.73	2.26	5.04	2.41	5.39	2.58	5.47	2.62	6.10	2.64	29.82	24.97
	50	3.30	1.58	3.67	1.76	3.88	1.86	3.98	1.91	4.76	1.93	15.18	13.38
	150	2.68	1.30	3.07	1.49	3.22	1.57	3.34	1.62	4.19	1.64	10.33	9.65
	Zinc												
	0	4.73	2.26	5.04	2.41	5.39	2.58	5.47	2.62	6.10	2.64	29.82	24.97
	50	3.91	1.89	4.26	2.06	4.65	2.25	4.75	2.29	5.48	2.31	21.90	19.25
	150	3.74	1.81	4.06	1.97	4.42	2.14	4.50	2.18	5.25	2.20	19.73	17.31

*explanations see Table 5

The indices calculated for soils contaminated with cadmium, copper and zinc which were cropped with spring oilseed rape (Table 3) were similar to the ones calculated for soil under oats. The BA_1 index indicated most evidently the differences between polluted and unpolluted soils. Its value in polluted treatments was 63% lower than in unpolluted ones. The indices BA_4, BA_5 and BA_9 followed.

Average differences in the range of 70% between values of the indices achieved for polluted and unpolluted soils under yellow lupine were shown by the indices BA_1, BA_4 - BA_6 and BA_9. (Table 4). The values of these indices showing the differences between polluted and unpolluted soils, as average ones for the whole experiment, were approximately the same, irrespective of the plant species and soil type. They all ranged between 65% to 68% and were positively correlated with yields of the crops (Table 8).

Similar values of the indices of soil biochemical activity pointed to a smaller difference between the biochemical activity of the soils polluted with heavy metals and unpolluted ones. In soils under oats (Table 5), spring oilseed rape (Table 6) and yellow lupine (Table 7), the largest differences in the soil enzymatic activity, reaching on average 53-60%, could be captured with the indices BA_{20} and BA_{21}. The index BA_{21} was positively correlated with yields of crops at $p=0.01$ and the index BA_{20} – at $p=0.05$ (Table 9).

Our analysis of the results suggests that the sensitivity of every index is primarily dependent on the activity of dehydrogenases and content of carbon. Consequently, the most sensitive were BA_1 among simple indices and BA_{20} and BA_{21} in the group of complex ones. BA_1 informs about an average, independent from the species of crop or type of soil, 68% depression in the biological activity of soil polluted with highest rates of heavy metals. The analogous percentages for the other two indices are 58% (BA_{20}) and 56% (BA_{21}). These two indices were the most sensitive ones among complex indicators, while the other ones in this group, when determined for the most seriously contaminated soil, declined in the range of 29 to 41%. And although they were positively correlated with the yield of plants, their poor sensitivity to contamination makes them less useful for soil quality assessment. Simple indices, despite their obvious advantage such as low cost, should be approached with caution, because they fail to account for a wider range of enzymes which together shape the total enzymatic activity of soil. In conclusion, indices B_{20} and B_{21} are recommended.

Table 8

Coefficients of the correlations between plant yields and simple biochemical indices of soil quality

Type of soil	Plant species	Simple biochemical indices of the quality of soil ***								
		BA_1	BA_2	BA_3	BA_4	BA_5	BA_6	BA_7	BA_8	BA_9
Loamy sand	oat	0.62	0.63	0.64	0.62	0.61	0.61	0.62	0.68	0.62
	oil seed rape	0.73*	0.78*	0.78*	0.30	0.29	0.30	0.30	0.41	0.30
	yellow lupine	0.69	0.60	0.61	0.48	0.47	0.49	0.45	0.52	0.47
Sandy loam	oat	0.73*	0.75*	0.76*	0.74*	0.71*	0.73*	0.74*	0.79*	0.74*
	oil seed rape	0.36	0.40	0.41	0.53	0.54	0.55	0.57	0.62	0.53
	yellow lupine	0.73*	0.67	0.66	0.43	0.43	0.43	0.44	0.46	0.42
Independent from the plant										
Loamy sand		0.61**	0.59**	0.59**	0.49*	0.48*	0.48*	0.47*	0.52*	0.48*
Sandy loam		0.44*	0.43*	0.44*	0.45*	0.41	0.45*	0.45*	0.46*	0.45*
Independent from the plant species and type of soil										
		0.50**	0.47**	0.17**	0.51**	0.43**	0.42**	0.45**	0.47**	0.37**

*correlation coefficients significant at p=0.05, ** at p=0.01
*** explanations see Table 2

Table 9

Coefficients of the correlations between plant yields and complex biochemical indices of soil quality

Type of soil	Plant species	Complex biochemical indices of soil quality ***											
		BA_{10}	BA_{11}	BA_{12}	BA_{13}	BA_{14}	BA_{15}	BA_{16}	BA_{17}	BA_{18}	BA_{19}	BA_{20}	BA_{21}
Loamy sand	oat	0.70	0.70	0.70	0.70	0.70	0.70	0.69	0.69	0.69	0.69	0.68	0.67
	oil seed rape	0.79*	0.81*	0.78*	0.80*	0.78*	0.80*	0.78*	0.80*	0.79*	0.80*	0.74*	0.77*
	yellow lupine	0.67	0.69	0.69	0.71*	0.70	0.72*	0.69	0.71*	0.71*	0.72*	0.74*	0.75*
Sandy loam	oat	0.79*	0.81*	0.79*	0.81*	0.76*	0.78*	0.75*	0.77*	0.77*	0.77*	0.73*	0.76*
	oil seed rape	0.43	0.45	0.45	0.48	0.46	0.48	0.47	0.49	0.48	0.49	0.42	0.46
	yellow lupine	0.80*	0.78*	0.80*	0.79*	0.76*	0.75*	0.76*	0.75*	0.76*	0.75*	0.79*	0.78*
Independent from the plant													
Loamy sand		0.63**	0.63**	0.62**	0.62**	0.62**	0.62**	0.61**	0.62**	0.61**	0.61**	0.62**	0.61**
Sandy loam		0.43*	0.45*	0.42*	0.45*	0.39	0.42*	0.39	0.42*	0.41	0.42*	0.39	0.42*
Independent from the plant species and type of soil													
		0.44**	0.11	0.42**	0.09	0.39**	0.08	0.38*	0.08	0.41**	0.07	0.38*	0.43**

*correlation coefficients significant at p=0.05, ** at p=0.01

*** explanations see Table 5

CONCLUSIONS

1. Activity of enzymes should be expressed in units of the product of a catalyzed reaction in 1 h time calculated per 1 kg d.m. of soil, i.e. the activity of dehydrogenases in ěmol TFT, catalase – mol O_2, alkaline phosphatase, acid phosphatase and arylsulphatase – nmol PNP, and urease – mmol $N\text{-}NH_4$. By using unified units we are able to compare results and to assess quality of different soils, regardless of the author and research centre.

2. Among the 21 analyzed biochemical indices of soil quality, the best ones were BA_{20} = %C × (Deh + Pal + Pac + Ure), calculated from the content of organic carbon and activity of four enzymes: dehydrogenases (Deh), alkaline phosphatase (Pal), acid phosphatase (Pac) and urease (Ure), as well as BA_{21} = Deh + Cat + Pal + Pac + Ure + Glu + Aryl, calculated from the activity of seven enzymes: dehydrogenases (Deh), catalase (cat), acid phosphatase (Pac), alkaline phosphatase (pal), urease (Ure), β-glucosidase (Glu) and arylsulphatase (Aryl).

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REVIEW PAPERS

ROLE OF SELENIUM IN PATHOPHYSIOLOGY OF ALCOHOL DEPENENCE – INDICATIONS FOR SUPPLEMENTATION

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Abstract

A significant increase of interest in the role of selenium in various biological processes may be noticed when reviewing recent literature related to many areas of science. Owing to various functions it fulfils, selenium is known as an essential component required for the proper functioning of human and animal organisms. A decrease in the concentration of selenium in the body of an alcohol dependent person may inhibit formation of selenoproteins, which in turn may impair the DNA repair, weaken the immunological and anti-inflammatory response, or adversely affect mechanisms preventing the development of diseases like hepatocirrhosis. The depressed activity of selenium in people addicted to alcohol may also cause poor semen quality leading to infertility in men. It is also held responsible for a higher risk of depression.

Key words: selenium, alcohol dependence.

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ROLA SELENU W PATOFIZJOLOGII UZALEŻNIENIA OD ALKOHOLU – WSKAZANIA DO SUPLEMENTACJI

Abstrakt

Dokonując przeglądu piśmiennictwa z ostatnich lat, można zauważyć istotny wzrost zainteresowania selenem w wielu dziedzinach nauki. Pierwiastek ten poprzez różnorakie funkcje, jakie spełnia jest nieodzownym składnikiem potrzebnym do właściwego funkcjonowania organizmów ludzkich i zwierzęcych. Zmniejszona zawartość selenu w organizmie pacjentów uzależnionych od alkoholu może hamować wytwarzanie selenoprotein, co z kolei może upośledzać naprawę DNA, wpływać na pogorszenie odpowiedzi odpornościowej i przeciwzapalnej oraz upośledzać mechanizmy chroniące przed rozwojem takich chorób, jak np. marskość wątroby. Niedobór selenu może także powodować złą jakość nasienia, a w konsekwencji niepłodność u mężczyzn oraz zwiększenie ryzyka wystąpienia depresji.

Słowa kluczowe: selen, uzależnienie od alkoholu.

INTRODUCTION

Selenium, one of the trace elements present in the human organism, has been known for about 200 years (SHER 2001). Over the years, selenium has become the subject of intense scientific research in numerous disciplines of science, e.g. biochemistry, geology or toxicology. Selenium is known as a two-faced element. On the one hand, it is essential for the proper functioning of living organisms. On the other hand, it is harmful in excessive quantities. The rising interest in the role of selenium and its compounds is driven not only by the need to clarify its functions in the human organism, but also by the fact that there is a very small difference between its essential and toxic levels. Until the mid-1950s, selenium had been known solely because of its toxicity to humans and animals. The toxic activity of selenium was mentioned as early as the 13th century (REID et al. 2004). It was not until 1957 that SCHWARTZ and FOLZ demonstrated positive effects of selenium, such as prevention of hepatic necrosis in rats with vitamin E deficiency (SCHWARZ, FOLZ 1957). In 1973, Rotruck and Flohe presented a wealth of evidence for better understanding of the biochemical role of selenium. For example, it was discovered that selenium was an integral component of the active center of glutathione peroxidase enzyme GSH-Px); 17 years later it was demonstrated that type 1 iodothyronine deiodase (D1, EC 1.97.1.10) contained selenocysteine (SeCys) in its active center. These discoveries were a breakthrough in the recognition of the role of selenium. Identification of different selenoproteins and selenoenzymes stimulated intense studies on the physiological role of selenium, nutritional problems connected to demand for this element, clinical symptoms of selenium deficiency or excess and the role of selenium deficiency in ethiology and course of pathological states (HOLBEN, SMITH 1999, ZAGRODZKI 2000, MISTRY et al. 2012).

Over the past years, disorders in the selenium management in organisms of alcohol addicts have been given growing attention. It was demonstrated that a low level of selenium considerably contributes to the increased incidence of depressions, phobic states and hostility in interpersonal contacts (Sher 2001). Various functions of selenium which may influence the development of alcohol dependence are due to selenoproteins in which selenium is present in as selenocysteine amino acid.

SELENIUM CONCENTRATION IN BLOOD OF ALCOHOL DEPENDENT PEOPLE

Intensive research conducted for a few decades now including the monitoring of selenium concentration in blood serum of alcohol dependent people has demonstrated a decrease in the selenium level compared to the control group. However, no relationship between selenium concentration and age of the patient or degree of alcohol addiction has been noted, indicating that the deficiency of selenium occurs at the initial stage of the disease and accompanies its development (Pilaczyńska et al. 1988). Total selenium concentrations in the serum of people with alcoholic hepatic insufficiency has been investigated recently. One of the aims was to try and assess the activity of the antioxidant system. The mean concentration of selenium in blood serum of alcohol dependent patients with liver impairment was considerably lower than in the control group of healthy people. One of the causes of alcoholic liver impairment is suspected to be peroxidation of lipids and proteins by free radical reactions causing. The activity of numerous antioxidants depends *inter alia* on the selenium concentration in an organism (González-Reimers et al. 2008, González-Reimers et al. 2009).

Only sixty-eight patients were followed up.

Other studies devoted to determination of the concentration of selenium in the blood serum of patients with alcoholic hepatocirrhosis demonstrated lowered concentrations of this element, decreasing parallel to the intensity of the disorder. Also, the activity of glutathione peroxidase in erythrocytes decreased as the extent of alcoholic hepatocirrhosis rose (Gerli, Locatelli 1992).

Table 1

Selenium concentration in serum of alcohol dependent patients (acc. to González-Reimers et al. 2008), µg l^{-1}, $p<0.05$

Examined group (n = 76)	Patients with hepatocirrhosis (n = 34)	Patients without hepatocirrhosis (n = 42)	Patients who recovered (n = 51)	Patients who died (n = 17)	Control group (n = 16)
73.6 (67.4-83.8)	70.18 (66.5-76.6)	77.12 (67.7-92.3)	76.2 (68.8-86.4)	68.2 (59.3-70.1)	81.9 (75.0-86.9)

THE ROLE OF SELENIUM IN PATHOPHYSIOLOGY OF ALCOHOL DEPENDENCE

Antioxidative function

Ethanol is a source and activator of free radicals synthesis. Higher intoxication with alcohol means more intensive formation of reactive oxygen species (ROS) in mitochondria. A decrease in the NAD/NADH ratio increases the generation of oxygen radicals via a decrease in the flow of electrons in the respiratory chain. Increased ROS formation impairs mitochondria, which aggravates oxidative stress, while higher oxygen consumption intensifies hypoxia of the organs. Most of the recognized selenoenzymes play a significant role in the protection of cellular membranes against their oxidative damage. The main contribution of selenoproteins is related to the antioxidant protection by glutathione peroxidase, thioredoxin reductase (TrxR, EC 1.8.1.9), selenoprotein P (SeP) and others (Jelski et al. 2006).

Glutathione peroxidases

Glutathione peroxidases are essential components of the antioxidant barrier in the cells, and their main enzymatic function is the reduction of hydrogen peroxide and organic peroxides with the contribution of reduced glutathione. This enzyme protects cellular membranes, hemoglobin and fatty acids against oxidation. Each GSH-Px sub-unit contains selenocysteine in position 47, i.e. cysteine analogue with a selenium atom instead of sulphur. The presence of selenocysteine enables glutathione oxidation without release of a free thiol radical of glutathione (Graczyk et al. 1994). The reaction follows three steps:

1) release of selenocysteine residue by hydrogen peroxide;

$$\text{GSH-Px} - \text{SeH} + H_2O_2 \rightarrow \text{GSH-Px} - \text{Se} - \text{OH} + H_2O$$

2) glutathione oxidation and selenosulphide formation;

$$\text{GSH-Px} - \text{SeOH} + \text{GSH} \rightarrow \text{GSH-Px} - \text{SeSG} + H_2O$$

3) selenosulphide reaction with a subsequent glutathione particle, regenerating selenocysteine;

$$\text{GSH-Px} - \text{SeSG} + \text{GSH} \rightarrow \text{GSH-Px} - \text{SeH} + \text{GSSG}$$

Five various GSH-Px forms are distinguished, each compound acting in a different compartment of the cell, e.g. cytosol, gastro-intestinal, extracellular, phospholipids and nuclei of spermatozoa (Kuhn, Borchert 2002). Selenium deficiency in people addicted to alcohol leads to defective GSH-Px activity, which may result in several dysfunctions of internal organs e.g. hepato cirrhosis. Inebriety induces increased formation of 4-HNE (4-hydroxynonenal), which lowers the amount of glutathione and inactivates GSH-Px, thus increasing the amount of free radicals (Miyamoto et al. 2003, Jelski et al. 2006).

Selenoprotein P

Selenoprotein P, described in the early 1980s, plays an antioxidant role (Motsenbocker, Tappel 1982), mainly as peroxynitrite reductase or lipid hydroperoxides GSH-Px. Selenoprotein P is a glycoprotein which binds up to 65% of selenium present in serum (Mostert 2000, Burk et al. 2003). Its antioxidant function can be verified by the fact that its decreasing concentration has been demonstrated in serum of people addicted to alcohol (Lai et al. 2009).

Thioredoxin reductase

Thioredoxin reductase (TrxR), one of selenoproteins, was characterized in the 1990s. TrxR is present in the form of three isoenzymes in mammalian cells: cytoplasmic (TrxR), mitochondrial (TrxR2) and as glutathione thioredoxin reductase (TrxR3). Selenium in the form of SeCys – one selenium atom in a protein particle, is present in the active center of TrxR. TrxR directly or indirectly catalyzes reduction of protein disulphide bridges. The reaction occurs in two steps:

1) $\mathrm{NADPH} + \mathrm{H}^+ + \mathrm{Trx\text{-}S}_2 \Leftrightarrow \mathrm{NADP}^+ + \mathrm{Trx\text{-}(SH)}_2$

2) $\mathrm{Trx\text{-}(SH)}_2 + \mathrm{protein\text{-}S}_2 \Leftrightarrow \mathrm{Trx\text{-}S}_2 + \mathrm{protein\text{-}(SH)}_2$

Thioredoxin reductase reduces also other low-molecular compounds, e.g. oxidized glutathione (GSSG), vitamin K, lipid peroxides. It may be thus concluded that Trx plays a very important role in the antioxidant protection of an organism (Jelski et al. 2006).

Table 2

Selenoproteins and their role in pathophysiology of alcohol dependence
(acc. to Mostert 2000, Kuhn, Borchert 2002, Savaskan et al. 2007, Paukert et al. 2011)

Selenoprotein	Functions
Cellular glutathione peroxidase (GSH-Px)	– protects against the oxidative stress – transforms harmful products of lipids and phospholipids peroxidation in harmless ones – water, alcohol – increases antioxidant activity of vitamin E
Lipid superoxide peroxidase	– plays a significant role in synthesis of prostaglandins and catecholamines. – protects phospholipids against their oxidative damage
Intestinal peroxidase	– facilitates vitamin E adsorption – protects cell membranes against their oxidative damage
Thioredoxin reductase	– protects against the oxidative stress – maintains intracellular redox homeostasis – reduces nucleotides during DNA synthesis
Selenoprotein P	– protects against the oxidative stress – transports selenium to erythrocytes – binds up to 60% of selenium contained in serum

There are just a few papers concerning an influence of selenium on cancers development in alcohol dependent patients published so far. In China, where the incidence of primary liver cancer is especially high, a study on a group of 130,471 patients was conducted. They were supplemented with selenium in the range from 30 mg to 50 µg of selenium per day for eight years. Over 50% decline in the incidence was noted: 27.2 cases per 100 000 examined subjects versus 50.4 per 100 000 in people who were given sodium chloride (Yu et al. 1997). Another study on people at risk of primary liver cancer has been reported in the literature. The patients were given selenium-fortified yeasts in the amount equal to a dose of 200 µg of selenium per day; the control group received placebo. This experiment lasted for two years. The signs of cancer were noted in 0.69% of the examined patients in the group receiving selenium compared to 1.26% in the control group. Based on these studies, it can be assumed with high probability that selenium supplementation in patients addicted to alcohol may decrease the risk of hepatocirrhosis. However, further studies are needed in order to recognize the influence of selenium on development of cancer in people addicted to alcohol. The suggested mechanisms of the anticarcinogenic activity of selenium include: influence on DNA repair, stimulating influence on apoptosis and decreased mutagenicity of carcinogenic factors (Whanger 2004).

The role of selenium in immunity of alcohol dependent people

Persistent alcohol consumption inhibits functions of the immunological system, which is reflected by an increased incidence of various infectious diseases. Impairment of immunity may also be caused by direct action of ethanol or by the indirect effects caused by its toxic metabolites (Waszkiewicz, Szulc 2010).

There are numerous studies supporting the assumption that selenium plays a significant role in the immunological system, both of animals and humans. Selenium produces multidirectional effects on the immunological system. It is essential for the proper course of humoral and cellular response. For example, it was observed that consumption of selenium in an amount of 200 µg per day as Na_2SeO_3 stimulates the cytotoxic activity of NKC cells (Natural Killer Cells), leads to elevated production of cytotoxic lymphocytes (mainly T ones) and macrophages, and appears to influence the process of gene transcription for the synthesis of immunoglobulins (Rayman 2000, Hardy 2004). Selenium is also contained in type II iodothyronine deiodase (D2, EC 1.97.1.10), an enzyme catalyzing transformation of thyroxin (T4) to triiodothyronine (T3) in the thymus, an organ essential for development of immunity. Therefore, the decreased activity of D2 caused by selenium deficiency in people addicted to alcohol adversely affects the maturation and functioning of cells in this organ (Arthur et al. 2003). The deficiency of selenium observed in people addicted to alcohol may lead to numerous disturbances in the immunological system, e.g.:

1) suppression of prostaglandins and immunoglobulins biosynthesis;
2) decrease in the number and activity of T lymphocytes, NK cells and macrophages;
3) decrease in the immunological response of the host to bacterial or viral infection;
4) increase in aggregation of blood platelets.

Alcohol abuse is also accompanied by an increased risk of sexually transmitted diseases, including AIDS. Considerably lower selenium concentrations in serum were observed in many studies on patients with HIV at an advanced stage of the disease compared to the control group or a group of patients in the early stage of the disease. In *in vitro* studies conducted on lymphocytes and macrophages collected from patients infected with HIV, partial inhibition of virus replication was obtained after selenium supplementation (Look et al. 1997, Stone et al. 2010).

Little is known about applicability of selenium-enriched supplements or a diet rich in selenium in the immunotherapy of alcohol dependent people. More detailed understanding of the contribution of selenium into mechanisms of the development of immunological disorders in alcoholics may allow rational administration of Se to control and treat diseases accompanying alcohol dependence, including HIV infections.

Selenium and sexual functions in alcohol dependent people

Chronic alcohol consumption may lead to depressed sexual activity of men and even to impotence. It has been demonstrated by ample studies that an increase of alcohol concentration in blood causes erection disorders, ejaculation delay and, in some individuals, testicular atrophy and sub-fertility. Selenium deficiency observed in people addicted to alcohol may deteriorate semen quality and in extreme situations lead to infertility. It is so because semen contains high concentrations of selenium, enclosed in the mitochondrial sheath of spermatozoa, and plays an important role in maintaining male sexual activity. Approximately 50% of selenium present in a man's body is localized in sexual glands and in semen. Selenium is also essential for the production of testosterone (Shafiei et al. 2011, Camejo et al. 2011). A chronically low testosterone level in men addicted to alcohol leads to hypogonadism and feminization. Administration of selenium with vitamin E decreases oxidative stress of semen. It has been demonstrated that an adequate diet including selenium supplementation increases the number of spermatozoa and improves their motility (Vezina et al. 1996, Nikolaev et al. 1999, Safarinejad 2009, Moslemi, Tavanbakhsh 2011). Thus, it may be supposed that selenium supplementation would be useful in treatment of subfertility in men addicted to alcohol. A strict correlation between the amelioration of the above changes subsiding and selenium supplementation has been noted. In the late 1990s, sperm nuclei glutathione peroxidase (SnGSH-Px) was identified and characterized in the nuclei of spermatozoa, which is a selenoprotein whose significant role in protection of DNA in spermatozoa may be proven by the occurrence of mechanical instability of mitochondrial matrix in the case of selenium deficiency (URSINI et al. 1999).

Selenium and depression

Selenium, except iodine, plays a significant role in the metabolism of hormones of the thyroid gland. Selenium is a component of 5'-iodothyronine deiodinase, an enzyme responsible for transformation of inactive thyroxin in triiodothyronine, which is the main biologically active thyroid gland hormone, or to an inactive rT_3 isomer. These disturbances of the above transformation may be observed accompanied by selenium deficit in an organism (KARLE et al. 2005, KÖHRLE, GÄRTNER 2009, SCHOMBURG 2011). It was demonstrated in numerous studies that am excessively low concentration of selenium is accompanied by a lowered level of both $T_{3,}$ and T_4. It is also known that thyroid gland hormones influence the proliferation of glial cells, myelination and synthesis of enzymes essential for neurotransmitter production. They also have a substantial impact on the cerebral metabolism, affecting various neurotransmission systems, e.g. noradrenergic, serotoninergic and GABAergic ones. Hormones excreted by the thyroid influence receptor changes in various brain structures (BAUER et al. 2002). Inadequate levels of hormones produced in the thyroid gland caused for example by selenium deficiency may favor the occurrence of depressions, phobic symptoms and cognitive deficiencies in alcohol dependent people. It has been proven that there is a strong relationship between alcohol consumption and depression. Moreover, the lower the selenium concentration in erythrocytes, the more frequent the occurrence of depressive disorders. A study devoted to the efficacy of selenium supplementation showed that an intake of about 100 µg of selenium per day effectively attenuates various symptoms, e.g. lowered mood, anxiety, fear, disorientation, hostile attitude to surroundings (RAYMAN 2000, SHER 2001, MOKHBER et al. 2011). It may be thus concluded that selenium-enriched food consumed by people addicted to alcohol may contribute to remission of depression symptoms.

Summary

Growing interest in selenium and its role in living organisms is notable in an overview of literature published in the recent years. This review of references and various investigations support the claim that selenium plays a significant role in the pathophysiology of alcohol dependence. It has been demonstrated that selenium has numerous functions, which makes it a very interesting object of medical examinations. A decrease in the concentration of selenium in an organism of alcohol dependent people may inhibit formation of selenoproteins, which in turn may impair DNA repair, induce deterioration of the immunological and anti-inflammatory response, and impair mechanisms protecting against the development of diseases like hepatocirrhosis. Selenium deficiency may also cause poor semen quality and consequently infertility in men. Selenium deficit is also held responsible for a higher risk of depression. With the current state of knowledge, it is impossible to conclude with absolute certainty how efficient supplementation of the human organism with selenium-enriched food is and what effects it has on the process of combating the addiction. Therefore, further long- term clinical studies are needed. Better recognition of the influence of selenium and its compounds on the body of an alcoholic would certainly contribute to the elaboration of new therapy methodology in treatment of diseases and disorders occurring in a course of alcohol dependence.

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EFFECT OF CADMIUM, COPPER AND ZINC ON PLANTS, SOIL MICROORGANISMS AND SOIL ENZYMES *

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Abstract

Heavy metals when present in amounts equal to the geochemical background do not interfere with the soil metabolism, which is associated with the growth and development of soil microorganisms as well as the processes of synthesis and re-synthesis, governed by intra- and extracellular enzymes. In the said concentrations, heavy metals do not cause undesirable changes in the development of plants. On the contrary, such elements as copper and zinc are essential constituents of physiological processes in all living organisms, including microorganisms and plants. Some soils suffer from zinc and copper deficits, which is why they are enriched with fertilizers containing copper or zinc to satisfy the nutritional requirements of crops. Cadmium is different in that its essential role in the proper functioning of living organisms has not been proven yet.

In Poland, soils contaminated with heavy metals, including cadmium, copper and zinc, occur only locally. The purpose of this study has been to discuss the characteristics of these elements in terms of the chemical properties and the role in the natural environment, the effect they produce on plants when present in excessive concentrations in soil and the response of soil microbes and enzymes to such contaminants.

Crops cultivated on soil with an elevated content of heavy metals typically present inhibited growth, reduced transpiration, chlorosis of leaves, limited germination of seeds and deformations of the root system. The effect induced by heavy metals is more pronounced in the early development of plants. Mobility and plant availability of heavy metals depend on a series of factors, for example the soil pH, content of organic matter, grain-size composition of soil, content of iron and manganese oxides, soil sorption capacity and the type of metal. Higher bioavailability of heavy metals is observed in soils with a low content of humic acids. As the soil pH increases (within 6.5-7.5), metals, especially zinc and – to a lesser degree – copper become less toxic to plants.

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The mechanism building tolerance of plants to heavy metals is closely connected with processes which reduce the uptake and transport of metals and with detoxification on cellular membranes and inside cells. An increase in the concentration of metals induces the synthesis of phytochelates, whose main function is to sustain the homeostasis of metals in the cell. These proteins also transport metal ions to vacuoles, where they can be bound by oxalates.

Excessive amounts of cadmium, copper and zinc disrupt the homeostasis of soil by interfering with the control mechanisms on the level of genes, thus inhibiting the activity of microbial enzymatic proteins. They cause damage to metabolic pathways, often resulting in the apoptosis of cells. Consequently, the counts and species diversity of soil microorganisms suffer. Such process as nitrification and ammonification are inhibited, alongside the activity of soil enzymes. The adverse influence of cadmium, copper and zinc on microorganisms and enzymes can be alleviated by application of organic and natural fertilizers. For soil phytoremediation, microorganisms resistant to these metals but enhancing their availability can be used.

Key words: cadmium, copper, zinc, plants, microorganism, enzymes.

ODDZIAŁYWANIE KADMU, MIEDZI I CYNKU NA ROŚLINY, DROBNOUSTROJE I ENZYMY GLEBOWE

Abstrakt

Metale ciężkie, gdy stanowią tło geochemiczne, nie powodują zakłóceń metabolizmu glebowego związanego z rozwojem organizmów glebowych oraz z procesami syntezy i resyntezy, o których decydują zarówno enzymy wewnątrzkomórkowe, jak i zewnątrzkomórkowe. Nie powodują także niekorzystnych zmian w rozwoju roślin, a wręcz przeciwnie, takie pierwiastki jak miedź i cynk są mikroskładnikami niezbędnymi w procesach fizjologicznych wszystkich organizmów, w tym także drobnoustrojów i roślin. W niektórych glebach stwierdza się niedobór cynku i miedzi, dlatego by uzupełnić potrzeby pokarmowe roślin wprowadza się nawozy zawierające miedź lub cynk. Inny charakter ma kadm, którego niezbędności w prawidłowym funkcjonowanie organizmów jak na razie nie udowodniono.

W Polsce lokalnie występują gleby zanieczyszczone metalami ciężkimi, w tym kadmem, miedzią i cynkiem, dlatego celem pracy było przedstawienie charakterystyki tych pierwiastków pod względem właściwości chemicznych i roli w środowisku przyrodniczym, ich oddziaływania na rośliny uprawiane w warunkach nadmiaru tych pierwiastków w glebie oraz odpowiedzi drobnoustrojów i enzymów glebowych na to zanieczyszczenie.

Rośliny uprawiane na glebach o podwyższonej zawartości metali ciężkich charakteryzują się zahamowaniem wzrostu, ograniczeniem transpiracji, chlorozą liści, ograniczeniem kiełkowania nasion oraz deformacją systemu korzeniowego. Odziaływanie to jest silniejsze we wczesnych fazach rozwojowych. Mobilność i dostępność metali ciężkich dla roślin jest uzależniona m.in. od pH gleby, zawartości materii organicznej, składu granulometrycznego gleby, zawartości tlenków żelaza i manganu, pojemności sorpcji oraz rodzaju metalu. Większą biodostępność metali ciężkich dla roślin obserwuje sie w glebach o niskiej zawartości kwasów humusowych. Wraz ze wzrostem pH gleb (6,5-7,5) zmniejsza się fitotoksyczne działanie, szczególnie cynku, w mniejszym stopniu miedzi.

Mechanizm tolerancji roślin na metale ciężkie związany jest z procesami ograniczającymi pobieranie i transport metali, procesami detoksykacji na błonach komórkowych i wewnątrz komórki. Wzrost stężenia metali indukuje syntezę fitochelatyn, których główną funkcją jest utrzymanie homeostazy metali w komórce. Białka te przenoszą również jony metali do wakuoli, gdzie mogą być wiązane przez szczawiany.

Nadmierne ilości kadmu, miedzi i cynku naruszają homeostazę gleby, zaburzając mechanizmy kontroli na poziomie genów, przez co hamują aktywność białek enzymatycznych drobnoustrojów. Wywołują uszkodzenia szlaków metabolicznych, niejednokrotnie prowadząc do apoptozy komórki. W konsekwencji zmianie ulega liczebność oraz różnorodność gatunkowa mikroorganizmów. Hamowane są takie procesy, jak nitryfikacja i amonifikacja oraz aktywność enzymów glebowych. Negatywne działanie kadmu, miedzi i cynku na drobnoustroje i enzymy można łagodzić stosując nawozy organiczne i naturalne, szczególnie efektywna jest substancja organiczna zasobna w kwasy huminowe. W fitoremediacji można wykorzystywać także drobnoustroje oporne na te metale, a jednocześnie zwiększające ich przyswajalność.

Słowa kluczowe: kadm, miedź, cynk, gleba, rośliny, mikroorganizmy, enzymy.

INTRODUCTION

Some heavy metals play an extremely important role in biochemical reactions which are significant for the growth and development of microorganisms, plants and animals (Kavamura, Esposito 2010). However, when present in excessive concentrations, then can form non-specific compounds, causing a cytotoxic effect (Nies 1999). Moreover, these metals perform a variety of functions. Such metals as cerium, tin, gallium, thorium and zircon do not play any biological roles. Iron, manganese and molybdenum are important micronutrients and are low in toxicity. Cobalt, copper, chromium, nickel, wolfram, vanadium and zinc belong to essential micronutrients but are toxic. Antimony, arsenic, cadmium, lead, mercury, silver and uranium are highly toxic. Their metabolic role is rather insignificant.

The IUNG Institute in Puławy (Siebielec et al. 2012) has proposed the following division into six degrees based on threshold levels of heavy metals in the 0 to 20 cm soil horizon:

0 – natural content,
I – elevated content,
II – weak contamination,
III – moderate contamination,
IV – strong contamination,
V – very strong contamination.

Depending on the soil grain-size distribution, pH and content of organic matter, the zinc threshold levels were established as follows: the 0 degree comprises soils with 50 to 100 mg Zn kg^{-1}, I – from 100 to 300 mg Zn kg^{-1}; II – from 300 to 1,000 mg Zn kg^{-1}, III from 700 to 3,000 mg Zn kg^{-1}, IV from 3,000 to 8,000 mg Zn kg^{-1} and V from over 3,000 to over 8,000 mg Zn kg^{-1}. For copper, the respective limits are: the 0 degree – from 15 to 40 mg Cu kg^{-1}, I – from 30 to 70 mg Cu kg^{-1}, II – from 50 to 100 mg Cu kg^{-1}, III – from 80 to 150 mg Cu kg^{-1}, IV – from 300 to 750 mg Cu kg^{-1} and V – from over 300 to over 750 mg Cu kg^{-1}. Finally, for cadmium, the set values are: 0 – from 0.03 to 1 mg Cd kg^{-1}, I – from 1 to 3 mg Cd kg^{-1}, II – from

2 to 5 mg Cd kg^{-1}, III – from 3 to 10 mg Cd kg^{-1}, IV – from 5 to 20 mg Cd kg^{-1}, V – from over 5 to over 20 mg Cd kg^{-1} (SIEBIELEC et al. 2012). The lower limits apply to very light soils, and the upper ones – to medium and heavy ones.

According to the Regulation of the Minister for the Environment of 9 September 2002 (Journal of Law 165, item 1359), the allowable concentrations of heavy metals in the topmost layer of soil from 0 to 30 cm are 4 mg Cd kg^{-1}, 150 mg Cu kg^{-1} and 300 mg Zn kg^{-1} of soil. In Poland, soil contamination with heavy metals is localized and appears predominantly in industrialized regions (SIEBIELEC et al. 2012). Globally, pollution of the natural environment has increased dramatically over the past century (FAIZ et al. 2009). The increase can be attributed to the rapid economic development, urbanization and industrialization (KELLY et al. 2003, MIKANOVA 2006, LIAO, XIE 2007, HELMREICH et al. 2010, DUONG, Lee 2011, KHAN et al. 2011, QIAO et al. 2011).

Ecologically, the accumulation of heavy metals in soils is extremely hazardous because soil is a major link in the natural cycling of chemical elements; it is also a primary component of the trophic chain, composed of soil – plants – animals – humans (FAIZ et al. 200, TAKÁČ 2009, BIELIŃSKA, MOCEK-PŁÓCINIAK 2010, LIU et al. 2012, SAGI, YIGIT 2012). Any disruption of the equilibrium between these components may have serious consequences in any of the links of this chain.

CHARACTERIZATION AND PRESENCE OF CADMIUM IN SOIL

Cadmium was discovered in 1817 by Stromeyer (CIBA et al. 1996, TRAN, POPOVA 2013). It is a transition metal, which belongs to group 12 of elements. Its atomic number is 48 and the relative atomic weight is 112,411. The density of the metal is 8,64 g cm^{-3}. The melting point is 321.11°C, and the boiling points equals 767°C. It appears in three oxidation states: Cd^0, Cd^+ and Cd^{2+}. There are 31 isotopes of cadmium with the atomic mass from 99 to 124. Its total content in the Earth's crust is $2 \cdot 10^{-5}\%$ by weight. Cadmium occurs as the mineral called greenockite (CdS). In addition, it appears as an impurity in zinc ores. It is a by-product of zinc metallurgic processes, added as a component to alloys for making telephone and telegraph cables. As a metal, cadmium is also used in nuclear reactors control rods to absorb neutrons. Cadmium compounds find applications in the manufacture of anti-corrosive coatings on plastic materials. The geochemical properties of cadmium are very similar to those of zinc, although it has a greater affinity to sulphur. Cadmium appears in simple and complex compounds. Its predominant form is bivalent, and the metal forms various complex ions (e.g. $CdOH^+$, $CdHCO_3^-$, $CdCl^-$, $Cd(OH)_4^{2-}$). It dissolves in mineral acids but is resistant to the effect of alkalines. It is a toxic element to humans and animals, acting as a strong carcinogenic agent. Cadmium accumulates in the body, in which it is responsible for skeletal deformations, causes kidney disorders and is a factor in diseases of the blood circulation system and in neoplasms (Ciba et al. 1996). The International Agency for Research on Cancer has classified cadmium to the first category of carcinogenic factors in human cancers. Because cadmium ions have a configuration of electrons analogous to that of zinc ions, they are able to supplant zinc in proteins (Beyersmann, Hartwig 2008, Tran, Popova 2013). Cadmium is transported by membrane transporters and cellular channels, where it forms complexes with thiol groups of biomolecules (Bridges, Zalups 2005).

In line with the Directive of the European Parliament and Council no 2006/11/EC, cadmium in included in the list I of families and groups of substances most toxic, persistent and most readily bioaccumulated (Official Journal of the EC 4.3.2006). The content of cadmium in soils worldwide ranges from 0.4 mg to 167 mg kg^{-1} of soil (Kabata-Pendias, Pendias 2001), and in the Polish soils its level varies from 0.04 mg to 57.50 mg kg^{-1} of soil, with an average content measured in 2010-2012 as 0.56 mg kg^{-1} of soil. In total, 98.6% of soils in Poland have a natural content of cadmium. Its highest amounts are detected in the southern parts of the country, encompassing the Provinces of Lower Silesia, Opole, Silesia and Małopolska (Siebielec et al. 2012).

CHARACTERIZATION AND PRESENCE OF COPPER IN SOIL

Copper is a metal known since ancient times (Ciba et al. 1996). It belongs to group 11 of elements. It is red in colour, but when exposed to humid air, it acquires a green layer of verdigris [$Cu(OH)_2$]. The atomic number of copper is 29 and atomic mass equals 63.546. The melting point is 1,084.87°C, and the boiling point is 2,567°C. The density of the metal is 8.92 g cm^{-3}. Copper occurs in five oxidation states: Cu^0, Cu^+, Cu^{2+}, Cu^{3+}, Cu^{4+}. There are 18 copper isotopes with the atomic masses from 58 to 73. Copper creates simple and complex compounds. It is resistant to hydrochloric and hydrofluoric acids, but dissolves in oxidizing acids (e.g. sulphuric and nitric acids). Its total content in the Earth's crust is $1 \cdot 10^{-2}$% by weight. It rarely occurs as native copper, but is more often found as a component of such minerals as chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S) and malachite ($Cu(OH)_2CuCO_3$). In Poland, the largest copper deposits are in Lubin. As a metal, copper is used to make electric wires and parts of various machines. There are many copper alloys, e.g. brass (Cu + Zn), bronze (Cu + Sn) and cupronickel (Cu + Ni). Copper compounds are used in manufacture of plant protection chemicals, dyes, pigments, artificial fertilizers (as a micronutrient) and catalysts. They are also employed for electroplating. Copper is an essential element to the proper functioning of all organisms. Copper belongs to the elements which are required by living organisms. It participates in photosynthesis and respiration of plants (Ashworth, Alloway 2004). "*Copper deficiency in humans causes anaemia, disorders of the nervous and circulation systems. Its excess may damage the liver, kidneys, cardiovascular vessels and brain tissue*" (Ciba et al. 1996).

The Directive of the European Parliament and Council no 2006/11/EC classifies copper into list II of families and groups of substances, which contains substances harmful to the aquatic environment, but whose influence can be limited to a given area (Official Journal of EC 4.3.2006). The content of copper in soils worldwide ranges from 13 mg to 3,700 mg kg^{-1} of soil (Kabata-Pendias, Pendias 2001), and in Poland, it varies from 1.53 mg to 271.73 mg kg^{-1} of soil, with the average concentration in Polish soils equal 10.2 mg kg^{-1} of soil. Most soils in Poland are characterized by the natural copper content. The first degree contamination (4 sites) occurs in the provinces of Małopolska, Lublin and Lower Silesia; the third degree contamination (1 site) can be detected in the Province of Lower Silesia and the fourth degree contamination (1 site) appears in the Province of Lower Silesia (Siebielec et al. 2012).

CHARACTERIZATION AND PRESENCE OF ZINC IN SOIL

Like copper, zinc was known in the ancient times (Ciba et al. 1996). This metal belongs to group 12 of elements. Its atomic number is 30 and the atomic mass equals 65.39. The melting point is 419.58°C, and the boiling point is 907°C. The density of the metal is 7.14 g cm^{-3}. Zinc occurs in three oxidation states: Zn^0, Zn^+, Zn^{2+}. Zinc forms simple and complex compounds. There are 23 zinc isotopes with the atomic weights from 57 to 78. Zinc dissolves in acids and alkalines, releasing hydrogen. It occurs in the following minerals: sphalerite (ZnS), smithsonite ($ZnCO_3$) and willemite (Zn_2SiO_4). Deposits of these minerals in Poland can be found in the area between Olkusz, Chrzanów and Bytom. The content of zinc in the Earth's crust is $7 \cdot 10^{-3}$% by weight. Zinc is used to make zinc sheets or zinc coating on iron and steel products. It is also a component of alloys, reducer in the metallurgy of noble metals and in organic chemistry. Zinc compounds are used to make paints, varnishes, cosmetics, plastics, artificial fertilizers (micronutrient). Zinc is an essential element for all living organisms (Ciba et al. 1996). It plays an important role in catalyzing biochemical reactions by participating in the formation of an enzyme-substrate system, protein translation, gene copying and multiplication of a genetic chain (Sekler 2007). Zinc deficiency causes changes in the bone system and in the chemical composition of blood, and may lead to the cardiac insufficiency or brain developmental defects; the excess of zinc is harmful to the body (Ciba et al. 1996). The toxicity of zinc stems from its interaction with other heavy metals. Zinc is responsible for disturbing the functions of mitochondria (Sekler et al. 2007).

The Directive of the European Parliament and Council no 2006/11/EC classifies zinc list II of families and groups of substances, containing substances harmful to the aquatic environment, whose effect can be limited to a given area (Official Journal of EC 4.3.2006).

The content of zinc in soils worldwide varies within a broad range from 35 mg to 12,400 mg kg^{-1} of soil (Kabata-Pendias, Pendias 2001); in the Polish soils, the range of zinc concentrations is from 10.27 mg do 5,805 mg kg^{-1} of soil, and its average content is 79.81 mg kg^{-1}. At present, there are three sites classified as the 2nd degree soil contamination and one site determined to represent the 4th soil pollution degree in Poland (Siebielec et al. 2012).

SOURCES OF HEAVY METALS IN SOIL

The natural content of heavy metals in soils is known as the biogeochemical background (Coskun et al. 2006, Castaldi et al. 2009, Takáč 2009, Kobierski, Dąbkowska-Naskręt 2012). The occurrence of heavy metals in soils is closely dependent on the chemical composition of parent rock (Kobierski, Dąbkowska-Naskręt 2012, Siebielec et al. 2012). Their concentrations in soils are negatively correlated with the depth of a soil profile. (Glina, Bogacz 2013). A higher content of heavy metals is observed in soils developed from flysch rocks and delluvial deposits (Tran, Popova 2013). The content of heavy metals in soils is shaped by both natural and anthropogenic factors. The natural conditions affecting the content of heavy metals in soils are: the parent rock, soil formation processes, grain-size distribution of a given soil, content of humus, oxydation/reduction potential, soil sorption capacity, soil reaction, plant cover (Burt et al. 2003, Covelo et al. 2007, Dragović et al. 2008, Jaworska, Dąbkowska-Naskręt 2012, Nadgórska-Socha et al. 2013, Skwierawska 2013, Tran, Popova 2013). Moreover, metals like cadmium, copper and zinc as soil pollutants can originate from geochemical processes evoked by volcanic eruptions or the weathering of parent rock (Kabata-Pendias 2004), from emissions by industries and motor transport (Kelly et al. 2003, Mikanova 2006, Liao, Xie 2007), from landfills (Szymańska-Pulikowska 2012), sewage sludge and all types of fertilizers made from waste (Xie et al. 2009, Achiba et al. 2010, Jakubus 2012, Sienkiewicz, Czarnecka 2012). Other sources of contamination include some mineral fertilizers and plant protection chemicals (Qiao et al. 2011). The highest quantities of heavy metals enter soils from the metallurgic and mining industries (Maiz et al. 2000, Wong 2003, Vásquez-Murrieta et al. 2006), and from transportation routes and emission of fumes (Liao, Xie 2007, Helmreich et al. 2010, Duong, Lee 2011, Khan et al. 2011, Qiao et al. 2011). Moreover, some amounts of heavy metals can permeate the environment from tyre manufacturing plants (Khan et al. 2011), petroleum refineries, leaks of petroleum products and lubricants from motor vehicles (Christoforidis, Stamatis 2009). Copper and zinc come from the same sources (Kobza 2005).

The fate of these contaminants depends on two groups of events. The first group comprises processes which aim at depressing their solubility and mobility; the other one encompasses processes stimulating the mobility of heavy metals, thus increasing their toxicity (Achiba et al. 2010).

EFFECT OF CADMIUM, COPPER AND ZINC ON PLANTS

The proper growth and development of plants above all depend on the availability of adequate amounts of nutrients. Apart from macroelements, there are also microelements such as copper and zinc which are necessary for maintaining proper functions of an organism (McCall et al. 2000). These elements may play a role of building blocks or catalysts. There are also such elements in the natural environment which do not have any physiological role, e.g. cadmium (Waalkes 2003, Tran, Popova 2013).

The average concentration of cadmium in plants ranges from 0.03 to 0.70 mg kg^{-1} d.m., copper from 1 do 16 mg kg^{-1}d.m., and zinc from 10 to 86 mg kg^{-1} d.m. (Kabata-Pendias, Pendias 2001). Both deficit and excess of these elements have a negative effect on plants, although their tolerance of copper or zinc deficits in soils is relatively high (Nadgórska-Socha et al. 2013, Tran, Popova 2013).

Many references, e.g. Wyszkowska, Kucharski (2003a), Wyszkowska et al. (2005), Wyszkowski, Wyszkowska (2006), Wyszkowska et al. (2006a), Wyszkowski, Wyszkowska (2009), Wyszkowska et al. (2010), Nadgórska-Socha et al. (2013), Tran, Popova (2013), state that soil contamination with heavy metals has an adverse influence on the growth and development of plants. Plants growing on soil contaminated with heavy metals may tend to take up more of these elements, which are then transferred to subsequent links in the feeding chain (Ciećko et al. 2001, Królak 2003, Zalewska 2012, Nadgórska-Socha et al. 2013). Two mechanisms of the uptake of trace elements by plant roots are distinguished: passive one, by diffusion, and active one, running against the gradient of concentrations and powered by metabolic energy (Rajkumar, Freitas 2008). Although cadmium is not essential for the growth and development of plants, it is readily taken up by the root system (Ciećko et al. 2001, Renella et al. 2004, Tran, Popova 2013), and therefore disturbs the uptake of other elements (Ciećko et al. 2004a, 2005).

Plant species or even cultivars might differ from one another in the tolerance to excessive quantities of cadmium, copper and zinc and in their ability to absorb these elements (Vig et al. 2003, Ciećko et al. 2001, Sekler et al. 2007, Beyersmann, Hartwig 2008, Zalewska 2012, Nadgórska-Socha et al. 2013). The most sensitive are papilionaceous plants, hop, grapevine, fruit (citrus) trees, cereals and spinach. The phytotoxicity of excess heavy metals is caused through the disturbance of physiological processes due to disorders in the uptake of micro- and macroelements that are necessary for the proper functioning of plants (Nadgórska-Socha et al. 2013, Tran, Popova 2013). Crops cultivated on soils with an elevated content of heavy metals are characterized by inhibited growth, reduced transpiration, chlorosis of leaves, limited seed germination and deformations of the root system (Nadgórska-Socha et al. 2013, Tran, Popova 2013). These effects are stronger during the early development stages (Vig et al. 2003, Sekler et al. 2007, Beyersmann, Hartwig 2008, Tran, Popova 2013). The mobility and plant availability of heavy metals depend on the soil pH, content of organic matter, grain-size composition of soil, content of iron and manganese oxides, soil sorption capacity, type of a metal and others (SEKLER et al. 2007, TAKÁČ 2009, ZALEWSKA 2012, TRAN, POPOVA 2013, GUALA et al. 2013). Heavy metals are more easily available to plants in soils with a low content of humic acids (BARANČÍKOVÁ, MAKOVNÍKOVÁ 2003, BORŮVKA, DRÁBEK 2004). As the pH of soils increases (from 6.5 to 7.5), the phytotoxic effect of heavy metals subsides, especially that of zinc and - to a lesser degree - of copper. The process is more intensive in soils containing elevated levels of these metals (CIEĆKO et al. 2001, FINŽGAR 2007, TAKÁČ 2009, WYSZKOWSKI,WYSZKOWSKA 2009, GUALA et al. 2013, NADGÓRSKA-SOCHA et al. 2013, TRAN, POPOVA 2013).

The mechanism building the tolerance of plants to heavy metals is associated with processes which restrain the uptake and transport of metals, processes of detoxication on cellular membranes and analogous processes inside cells (SEKLER et al. 2007, BEYERSMANN, HARTWIG 2008). An increase in the concentration of metals induces the synthesis of phytochelates, whose main function is to maintain the homeostasis of metals in cells. These proteins also transfer metal ions to vacuoles, where they can be bound by oxalates (TEKLIĆ et al. 2008, TRAN, POPOVA 2013).

A useful test indirectly demonstrating changes in the microbiological and biochemical properties is simultaneous assessment of the effects of heavy metals on the growth and development of plants (BELYAEVA et al. 2005, TRAN, POPOVA 2013). Such complex comparisons enable us to appreciate fully the harmful impact of heavy metals on the soil environment (WYSZKOWSKA, KUCHARSKI 2003a, WYSZKOWSKI, WYSZKOWSKA et al. 2006a, WYSZKOWSKA 2009).

Plants became more sensitive as the degree of soil contamination with copper increased. The said sensitivity was a species-specific trait. Yellow lupine was the most sensitive to excess copper; spring canola, especially grown on a more compact soil, i.e. on sandy loam, was the least sensitive. Oat demonstrated an intermediate sensitivity, regardless the soil on which it had been sown (WYSZKOWSKI, WYSZKOWSKA 2004, WYSZKOWSKA et al. 2010). Also, WYSZKOWSKA et al. (2009) confirmed empirically that an excessive content of copper in soil had a negative influence on yields of oat, spring canola and yellow lupine.

The assimilability of heavy metals by plants is also shaped by the antagonistic and synergistic effects of elements. According to BADORA (2002), zinc inhibits the accumulation of cadmium, whereas aluminum is an essential element for the process of zinc immobilization in soil. RENGEL (2000) showed that more intensive fertilization of soil with zinc not only raised the content of that element in *Holcus lanatus*, but also lowered its concentrations of iron, manganese and copper. In turn, CIEĆKO et al. (2004b and 2006) concluded that soil contamination with cadmium depressed the content of lead in aerial parts of maize and roots of radish, or potassium in oat grain and in the aerial parts and roots of yellow lupine and radish.

In treatments where copper had been applied simultaneously with another heavy metal, toxicity of the applied mixtures of metals decreased in the following order: CuNi >CuPb >CuZn>CuCr>CuCd; with two metals: CuZnNi >CuZnPb>CuZnCd >CuZnCr; and with three metals: CuZnNiCr>CuZnNiPb>CuZnNiCr (WYSZKOWSKA et al. 2006a). In another study, WYSZKOWSKA et al. (2007) concluded that the yield of oat had declined significantly on soil polluted with a mixture of metals (NiZnCuPbCdCr).

EFFECT OF CADMIUM, COPPER AND ZINC ON SOIL MICROORGANISMS

Among the factors which influence life in soils are heavy metals (HUANG, SHINDO 2000), which permeate into the soil environment from a variety of sources (KHAN, SCULLION 2002) and substantially modify soil properties. For this reason, they are a severe problem to the whole ecosystem and to organisms which live in it (BELYAEVA et al. 2005).

Ecologically, the accumulation of elements in soil is dangerous because of their possible delayed re-mobilization (OLIVEIRA, PAMPULHA 2006, DE BRUWERE et al. 2007, MERTENS et al. 2007). Toxicity and bioavailability of heavy metals depends on their chemical form and quantities present in a given habitat (LEIROS et al. 1999, LOSKA, WIECHUŁA 2000). Other factors are the temperature, oxidative and reductive potential, presence of anions and cations of other metals and pH (SŁABA, DŁUGOŃSKI 2002).

The results reported by EGLI et al. (2010) and JIANG et al. (2010) indicate that cadmium, copper and zinc can disrupt the microbiological equilibrium of soil. Diverse effects produced by these heavy metals on individual groups of microbes result from specific physiological, morphological and genetic characteristics of the former (CHMIELOWSKI, KŁAPCIŃSKA 1984, BINET et al. 2003, RENELLA et al. 2006, PAUL et al. 2007).

Regarding the metals essential for the proper course of cellular processes, such as copper, zinc or iron, there are mechanisms which regulate their cellular capture. Toxic metals, however, like mercury, cadmium or lead, do not have any specific transport methods. BRIDGES and ZALPUS (2005) explain the mechanism engaged in the penetration of some heavy metals into cells according to the concept of molecular mimicry, in which metal ions are bound by biomolecules. Molecular mechanisms depend strongly on proteins characterized by specific affinity for copper and cadmium.

Disturbances of the biological balance of soil caused by excess of cadmium, copper and zinc might be attributed to the disruption of physiological functions, denaturation of proteins and destruction of cellular membranes of soil microorganisms (CHMIELOWSKI, KŁAPCIŃSKA 1984, ULBERG 1997, KUCHARSKI et al. 2000, LEDIN 2000, BINET et al. 2003, KUCHARSKI, WYSZKOWSKA 2004, RENELLA et al. 2006, ZABOROWSKA et al. 2006). On the one hand, soil bacteria immobilize heavy metals. On the other hand, they contribute to the enhanced mobility of heavy metals, a result caused mainly by microbial metabolites (GILLER et al. 1998, DUMESTRE et al. 1999, LUGAUSKAS et al. 2005, KUFFNER et al. 2008, HE et al. 2010a, b), which is why certain strains of microorganisms are increasingly often employed in phytoremediation (PAUL et al. 2007, RAJKUMAR, FREITAS 2008, HE et al. 2010a, b).

Soil pollution with heavy metals in different quantities and forms causes changes in the counts of microorganisms and activity of microbial enzymes, which is a true reflection of the actual microbiological condition of soil (DICK et al. 2000, TRASAR-CEPEDA et al. 2000, WYSZKOWSKA et al. 2007).

Heavy metals create abiotic stresses (GILLER et al. 1998, LUGAUSKAS et al. 2005, HE et al. 2010a, b) by inducing disorders in the metabolism of microorganims. They can cause the denaturation of proteins and disintegration of cellular membranes (BROOKES et al. 1984). The destructive effect of metals involves some damage to the control systems regulated by regulatory and signal proteins, including the cell's development, apoptosis and regulation of the cellular cycle (BEYERSMANN, HARTWIG 2008). According to NIES (1999) and SCHMIDT et al. (2005), the toxic effect of metals could be due to the blocking of enzymatic active centres and driving away cations important for the functioning of a cell, supplanting their functions, e.g. discontinuation of the cell-to-cell adhesion (cadmium), direct binding with the DNA (chromium), interacting with the binding sites of protein phosphatases (vanadium) (BEYERSMANN, HARTWIG 2008). According to SKŁODOWSKA (2000), cadmium can supplant zinc, while zinc can replace magnesium in cellular structures of microorganisms. These processes might cause mutations. This effect was verified for cadmium acting on *Bacillus subtilis* (PACHA, GALIMSKA-STYPA 1986).

Microorganisms are characterized by high adaptability to undesirable environmental conditions. Tolerant species demonstrate higher resistance to stress factors than sensitive ones (RENSING et al. 2002). Their tolerance is associated with such metabolic functions as:

1) specific transport of metal ions which involves permeases localized in the cytoplasmic membrane (CHMIELOWSKI, KŁAPCIŃSKA 1984, ULBERG 1997, BINET et al. 2003);
2) synthesis and excretion to the environment chelating compounds, which bind and transport ions dissolved in the environment (CHMIELOWSKI, KŁAPCIŃSKA 1984, RENELLA et al. 2006, PAUL et al. 2007);
3) non-specific accumulation of metals: sorption of ions onto mucosal surfaces and the binding by bio-polymers of the wall and membrane complex (CHMIELOWSKI, KŁAPCIŃSKA 1984, LEDIN 2000);
4) presence of plasmids in a bacterial cell, which enable it to acquire resistance to toxic elements: Ag, As, Cd, Cr, Hg, Ni, Sb, Te (ZHANG et al. 2001, MEGURO et al. 2005). The *Rhizobium* bacteria possessing more plasmids are more tolerant to heavy metals than cells of the same species with fewer plasmids (LAKZIAN et al. 2002).

There can be different target sites of accumulation inside cells. Blue-green algae and yeasts accumulate metals in vacuoles, often in the form of polyphosphate granules (Chmielowski 1991, Binet et al. 2003). The transfer of metals in the form of a PC (*phytochelatina*) - CdS complex into vacuoles is associated with the occurrence of an ABC family transporter, coded by the htm (heavy metal tolerance) 1 gene (Ow 1996). Microorganisms can also disarm heavy metals by changing their oxidation degree or converting into a volatile form through methylation (Chmielowski 1991, Binet et al. 2003).

Many researchers (Giller et al. 1998, Kucharski, Wyszkowska 2004, Lugauskas et al. 2005, Zaborowska et al. 2006, Wyszkowska et al. 2008, Boros et al. 2011) demonstrated that cadmium, copper and zinc, when present in excessive quantities in soil, cause disorders in the microbiological balance of soil. Most common are decreasing counts and diminishing diversity of microorganisms (Kucharski 1992, Moffett et al. 2003, Renella et al. 2005a,b, Khan et al. 2006, Lorenz et al. 2006, Xie et al. 2009, Wyszkowski, Wyszkowska 2009, Wakelin et al. 2010). Moffet et al. (2003) found 25% lower biodiversity of taxonomic groups in soil contaminated with 400 mg Zn kg^{-1} versus soil with the natural content of zinc (57 mg kg^{-1} of soil). Several references, e.g. Giller et al. 1998, Singha et al. 1998, Sauve et al. 1999, Cela, Sumner 2002, Wyszkowska et al. 2006a, Wyszkowska et al. 2007, indicate that nitrifying bacteria, symbiotic nitrogen-fixing bacteria and *Azotobacter* spp are the microorganisms most susceptible to heavy metals. Similar conclusions were drawn by Lugauskas et al. (2005), Borowik et al. (2013) as well as Oliveir and Pampulh (2006). Heavy metals produce a stronger effect on *Azotobacter* cells than organotrophic bacteria mainly because richer communities of microbes are more resistant to heavy metals than single species and genera (Loc, Janssen 2005, Mertens et al. 2010).

Singha et al. (1998) report that cadmium is a 6- to 8-fold stronger inhibitor of nitrification and ammonification than zinc. The power of nitrification was reduced to 86.1%, under the influence of heavy metals while ammonification was depressed down to 44.2% of the unaffected level. Gupta and Chaudhry (1994) proved that conversion of $N\text{-}NH_4$ to $N\text{-}NO_3$ was inhibited by metal ions in the following order Hg > Zn > Ni > Pb. The negative influence of zinc on nitrification is most probably due to the direct toxic effect of excess zinc on nitrifying bacteria (Ruyters et al. 2010) as well as the toxic influence of the metal on enzymes responsible for nitrification (Trevisan et al. 2012). The adverse effect of zinc, copper and cadmium on autochthonic soil microorganisms is confirmed by a wealth of references (Kucharski, Wyszkowska 2004, Zaborowska et al. 2006, Wyszkowska et al. 2007, Wyszkowska et al. 2008, Ruyters et al. 2010).

According to Wyszkowska and Kucharski (2003a), the inhibitory effect of heavy metals on soil microorganisms can be represented as follows:

oligotrophic bacteria: (Ni > Pb > $Cr_{(III)}$ > Cu > Zn > Cd),
copiotrophic bacteria: (Cd > Ni > $Cr_{(III)}$ > Zn > Cu),

ammonifying bacteria: (Ni > Pb > $Cr_{(III)}$ > Cd > Zn > Hg),
nitrogen immobilizing bacteria: (Zn > $Cr_{(III)}$ > Hg > Cu),
actinomycetes: (Cu > $Cr_{(III)}$ > Ni > Zn > Pb).

Also, SMYŁŁA (1995) established series of metals according to their toxic effect on actinomycetes of the genus *Streptomyces* (Hg > Cd > Cu > Zn > Ni > Pb).

There are also reports (LOC, JANSSEN 2005, LIU et al. 2007) suggesting that under certain conditions heavy metals can stimulate higher counts of microbial cells in soil, which may be a result of the succession of microorganisms. Such contrary effects of heavy metals on microorganisms can be a result of the varied composition of metabolites produced by microorganisms (MEGURO et al. 2005, WYSZKOWSKA et al. 2007). Products of metabolism form chelates with different metals or change into permanent deposits.

According to LIU et al. (2007), the way heavy metals act depends on their type and rate. In an experiment reported by the cited authors, cadmium applied in rates from 1 to 200 mg kg^{-1} raised counts of actinomycetes and fungi, while depressing numbers of bacteria. According to LIU et al. (2007) and KHAN et al. (2010), bacteria are more sensitive to heavy metals than actinomycetes or fungi. However, lead doses above 50 mg kg^{-1} decreased counts of bacteria, actinomycetes as well as fungi. WYSZKOWSKA and KUCHARSKI (2003a) demonstrated a significant increase in counts of fungi in soil contaminated with zinc and copper at a rate of 500 mg kg^{-1} of soil, while WAKELIN et al. (2010) identified changes in the structure of a bacterial community dwelling in soil with excess copper.

Many references (WYSZKOWSKA, WYSZKOWSKI 2002, YANG et al. 2007, CASTALDI et al. 2009) prove that counts of soil microbes can also be determined by species of grown crops. YANG et al. (2007), VOGELER et al. (2008) and CASTALDI et al. (2009) provide evidence that crops can moderate the influence of heavy metals on soil microbes. The experiments reported by the quoted researchers unquestionably show that crops improve the microbiological activity of soil, mainly owing to substances secreted by roots. RENELLA et al. (2006) created a simplified, artificial rhizosphere, which enabled them to demonstrate that root secreta such as glucose, glutamic acid, citric acid, oxalic acid and their mixtures have a significant albeit varied effect on the growth of microorganisms.

RAJKUMAR and FREITAS (2008) concluded that bacteria resistant to heavy metals can be more successfully used in phytoremediation because they add to a better solubility of heavy metals and can be taken up in larger quantities by plants known as hyperaccumulators. Similar conclusions have been arrived at by HE et al. (2010a, b) and PAUL et al. (2007).

YUANGEN et al. (2006) state that cadmium, copper and zinc cause disorders in the soil respiration and depress the biomass of microorganisms. These researchers suggest that both parameters can be useful for evaluation of the degree of soil contamination with the metals TEJADA (2009) demonstrates that the biomass of microorganisms, mass of earthworms and number of nematodes decrease in soils polluted with cadmium in doses from 100 to 1000 mg kg^{-1} of soil. The negative influence of cadmium on microorganisms was alleviated by application of organic and natural fertilizers. The latter research suggests that organic substance is a good, strategic element in remediation of soils polluted with heavy metals. Particularly helpful for soil remediation is organic matter with a high content of humic acids. The important role of organic substance in remediation of soils polluted with heavy metals has been pointed to by other scholars as well (MARZADORI et al. 2000, RENELLA et al. 2005a, RENELLA et al. 2006, PÉREZ-DE-MORA et al. 2006, TEJDA et al. 2008, TRASAR-CEPEDA et al. 2008, CASTALDI et al. 2009, LI et al. 2009, MORENO et al. 2009, EGLI et al. 2010).

A study conducted by KELLY et al. (2003) in the vicinity of a zinc plant proves that heavy metal contamination has a negative effect on mycorrhizal fungi, Gram positive bacteria and other fungi and actinomycetes. The results suggest that metals also change the structure of microorganisms, which can be restored by effective soil remediation.

LOC and JANSSEN (2005) prove that soil contamination with zinc causes disappearance of sensitive microorganisms, thus raising the counts of zinc tolerant cells. In their study, the physiological diversity of microbial communities decreased as the zinc contamination degree increased. According to WANG et al. (2010), Gram positive bacteria are more susceptible to heavy metals than Gram negative ones. These authors ranked heavy metals with respect to their toxicity towards microorganisms as follows: Cr > Pb > As > Co > Zn > Cd > Cu. On the other hand, GRABOWSKI et al. (1997) decided that the negative influence of heavy metals on microorganisms should be ordered in the following series, according to the increasing toxicity of each subsequent metal: Cu > Pb > Zn > Cd > Hg > Ni > Co > $Cr_{(VI)}$.

The above information univocally proves that excessive rates of cadmium, copper and zinc interfere with the homeostasis of soil, disturbing the control mechanisms on the level of genes, thus inhibiting the activity of enzymatic proteins. Rates of heavy metals above the norm cause damage to metabolic pathways, often resulting in apoptosis of cells. Consequently, counts and diversity of macro- and microorganisms change. Counts of microorganisms in soil are an indirect indicator of the soil's biological activity (WYSZKOWSKA, KUCHARSKI 2003a, LUGAUSKAS et al. 2005, RENELLA et al. 2006). Heavy metals decrease biomass of microorganisms and reduce their activity in soil (WYSZKOWSKI 2002, LUGAUSKAS et al. 2005, MIN et al. 2005, WYSZKOWSKA, WYSZKOWSKA et al. 2008). In cases when they do not lower counts of microorganisms, they still reduce their diversity (MOFFETT et al. 2003, RENELLA et al. 2005a, b, KHAN et al. 2006, LORENZ et al. 2006, WANG et al. 2007, XIE et al. 2009, WAKELIN et al. 2010). LOC and JANSSENA (2005) claim that although the tolerance of microorganisms to soil pollution with heavy metals is a new concept in ecotoxicology and the mechanism involved in this phenomenon has not been completely recognized, it is undeniable that the physiological diversity of microorganisms decreases as the contamination with heavy metals increases. The reason is higher morbidity of sensitive cells under stress conditions, which favours an increase in counts of more tolerant microbes. The research reported by LORENZ et al. (2006), KHAN et al. (2010) or WAKELIN et al. (2010) also shows that heavy metals alter the structure of bacterial communities in soils.

EFFECT OF CADMIUM, COPPER AND ZINC ON SOIL ENZYMES

Soil contamination with heavy metals alters counts and diversity of microorganisms, but also changes the enzymatic activity of soil, which – as many authors claim (KUCHARSKI 1997, DICK et al. 2000, TRASAR- CEPEDA et al. 2000, WYSZKOWSKA et al. 2005a, b, LIU et al. 2007, GULSER, ERDROGAN 2008, VOGELER et al. 2008, MORENO et al. 2009, FU et al. 2009, LEE et al. 2009, XIE et al. 2009, JIANG et al. 2010, WYSZKOWSKA et al. 2013) – is an objective manifestation of the biological status of soil. Among enzymes secreted by microorganisms to soil important are the ones which take part in degradation of plant residues and in transformations of nitrogen, phosphorus and sulphur compounds (KUCHARSKI 1997, WYSZKOWSKA, WYSZKOWSKI 2003, WYSZKOWSKA et al. 2005b, KUCHARSKI, WYSZKOWSKA 2010, BIELIŃSKA et al. 2013). In the environment, the most important functions are performed by the enzymes which belong to oxidoreducates: dehydrogenases and catalase, and to hydrolases: acid phosphatase, alkaline phosphatase, urease, arylsulphatase and β-glucosidase (KUCHARSKI 1997, RENELLA et al. 2006, KUMPIENE et al. 2009, WYSZKOWSKI, WYSZKOWSKA 2009, DICK et al. 2000).

Some heavy metals are essential for enzymes to function properly. Zinc appears in over 300 enzymes, which belong to six classes (MCCALL et al. 2000). Trace elements in enzymes play a triple function: catalytic, structural and regulatory. Many intracellular enzymes could not function well without zinc. Such enzymes include carbon anhydrase, carboxypeptidase, thermolysine, alkaline phosphatase, dehydrogenases (glyceraldehyde-3-phosphate, alcohol, glutamine), fructo-diphosphate aldolases, superoxide dismutase, DNA and RNA polymerase, tRNA transferase. Zinc can stabilize their protein structure, or else act as its activator or inhibitor (CORDOVA, ALVAREZ-MON 1995). The effect of heavy metals on soil enzymes can be direct or indirect. The direct influence consists in changing the activity of free, extracellular enzymes; the indirect influence is produced by affecting the biosynthesis of enzymes by microorganisms, composition of soil microorganisms, mycorrhizae, production of root excreta or release of enzymes from dead roots (CORDOVA, ALVAREZ-MON 1995, MCCALL et al. 2000, HINOJOSA et al. 2008).

These natural functions of zinc can be distorted when the metal appears in excessive amounts. Moreover, cadmium demonstrates a high degree of similarity to zinc ions, which means it can replace zinc in many biocomplexes and change their biological activity (VIG et al. 2003).

In general, heavy metals, including cadmium, copper and zinc, depress the activity of soil metals if present in excessive amounts (Djukic, Mandic 2006, Wyszkowska et al. 2006b, Wyszkowski, Wyszkowska 2006, Gulser, Erdrogan 2008, Vogeler et al. 2008, Kucharski et al. 2009, Lee et al. 2009, Wyszkowska et al. 2010, Jiang et al. 2010, Kucharski et al. 2011), although there are exceptions. For example, Kýzýkaya et al. (2004) reported an experiment in which the activity of dehydrogenases and catalase as well as respiration declined under the influence of excess cadmium and copper, but the activity of urease remained unchanged. Chaperon and Sauve (2008) showed that both cadmium and copper inhibit the activity of dehydrogenases as well as urease. Cadmium applied in doses of 1, 10, 20 and 50 mg Cd kg^{-1} and copper in doses of 50, 250, 500 and 1000 mg kg^{-1} produced a stronger inhibitory effect when applied singly than in conjunction. This observation is not always confirmed, as the study completed by Yang et al. (2006) revealed that cadmium and zinc produced a synergic effect on urease, catalase and alkaline phosphatase. Urease, in turn, is the most sensitive enzyme with respect to the tested metals. Khan et al. (2006) noticed that cadmium and lead are of little importance in respect of the activity of soil enzymes (dehydrogenases, alkaline phosphatase, catalase). This conclusion was most probably formulated because of the small scale of contamination in the cited experiment, which was 1,5 mg, 3 mg and 5 mg Zn kg^{-1}, and 150 mg, 300 mg and 500 mg Pb kg^{-1}.

Kunito et al. (2001) drew our attention to the fact that different metals inhibited enzymatic activity in different ways. The activity of dehydroegnases, urease and β-glucosidase was more strongly inhibited by zinc fractions extracted by nitric acid than by copper. In an experiment run by Wang et al. (2007), the activity of dehydrogenase declined in soil contaminated with 10 mg Cd kg^{-1}, whereas the same metal did not inhibit urease. Suve et al. (1999) verified that soil contamination with lead and copper may also retard nitrification. Such undesirable events can be prevented by introducing to soil phytostabilizing substances, e.g. zeolite and lime (Castaldi et al. 2009, Kumpiene in. 2009).

According to Renell et al. (2005a), the activity of enzymes in soils polluted with heavy metals depends on the structure of pollutants. They arrived at this conclusion based on experiments which involved soil fertilization with sludge polluted with nickel and cadmium or manganese and zinc. Sludge containing nickel and cadmium depressed the activity of phosphatases, β-glucosidase and arylsulphatase, while sludge polluted with manganese and zinc lowered the activity of arylphosphatase alone. Both types of sludge had a stimulating effect on protease, while the activity of urease was unaffected by either type of soil amending substance. The cited authors also noticed that presence of some heavy metals in sludge could be a serious obstacle to its utilization. Similar conclusions were presented by Viog et al. (2003).

Epelde et al. (2008) found out that by growing the hyperaccumulating plant called *Thlaspi caerulescens* on soil contaminated by zinc and cadmium, it was possible to attain a higher activity of β-glucosidase, arylsulphatase, acid phosphatase, alkaline phosphatase and urease, although the two heavy metals did not cause unambiguous inhibition of the mentioned enzymes in uncropped soil.

A study conducted by Castaldi et al. (2004) proves that the activity of dehydrogenases, sulphatase, glucosidase and the respiratory activity in soil decreased exponentially as the content of heavy metals in soil increased, while the activity of protease and urease was not significantly correlated with the content of these metals.

Wang et al. (2006) observed that the activity of alkaline phosphatase, arylsulphatase, nitrification and respiration was significantly negatively correlated with the content of cadmium and zinc, or aluminum and manganese, while being positively correlated with the content of calcium and level of pH. The activity of acid phosphatase was negatively correlated with the content of calcium, magnesium and pH but positively – with the content of aluminum, cadmium, manganese and zinc. Chaperon and Sauve (2007) concluded that copper and zinc are inhibitors of dehydrogenases and urease, but were even more strongly affected by silver and mercury. Khan et al. (2010) demonstrated that cadmium had a negative effect on both microorganisms and the activity of phosohatases and urease. In turn, Speir et al. (1999) proved that cadmium and nickel are stronger inhibitors than copper, zinc and chromium (III).

Liu et al. (2007) tested cadmium applied in doses from 5 to 200 mg kg^{-1} and noticed that it inhibited the activity of urease and phosphatases; however, in the doses of 5 and 10 mg kg^{-1}, the metal stimulated the activity of catalase, causing an evident inhibition of that enzyme only when introduced to soil at a rate of 200 mg kg^{-1}. Cadmium added to soil in doses from 5 to 100 mg kg^{-1} stimulated the activity of invertase, but inhibited that enzyme when applied in higher doses. Lorenz et al. (2006) and Kucharski et al. (2011) claim that the adverse effect of heavy metals on the microbiological and biochemical activity of soil is persistent. Twenty-five years after polluting soil with cadmium in amounts of 50 and 250 mg kg^{-1}, it was found to contain 34 and 134 mg Cd kg^{-1}, respectively. The PCR analysis showed that the structure of bacteria was different from that observed in unpolluted soil. Under the influence of cadmium, the activity of alkaline phosphatase, arylphosphatase, protease and urease declined. In contrast, the activity of xylanase either did not change or increased, being correlated to the content of fungal quinones and *Proteobacteria*. Microorganisms were exceptional in that they did not respond negatively to the contamination (Lorenz et al. 2006).

Marzadori et al. (2000) claim that the destructive influence of copper can be alleviated by humic acids with a high molecular weight (100-300 kDa). Such acids proved to be good stabilizers of the activity of urease. They also protected that enzyme from attacks by proteases. In general, the activity of soil enzymes was higher in cropped than in uncropped soil (Pérez-de-Mora et al. 2006, Castaldi et al. 2009). This regularity is attributed to the positive role played by substances exerted by roots, which moderate effects of heavy metals on soil's enzymatic performance (Renella et al. 2005a, Pérez-de-Mora et al. 2006, Tejada et al. 2008, Wyszkowska et al. 2009). Another reason is the uptake of metals by plants (Rajkumar, Freitas 2008). The ameliorating influence of plants on heavy metals affecting the soil metabolism has been implied by other researchers (Renella et al. 2006, Epelde et al. 2008, Jiang et al. 2010, Wyszkowska et al. 2010). Also, a study by Chaudhuri et al. (2003) prove that introduction of organic substance to soil limits the extent of the negative effect of heavy metals on activity of dehydrogenases, urease, acid phosphatase and arylsulphatase.

A review of the relevant literature shows that many researchers (Welp 1999, Nowak et al. 2003, Wyszkowska, Kucharski 2003b, Wyszkowska et al. 2006a) have attempted, with a different degree of success, to determine series of enzymes with respect to their sensitivity to heavy metals. Below are some of the results. Sensitivity of:

– dehydrogenases, according to Welp (1999) is: Hg (2 mg) > Cu (35 mg) > Cr^{6+} (71 mg) > Cr^{3+} (75 mg) > Cd^{2+} (90 mg) > Ni^{2+} (100 mg) > Zn^{2+} (115 mg) > As^{3+} (168 mg) > Co^{2+} (582 mg) > Pb^{2+} (652 mg kg^{-1}), and according to Wyszkowska and Kucharski (2003b): Cu^{2+} > Zn^{2+} > Cr^{6+}> Hg^{2+} > Ni^{2+} > Cd^{2+} > Cr^{3+}, Wyszkowska et al. (2006a): Cr^{6+}>Cd^{2+}>Zn^{2+}>Pb^{2+}>Cu^{2+}>Ni^{2+}.;

– acid phosphatase – according to Nowak et al. (2003): Cu^{2+} > Al^{3+} > Cd^{2+} > Zn^{2+} > Fe^{3+}> Ni^{2+} > Pb^{2+} > Sn^{2+} > Fe^{2+} > Co^{2+}, and according to Wyszkowska and Kucharski (2003b): Cu^{2+} > Ni^{2+} > Zn^{2+} > Cd^{2+} > Cr^{3+} > Cr^{6+} > Hg^{2+}; Wyszkowska et al. (2006a): Cr^{6+}> Ni^{2+}>$Cu^{2+}$$Cd^{2+}$> Pb^{2+}>Zn^{2+};

– alkaline phosphatase – according to Nowak et al. (2003): Cd^{2+} > Al^{3+} > Zn^{2+} > Fe^{3+} > Cu^{2+} > Pb^{2+} > Ni^{2+} > Fe^{2+} > Se^{2+} > Co^{2+}, and according to Wyszkowska and Kucharski (2003b): Zn > Cu^{2+} > Ni^{2+} > Hg^{2+} > Cr^{6+}, Wyszkowska et al. (2006a): Cd^{2+}>Ni^{2+}>Cu^{2+}> Zn^{2+}>Cr^{6++}>Pb^{2}.

Differences in the above series may have been caused by differences in the content of the silt fraction in analyzed soils, which absorbed different amounts of both heavy metals and some of the enzymes (Boyd, Mortland 1985). Another reason could have been the different degrees of soil contamination analyzed by the cited authors.

All the factors which alter the activity of soil enzymes also modify enzymes responsible for oxidation of ammonia nitrogen (Trevisan et al. 2012). The same effect is produced by heavy metals (Mertens et al. 2010), but in that case it is accompanied by the toxic influence of heavy metals on nitrifying bacteria (He et al. 2012). The adverse effect of heavy metals on the ammonifying process has also been indicated by Antil et al. (2001) or Hund-Rinke and Simon (2008), while Suve et al. (1999), Yin et al. (2003), Hund-Rinke and Simon (2008) and Vogeler et al. (2008) reported their adverse influence on nitrification.

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