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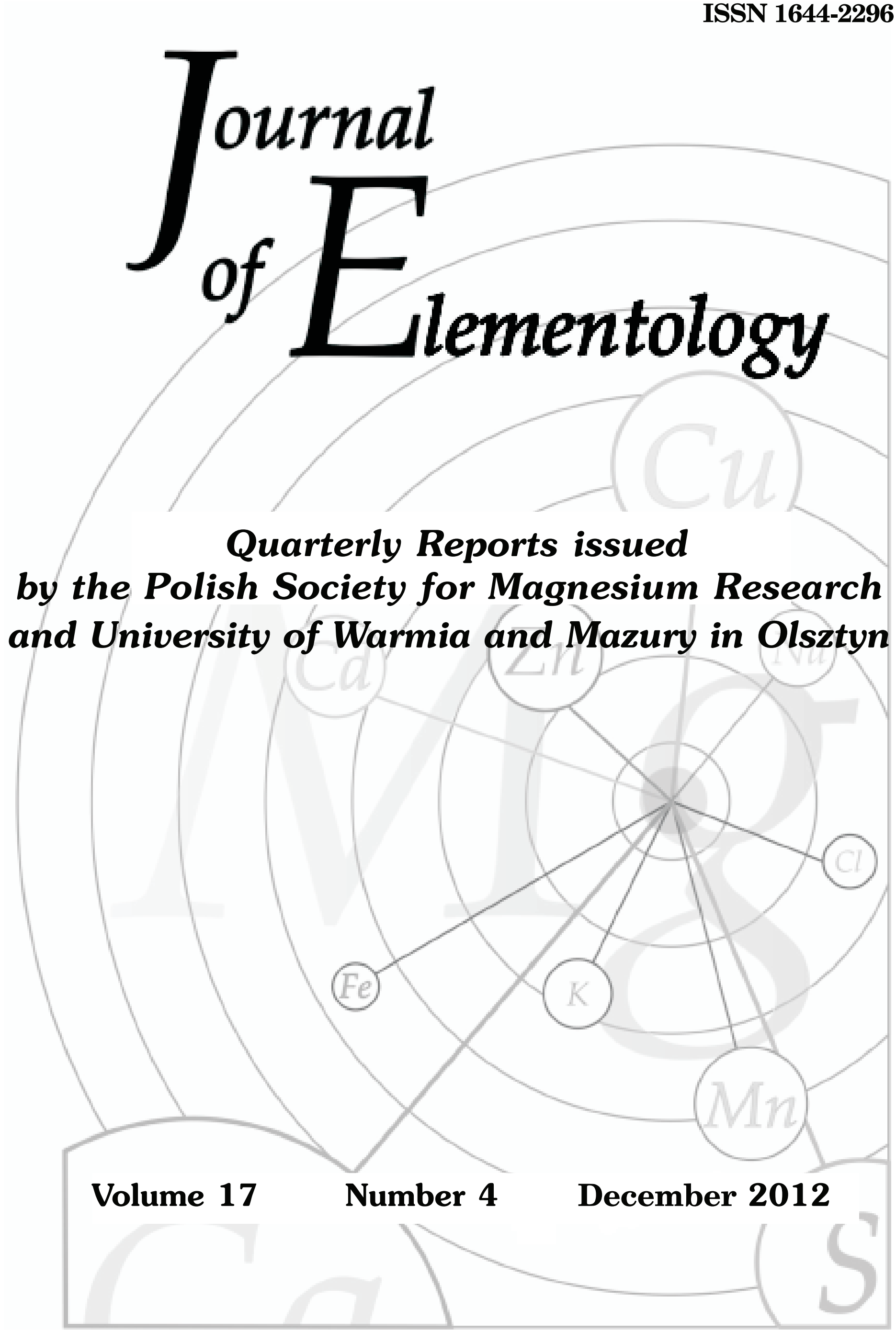

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Contents

Spis treści

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INFLUENCE OF LIQUID MANURE AND NPK ON SELECTED SORPTION PROPERTIES OF SOIL

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Abstract

The aim of the experiment was to assess the effect of long-term fertilization with liquid manure applied annually and biennially and with mineral fertilizers on selected sorption properties of soil, i.e. the content of exchangeable magnesium, potassium, calcium, sodium and as well as the value of hydrolytic acidity, sorption capacity and base cation saturation ratio. It was found that fertilization with liquid manure, in contrast to NPK application (N as NH_4NO_3, P as triple super phosphate, K as KCl), led to a significant rise in the content of exchangeable magnesium in soil, whereas application of liquid manure or mineral fertilizers contributed to a significant increase in the potassium content in soil. Application of liquid manure did not cause significant changes in the value of hydrolytic acidity between the lowest and the highest dose used, while mineral fertilization contributed to an increase in the value of hydrolytic acidity. Application of liquid manure reduced the value of hydrolytic acidity as deep as the third layer of the soil profile. Fertilization with liquid manure and mineral fertilizers did not affect the content of exchangeable calcium in the whole soil profile, although the calcium content in the soil profile increased to the 51-75 cm layer. Application of liquid manure and NPK did not alter significantly the content of exchangeable sodium in soil. Upon application of both liquid manure and NPK, no significant changes were observed in the total base exchangeable cations in soil (S) or in the sorption complex saturation (V). However, S and V significantly increased along the depth of sampling. It was found that fertilization with both liquid manure and mineral fertilizers did not induce substantial changes in the soil sorption capacity, although there was an increase in the total sorption capacity in two layers (51-75 cm and 76-100 cm) of the soil profile.

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Key words: liquid manure; natural fertilizers; soil sorption properties; long-term fertilization.

WPŁYW GNOJOWICY I NPK NA WYBRANE WŁAŚCIWOŚCI SORPCYJNE GLEBY

Abstrakt

Celem eksperymentu było określenie wpływu wieloletniego nawożenia gnojowicą, stosowaną co rok i co dwa lata, oraz nawozami mineralnymi na wybrane właściwości sorpcyjne gleby, tj. zawartość wymiennego magnezu, potasu, wapnia, sodu oraz wartość kwasowości hydrolitycznej, pojemność sorpcyjną i stopień wysycenia kationami zasadowymi. Stwierdzono, że nawożenie gnojowicą spowodowało istotne zwiększenie zawartości magnezu wymiennego w glebie w przeciwieństwie do nawożenia NPK (N jako NH_4NO_3; P jako superfosfat potrójny; K jako KCl). Natomiast zarówno nawożenie gnojowicą, jak i nawożenie mineralne przyczyniło się do istotnego przyrostu zawartości potasu w glebie. Nawożenie gnojowicą nie spowodowało istotnych zmian wartości kwasowości hydrolitycznej między najmniejszą a największą zastosowaną dawką, zaś nawożenie nawozami mineralnymi przyczyniło się do wzrostu wartości kwasowości hydrolitycznej. Zastosowanie gnojowicy zmniejszyło wartość kwasowości hydrolitycznej do trzeciego poziomu w profilu glebowym. Nawożenie dawkami gnojowicy i nawozów mineralnych nie spowodowało zmian zawartości wapnia wymiennego w glebie, natomiast zawartość wapnia w profilu glebowym wzrastała do warstwy 51-75 cm. Nawożenie gnojowicą i NPK nie spowodowało istotnych zmian w zawartości sodu wymiennego w glebie. Zarówno po zastosowaniu gnojowicy, jak i NPK nie zaobserwowano istotnych zmian w całkowitej zawartości kationów zasadowych w glebie (S), a także stopnia wysycenia nimi kompleksu sorpcyjnego (V). Natomiast parametry S i V istotnie wzrastały wraz z głębokością pobrania próby. Stwierdzono, że nawożenie zarówno gnojowicą, jak i nawozami mineralnymi nie spowodowało istotnych zmian pojemności sorpcyjnej gleby, ale wykazano przyrost całkowitej pojemności sorpcyjnej w dwóch warstwach profilu gleby, 51-75 cm i 76-100 cm.

Słowa kluczowe: gnojowica, nawożenie mineralne, właściwości sorpcyjne gleby, nawożenie długoletnie.

INTRODUCTION

The 2002 agriculture census showed that farms in Poland produced 31.5 million m^3 of liquid manure, which was equivalent to approximately 23 kg NPK per 1 ha of arable land. It can be assumed that production of this fertilizer will increase as more livestock, especially cattle and swine, is reared. Increasing application of liquid manure may induce specific changes in the natural environment (Mazur, Sądej 1989, Choudhary et al. 1996, Qian et al. 2005, Popers et al. 2011). Therefore, constant monitoring of the impact of the fertilizer on soil, water, plants and air is strongly advisable. Long-term fertilization experiments may provide many interesting and reliable results (Maćkowiak 2000, Potarzycki 2000, Walker, Bernal 2004, Gondek And Filipek-Mazur 2005). Analysis of the impact of a type and dose of fertilizer on selected soil parameters, of which the most important are the grain-size distribution, pH, organic carbon content and sorption capacity, is crucial for optimization of growing conditions and environmental protection (Potarzycki 2000).

The aim of the study was to estimate the effect of long-term fertilization with liquid manure and NPK on the sorption properties of soil, an important element of the natural environment.

MATERIAL AND METHODS

The paper is based on results of chemical analyses of soil samples collected in the spring 2002 from an experiment conducted by Maćkowiak (2000) since 1973 and used in this study with the author's consent. The experiment was performed on fenced 1 m^2 plots located in the vicinity of a vegetation hall at the IUNG Institute of Soil Science and Plant Cultivation, Puławy, Poland. The plots were filled with soil to 1 m depth. The soil was taken from an arable field of the IUNG Experimental Station in Grabów, Poland; its grain-size composition corresponded to loamy sand and loam in the subsoil, where the natural layers of the soil profile were preserved. It was only in the 0–25 cm layer that the soil had been mixed in a 2:1 ratio with low-clay humic sandy loam, sampled from the topsoil of an arable field at the Experimental Station of the IUNG in Sadłowice, Poland. The content of available nutrients in the topsoil was: 2.09 mg kg^{-1} P and 6.47 mg kg^{-1} K; the pH_{KCl} was 5.6 and the content of organic carbon equalled 1.06%.

Bovine liquid manure was applied annually at doses of 25, 50, 100 and 200 m^3 ha^{-1} and biennially at double doses. Liquid manure was used in the above doses during the successive seventeen years, i.e. until 1989. On average, the fertilizer contained 9.10% d.m., and 0.31% N, 0.07% P, 0.34% K, 0.16% Ca and 0.03% Mg in fresh weight. In 1990, the application of liquid manure was discontinued and its subsequent effect was investigated until 1999. In order to compare liquid manure and mineral fertilizers, the experiment included four treatments fertilized only with NPK in the form of mineral fertilizers at a dose approximately corresponding to half the dose of each of these components incorporated into soil in the respective treatments where liquid manure was applied annually. The NPK doses were: 50, 100, 200 and 400 kg ha^{-1} N (as NH_4NO_3; 34% N); 10.9; 21.8; 43.6 and 87.2 kg ha^{-1} P (20% P); and 41.5; 83; 166 and 332 kg ha^{-1} K (as KCl; 47.3% K). No liming was applied throughout the whole experiment. The details on the methodology of the experiment can be found in Bednarek et al. (2012).

Chemical analyses were performed at the Regional Chemical and Agricultural Station laboratory, Lublin, Poland, with the following methods: exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) after extraction with 1 mol CH_3COONH_4 dm^{-3}, pH 7.0 from the soil and quantitative determination in the filtrate performed with the ASA method; the content of organic carbon was determined according to Tiurin's method; pH was assessed in 1 mol KCl dm^{-3} and hydrolytic acidity was determined by Kappen's method after extraction with 1 mol CH_3COONa dm^{-3} from soil (*Katalogue*... 2007). Statistical analyses were employed such as variance analysis with Tukey's confidence semi-intervals (α = 0.05).

RESULTS AND DISCUSSION

Annual application of liquid manure and biennial treatment with double doses did not cause significant changes in the value of hydrolytic acidity between the lowest and highest doses (Table 1). It was found that hydrolytic acidity in the soil profile significantly declined reaching the third layer. Fertilization with NPK resulted in a steady rise in hydrolytic acidity from 10.8 (1 NPK) to 12.8 mmol(+) kg^{-1} (4 NPK), creating a significant difference between the extreme doses. Similarly, POTARZYCKI (2000) reported that 20-year-long application of cattle manure led to an evident decline in exchangeable hydrolytic acidity (60.0 mmol(+) kg^{-1}) in soil compared to treatments fertilized with NPK (70.0 mmol(+) kg^{-1}). In contrast, MAZUR and SĄDEJ (1989) found that a lower dose of swine manure (46.7 t ha^{-1}) raised hydrolytic acidity (Hh) (33.7 mmol(+) kg^{-1}), whereas a higher dose (126.4 t ha^{-1}) failed to induce such a change so that the value of this parameter was the same as in a control, non-fertilized treatment (26.2 mmol(+) kg^{-1}). Elevated hydrolytic acidity was reported by GONDEK and FILIPEK-MAZUR (2005) in their study on mineral, organic and organic-mineral fertilizers.

Application of increasing doses of liquid manure and mineral fertilizers did not produce regular changes in the content of exchangeable calcium in soil (Table 1). However, the Ca^{2+} content in the soil profile increased steadily, particularly as deep as the third 51-75 cm layer. Literature provides discrepant results of studies on the calcium content in soil, i.e. CHOUDCHARY et al. (1996) reported its increase on application of swine manure, while QIAN et al. (2005) found either no changes or a decline in the content of this element. POTARZYCKI (2000) reported a significant increase in the content of exchangeable calcium in the sorption complex of soil fertilized with liquid manure (36.6 mmol(+) kg^{-1} compared to soil supplemented with NPK (11.6 mmol(+) kg^{-1}). Similar regularities were found by MAZUR and SĄDEJ (1989), who reported a substantial increase in the content of exchangeable calcium (57.0 mmol(+) kg^{-1}), particularly when a lower dose of swine manure was applied, compared to the control treatment (44.1 mmol(+) kg^{-1}). Soil properties, e.g. the content of exchangeable K, Mg, and Ca, were also analysed by PYPERS et al. (2011) in their study at the 15 cm topsoil layer.

Table 1

The value of soil hydrolytic acidity and the content of calcium in soil fertilized with liquid manure and NPK

Soil layer (cm)	Liquid manure (m^3 ha^{-1})										NPK (kg ha^{-1})					Mean
	annually					biennially										
	25	50	100	200	mean	50	100	200	400	mean	1	2	3	4	mean	
Hydrolytic acidity [mmol(+) kg^{-1} d.w.]																
0-25	17.8	16.3	20.3	19.4	18.5	15.9	17.7	18.5	22.6	18.7	18.1	18.2	18.4	19.9	18.6	18.6
26-50	11.7	11.1	11.3	10.5	11.1	11.6	11.7	12.8	12.0	12.0	10.7	12.8	14.4	16.6	13.6	12.3
51-75	6.90	6.55	6.61	6.78	6.71	6.48	7.28	7.05	6.30	6.78	7.20	6.48	6.32	6.80	6.71	6.73
76-100	7.27	7.33	7.90	6.68	7.30	7.15	8.07	7.83	6.62	7.42	7.25	6.43	6.75	7.73	7.04	7.25
Mean	10.9	10.3	11.5	10.8	10.9	10.3	11.2	11.5	11.9	11.2	10.8	11.0	11.5	12.8	11.5	11.2
HSD (0.05) (fertilization type – 0.66; (depth×fertilzation type) – 1.87; (fertilization type×fertilization rate) – 4.99; (depth×fertilization type×fertilization rate) – 3.97																
Ca^{2+} [mmol(+) kg^{-1} d.w.]																
0-25	18.5	22.8	23.6	29.9	23.7	19.7	18.5	21.8	25.5	21.4	18.4	16.1	17.0	17.0	17.1	20.7
26-50	26.9	29.2	28.4	37.6	30.5	29.9	26.7	26.4	22.6	26.4	32.0	24.7	25.9	24.0	26.7	27.8
51-75	70.9	73.9	84.8	68.3	74.5	80.4	70.7	87.5	81.7	80.0	81.6	85.1	101.6	97.5	91.4	82.0
76-100	77.8	62.2	65.3	66.0	67.8	77.8	62.7	92.8	76.8	77.5	68.2	83.3	88.6	78.1	79.5	75.0
Mean	48.5	47.0	50.5	50.4	49.1	51.9	44.7	57.1	51.6	51.3	50.0	52.3	58.3	54.1	53.7	51.4
HSD (0.05) (fertilization type) – 6.1; (depth×fertilzation type) – 16.9; (fertilization type×fertilization rate) – 31.6; (depth×fertilization type×fertilization rate) – 41.6																

Annual fertilization with increasing doses of liquid manure resulted in a significant rise in the exchangeable magnesium content, especially between the extreme doses (Table 2). A statistically confirmed increase in the Mg form was also found in the soil profile. Similar relationships were also observed at the biennial application of double doses of liquid manure. These results are in agreement with the ones obtained in the study on swine manure conducted by CHOUDHARY et al. (1996) and in investigations carried out by WALKER et al. (2004) on application of poultry manure, in which an increased content of magnesium was reported. Similarly, POTARZYCKI (2000) found that 20-year-long application of cattle manure, compared to NKP fertilization, produced an increase in the exchangeable magnesium content in the sorption complex. A similar conclusion was drawn by MAZUR and SĄDEJ (1989), who observed a similar tendency during 13-year-long application of swine manure, in contrast to a non-fertilized treatment. Different results were obtained by QIAN et al. (2005), who reported that the Mg^{2+}content in soil remained on the same level or declined in response to manure fertilization. However, application of increasing NPK doses resulted in a gradual fall in the Mg^{2+} content; a reverse phenomenon was observed in the soil profile where the content of this element significantly increased down to the 51-75 cm layer.

Annual treatment with liquid manure and its double doses applied biennially resulted in a steady and significant increase (particularly between the extreme doses) in the exchangeable potassium content (Table 2). The results correspond well with those presented by other authors, who measured a rise in the potassium content in soil after swine manure fertilization (CHOUDHARY et al. 1996, QIAN et al. 2005) and poultry manure application (WALKER et al. 2004). Similarly, POTARZYCKI (2000) reported a certain increase in the K^+ content in the sorption complex of soil fertilized with cattle manure, in comparison to mineral fertilization. In soil fertilized with swine manure, the increase was particularly high at application of a double dose 126.4 t ha^{-1} (MAZUR, SĄDEJ 1989). In the topsoil and the 51-75 and 76-100 cm layers, the K^+ content was significantly higher than in the 26-50 cm layer. Also, mineral fertilization produced a steady and significant increase in the K^+ content, particularly between the extreme doses. The K^+ content in the soil profile was similar to that found at application of liquid manure.

The annual application of liquid manure and biennial use of its double doses as well as NPK treatment did not alter significantly the content of exchangeable sodium in soil (Table 3). However, a steady increase in the content of this form of sodium was found in the soil profile, particularly in the third layer (51-75 cm). The investigations performed by CHOUADHARY et al. (1996) indicated an increased content of sodium in soil treated with swine manure. This was consistent with the results obtained by MAZUR and SĄDEJ (1989), who reported the sodium content of 1.4 mmol(+) kg^{-1} in soil fertilized

Table 2

The content of magnesium and potassium in soil fertilized with liquid manure and NPK

Soil layer (cm)	Liquid manure (m^3 ha^{-1})										NPK (kg ha^{-1})					Mean
	annually					biennially										
	25	50	100	200	mean	50	100	200	400	mean	1	2	3	4	mean	
Mg^{2+} [mmol(+) kg^{-1} d.w.]																
0-25	1.23	1.65	1.78	3.02	1.92	1.30	1.23	1.58	2.02	1.53	1.85	2.23	2.33	3.09	2.37	1.94
26-50	2.95	3.46	5.01	6.93	4.59	3.09	3.05	3.91	4.15	3.55	3.67	3.36	3.53	4.94	3.87	4.00
51-75	8.09	8.50	9.57	10.5	9.15	8.13	7.99	8.45	10.3	8.81	8.54	8.26	7.85	6.10	7.69	8.55
76-100	9.02	7.65	9.84	9.12	8.91	9.36	7.95	8.19	8.78	8.57	9.43	9.87	9.02	7.58	8.97	8.82
Mean	5.32	5.31	6.55	7.39	6.14	5.47	5.06	5.63	6.31	5.62	5.87	5.93	5.68	5.43	5.73	5.83
HSD (0.05) (fertilization type – 0.37; (depth×fertilzation type) – 1.04; (fertilization type×fertilization rate) – 3.02; (depth×fertilization type×fertilization rate) – 1.77																
K^+ [mmol(+) kg^{-1} d.w.]																
0-25	1.73	1.98	2.15	3.18	2.26	1.84	1.82	2.18	2.89	2.19	1.61	1.89	1.87	2.80	2.04	2.16
26-50	1.11	1.31	1.87	2.72	1.75	1.03	1.14	1.91	2.36	1.61	0.96	1.01	1.39	1.74	1.27	1.54
51-75	2.30	2.41	3.10	6.17	3.46	2.01	2.20	3.13	7.12	3.62	2.00	2.27	2.41	3.04	2.43	3.18
76-100	2.32	2.30	2.56	4.00	2.79	2.21	2.05	2.28	5.82	3.09	2.24	2.42	2.47	2.23	2.34	2.74
Mean	1.87	2.00	2.42	4.01	2.58	1.78	1.80	2.38	4.55	2.62	1.70	1.90	2.03	2.45	2.02	2.41
HSD (0.05) (fertilization type) – 0.24; (depth×fertilzation type) – 0.99; (fertilization type×fertilization rate) – 0.84; (depth×fertilization type×fertilization rate) – 0.73																

Table 3

The content of sodium and total base exchangeable cations (S) in soil fertilized with liquid manure and NPK

Soil layer (cm)	Liquid manure (m^3 ha^{-1})										NPK (kg ha^{-1})					Mean
	annually					biennially										
	25	50	100	200	mean	50	100	200	400	mean	1	2	3	4	mean	
Na^+ [mmol(+) kg^{-1} d.w.]																
0-25	0.80	0.96	1.05	1.35	1.04	0.80	0.78	0.92	1.15	0.91	0.78	0.67	0.63	0.71	0.70	0.88
26-50	1.09	1.12	1.24	1.51	1.24	1.16	1.04	1.19	1.16	1.13	1.12	0.95	0.91	0.86	0.96	1.11
51-75	2.57	2.59	3.09	2.59	2.71	2.95	2.58	3.29	3.02	2.96	2.67	2.77	3.45	3.14	3.00	2.89
76-100	2.81	2.21	2.63	2.53	2.54	2.87	2.37	3.41	2.67	2.83	2.29	2.93	3.01	2.71	2.74	2.70
Mean	1.82	1.72	2.00	1.99	1.88	1.95	1.69	2.20	1.99	1.96	1.72	1.83	2.00	1.85	1.85	1.90
HSD (0.05) (fertilization type) – 0.19; (depth×fertilzation type) – 0.54; (fertilization type×fertilization rate) – 1.03; (depth×fertilization type×fertilization rate) –1.31																
Total alkaline exchangeable cations (S) [mmol(+) kg^{-1} d.w.]																
0-25	22.2	27.4	28.6	37.4	28.9	23.7	22.4	26.5	31.6	26.0	22.6	20.8	21.9	23.6	22.2	25.7
26-50	32.0	35.0	36.6	48.7	38.1	35.2	31.9	33.4	30.3	32.7	37.8	30.0	31.7	31.6	32.8	34.5
51-75	83.8	87.4	100.6	87.5	89.8	93.5	83.5	102.8	102.1	95.5	94.8	98.4	115.3	109.8	104.6	96.6
76-100	92.0	74.4	80.3	81.6	82.1	92.2	75.1	106.7	94.0	92.0	82.2	98.5	103.1	90.6	93.6	89.2
Mean	57.5	56.1	61.5	63.8	59.7	61.2	53.2	67.3	64.5	61.6	59.4	61.9	68.0	63.9	63.3	61.5
HSD (0.05) (fertilization type) – 6.41; (depth×fertilzation type) – 17.83; (fertilization type×fertilization rate) – 35.58; (depth×fertilization type×fertilization rate) – 43.77																

Table 4

Sorption capacity (T) and base cation saturation ratio (V) in soil fertilized with liquid manure and NPK

Soil layer (cm)	Liquid manure (m^3 ha^{-1})										NPK (kg ha^{-1})					Mean
	annually					biennially										
	25	50	100	200	mean	50	100	200	400	mean	1	2	3	4	mean	
Sorption capacity (T) [mmol(+) kg^{-1}]																
0-25	40.0	43.7	48.9	56.8	47.4	39.6	40.1	45.0	54.2	44.7	40.7	39.0	40.2	43.5	40.8	44.3
26-50	43.7	46.2	47.8	59.3	49.2	46.8	43.6	46.1	42.3	44.7	48.4	42.8	46.1	48.2	46.4	46.8
51-75	90.7	94.0	107.2	94.3	96.6	100.0	90.7	109.8	108.4	102.2	102.0	104.9	121.7	116.6	111.3	103.4
76-100	99.2	81.7	88.2	88.3	89.4	99.4	83.2	114.5	100.6	99.4	89.4	104.9	109.8	98.3	100.6	96.5
Mean	68.4	66.4	73.0	74.7	70.7	71.5	64.4	78.9	76.4	72.8	70.1	72.9	79.5	76.7	74.8	72.8
HSD (0.05) (fertilization type) – 6.13; (depth×fertilization type) – 17.19; (fertilization type×fertilization rate) – 31.58; (depth×fertilization type×fertilization rate) – 41.91																
The degree of saturation with alkaline cations (V) [%]																
0-25	50.8	56.4	53.1	58.0	54.6	54.2	50.9	53.4	52.3	52.7	50.2	47.3	48.6	47.0	48.3	51.9
26-50	65.6	67.1	65.0	67.7	66.4	67.1	65.4	62.8	60.6	64.0	69.5	62.5	60.8	56.7	62.4	64.2
51-75	80.2	81.1	81.8	76.9	80.0	82.4	80.6	81.1	78.6	80.7	81.5	83.1	84.4	84.5	83.4	81.4
76-100	79.6	79.2	77.7	78.4	78.7	80.3	78.0	80.2	78.4	79.2	78.9	81.6	82.0	81.1	80.9	79.6
Mean	69.1	71.0	69.4	70.3	69.9	71.0	68.7	69.4	67.5	69.2	70.0	68.6	69.0	67.3	68.8	69.3
HSD (0.05) (fertilization type) – 1.55; (depth×fertilization type) – 5.18; (fertilization type×fertilization rate) –12.48; (depth×fertilization type×fertilization rate) – 9.26																

with a lower dose of swine manure and 1.6 mmol(+) kg^{-1} with a higher dose, compared to 1.3 mmol(+) kg^{-1} in the control treatment. In contrast, Potarzycki (2000) did not find such a tendency: the Na^+ content in soil fertilized only with cattle manure and with NPK was identical (0.5 mmol(+) kg^{-1}).

Fertilization with liquid manure caused a slight increase in the sorption capacity in the topsoil (Table 4). Additionally, a steady increase in the total sorption capacity in the soil profile was found, especially in the 26-50 cm and above all in the 51-75 cm layers. Potarzycki (2000) reported that the total sorption capacity in soil fertilized with cattle manure for 20 years was 101.3 mmol(+) kg^{-1}, compared to just 85.3 mmol(+) kg^{-1} in response to fertilization with NPK. Mazur and Sądej (1989) found that the total sorption capacity in soil fertilized with a lower dose of swine manure was 105.7, with a higher dose – 90.2, and in non-fertilized soil (control treatment) – only 78.2 mmol(+) kg^{-1}.

Annual application of increasing manure doses, biennial treatment with double doses, and use of mineral fertilizers did not cause pronounced changes in the percentage of base cations (V) in the total sorption capacity (Table 4). In contrast, there was a significant increase in the value of S in the 26-50 and 51-75 cm layers of the soil profile, compared to the topsoil (0-25 cm). In his studies, Potarzycki (2000) found that the contribution of S to the total sorption capacity was 41%, and only 18% in soil fertilized with NPK. Similarly, Mazur and Sądej (1989) found an increase in the share of base cations in the total sorption capacity in soil treated with a lower (68.1%) and higher dose of swine manure (approximately 71%), in comparison to the control, non-fertilized treatment (66.5%).

The values of S, T, and V increased to the third layer, whereas the Hh value declined gradually. The increase in the content of exchangeable cations in the deeper layers of the soil profile was primarily caused by some leaching of individual cations, which were not taken up by crops and did not undergo exchangeable sorption. Another reason was that clay, present on the surface of the examined soil, has a higher sorption potential than sand.

CONCLUSIONS

1. Annual application of increasing doses of cattle manure resulted in a steady increase in the content of Mg^{2+}, K^+, Ca^{2+}, Na^+, S, and T and an irregular increase in the H^+ and V content in the arable layer (0-25 cm) and, to a lesser extent, in the other layers of soil.

2. Biennial fertilization with double liquid manure doses led to an increase in the content of Mg^{2+}, K^+, H^+, Ca^{2+}, Na^+, S, and T in the 0-25 cm layer (and in the other soil layers). The degree of saturation of the soil with base cations remained on a relatively stable level of 50.9-54.2%, irrespective of the dose of the fertilizer used.

3. Application of increasing doses of mineral (NPK) resulted in an increase in the content of Mg^{2+}, K^+, H^+, S, and T in the 0-25 cm layer and, to a lesser extent, in the other soil layers, but it did not considerably affect the Ca^{2+} and Na^+ content, although it lowered the V value.

4. Annual application of increasing doses of cattle manure and biennial use of its double doses as well as the NPK treatment increased the content of Mg^{2+}, Ca^{2+}, Na^+, S, T, and V in the soil profile and decreased the content of H^+ ions in the 51-75 cm layer.

REFERENCES

Bednarek W., Dresler S., Tkaczyk P., Hanaka A. 2012. *Available forms of nutrients in soil fertilized with liquid manure and NPK.* J. Elem. (in press).

Catalogue of agrichemical research methods in agri-chemical stations 2007. OSCH-R Lublin, 1-19. (in Polish)

Choudhary M., Bailey L.D., Grant C.A. 1996. *Review of the use of swine manure in crop production: Effects on yield and composition and on soil and water quality.* Waste Manage. Res., 14(6): 581-595.

Gondek K., Filipek-Mazur B. 2005. *The effects of mineral treatment and the amendments by organic and organomineral fertilisers on the crop yield, plant nutrient status and soil properties.* Plant Soil Environ., 51(1): 34-45.

Gondek K., Kopeć M. 2008. *Effect of fertilization on leaching of plant nutrients in a pot experiment.* Acta Agroph., 12(1): 79-89. (in Polish)

Maćkowiak C. 2000. *Comparison of the fertilizng effect of liquid manure and mineral fertilizers.* Nawozy i Nawożenie, 4: 110-118. (in Polish)

Mazur T., Sądej W. 1989. *Effect of long-term fertilization with liquid manure, FYM and NPK on some chemical and physicochemical properties of soil.* Rocz. Glebozn., 40 (1): 147-153.

Potarzycki J. 2000. *Chemical properties of soils after twenty-year-long fertilization with cattle slurry and NPK.* Fol. Univ. Agric. Stetin. 211, Agricultura, 84: 405-410. (in Polish)

Pypers P., Sanginga J.-M., Kasereka B., Walangululu M., Vanlauwe B. 2011. *Increased productivity through integrated soil fertility management in cassava-legume intercropping systems in the highlands of Sud-Kivu, DR Congo.* Field Crops Res., 120: 76-85.

Qian P., Schoenau J.J., King T., Japp M. 2005. *Effect of repeated manure application on potassium, calcium and magnesium in soil and cereal crops in Saskatchewan.* Can. J. Soil Sci., 85: 397-403.

Walker D.J., Bernal M.P. 2004. *Plant mineral nutrition and growth in a saline Mediterranean soil amended with organic wastes.* Commun. Soil Sci. Plant Anal., 35(17-18): 2495--2514.

J. Elem. s. 559–585 DOI: 10.5601/jelem.2012.17.4.02

LOCAL BACKGROUND CONCENTRATION OF HEAVY METALS IN VARIOUS SOIL TYPES FORMED FROM GLACIAL TILL OF THE INOWROCŁAWSKA PLAIN

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Abstract

In the present study, local background concentrations of heavy metals were determined in soils which have been minimally influenced by human activities. The ground moraine landscape of the Inowrocławska Plain is dominated by Phaeozems, which occur in numerous associations with Luvisols and Cambisols. Four profiles of Phaeozems, three profiles of Luvisols and two profiles of Cambisols had been researched earlier, especially their morphology, selected physicochemical properties, texture and mineralogical composition. Selected properties were also measured to determine their influence on the content and distribution of trace elements in soil profiles. For determination of the concentration of metals in the soil profiles, the following indices were used: distribution factor (DI), enrichment factor (EF) and transfer factor (TF). The total content of metals in the genetic horizons and the local geochemical background level of metals in soils were determined. With the knowledge of the natural content of elements in the parent material, assumed to be the geochemical background, a degree of contamination of surface soil horizons can be evaluated. The content of Zn, Cu, Ni, Pb, Cr, Mn (mg kg^{-1}) and Fe and Ti (g kg^{-1}), which was defined as the content of the local background, was: 40.6; 12.6; 14.3; 12.9; 5.5; 309.9 and 16.5; 1.4, respectively. The distribution of Ni, Cr, Cu, Mn and Fe in the soil profiles can be explained as the effect of pedogenic factors, although in the case of, a tendency towards accumulation of Zn and Pb in the humus horizons as a result of anthropogenic input was observed. The distribution of pedogenic Ni, Cu, Cr and Mn was influenced by specific adsorption of metals on Fe oxides. The results indicate that the metals were bound more strongly to iron oxides than to organic matter. Based on the results of geochemical studies on selected arable soils from the Iowrocławska Plain, an integrated method was applied to assess the local background using an iterative 2s-technique (mean + 2 standard deviation). The relationships between trace elements and the conservative element such as Fe were

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used to predict the expected values of trace elements in topsoil. The results substantiate the importance of determining local background concentrations.

Key words: geochemical background, trace elements, arable soils.

LOKALNA ZAWARTOŚĆ TŁA GEOCHEMICZNEGO METALI CIĘŻKICH W RÓŻNYCH TYPACH GLEB WYTWORZONYCH Z GLINY ZWAŁOWEJ RÓWNINY INOWROCŁAWSKIEJ

Abstrakt

W badaniach określono zawartości tła geochemicznego metali ciężkich w glebach, które były przekształcone przez człowieka w niewielkim stopniu. W krajobrazie moreny dennej Równiny Inowrocławskiej dominują czarne ziemie będące w licznych asocjacjach glebowych z glebami płowymi i brunatnymi. Cztery profile czarnych ziem, trzy profile gleb płowych i dwa profile gleb brunatnych były przedmiotem wcześniejszych badań, które dotyczyły ich budowy morfologicznej, wybranych właściwości fizykochemicznych, uziarnienia oraz składu mineralogicznego. Oznaczono wybrane właściwości gleb mające wpływ na zawartość metali oraz ich dystrybucję w profilu glebowym. Określając koncentrację metali w profilach gleb, wykorzystano następujące wskaźniki: wskaźnik dystrybucji (DI), wskaźnik wzbogacenia (EF) oraz wskaźnik przemieszczenia (TF). Oznaczono całkowitą zawartość metali w poziomach genetycznych oraz określono zawartości lokalnego tła geochemicznego. Naturalne zawartości metali w skale macierzystej, uznane jako tło geochemiczne, są niezbędne do oceny stopnia zanieczyszczenia poziomów powierzchniowych gleby. Zawartości lokalnego tła geochemicznego dla Zn, Cu, Ni, Pb, Cr, Mn (mg kg^{-1}) oraz Fe i Ti (g kg^{-1}) wynoszą odpowiednio: 40,6; 12,6; 14,3; 12,9; 5,5; 309,9 oraz 16,5; 1,4. Rozmieszczenie Ni, Cr, Cu, Mn i Fe w profilach badanych gleb należy tłumaczyć wpływem czynników pedogenicznych, natomiast w przypadku Pb i Zn zaobserwowano tendencję ich akumulacji w poziomach próchnicznych w wyniku antropogenezy. Rozmieszczenie Ni, Cu Cr oraz Mn było spowodowane specyficzną adsorpcją na tlenkach żelaza. Wyniki badań wskazują, że więcej metali było związanych z tlenkami żelaza aniżeli z materią organiczną.

Opracowując wyniki badań gleb uprawnych Równiny Inowrocławskiej, zastosowano zintegrowaną metodę do oszacowania tła geochemicznego. W tym celu wykorzystano metodę iteratywną 2 s (średnia + 2 wartości odchylenia standardowego). Relacje między metalami a konserwatywnym elementem, jakim jest Fe, wykorzystano w celu oszacowania spodziewanych zawartości pierwiastków śladowych w poziomach wierzchnich gleb. Wyniki badań wskazują na potrzebę określenia lokalnego tła geochemicznego.

Słowa kluczowe: tło geochemiczne, pierwiastki śladowe, gleby uprawne.

INTRODUCTION

Origin of trace elements in soils

Trace elements enter into an agricultural ecosystem through natural and anthropogenic processes. The content of trace metals in soils depends on the mineralogical composition of the geologic parent material and on the weathering processes to which the soil forming materials have been subjected (Adriano 1986). The behaviour of trace metals in soils is related to their origin and chemical forms at the time of impaction (Allaway 1990). Lithogenic metals are primarily inherited from the parent material. Pedogenic metals are of lithogenic and anthropogenic origins but their distribution in soil profiles changes due to mineral transformation and other pedogenic processes. Anthropogenic heavy metals are deposited into soils as a result of human activities (Kabata-Pendias 2004). Colloidal materials and clay minerals are generally surface-active and iron/manganese oxide surface coatings play an important role in the distribution of trace metals in soil profile. Metals fixed by Al, Fe and Mn hydrous oxides and other crystalline solid components are hardly mobile. The behaviour of pedogenic metals reflects several soil properties, of which pH and redox potential are the most important parameters. Anthropogenic metals are generally more mobile than lithogenic and pedogenic ones, whose origins are difficult to distinguish (Kabata-Pendias 1993). The duration and intensity of pedogenic processes, and parent material characteristics significantly determine the type and distribution of soil in landscape. Pedogenic processes clearly modify soil material by weathering and leaching minerals, adding organic matter, redistributing (clay illuviation) and transforming material (*in situ* clay formation). The pedological factors involved in distribution of trace elements in a soil profile include surface enrichment, leaching, translocation and mobilization (Alloway 1990). The distribution of pedogenic trace metals is influenced by the specific adsorption of metals on various soil constituents. Generally, soil horizons rich in clay fraction or organic matter have higher contents of trace elements than sandy horizons. Metals accumulate in fine-particle size fraction in soils due to high surface areas and negative charges associated with clay minerals (e.g. smectite and vermiculite) and humic substances. Metal content in soil samples are commonly used to assess contamination, however, the accumulation of metals in soils increases with the decreasing particle size (Acosta et al. 2009). The main factors controlling the behaviour of trace metals in soils are organic matter content, iron and manganese hydroxides and redox potential. Kabata-Pendias (1993) referred to minerals from the topsoil being richer in Cu and Cr than the same minerals from the parent material while Ni – more concentrated in minerals from the parent material than from the surface horizon of soil, which may reflect a higher mobility of Cr and Cu under chemical weathering. Certain elements, e.g. Mn, tend to accumulate in surface horizons, while others, like Cr, are often higher in subsurface horizons. The content of trace metals in topsoil are likely to increase with growing industrial and agricultural activities. Due to many anthropogenic sources of pollution, it is important to estimate the geochemical background concentration of trace elements in soils. The geochemical background levels of trace metals should represent their natural concentration. The atmosphere plays a key role in global metal cycles as it receives inputs from many sources. Trace metals are transported and deposited having been released into the atmosphere due to natural processes and anthropogenic activity (Pacyna, Pacyna 2001). Assessment of concentrations of trace metals in the geological parent material, generally accepted as the geochemical background, is necessary for determining the pollution degree of topsoil. The origin of parent materials and their texture determines the amount of heavy metals in Polish soils (Table1).

Trace elements in farmlands

Concentration of trace elements in soils is associated with geochemical and biological cycles and generally is influenced by anthropogenic factors such as agricultural practices, industrial activities, and waste treatment (Kabata-Pendias 2004). Contamination of soils with trace elements is caused by atmospheric deposition or by direct disposal on the soil, which includes industrial waste disposal, impurities in fertilizers and manure, sewage sludge and pesticides containing heavy metals. Repeated use of fertilizers and sewage sludge containing trace amounts of metals may cause contamination of soils on a large scale (He et al. 2005). Trace elements enter agroecosystems through anthropogenic processes, including input of heavy metals from fertilizers, organic manures, irrigation and wet or dry deposits, which depend on the nature of and distance to point sources. On farmland, the presence of lithogenic trace metals in soils results from vertical (parent material weathering and pedogenesis) and also lateral redistribution processes, including tillage homogenisation and erosion. The long-term effect of metal-enriched substances application (fungicides, pesticides, and herbicides, phosphates containing of Cd and Pb, farm manures, biosolids) may cause contamination on a large scale (McBride, Spiers 2001). Fungicides and pesticides containing Cu and Zn are widely used to protect plants, especially in orchards. Phosphate fertilizers are among other sources of heavy metal input into agricultural systems. On farmland with a long history of crop production, the concentration of trace elements in the soil upper horizon can be higher than that found in the parent materials. In assessments of soil contamination, especially in risk assessment procedures, more parameters, such as total carbon content, cation exchange capacity and soil reaction, are often necessary.

Terms and definitions of background values of trace elements concentration

The influence of the geological substrate on trace element concentrations of soils can cause developed on specific substrates to demonstrate aberrant trace elements loads. Commonly, the terms *background* and *baseline* are often used as synonymous. *Environmental background* is considered to be descriptive of the natural tendency of an environmental material in the absence of human influences, while an ‘environmental baseline’ is a summary of existing conditions and can include influences of human activities such as land use. Baize and Sterckemann (2001) applicate the term ‘pedogeochemical background’ in relation to natural concentration of elements in

Table 1

Arithmetic mean and/or range of total content of metals

Soil materials	Zn	Cu	Pb	Cr	Ni	Mn	Fe	References
	(mg kg^{-1})						(g kg^{-1})	
Boulder loams (middle texture) in northern and central Poland	30-51 (41.0)	4.0-13.6 (10.0)	6-16 (10.7)	20.0-48 (37.0)	12.6-20.-6 (13.7)	228-457 (344.0)	1.51-2.42 (2.03)	CZARNOWSKA (1996)
Bed-rocks in northern and central Poland	5-59 (30.0)	0.4-23.5 (7.1)	0.5-21 (9.8)	2-64 (27.0)	0.5-28.5 (10.2)	15-1040 (289.0)	0.11-3.70 (1.29)	CZARNOWSKA (1996)
Sedimentary rocks of glacial origin in Poland	(36.0)	(8.8)	(10.3)	(30.0)	(12.7)	(322.0)		CZARNOWSKA, GWOREK (1990)
Soils in Poland 0-20 cm layer	(32.5)	(6.6)	(13.70		(6.3)			PIOTROWSKA TERELAK (1997)
Soils in the Kujawy and Pomorze region 0-20 cm layer	(23.8)	(4.7)	(12.6)		(6.3)			PIOTROWSKA TERELAK (1997)
Soils developed from glacial tills in WNP horizon-B – samples <0.06 mm	(19.0)	(3.0)				(300.0)		GAŁUSZKA (2007)

WNP – Wigierski National Park

soils. In contrast, another term ‘anthropogenic background’ used by Portier (2001) defined concentrations typically observed in an area affected by human activities but that are not associated with a specific contamination activity. The natural abundance of an element in a rock, sediments, soil with references to a particular area is the most common definition of the term ‘geochemical backgrund’. The concept of ‘geochemical background’ comes originally from exploration geochemistry and was introduced to differentiate between normal concentration of element and anomalies, which might be indicative of an ore existence. Traditionally, geochemical anomaly is an aberration from the geochemical patterns that are normal for a given area. The term ‘threshold’, which describes the limit of normal background variation, was introduced to determine the difference between background and anomaly. The geochemical anomalies have been identified by setting threshold values, which mark the upper and lower limits of normal variation for a particular element. Values within the threshold values are referred to as background values and those above or below as anomalies but usually expressed as a single value showing the limit between anomalous and background concentrations. This definition is used mainly in exploration, and is not appropriate for environmental purposes. In environmental geochemistry, there are also problems with the definition of natural background concentration of trace metals. In the ISO document (ISO 2005) the term ‘ambient background concentration’ (ABC) has been used with the same meaning as that of ‘usual background concentration’. Ambient background concentration (ABC) of a trace metals may vary depending on soil types and land uses but consists of both a natural pedo-geochemical fraction and an anthropogenic fraction (Zhao et al. 2007). Soil parent materials and pedogenic processes clearly determined ambient background concentration of trace metals in soils. Reiman and Garrett (2005) introduced the term ‘ambient background’ that defines the unmeasurable perturbed and no longer original natural background. Many slightly elevated horizons or levels in soils reflect ambient background and are no longer pristine natural since natural background no longer exists. The term ‘natural background’ is widely used to describe background levels reflecting natural processes unaffected by human activities. Both effects will modify the ‘natural background’ at the location where the material is deposited, independent of natural or anthropogenic origin (Reimann, Garrett 2005). Therefore, the term natural background could be used as long as natural processes can still be noticed. Gałuszka et al. (2007) described that the background values are different for remote areas, and are governed primarily by the geologic setting of the region and it may be assessed only on a local or regional scale. Blaser et al. (2000) suggest that element concentration measured in a deeper soil horizon could be taken as a ‘local background’ for the surface horizon that is more possibly affected by anthropogenic contamination. Greatly higher element values in upper soil horizons are usually interpreted as evidence of anthropogenic input. A specific TOP/BOT ratio as an index of relative enrichment or depletion of metals in the topsoil horizon may, to certain extent, make sense on farmland, whereas, even for arable soil, a high value in the TOP/BOT-ratio is no evidence of contamination since the TOP-horizon is not 1:1 comparable to the BOT-horizon. The surface horizons are relatively depleted of the colloidal fraction. Clay fraction and oxyhydroxides commonly demonstrate much higher metals concentration than the coarser soil size fraction. REIMANN et al. (2005), demonstrated that only several elements showed a general enrichment in the TOP-layer. Researchers have suggested that contamination is not the most likely explanation for enrichment. Zinc and manganese are often correlated to the abundance of organic matter and for lead the explanation that the enrichment is due to atmospheric input may be suggested. It is very important that regulators recognize that 'background' depends on location and scale. It changes from region to region and with scale of the area investigated. In another method, trace metals are measured in deeper soil horizon as the local background for the upper horizons, usually after correction for the variation of a reference element such as Zr. The relationships between trace elements and semi-conservative elements such as Fe, Al and Mn were used to predict the expected values of trace elements in topsoils.

The term 'geochemical baseline' is often used interchangeably with the term geochemical background, particularly when is used in environmental contexts and is very important in environmental legislation, which prescribes limits for heavy metals uncontaminated area. Baseline studies allow investigators to assess chemical pattern changes in the environment resulting from anthropogenic activity. A 'geochemical baseline' represents a measure of a given soil samples in specific location and time (LEE, HELSEL 2005) and refers to the prevailing variation in the concentration of an element in the surface environment. Thus baselines must always be verified in any assessment of sites for contamination. Therefore, a 'geochemical baseline' or background should be described by regional variability and it is a function of time. The baseline concentrations, usually express as an observed or 95% expected range, represent a measure of a given sample in a singular location and time (MATSCHULLAT et al. 1999). They vary in regions of different pollution, but in pristine areas, geochemical baseline concentrations are close to background values. The normalization procedure is a widely used method to obtain the regional geochemical baseline. The measured trace elements have to be fitted with the references element not influenced by human activities. The baseline can be estimated for every single point that confirms to the linear regression conditions. Iron and alluminum as a constituent of aluminosilicate mineral are widely used as the reference elements (DONOGHUE et al. 1997, ZHANG et al. 2007). Titanium and Sc have also been used as reference elements due to their characteristics of geochemical stability in the environmental compartments. A 'geochemical baseline' provides the means to distinguish between the pedogenic and the anthropogenic origin of trace element in the environmental compartments (ZHANG et al. 2007). The term ‘geochemical baseline’ has not yet been defined clearly and most commonly it referred to the natural abundance of an element in particular material such as rock, sediments, and soil respect to a particular land. However, this term was used mainly in exploration rather than environment as it generally refers to a single value. Many times natural variation of element concentration in different environmental sample materials is often so large that it is difficult to identify anthropogenic addition and contamination. These should be restricted to the local scale (Reimann, Garrett 2005). A statistically determined background value and associated range cannot be used alone to detect anthropogenic influences on the environment; geochemical mapping at an appropriate scale is essential. To define «background» or «normal abundance», a considerable number of samples have to be collected over a sufficiently large area to be able to differentiate between different possible natural and anthropogenic sources. Jarva et al. (2010) concluded that geochemical properties to a great extent determine the distribution of metals in soil. Regional variations in the fine-fraction content of different tills reflecting glaciation processes define element concentration. An anthropogenic impact on baseline levels can be detected particularly in industrialised areas. Reinman and Garrett (2005) claimed that geochemical mapping at an appropriate scale is indispensable to create a map showing areas of relatively homogenous geochemistry. A particular map of geochemically homogeneous areas with other relevant information can be used to deduct the natural and anthropogenic processes that determine the distribution of metals.

Modes of chemical normalization

Since the background value for particular element is theoretical, different methods of assessing it, i.e. geochemical, statistical, and integrated methods, may be applied. Direct (empirical) and indirect (statistical) methods are used for assessing background concentrations of elements in specific area. The direct (geochemical) method of assessing background concentration bears on studies of samples not influenced by anthropogenic activities or to relatively pristine areas (Horckmans et al. 2005). There are selected statistical methods used not only for assessing background concentration but also for the separation of geochemical anomalies from the geochemical background. In statistical methods: the regression analysis, probability plots, and fractal method have been applied (Matschullat 2000). Some selected tests (iterative 2s-technic and calculated distribution function; 4σ-outlier test; Lepeltier method), were used to quantify the background concentration of trace metals in soils. Most statistical tests are only helpful in eliminating the so-called outliers, and thus to reduce the original detaset to a clean data collective. From reduced dataset the essential descriptive statistical parameters can be calculated. The normal range of a sample is defined by the mean $\pm 2\sigma$, this means that ca. 95% of the samples come within the range. The upper values describe the threshold level (mean $+2\sigma$), which means that ca.

97% of the samples lie below this value. The iterative 2σ-technic is also an appropriate method of background assessment and it corresponds to widely used threshold calculation with the formula: upper limit of mean ±2σ range (Li et al. 2003). Mean and standard deviation (σ) are calculated for the original dataset. All values beyond the mean ±2σ interval are omitted. The mean ±2σ calculated from the resulting sub-collective is considered to reflect the geoegenic background (Matschullat 2000). The definition of the background as «the normal range of a sample» (mean ±2σ) requests the assumption of a long-normal distribution.The linear relationships between the content of individual metal of concern and a conservative element (e.g. Fe, Al) content were defined in the form of $y=ax+b$, where y is the value of the metal, x is the Fe, Al content in the samples. The data lying out of the 95% confidence band were eliminated, and then there was created a new linear equation with the updated dataset where all the data were within the 95% confidence band. The measured element concentration has to be fitted with conservative elements that are not influenced by anthropogenic activities. The measured e.g. Fe, Al or Zr contents are predicted value for each element at each samples location was computed. The predicted mean value as the critical value and mean ±2σ as the range of the geochemical baseline for each element, where s is the standard deviation of predicted dataset (Covelli, Fontolan 1997). The study allowed estimating the amount of anthropogenically-introduced metals into the arable soils.

Chemical normalization was performed by comparing the analysed samples, to the nearby non-contaminated samples of similar texture, chemical and mineralogical composition. Background concentration of non-contaminated samples can be established from parent material samples (C-horizon), below the level of anthropogenic influences, of the same region. The level of contamination of soil with heavy metal is expressed in terms of a distribution index (DI) calculated as follows:

$$\text{Distribution Index (DI)} = \frac{\text{metal content in the solum horizon}}{\text{metal content in parent material}} \qquad (1)$$

where: DI < 1 refers to low contamination, 1 ≥ DI ≥ 3 means moderate contamination, 3 ≥ DI ≤ 6 indicates considerable contamination and DI > 6 indicates very high contamination. Potential pollutant concentrations are to be compared with background averages in order to calculate the enrichment factor (EF). To identify anomalous metal concentration, geochemical normalization of the trace elements data to a conservative element, such as Fe, Al, was applied (Bourennane et al. 2010). In this study iron has also been used as a conservative tracer to differentiate natural from anthropogenic components.

Enrichment factor (EF) is defined as follows:

$$\mathrm{EF} = \frac{\left(\frac{M}{\mathrm{Fe}}\right)sample}{\left(\frac{M}{\mathrm{Fe}}\right)background} \tag{2}$$

where: M stands for 'metal'.

Zhang and Liu (2002) proposed that EF values between 0.5 and 1.5 indicate the metal is entirely from crustal or natural processes, whereas values greater than 1.5 suggest the possible anthropogenic impact in soils. Transfer factor (TF) is defined as follow:

$$\mathrm{TF_h = (X_h / X_p) / (Y_h / Y_p)} \tag{3}$$

where X_h stands for the concentration of the metal in bulk soil sample in genetic horizon, X_p is the concentration of the Ti in bulk soil sample in genetic horizon; Y_h is the concentration of metal in parent material, Y_p stands for the concentration of Ti in parent material (Acosta et al. 2011). Values >1.0 indicate the accumulation of metals in soil profile due to soil forming processes. However values of 1.0 and <1.0 show that enrichment in metals is not detectable and loss of metals is due to pedogenic processes.

MATERIAL AND METHODS

Geological background concentrations of trace elements were determined for arable soils in the Inowrocławska Plain mezoregion, which are developed on the poorly sorted glacial till. The nine representative pedons were selected for research (Table 2). Soils developed from the Baltic glacial till, similar in age, and the same origin, with different water regime. In addition, there is no natural geochenical anomaly. Detailed characteristics of soils and their morphology, selected properties were given in the previous papers (Kobierski, Dąbkowska-Naskręt 2003a,b, Kobierski et al. 2009). According to WRB taxonomy (IUSS WRB 2007), the soils from the region represent Phaeozems (profiles I, II, III, IV), Luvisols (profiles V, VI, VII) and Cambisols (profiles VIII, IX). Sampling sites with relatively small impacts of human activity were selected, and soil profile samples were analysed for Zn, Cu, Pb, Cr, Ni, Fe and Ti content. Moreover, usual data for soil characterization of some properties (e.g. pH, hydrolytic acidity; CEC, texture, organic carbon, total carbonates) were used. The soil was sampled from a particular genetic horizon, air-dried and sieved through a 2 mm screen. The depth of the horizons varied according to the soil development. In the Inowrocławska Plain region the soil-forming processes have greatly affected the initial composition inherited from parent material. The mean depth of the parent material in Luvisols and Cambisols was deeper than in Phaeozems. The following physical and chemi-

Table 2

Pedons location in the Inowrocławska Plain

No	GPS positioning		
I	Szadłowice	52° 50' 35.2" N	18° 20' 14.9" E
II	Cieślin	52° 48' 08.9" N	18° 12' 09.2" E
III	Gnojno	52° 59' 38.2" N	18° 15' 59.6" E
IV	Dobre	52° 41' 10.8" N	18° 35' 04.6" E
V	Liszkowo	52° 53' 26.2" N	18° 13' 53.5" E
VI	Zduny	52° 49' 35.9" N	18° 33' 22.9" E
VII	Rucewko	52° 44' 14.2" N	18° 05' 37.2" E
VIII	Batkowo	52° 45' 58.7" N	18° 12' 13.1" E
IX	Pęchowo	52° 56' 12.5" N	18° 04' 19.9" E

cal properties of soil were determined by standard methods used in analytical work: granulometric composition by Cassegrande method, pH in 1 M KCl dm^{-3} – potentiometrically, organic carbon (TOC) using TOCN Primacs Skalar Analyser, cation exchange capacity (CEC) was calculated on the basis of hydrolytic acidity and exchangeable bases determined with $BaCl_2$. Total Zn and Cu content were defined after HF and $HClO_4$ mineralization of homogenised soil samples according to Crock and Severson (1987). The analyses of element concentrations were conducted using atomic absorption spectrometer (Philips PU 9100X). The descriptive statistical analysis such as standard deviation, standard error, regression analysis mean for metals were calculated using Statistica 6.0 computer package. The relationships between the elemental composition were determined using correlation and cluster analysis. The cluster was performed with the method of weighted average linkage between the groups. Person's correlation method for the cluster intervals and the elements showing a close correlation were identified. Reference soil (SO-4) from the Canadian Certified Reference Materials Project (Bowman et al. 1979) and reagent blanks; replications were used as the quality control samples during the analysis. The results showed no sign of contamination and revealed that the precision and bias of the analysis were generally below 7%. The recovery rates for the metals in the standard reference material ranged from 89 to 112%. The recovery of metals in the analysis was within <5.0%, as compared to the reference soil material.

Soil research results for the Inowrocławska Plain

The original stage of soil formation is the accumulation of the parent materials. The northern part of the Polish land mass was glaciated during the last Baltic glacial episode, and therefore the majority of parent materials in mesoregions are of glacial origin and relatively young. Glacial till deposits are poorly sorted and heterogeneous mixtures of gravel, sand, silt, and clay with varying texture and mineralogy. The nature, properties and mineralogical composition of the parent materials exert a strong subsequent control on the pathways of soil genesis. Different types of soils from the Inowrocławska plain were formed from glacial sediments and they may further undergo transformations (KOBIERSKI et al. 2009). Granulometric indices confirm the presence of typical glacial till. In the upper horizons of soil fine sandy loam was dominated, however, in the parent material there was observed homogenous sandy loam (KOBIERSKI, DĄBKOWSKA-NASKRĘT 2003a, KOBIERSKI 2010b). Phaeozems and Cambisols from this region were formed under at least two processes including lessivage processes. The downward leaching of soluble ions and translocation of clays in soil profiles is related to the average depth to which water penetrates the soil. The content of calcium carbonate in parent material is very essential to buffering as $CaCO_3$ is able to neutralize soil acidity. Clay minerals and organic matter contents are also important to buffering and cation exchangeable capacities. Illite dominated the clay mineralogy in the soil analysed, other clay minerals such as smectite, chlorite and interstratified minerals (illit-smectie, chlorite-smectite) were also identified by XRD (KOBIERSKI, DĄBKOWSKA-NASKRĘT 2003b, KOBIERSKI et al. 2009).

Soils formed as a result of the interaction of soil factors and specific soil-formation processes currently undergo strong anthropogenic effect. An intensive agricultural cultivation of Luvisols in the Inowrocław Plain resulted in irreversible changes in their morphology, seen by including the Et horizon to Ap horizon. The evaluation of iron forms distribution in soil profiles show that it can be concluded that the Cambisols researched show qualities of stratified Luvisols (KOBIERSKI 2010a). The Fed/clay ratio profile distribution indicates comigration of clay fraction and free iron oxides in Phaeozems and Luvisols. Neutral and alkaline reaction and a considerable content of organic matter do not facilitate metals mobilisation, unlike the soils in which the degree of oxidation of iron is low under the conditions of low redox potential. A low content of active iron in the parent material of the soils researched can be referred to a relatively high content of calcium carbonate, which constitutes a geochemical barrier, decreasing the mobility of that metal (KOBIERSKI 2010a). A study of morphology of Phaeozems provides a clearer picture of recent changes in mineral phase due to soil texture, which influences the water regime in soil. Luvisols and Cambisols do not show any gleyic process traits.

RESULTS AND DISSCUSION

Some properties of the investigated arable soils are included in Table 3. The soils were neutral or alkaline, with the pH in KCl ranging from 6.70 to 7.90. Soils differed in texture and organic matter content, mean values for clay particles and organic matter contents were found in the range 7-17% and 7.9-15.2 mg kg^{-1} in humus horizon, respectively. Illuvial horizons of all the soils analysed were enriched in clay fraction. The cation exchange capacity (CEC) ranged from 29.6 to 194.5 mmol(+) kg^{-1} of soil. The lowest amounts of exchangeable cations were found in horizons Et but the soils were sorption-saturated. The horizons rich in clay fraction contained higher content of cations.

The mobility of trace elements depends not only on the total concentration in the soil but also on soil properties, and environmental factors. Metals accumulate in soil in various forms: water soluble, exchangeable, carbonate associated and Fe-Mn oxides associated, organic matter associated and residual forms. Experimental datasets have been tested by statistical analysis in order to evaluate the possibility of differentiating the sampling sites to identify possible correlations existing among metals and to assess relationships between the chemical composition and the anthropogenic influences. Geochemical association of trace metals in the soil is mostly determined by the local geological features, pedogenesis and the characteristics of trace elements. Soil forming processes cause variation in the distribution of elements. The different mineral composition and differences in soil texture are factors determining metals concentrations. Cu, Ni, Cr, Mn and Fe partly leach from the minerals and precipitate as colloidal particles. We have observed an accumulation of metals at 42-95 cm in soil profiles. Clay fraction and organic carbon content is generally considered to be important factors in evaluating the trace element concentration in soils (LIVENS 1991, SPARK et al. 1997, TACK et al. 1997). In this study no clear positive relationships between trace elements and organic carbon content was apparent but a negative limited association of Ni and Fe with organic carbon content could be seen (Table 4). A fairly linear relation was observed between Fe and metals such as Cu, Ni, Cr, Mn (Table 5). The correlation between Fe and trace elements content may be a relevant factor when determining background concentration levels. Figure 1 is the result of the cluster analysis for the metals of the topsoil and two main groups were distinguished in the dendrogram.

Cluster 1 consisted of Ni, Fe, Cu, Cr, Mn and Zn and cluster 2 included Pb. One can observe four sub-clusters for cluster 1 (a) Ni, Fe; (b) Cu; (c) Cr, Mn; (d) Zn, which indicated that Ni, Cu, Cr and Zn were mainly associated with Fe-Mn oxides and were relatively immobile in geochemistry. This implied that the first geochemical association was mainly influenced by local geological features. The accumulation could reflect an illuvial horizon loca-

Table 3

Selected properties of analyzed soils

No	Horizon	Depth (cm)	pH KCl	Corg (g kg^{-1})	Hh	S	CEC	Clay fraction (%)
					[mmol(+) kg^{-1}]			
I	Ap	0-32	7.27	15.1	2.5	146.2	148.7	14
	A2	32-56	7.90	10.2	0.2	158.5	158.7	14
II	Ap	0-28	7.05	10.5	3.3	140.6	143.9	13
	A2	28-40	6.97	10.2	3.9	136.2	140.1	12
	AB	40-55	7.44	7.3	0.2	161.4	161.6	16
	Bt	55-75	7.36	2.9	1.9	159.2	161.1	20
III	Ap	0-28	7.23	15.2	2.4	182.7	185.1	13
	A2	28-49	7.26	14.8	3.2	191.3	194.5	14
IV	Ap	0-26	7.28	12.2	3.0	153.5	156.5	13
	A2	26-42	7.77	10.6	1.1	185.9	187.0	14
	Bt	42-60	7.67	2.6	2.5	168.0	170.5	21
V	Ap	0-25	7.21	9.3	2.7	108.3	111.0	12
	Et	25-45	6.96	1.7	5.3	38.9	44.2	7
	Bt	45-95	7.03	1.5	3.9	138.8	142.7	24
VI	Ap	0-32	7.15	7.9	2.4	70.3	72.7	7
	Et	32-50	6.96	1.3	8.0	21.6	29.6	5
	Bt	50-89	7.16	1.8	6.4	154.4	160.8	24
VII	Ap	0-29	7.07	8.9	4.0	135.2	139.2	13
	Bt1	29-49	6.98	4.2	3.0	126.0	129.0	19
	Bt2	49-78	7.04	2.8	2.4	131.0	133.4	22
VIII	Ap	0-28	7.89	9.3	7.6	120.8	128.4	17
	Bw(t)1	28-50	7.86	4.1	8.3	144.4	152.7	23
	Bw(t)2	50-70	7.18	2.7	4.0	132.1	136.1	22
IX	Ap	0-26	7.17	9.0	3.0	109.6	112.6	12
	Bw(t)1	26-50	6.72	3.5	8.3	124.2	132.5	21
	Bw(t)2	50-70	6.70	3.0	6.7	119.9	126.6	23

Hh – hydrolytic acidity, S – base saturation, CEC – cation exchange capacity Bw(t) weakly developed luvic features in cambic horizon

Table 4

Correlation coefficients significant at $p<0.05$

Parameters	Zn	Cu	Ni	Cr	Mn	Fe	Ti
Corg			-0.54			-0.54	-0.70
Clay		0.81	0.94	0.53	0.40	0.95	0.70
CEC	0.57	0.64		0.40			
Zn		0.48					
Cu			0.79			0.74	0.70
Ni				0.50	0.41	0.95	0.79
Pb					0.50		
Cr					0.58	0.60	
Mn						0.52	
Fe							0.78

Table 5

Linear relationship between metals and Fe from topsoil

Equation	SEE	r	p
Cu = 0.37 Fe + 5.97	1.87	0.74	<0.001
Ni = 0.93 Fe - 1.43	1.75	0.95	<0.001
Mn = 4.83 Fe + 226.72	44.77	0.52	<0.001
Cr = 0.17 Fe + 2.73	1.27	0.60	<0.002

SEE indicates the standard error of Y extimation

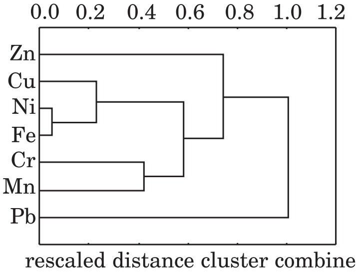

Fig. 1. Dendrogram using average linkage (between groups) to display the elements association in the soils

ted here in as much as trace elements are mostly associated with the clay fraction of soils. Therefore, it was attempted to use Fe as a proxy for clay fraction content in the clay-rich soil materials. A strong correlation was found between Fe and clay content for analysed profiles and so the comparison was properly used. Finally, the trace elements could also be translocated and then precipitated due to an increase in pH. In well-aerated acid soils several metals (especially Zn) are substantially more mobile and bioavailable than in poorly aerated neutral or alkaline soils (TACK, VERLOO 1995).

It is very important to determine the total metals content in arable soils in order to assess their quality and the potential risk of contamination. Determination of the total concentration should be complemented by the measurement of the labile fraction available to plants (KABATA-PENDIAS, PENDIAS 2001). Lead is generally considered to be a fairly immobile element, as well as a common pollutant, resulting from the long-range transport of aerial contaminants (PACYNA, PACYNA 2001). The limited mobility and strong complexation of Pb by organic matter result in the bioaccumulation of the element in humus surface horizons of soils. The highest amounts of metals found in the upper horizon (humus horizon) in comparison with subsurface horizons indicated a chelating role of organic matter in relation to metals (LIVENS 1991). Humic phenolic hydroxyl and carboxyl groups are mainly involved in the formation of metal-humic complexes (SPARK at al. 1997, COLES, YONG 2006).

The influence of the geological substrate on the content of trace metals is very important and can make soils developed on specific substrates demonstrate divergent trace elements loads. HORCKMANS et al. (2005) confirm the importance of using appropriate background levels for the assessment of contaminated soils and the necessity of determining background concentrations locally. Specific substrates with possibly unpredictable trace element loads can be present over extensive areas, designating it necessary to know the background concentrations before defining any conclusions as to whether elevated element concentrations in soil are lithogenic or anthropogenic. The geochemical baseline takes into account the geochemical variation in basic geology, the different geologic units and the influence of soil-forming processes but it also influences mineralization and anthropogenic effects (SALMINEN, GREGORAUSKIENE 2000).

A level of contamination of farmland in Poland was evaluated in two countrywide programs (TERELAK et al. 2000). The results of study indicated that 80% of agricultural land is characterised by natural and 17.6% – slightly elevated content of heavy metals. About 3% of arable soils are contaminated with heavy metals to various degrees. The expected range of metals content in farmland soils in Poland differs significantly from the state range, which clearly indicates that low as well as high metal concentrations in arable soil in Poland may appear infrequently (CZARNOWSKA, GWOREK 1990, CZARNOWSKA 1996, PIOTROWSKA, TERELAK 1997). The average Zn, Cu, Ni and Pb contents in farmland soils in Poland are 32.4, 6.5, 6.2, 13.6 mg kg^{-1} respectively (Oleszek et al. 2003).

The distribution index (DI) values ranged from 0.3 to 1.7, while enrichment factor (EF) values were higher and ranged from 0.6 to 2.5 (Tables 6, 7). In the Ap horizon most profiles and in a few cases in Et and Bt horizons there were found the highest values of both indices for respective metals. There was reported no anthropogenic effect on the content of nickel and an slight effect on the content of the other metals. Soil contamination assessment was carried out using enrichment factor and transfer factor (Figures 2, 3). The calculation of enrichment factors showed that Mn, Cu, Cr in humus horizon ranged from 0.8 to 2.0 and Ni, and Fe from 0.8 to 1.0, and Pb from 1.1 to 2.4 whereas Zn ranged from 0.9 to 2.4. Some of the elevated concentrations of Zn are probably due to anthropogenic sources, mainly fertilizers and pesticides used in agriculture. A long-term agricultural use of analysed soils resulted in a slight increase in the Pb, Zn, Cu content in the profiles of the soils, as compared with the contents determining the geochemical background. The pedogenic formation of Fe oxides seems to be a factor determining the distribution of trace metals in the depth of soil profile. The distribution of pedogenic Cu, Zn, Ni and Pb is influenced by the specific adsorption of metals on Fe oxides. There was found an effect of pedogenesis on the distribution of metals in the profiles of the soils, which refers to Ni and Fe since the transfer factor (TF) scored highest in subsurface horizons and the illuvial horizon of II, IV, V, VI, VIII, IX profiles (Figures 2, 3). The highest TF values for lead were reported in the Ap horizon in profiles V, VI, IX, which points to the effect of anthropogenic factors on the accumulation of that metal. The highest TF values in horizon Ap of soils I, II, III demonstrates a tendency to accumulate Zn, Cu and Pb as a result of a long-term agricultural use of soils of the Inowrocławska Plain. The distribution of metals in the soil profiles was most affected by the content of clay fraction, which was confirmed by the statistical analysis (Table 4). The distribution pattern of Zn and Cu in soil profiles of Phaeozems suggested an accumulation of these metals in the surface horizon but Cr and Ni are gradually leached down and accumulated in Bt horizon, which is conventional view for the mechanism of metals distribution in soil profiles. The clay fraction proved a strong mobility to fix the metals on their surface. The content of metals (arithmetic means) in the parent material of analyzed soils were considered to be the content of the geochemical background (Table 8). Respectively higher content for respective metals were found after the application of one of the statistical methods (mean + 2σ range).

The content of metals were similar to the ones presented by other authors describing the content of the geochemical background for respective metals in arable soils in analyzed region (Dąbkowska-Naskręt et al. 2000, 2006).

Table 6

Content of Zn, Cu, Ni, Pb (mg kg^{-1})

No	Horizon	AM / s.e.	Zn	DI	EF	Cu	DI	EF	Ni	DI	EF	Pb	DI	EF
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
I	Ap		67.4	1.1	2.1	12.6	1.1	1.3	10.1	0.7	0.9	12.1	1.0	1.2
	A2		57.4	1.0	1.7	12.4	1.0	1.2	11.3	0.8	0.9	11.7	1.0	1.1
	Ckg	(*n*=3) s.e.	58.5 3.5			11.9 0.3			14.2 1.0			11.7 0.7		
II	Ap		72.7	1.7	2.4	10.8	0.9	1.1	9.1	0.7	0.8	13.1	0.9	1.3
	A2		52.2	1.2	1.7	10.1	0.9	1.0	9.7	0.7	0.8	12.6	0.9	1.2
	AB		55.3	1.3	1.4	10.7	0.9	0.9	13.1	0.9	0.9	14.7	1.0	1.1
	Bt		58.7	1.3	1.2	12.0	1.0	0.8	17.2	1.2	1.0	12.2	0.8	0.8
	Ckg	(*n*=3) s.e.	43.5 5.1			11.6 1.0			13.7 1.3			14.5 1.2		
III	Ap		45.4	1.2	1.6	14.2	1.2	1.6	10.8	0.8	1.0	13.0	1.0	1.4
	A2		44.2	1.1	1.5	13.8	1.2	1.6	9.3	0.7	0.9	12.8	1.0	1.4
	Ckg	(*n*=4) s.e.	39.1 3,0			11.3 0.7			13.6 0.5			12.4 0.3		
IV	Ap		40.0	1.0	1.2	12.3	0.8	1.2	12.8	0.8	1.0	13.5	1.1	1.3
	A2		33.8	0.9	1.1	10.7	0.7	1.2	10.2	0.6	0.9	12.4	1.0	1.3
	Bt		39.5	1.1	0.7	10.2	0.6	0.6	17.3	1.1	0.9	12.4	1.0	0.7
	Ckg	(*n*=2) s.e.	37.3 0.6			15.9 2.1			16.0 0.6			12.5 0.2		
V	Ap		34.7	0.9	1.4	9.8	0.6	1.3	7.7	0.5	0.8	15.6	1.1	1.3
	Et		23.5	0.6	1.1	6.9	0.4	1.0	7.2	0.5	0.7	14.3	1.0	2.1
	Bt		49.5	1.3	0.8	16.9	1.1	0.9	20.7	1.4	0.9	13.3	1.0	0.7
	Ck	(*n*=2) s.e.	39.3 0.9			15.6 3.3			14.3 0.8			13.6 0.3		

cont. Table 6

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
VI	Ap		30.0	0.9	1.5	7.2	0.6	1.2	5.6	0.4	0.8	14.8	1.1	2.4
	Et		20.0	0.6	1.1	5.7	0.6	1.1	4.5	0.3	0.7	14.1	1.1	2.5
	Bt		50.9	1.5	0.9	16.5	1.5	0.9	23.6	1.7	1.1	14.8	1.1	0.8
	Ck	(*n*=2) s.e.	33.6 0.3			11.3 0.4			14.0 0.6			13.4 0.5		
VII	Ap		42.5	1.1	0.9	11.5	1.0	0.8	14.6	0.9	0.9	16.5	1.1	1.1
	Bt1		49.5	1.3	0.9	12.6	1.1	0.8	15.9	1.0	0.8	17.5	1.2	1.0
	Bt2		45.6	1.2	0.9	14.2	1.2	0.8	17.5	1.1	0.8	18.2	1.2	1.0
	Ck	(*n*=2) s.e	37.5 0.6			15.3 0.4			11.7 0.5			14.9 0.3		
VIII	Ap		38.5	1.2	1.0	11.0	1.0	0.9	14.1	0.9	1.0	15.1	1.2	1.2
	Bw(t)1		42.8	1.3	0.8	13.3	1.2	0.8	16.8	1.1	0.9	13.6	1.1	0.8
	Bw(t)2		42.1	1.3	0.8	14.0	1.3	0.9	21.8	1.5	1.1	13.9	1.1	0.8
	Ck	(*n*=2) s.e.	32.8 2.1			11.0 0.7			15.0 1.6			12. 0.05		
IX	Ap		39.0	1.0	1.2	12.3	1.0	1.2	12.5	0.7	1.0	15.4	1.3	1.4
	Bw(t)1		45.0	1.2	0.8	14.6	1.2	0.8	19.9	1.2	0.9	14.0	1.2	0.7
	Bw(t)2		44.7	1.2	0.8	15.3	1.3	0.9	23.4	1.4	1.2	15.0	1.3	0.9
	Ck	(*n*=3) s.e.	37.1 0.9			11.9 0.3			16.6 0.5			11.7 1.1		

DI – Index of distribution in soil profile calculated from the average content of metal in parent material to the content of metal in analyzed horizons;
EF – Enrichment factor; AM – arithmetic mean;
s.e. – standard error.

Table 7

Content of Cr, Mn (mg kg^{-1}), and Fe, Ti (g kg^{-1})

No	Horizon	AM / s.e.	Cr	DI	EF	Mn	DI	EF	Fe	DI	Ti	DI	EF
1	2	3	4	5	6	7	8	9	10	11	12	13	14
I	Ap		3.2	0.7	0.8	335.4	1.1	1.4	12.6	0.8	1.0	0.8	1.0
	A2		3.2	0.7	0.7	304.2	1.0	1.2	13.9	0.9	1.3	1.0	1.2
	Ckg	(*n*=3) s.e.	4.3 0.5			300.8 20.3			16.1 0.5		1.3 0.0		
II	Ap		3.9	0.5	0.9	290.5	0.9	1.2	12.6	0.8	1.3	0.8	1.3
	A2		4.9	0.9	1.1	302.5	1.0	1.2	12.9	0.8	1.2	0.7	1.2
	AB		5.2	0.9	1.0	303.4	1.0	1.0	16.5	1.0	1.3	0.8	1.0
	Bt		5.9	1.0	0.9	298.2	1.0	0.8	19.9	1.2	1.3	0.8	0.8
	Ckg	(*n*=3) s.e.	5.7 0.2			306.9 39.3			16.6 1.1		1.6 0.2		
III	Ap		5.6	0.8	1.4	252.2	0.8	1.1	11.8	0.7	1.1	0.9	1.2
	A2		5.9	0.9	1.5	252.5	0.8	1.1	11.8	0.7	1.1	0.9	1.2
	Ckg	(*n*=4) s.e.	6.8 0.1			333.4 36.9			16.9 1.7		1.2 0.04		
IV	Ap		7.5	1.1	1.7	308.0	1.1	1.2	13.4	0.8	1.2	0.8	1.1
	A2		6.4	1.0	1.6	307.9	1.1	1.3	12.3	0.7	1.2	0.8	1.2
	Bt		9.0	1.4	1.2	340.6	1.2	0.8	21.8	1.3	1.3	0.9	0.8
	Ckg	(*n*=2) s.e	6.5 1.5			290.6 80.7			17.1 0.5		1.4 0.05		
V	Ap		4.1	0.7	1.2	265.5	0.8	1.4	10.0	0.6	1.1	0.8	1.4
	Et		4.0	0.7	1.4	298.0	0.9	1.8	8.8	0.5	1.3	1.0	1.9
	Bt		6.7	1.3	0.8	307.8	0.9	0.7	24.2	1.4	1.6	1.2	0.8
	Ck	(*n*=2) s.e.	6.0 0.1			345.3 16.6			16.8 0.8		1.3 0.05		

cont. Table 7

1	2	3	4	5	6	7	8	9	10	11	12	13	14
VI	Ap		4.0	0.8	1.5	295.3	1.1	2.0	7.9	0.5	1.1	0.8	1.8
	Et		3.9	0.8	1.7	191.0	0.7	1.4	7.1	0.4	1.2	0.9	2.1
	Bt		6.0	1.3	0.8	347.5	1.4	0.8	23.8	1.5	1.6	1.2	0.9
	Ck	(*n*=2) s.e.	4.8 0.2			254.8 11.9			16.1 0.5		1.3 0.05		
VII	Ap		6.4	1.2	1.0	356.2	0.9	1.0	18.6	1.1	1.2	0.9	0.8
	Bt1		9.1	1.7	1.3	451.2	1.2	1.1	21.5	1.2	1.6	1.2	0.9
	Bt2		7.4	1.4	0.9	412.3	1.1	0.9	23.8	1.4	1.7	1.3	0.9
	Ck	(*n*=3) s.e.	5.2 0.2			384.0 2.1			17.2 0.2		1.3 0.05		
VIII	Ap		4.8	1.1	0.9	274.2	1.1	0.9	15.6	1.0	1.3	1.0	1,1
	Bw(t)1		5.3	1.2	0.8	250,8	1.0	0.6	21.2	1.4	1.4	1.1	0.8
	Bw(t)2		5.1	1.2	0.6	311.1	1.2	0.8	21.6	1.4	1.7	1.3	0.8
	Ck	(*n*=2) s.e.	4.5 0.05			258.9 22.2			15.0 0.0		1.3 0.05		
IX	Ap		4.5	0.8	1.0	296.7	1.0	1.1	13.9	0.8	1.4	1.0	1.3
	Bw(t)1		5.8	1.0	0.7	267.2	0.9	0.8	23.5	1.4	1.7	1.2	0.9
	Bw(t)2		5.8	1.0	0.8	317.7	1.0	0.8	21.8	1.3	1.7	1.2	1.0
	Ck	(*n*=3) s.e.	5.6 0.3			300.9 8.0			16.8 0.1		1.4 0.0		

DI – Index of distribution in soil profile calculated from the average content of metal in parent material to the content of metal in analyzed horizons;
EF – Enrichment factor; AM – arithmetic mean;
s.e. – standard error.

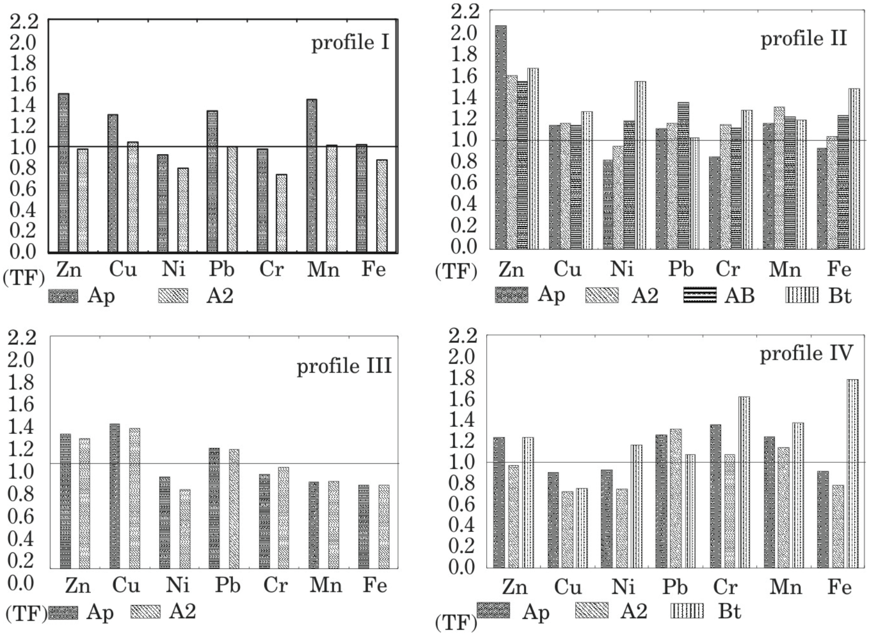

Fig. 2. Transfer factor (TF) calculated for Phaeozems

Table 8

Metal content and selected statistics for parent material of arable soils from the Inowrocławska Plain

Glacial till (n=23) Phaeozems Luvisols Cambisols		Zn	Cu	Ni	Pb	Cr	Mn	Fe	Ti
		(mg kg^{-1})						(g kg^{-1})	
	AM	40.6	12.6	14.3	12.9	5.5	309.9	16.5	1.4
	median	38.1	12.1	13.6	12.7	5.4	316.9	16.6	1.3
	SD (σ)	8.66	2.33	1.74	1.47	1.04	57.1	1.58	0.16
	upper limits the mean + 2σ range	57.9	17.3	17.8	15.8	7.6	424.1	19.7	1.7

AM – arithmetic mean, SD (σ) – standard deviation

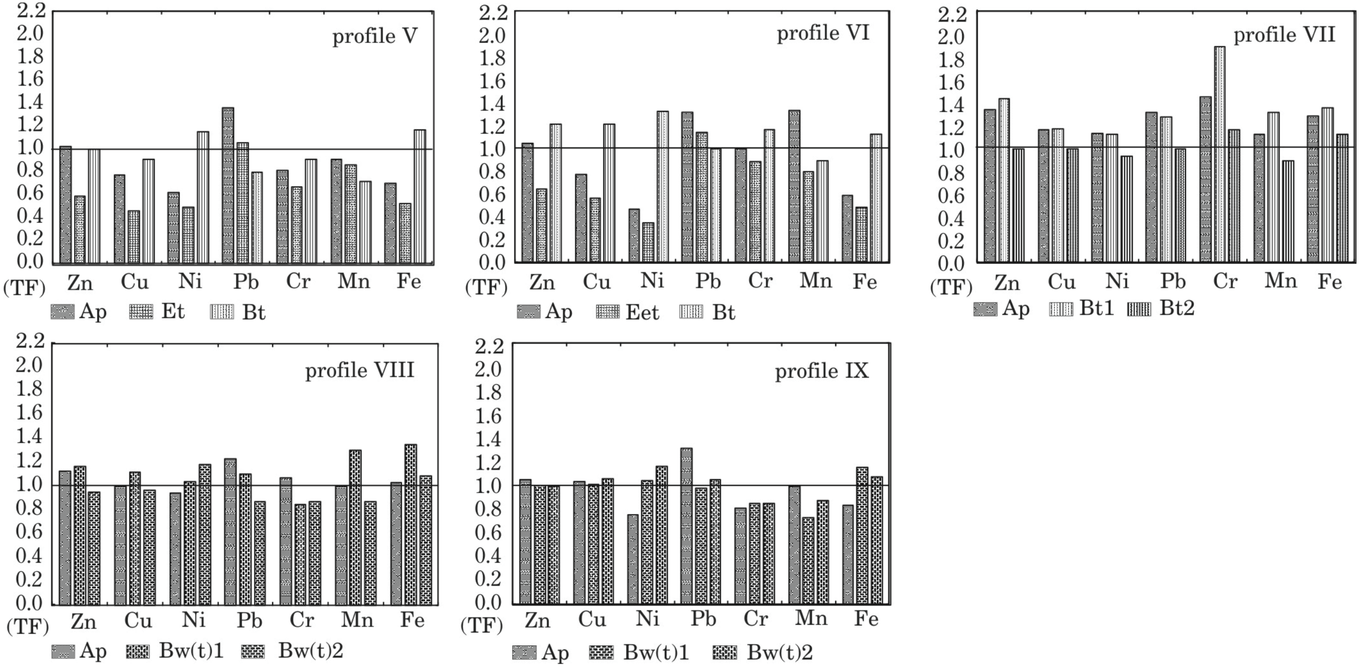

Fig. 3. Transfer factor (TF) calculated for Luvisols (profiles V, VI, VII) and Cambisols (profiles VIII, IX)

CONCLUSIONS

1. The distribution of Ni, Cr, Cu, Mn and Fe in the soil profiles must be the effect of pedogenic factors, however in the case of Zn and Pb, there was observed their tendency to accumulation in the humus horizons as a result of anthropogenic inputs.

2. The pedogenic formation of Fe oxides seems to be a factor determined the distribution of trace metals in the depth of soil profile. The distribution of pedogenic Ni, Cu, Cr and Mn was influenced by the specific adsorption of metals on Fe oxides. The results indicate that metals were bound more strongly to iron oxides then to the organic matter.

3. The distribution of metals in the soil profiles was most affected by the content of clay fraction, which was confirmed by the statistical analysis.

4. The content of Zn, Cu, Ni, Pb, Cr, Mn and Fe, Ti was defined as the content of the local background: 40.6; 12.6; 14.3; 12.9; 5.5; 309.9 (mg kg^{-1}) and 16.5; 1.4 (g kg^{-1}), respectively. A long-term agricultural use of soils in the Inowrocławska Plain resulted in a slight increase in the Pb, Zn, Cu content in comparison with the geochemical background.

5. The results confirm the importance of determining background concentrations locally. With the knowledge of the natural content of elements in the parent material, assumed as the geochemical background, one can evaluate the degree of contamination of topsoil.

REFERENCES

Acosta J.A., Faz Cano A., Arocena J.M., Debela F., Martínez-Martínez S. 2009. *Distribution of metals in soil particle size fractions and its implication to risk assessment of playgrounds in Murcia City (Spain).* Geoderma, 149: 101-109.

Acosta J.A., Martínez-Martínez S., Faz A., Arocena J. 2011. *Accumulation of major and trace elements in particle size fraction of soils on eight different parent materials*. Geoderma, 161: 30-42.

Adriano D.C. 1986. *Trace elements in the terrestrial environment*. New York. Springer, 533 pp.

Allaway B.J. 1990. *Heavy metals in soils*. Glasgow, Scotland/New York: Blackie/Wiley, 339 pp.

Baize D., Sterckeman T. 2001. *Of the necessity of knowledge of the natural pedo-geochemical background content in the evaluation of the contamination of soils by trace elements*. Sci. Total Environ., 264: 127-139.

Blaser P., Zimmermann S., Luster J., Shotyk W. 2000. *Critical examination of trace element enrichments and depletions in soils: As, Cr, Cu, Ni, Pb, and Zn in Swiss forest soils*. Sci. Total Environ. 249: 257-280.

Bourennane H., Douay F., Sterckeman T., Villanneau E., Ciesielski H., King D., Baize D. 2010. *Mapping of anthropogenic trace elements inputs in agricultural topsoil from Northern France using enrichment factors*. Geoderma, 157: 165-174.

Bowman W.S., Faye G.H., Sutarno R., McKeague J.A., Kodama H. 1979. *Soil Samples SO-1, SO-2, SO-2 and SO-4 – Certified Reference Materials*. CANMET Report 79-3. CANMET Mining and Mineral Sciences Laboratories, Ottawa, pp. 34.

Coles C. Yong R.N. 2006. *Humic acid preparation, properties and interaction with metals lead and cadmium*. Engin. Geol., 85: 26-32.

Covelli S., Fontolan G. 1997. *Application of a normalization procedure in determining regional geochemical baselines*. Environ. Geol., 30: 34-45.

Crock J.G., Severson R. 1987. *Four reference soil and rock samples for measuring element availability in the Western Energy Regions:* U.S. Geochemical Survey circular 841. pp. 16 .

Czarnowska K. 1996. *Total content of heavy metals in parent material as geochemical soil background*. Soil Sci. Ann. Supl., 47: 43-50. (in Polish, English abstr)

Czarnowska K., Gworek B. 1990. *Geochemical background values for trace elements in arable soils developed from sedimentary rocks of glacial origin*. Eviron. Geochem. Health., 12: 289-290.

Dąbkowska-Naskręt H., Długosz J., Jaworska H., Kobierski M., Malczyk P., Bartkowiak A., Różański S. 2006. *Zinc contents in surface horizons of soils from the eastern part of the Wielkopolska Lake District*. Part 1. Pol. J. Environ. Stud., 15(2a): 52-55.

Dąbkowska-Naskręt H., Jaworska H., Kobierski M. 2000. *Contents of total and plant available microelement forms in selected arable black earths types of Kujawy region*. Zesz. Probl. Post. Nauk Rol., 471(1): 237-245. (in Polish, English abstr)

Donoghue J.F., Ragland P.C., Chen Z.Q. Trimble C.A. 1997. *Standardization of metal concentrations in sediments using regression residuals: an example from a large lake in Florida, USA*. Environ Geol., 361(1-2): 65-76.

Gałuszka A. 2007. *A review of geochemical background concepts and an axamples using data from Poland*. Environ. Geol., 52: 861-870.

He Z. L., Yang X. E., Stoffella P.J. 2005. *Trace elements in agroecosystems and impacts on the enevironment*. J. Trace Elements Medic. Biol., 19: 125-140.

Horckmans L., Swennen R., Deckers J., Maquil R. 2005. *Local background concentration of trace elements in soils: a case study in the Grand Duchy of Luxembourg*. Catena, 59: 279-304.

ISO 2005. *Soil quality: Guidance on the determination of background values.* ISO 19258: 2005. International Organization for Standardization.

IUSS WORKING GROUP WRB. 2007. World References Base for soil resources 2006. World Soil Resoueces Reports. No. 103, FAO, Rome.

Jarva J., Tarvainen T., Reinikainen J., Eklund M. 2010. *TAPIR – Finnish national geochemical baseline database*. Sci. Total Environ., 408: 4385-4395.

Kabata-Penadias A. 1993. *Behavioral properties of trace metals in soil*. Appl. Geochem, Suppl., 2: 3-9.

Kabata-Pendias A. 2004. *Soil-plant transfer of trace elements – an environmental issue*. Geoderma, 122: 143-149.

Kabata-Pendias A., Pendias H. 2001. *Trace element in soil and plants*. 2th edition. Boca Raton, Florida. pp. 396.

Kobierski M. 2010a. *Profile distribution of iron in arable soils formed on glaciall till from Inowrocławska Plain*. In: *Physical, chemical, and biological processes in soils.* Szajdak L.W., Karabanov A.K. (ed.). Institute for Agricultural and Forest Environment, Pol. Acad. of Sci., The Committe on Land Reclamation and Agricultural Environment Engineering, Polish Academy of Sciences, pp. 295-309.

Kobierski M. 2010b. *Texture of different types of soils formed from glacial till in the aspect of PTG 2008 classification system*. Soil Sci. Ann., 61(3): 67-76. (in Polish, English abstr)

Kobierski M., Długosz J., Bartkowiak A. 2009. *Clay minerals in different soil types from the Kuyavian-Pomeranian Province*. In: *Understanding the requirements for development agricultural production and of rural areas in the Kuyavian-Pomeranian province as a result of scientific research*. Śliwińska E., Spychaj-Fabisiak E. (ed.) UTP Bydgoszcz, pp. 183-197.

Kobierski M., Dąbkowska-Naskręt H. 2003a. *Mineralogical composition and chosen physiochemical properties of the typologically diversified soils of the Inowrocławska Plain.* Part I. *Morphology and physical and chemical properties of selected soils.* Soil Sci. Ann., 54(4): 17–27. (in Polish, English abstr.)

Kobierski M., Dąbkowska-Naskręt H. 2003b. *Mineralogical composition and chosen physiochemical properties of the typologically diversified soils of the Inowrocławska Plain.* Part II. *Mineralogical composition of clay fraction.* Soil Sci. Ann., 54(4): 29–44. (in Polish, English abstr.)

Lee L., Helsel D. 2005. *Baseline models of trace elements in major aquifers of the United States*. Appl. Geochem., 20: 1560-1570.

Li Ch., Ma T., Junfa S. 2003. *Application of a fractal method relating concentration and distances for separation of geochemical anomalies from background*. J. Geochem. Explor., 77: 167-175.

Livens F. 1991. *Chemical reaction of metals with humic material*. Environ. Pollut., 70: 183--208.

Matschullat J., Ottenstein R., Reimann C. 2000. *Geochemical background – can we calculate it?* Environ. Geol., 39(9): 990-1000.

McBride M. B., Spiers G. 2001. *Trace element content of selected fertilizers and dairy manures as determined by ICP-MS*. Commun. Soil Sci. Plant Anal., 32(1,2): 139-156.

Oleszek W., Terelak H., Maliszewska-Kordybach B., Kukuła S. 2003. *Soil, food and agroproduct contamination monitoring in Poland*. Pol. J. Environ. Stud., 12(3): 261-268.

Pacyna J.M., Pacyna E.G. 2001. *Assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide*. Canad. J. Environ. Rev., 9(4): 269-298.

Piotrowska M., Terelak H. 1997. *Heavy metals in soils of the Pomorze and Kujawy against its occurrence at home*. Conf. Proc.: Monitorowanie i ochrona gleb Pomorza i Kujaw. Przysiek/Toruń, 6-19. (in Polish)

Portier K.M. 2001. *Statistical issues in assessing anthropogenic background for arsenic*. Environ. Forensics, 2: 155-160.

Reimann C., Filzmoser P., Garrett R.G. 2005. *Background and threshold: critical comparison of methods of determination*. Sci. Total Environ., 346: 1-16.

Reimann C., Garrett R.G. 2005. *Geochemical background – concept and reality*. Sci. Total Environ, 350: 12-27.

Salminen R., Gregorauskienė V. 2000. *Considerations regarding the definition of a geochemical baseline of elements in the surficial materials in areas differing in basic geology*. Appl. Geochem., 15: 647-653.

Spark K.M., Wells J.D., Johnson B.B. 1997. *The interaction of humic acid with heavy metals*. Aust. J. Soil Res., 35: 89-101.

Tack F.M.G., Verloo M.G. 1995. *Chemical speciation and fractionation in soil and sediment heavy metal analysis: a review*. Int. J. Environ. Anal. Chem., 59: 225-238.

Tack F.M.G., Verloo M.G., Van Mechelen L., Van Ranst E. 1997. *Baseline concentration of trace elements as a function of clay and organic carbon contents in soils in Flanders (Belgium)*. Sci. Total Environ., 201: 113-123.

TERELAK H., MOTOWICKA-TERELAK., STUCZYŃSKI T., PIETRUCH C. 2000. *Trace elements (Cd, Cu, Ni, Pb, Zn) in Polish farmland soils*. Bibl. Monit. Środ., pp. 1-69.

ZHANG H.B., LUO Y.M., WONG M.H., ZHAO Q.G., ZHANG G.L. 2007. *Defining the geochemical baseline: a case of Hong Kong soils*. Environ. Geol., 52: 843-851.

ZHANG J., LIU C.L. 2002. *Riverine composition and estuarine geochemistry of particulate metals in China – Weathering features anthropogenic impact and chemical fluxes*. Estuar. Coast. Shelf Sci., 54(6): 1051-1070.

ZHAO F.J., MCGRATH S.P., MERRINGTON G. 2007. *Estimates of ambient background concentrations of trace metals in soils for risk assessment.* Environ. Pollut., 148: 221-229.

J. Elem. s. 587–596 DOI: 10.5601/jelem.2012.17.4.03

YIELD AND NUTRITIONAL VALUE OF JAPANESE BUNCHING ONION (*ALLIUM FISTULOSUM* L.) DEPENDING ON THE GROWING SEASON AND PLANT MATURATION STAGE*

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Abstract

Japanese bunching onion remains a less common crop in Poland, where it is mostly grown in home gardens as a perennial plant species and used as cut foliage in early spring. Recently, some pseudostem type cultivars producing few tillers have been developed, which can be grown for the use of whole plants in early growth stages or for blanched pseudostems like leek species. This study, comprising two field trials, has been undertaken to evaluate the yield, its quality and nutritional value of cv. Performer Japanese bunching onion as affected by the growing season and age of plants. Seed propagated transplants were grown for a similar period of time from 5 Apr to 8 Jun, 6 May-8 Jul, 7 Jun-10 Aug, 8 Jul-10 Sep, 5 Aug-11 Oct or harvested 60, 75, 90, 105, 120, 135 and 150 days after planting. Plants grown for 135 and 150 days were blanched by mounding soil around the lower leaf bases.

The results of the study indicated that plants of the same age harvested in monthly intervals since early June to September produced similar yields, while those in October gave lower yields, with a gradual decrease of dry matter, carotenoids, sugars, volatile oils and nitrates (V). The delay of harvest date from 60 to 120 days after planting resulted in a substantial yield increment with a simultaneous depletion of vitamin C, carotenoids, chlorophyll a+b, sugars, volatile oils, nitrates, total N, K and Ca content. Blanched plants were characterized by a considerably longer white portion of the pseudostem, lower content of chlorophyll a+b, and carotenoids, and higher amounts of vitamin C.

Key words: growing period, harvest date, yield, plant composition.

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PLON I WARTOŚĆ ODŻYWCZA CEBULI SIEDMIOLATKI W ZALEŻNOŚCI OD TERMINU UPRAWY I STOPNIA DOJRZAŁOŚCI ROŚLIN

Abstrakt

Cebula siedmiolatka jest w Polsce warzywem o małym znaczeniu gospodarczym, uprawianym jedynie amatorsko jako roślina wieloletnia, której częścią użytkową jest szczypior zbierany na wiosnę. Uzyskane w ostatnich latach odmiany tej rośliny tworzące małą liczbę odrostów mogą być uprawiane na zbiór pęczkowy całych roślin lub bardziej zaawansowane we wzroście, po wybieleniu łodygi rzekomej, podobnie jak por. Celem badań obejmujących dwa doświadczenia polowe była ocena plonowania oraz wartości biologicznej cebuli siedmiolatki odmiany Performer w zależności od okresu uprawy i terminu zbioru. Rośliny uprawiano przez taki sam okres w następujących terminach: 5 IV - 8 VI, 6 V - 8 VII, 7 VI - 10 VIII, 8 VII - 10 IX i 5 VIII - 11 X, lub zbierano odpowiednio po 60, 75, 90, 105, 120, 135 i 150 dniach po posadzeniu rozsady. Rośliny uprawiane przez okres 135 i 150 dni były bielone przez podsypanie ziemią dolnej części łodygi rzekomej.

Wyniki badań dowiodły, że rośliny w takiej samej fazie wzrostu zbierane w miesięcznych odstępach od początku czerwca do września plonowały na zbliżonym poziomie, w październiku zaś na niższym. W tym okresie następował systematyczny spadek zawartości suchej masy, karotenoidów, cukrów, olejków lotnych i azotanów. Opóźnienie terminu zbioru z 60 do 120 dni od posadzenia roślin przyczyniło się do znacznego wzrostu plonu a jednocześnie spadku zawartości witaminy C, karotenoidów, chlorofilu a+b, cukrów, olejków lotnych, azotanów (V), N ogólnego, K i Ca. Rośliny charakteryzujące się większym udziałem części wybielonej zawierały mniej chlorofilu a+b oraz karetonoidów, więcej zaś witaminy C.

Słowa kluczowe: termin uprawy, data zbioru, plon, skład chemiczny.

INTRODUCTION

Genus *Allium* includes about 780 plant species, of which onion, Japanese bunching onion, leek and garlic are the most important edible onion plants. Onion, the principal *Allium* representative, ranks second after tomato in the scale of production among cultivated vegetable crops worldwide and is grown in most regions of the world (Rabinowitch, Currah 2002). Regional preferences shape the popularity of other *Allium* crops, e.g. leek is popular in Western Europe and Japanese bunching onion (syn. Welsh onion) is common in China, Japan, Korea and Taiwan (Wang et al. 2005). The latter is still a less popular crop in Poland, mostly grown as a perennial plant in home gardens to use as cut foliage in early spring (Kotlińska, Kojima 2000, Tendaj, Mysiak 2007a).

The advantages of Japanese bunching onion are its high resistance to low temperatures, little soil requirements (Kotlińska, Kojima 2000, Yamasaki et al. 2003, Su et al. 2007), high nutritive value and unique flavour (He et al. 1989, Lazić et al. 2002, Štainer et al. 2006, Tendaj, Mysiak 2007b). According to Kotlińska and Kojima (2000) and Higashio et al. (2007), Japanese bunching onion is particularly abundant in vitamin C, but also contains other valuable compounds such as carotenoids, macro- and micronutrients, especially Ca and K, as well as flavonoids, which are potent antioxidants (AOYAMA, YAMAMOTO 2007, MYSIAK, TENDAJ 2006, 2008). Normally, leaf blades contain more vitamin C, carotenoids, vitamins B_1, B_2, niacin and minerals than the pseudostem (WARADE, SHINDE 1998). The specific odour of the crop is attributed to volatile allyl sulphides.

In Eastern Asia, where bunching onion receives considerable attention, many cultivars are available for different latitudes and climatic conditions (RUBATZKY, YAMAGUCHI 1997). In Poland, a local population called Siedmiolatka is widely grown in home gardens (TENDAJ, MYSIAK 2011). Lately however, two new cultivars called Kroll and Wita have been developed. They are both resistant to bolting, produce numerous tillers and abundant foliage, thus they are suitable for harvest of green tops (Polish National List of Vegetable Plant Varieties 2010). Bejo Zaden, a Dutch seed company, has recently developed some pseudostem type cultivars producing few tillers, which can be grown for the use of whole plants in early stages of the growth or – like leek species – for blanched pseudostems, usually harvested in September (KOTLIŃSKA, KOJIMA 2000, KOTLIŃSKA et al. 2005). The cultivars Parade and Performer belong to this type of cultivars and, having a short growing period, they may be considered as an alternative green bunching onion to bulb onion normally used for this purpose (GREVSEN 1989). The advantage of *Allium fistulosum* L. is its resistance to many diseases and pests of bulb onion, including pink root (MARTINEZ 2005).

The common practice is to use seed propagated transplants in order to shorten a long growth period and to enhance the blanched part of the pseudostem. Plants with green leaves can be harvested 2-3 month after transplanting, but it may take 6 to 9 months before plants are ready to harvest for blanched pseudostems (RUBATZKY, YAMAGUCHI 1997). Under favourable weather conditions, Japanese bunching onion may be grown all year round for green tops (INDEN, ASAHIRA 1990).

The aim of this study has been to evaluate the volume and biological value of the yield of Japanese bunching onion cultivated and harvested on different dates during a growing season.

MATERIAL AND METHODS

The study comprising two field experiments was conducted in 2009-2010 on sandy clay soil with pH 7.1 and organic matter content 1.8%. In experiment I, the effects of different dates of growing the plants on field on yield, its market quality and nutritional value of the crop were evaluated (5 Apr to 08 Jun, 6 May-8 Jul, 7 Jun-10 Aug, 8 Jul-10 Sep, 5 Aug-11 Okt). In experiment II, seedlings in all treatments were planted on 3-4 of April, while harvest for bunches started 60 days after transplanting and continued in

15-day intervals until 150 days after planting. Both trials were established in a single factor design, with four replications and the plot area of 6 m^2 (1.5 x 4.0 m).

Seeds of the cultivar Performer Japanese bunching onion were sown in multicell trays filled with standard peat moss substrate. 3-4 seeds were put into each cell containing 54 cm^3 of the medium. After emergence, the number of seedlings was reduced to two. Seedlings for experiment II and for the first two plantings in experiment I were produced in a greenhouse and hardened in a non-heated foil tunnel for the last ten days, while seedlings for the later plantings were raised in a high plastic tunnel. Well-developed seedlings with 2-3 true leaves were transplanted into a field at 30x15 cm spacing, which provided the population of 44 plants per 1 m^2.

Available forms of phosphorus and potassium were elevated to 80 mg P and 200 mg K per 1 dm^3 of soil by early spring fertilization with triple superphosphate and potassium chloride. Nitrogen in the form of ammonium nitrate had been supplied in a preplant dose of 150 kg ha^{-1} shortly before seedlings were transplanted, and mixed with the soil by harrowing. Crop management included hand weeding of plots and irrigation of plants in a rainfall deficiency period. In treatments harvested after 135 and 150 days from the planting date, pseudostems of plants were blanched by mounding soil around the lower leaf bases to a height of about 20 cm. The treatment was conducted twice: six and three weeks before the harvest date.

Single harvest on an appropriate date for each treatment was conducted manually. Whole plants with removed roots and yellowing leaves and pseudostems of the diameter >10 mm were considered marketable yield of Japanese bunching onion. The leaves of blanched plants were trimmed to 2/3 of total length. A sample of 10 plants from each plot was collected for evaluation of such morphological traits as the mean weight and total length of plant, total length of the pseudostem and its blanched portion, number of leaves and bulb diameter.

The following were determined in samples of whole plants taken separately from 3 replications: the content of dry matter (by drying at 105°C to the constant weight), vitamin C (Tillman's method), total and reducing sugars (Loof-Shoorl method), chlorophyll a+b (spectrophotometric method), carotenoids (colorimetric method), sum of volatile oils (Farmakopea Polska VI 2002), total N (Kjeldahl´s method), nitrates (V) expressed by the amount of $NO_3 - N$ in f.w. (ion selective electrode, Orions method), P and Mg (colorimetrically), Ca and K (photometric method).

The results of the field experiment and chemical analysis were evaluated statistically using analysis of variance for a single-factor design and the least significant differences were calculated by Tukey's test at α=0.05.

RESULTS AND DISCUSSION

The relationships between the examined factors, yield and nutritive value of the crop were similar in both years, hence the data are shown as means for 2010-2011. The results suggest high suitability of the tested cultivar Performer for production of whole plants for bunches owing to its rapid growth, long pseudostem and low tendency to tillering. Similar, favourable features of this cultivar were also noted in a study conducted by Tendaj and Mysiak (2011).

In experiment I, where the plants were harvested in approximately one-month intervals since 8 June to 10 September, usually 63-64 days after transplanting, they produced the marketable yield in the range of 13.20-16.14 t ha^{-1} with an average weight of a plant equal 48.08-50.89 g (Table 1). Such yield is generally considered satisfactory when plants are harvested within 2.5 and 3 months after planting (Grubben 1994). A significant decrease in the crop yield to 8.72 t ha^{-1} and in the mean plant weight to 33.32 g occurred only in the treatment where seedlings were planted on 5 August and harvested on 11 October. This was due to the unfavourable weather conditions, and especially a high drop in the daily air temperature.

Table 1

The effects of growing term on yield and morphological characteristics of plants at harvest time (mean for 2010-2011)

Growing term	Marketable yield (t ha^{-1})	Mean weight of plant (g)	Height of plant (cm)	Number of leaves per plant	Legth of pseudostem (cm)	Legth of blanched pseudostem (cm)	Bulb diameter (mm)
5 Apr-8 Jun	16.14	48.08	50.2	5.4	13.3	4.5	17.3
6 May-8 Jul	13.96	50.89	48.4	5.4	12.3	3.5	17.2
7 Jun-10 Aug	14.18	48.56	53.7	5.7	12.9	3.3	14.5
9 Jul-10 Sep	13.20	49.40	53.4	4.9	14.1	3.8	13.9
5 Aug-11 Oct	8.72	33.32	46.1	4.1	12.2	4.0	13.9
Mean	13.24	46.05	50.4	5.1	13.0	3.8	15.4
LSD $\alpha = 0.05$	3.14	5.86	2.1	0.3	0.6	0.4	1.2

Generally, plants harvested from early June until September were not differentiated in the total height, number of leaves or length of the pseudostem, while the plants collected in October demonstrated significant growth reduction expressed in all the above measures. The bulb diameter of plants from the first two growing terms was significantly higher than from the later terms.

By postponing the growing period from 5 Apr-8 Jun to 5 Aug-11 Oct, a decrease of dry matter, carotenoids, total and reducing sugars, volatile oils and nitrates content was induced (Tables 2, 3). Plants harvested in early June contained 3929 mg NO_3-N kg^{-1} f.w. A substantial decrement in the nitrate content, down to 3378 mg kg^{-1} f.w., was found in Japanese bunching onion harvested in July. Further delay of the plant cultivation period was ineffective in accumulating NO_3-N, which varied from 3001 to 3120 mg kg^{-1} f.w. Cultivation of plants in the summer months, from 7 June to 10 August, appeared to be beneficial for the content of chlorophyll, total nitrogen, potassium and calcium.

The content of vitamin C was rather stable and not influenced by the cultivation term. The only exception was a high increment of this compound in plants harvested in October. Similar, positive effects of the autumn growing season on vitamin C was observed in Swiss chard (Kołota et al. 2010).

Table 2

Content of dry matter and selected organic compounds in Japanese bunching onion in relation to the growing term (mean for 2010-2011)

Growing term	Dry matter (%)	Vitamin C (mg %)	Carotenoids (mg 100 g^{-1} f.w.)	Chlorophyl a+b (mg 100 g^{-1} f.w.)	Total sugars (%)	Reducing sugars (%)	Volatile oils (mg 100 g^{-1} f.w.)
5 Apr-8 Jun	10.87	27.80	192.28	85.98	5.45	5.13	0.76
6 May-8 Jul	10.90	28.42	185.37	90.08	4.88	4.39	0.68
7 Jun-10 Aug	9.85	28.69	184.52	94.00	4.52	3.91	0.58
9 Jul-10 Sep	9.79	26.33	177.47	84.15	4.24	3.84	0.55
5 Aug-11 Oct	9.75	36.27	122.57	75.05	3.52	3.04	0.58
Mean	10.23	29.50	172.44	85.35	4.52	4.06	0.63
LSD α = 0.05	0.57	3.48	14.37	10.43	0.66	0.64	-

Table 3

Content of macronutrients in Japanese bunching onion in relation to the growing term (mean for 2010-2011)

Growing term	NO_3–N (mg kg^{-1} f.w.)	Total N (%)	P (%)	K (%)	Mg (%)	Ca (%)
5 Apr-8 Jun	3929	1.87	0.24	2.63	0.15	0.97
6 May-8 Jul	3378	2.32	0.22	2.51	0.13	0.85
7 Jun-10 Aug	3120	2.68	0.23	2.92	0.16	1.16
9 Jul-10 Sep	3001	1.75	0.25	2.47	0.17	1.01
5 Aug-11 Oct	3053	1.92	0.22	2.12	0.15	0.77
Mean	3296	2.31	0.23	2.53	0.15	0.95
LSD α = 0.05	3381	0.35	n.s.	0.25	0.08	0.12

This phenomenon can be probably explained by an earlier stage of plant maturation during harvest conducted in October. This explanation is somewhat supported by our previous studies on leek, kohlrabi and zucchini (Biesiada et al. 2007), where plants harvested earlier or smaller fruits were a richer source of vitamin C than plants or fruits more advanced in maturation.

The data from experiment II indicate a high and significant increment of crop yield associated with a later harvest date, postponed from 60 to 120 days after transplanting (Table 4). Within this period of time, the marketable yield of Japanese bunching onion increased more than 4.5-fold and the mean weight of plant rose from 42.75 g to 196.25 g, with significant enhancement of the plant height, number of leaves, length of the pseudostem and bulb diameter. The size of plants obtained in the study satisfies the requirements described by Wonneberger et al. (2004), such as 40-50 g for early harvested bunching onions and at least 150 g or for those obtained from prolonged cultivation. Plants harvested after 130 and 150 days after transplanting were blanched for 6 weeks by mounding soil around plants and tops of leaves were cut off at harvest. As a result, the length of the pseudostem's white portion increased from the maximum of 5.2 cm in plants harvested after 120 days of growing up to 10.0 cm and 11.0 cm, respectively. The yield and mean weight of plant Japanese bunching onion did not change significantly in comparison to the plants harvested 120 days after transplanting. The length of the blanched portion of the pseudostem is an important market quality parameter and according to Rubatzky and Yamaguchi (1997) should reach from a quarter to a third of the total length. This requirement was fulfilled by plants whose leaved had been trimmed by 1/3.

Table 4

Yield and morphological traits of Japanese bunching onion at harvest time in relation to the stage of maturity (mean for 2010-2011)

Term of harvest (days after planting)	Marketable yield (t ha^{-1})	Mean weight of plant (g)	Height of plant (cm)	Number of leaves per plant	Legth of pseudostem (cm)	Legth of blanched pseudostem (cm)	Bulb diameter (mm)
60	14.25	42.75	48.5	5.2	12.9	4.2	16.4
75	19.70	67.93	52.8	5.5	15.5	4.5	19.4
90	33.47	116.82	55.1	6.1	17.3	4.7	21.5
105	45.83	174.15	64.6	7.1	20.9	5.1	25.4
120	64.38	196.25	67.7	8.5	22.2	5.2	26.4
135	65.71	211.35	72.1	8.6	22.2	10.0	25.1
150	69.62	195.07	69.1	7.7	24.4	11.0	24.9
LSD $\alpha = 0.05$	6.63	18.53	3.6	0.7	2.7	0.9	1.2

The chemical analysis of edible parts showed that the content of most of the investigated compounds, e.g. vitamin C, carotenoids, total and reducing sugars, volatile oils, total nitrogen, potassium and calcium, in early harvested plants was higher then in those obtained during harvest delayed up to 120 days after transplanting (Tables 5, 6). Japanese bunching onion grown for 60 days contained the highest amount of chlorophyll a+b. High accumulation of nitrates, exceeding 4000 mg, found in plants harvested 60 and 75 days after transplanting was substantially reduced in plants harvested later. In treatments harvested after 105 and 120 days as well as in plants with blanched pseudostems, the accumulation of nitrates oscillated within 2527-2771 mg kg^{-1} f.w., and the differences were not significant. A similar relationship between the age of harvested plants and NO_3–N content was found in kohlrabi and leek (Biesiada et al. 2007). Considering the data obtained from both field trials, we could not confirm the claim expressed by Zhu et

Table 5

Content of dry matter and selected organic compounds in Japanese bunching onion in relation to the stage of maturity (mean for 2010-2011)

Term of harvest (days after planting)	Dry matter (%)	Vitamin C (mg%)	Carotenoids (mg 100 g^{-1} f.w.)	Chlorophyll a+b (mg 100 g^{-1} f.w.)	Total sugars (%)	Reducing sugars (%)	Volatile oils (mg 100 g^{-1} f.w.)
60	10.88	20.95	177.99	98.43	5.31	4.97	0.79
75	11.65	22.93	137.63	80.01	5.31	4.64	0.67
90	10.45	15.15	116.56	79.11	5.09	4.64	0.55
105	10.25	17.15	115.91	83.88	4.96	4.24	0.49
120	10.60	16.58	117.85	85.89	5.20	4.69	0.50
135	11.58	24.96	109.57	45.69	5.38	5.05	0.62
150	10.62	31.95	111.94	48.02	5.45	4.95	0.68
LSD α= 0.05	n.s	3.66	19.04	10.11	0.25	0.23	-

Table 6

Content of macronutrients in Japanese bunching onion in relation to the stage of maturity (mean for 2010-2011)

Term of harvest (in days after planting)	NO_3–N (mg kg^{-1} f.w.)	Total N (%)	P (%)	K (%)	Mg (%)	Ca (%)
60	4173	2.42	0.21	2.71	0.12	0.96
75	4101	1.74	0.20	2.12	0.12	0.87
90	3290	1.79	0.21	2.04	0.12	0.70
105	2771	1.67	0.21	1.99	0.13	0.73
120	2527	1.62	0.24	1.85	0.14	0.59
135	2605	1.79	0.24	1.80	0.12	0.58
150	2560	1.74	0.26	2.55	0.15	0.80
LSD α = 0.05	3.94	0.19	0.03	0.23	n.s.	0.11

al. (1998) regarding a weak tendency of this vegetable crop to accumulate nitrates, irrespective of the stage of plant maturation.

Japanese bunching onion with a blanched pseudostem contained more vitamin C, sugars and volatile oils in comparison to plants harvested 120 days after transplanting. Adverse effects of this treatment were observed in the case of chlorophyll a+b and carotenoids.

CONCLUSIONS

1. The results of this study indicate that Japanese bunching onion can be produced for fresh market supply from June until October by selecting differet dates for planting seed propagated transplants or by harvesting plants at different stages of growth within 60 and 150 days of cultivation on a field.

2. Plants of the some age harvested in on-month intervals from June to September produced similar yields and themean weight of plant, unlike plants harvested in October, when these parameters were significantly lower.

3. The delay of a growing season from April – June to August – October resulted in a decreased content of dry matter, carotenoids, total and reducing sugars, volatile oils and nitrates in Japanese bunching onion plants.

4. The yield of plants was highly dependent on their age at harvest time and increased from 14.25 t ha^{-1} after 60 days to 64.38 t ha^{-1} after 120 days from transplanting. The mean weight of plant improved during that time from 42.75 g to 196.25 g.

5. A more advanced stage of plant development at harvest time was associated with a decrease of vitamin C, carotenoids, chlorophyll a+b, sugars, volatile oils, nitrates, total nitrogen, potassium and calcium content.

6. Japanese bunching onion grown for 135 or 150 days whose pseudostems were blanched produced similar marketable yields as those harvested after 120 days without blanching. Blanched plants had a substantially longer white portion of the pseudostem, a lower content of chlorophyll a+b and carotenoids but more vitamin C.

REFERENCES

Aoyama S., Yamamoto Y. 2007. *Antioxidant activity and flavonoid content of Welsh onion (Allium fistulosum) and the effect of thermal treatment*. Food Sci. Technol. Res., 13(1): 67-72.

Biesiada A., Kołota E., Adamczewska-Sowińska K. 2007. *The effect of maturity stage on nutritional value of leek, zucchini and kohlrabi.* Veget. Crops Res. Bull., 66: 39-45.

Grevsen K. 1989. *Effects of sowing dates on different varieties of Welsh onion (Allium fistulosum* L.*) under temperate coastal climate*. Acta Hortic., 242: 319-324.

Grubben G.J.H. 1994. *Constraints for shallot, garlic, and Welsh onion in Indonesia: A case study on the evolution of Allium crops in the equatorial tropics.* Acta Hortic., 358: 333-339.

HE Q-W., SU D.S., ZHAO D.-W. 1989. *Shandong famous vegetables*. Tsinan: Shandong Sci. Technol. Press, 165-217.

HIGASHIO H., HIROKANE H., SATO F., TOKUDA S., URAGAMI A. 2007. *Enhancement of Functional Compounds in Allium Vegetables with UV Radiation.* Acta Hortic., 744: 357-361.

KOŁOTA E., ADAMCZEWSKA-SOWIŃSKA K., CZERNIAK K. 2010. *Yield and nutritional value of Swiss chard grown for summer and autumn harvest.* J. Agr. Sci., 2(4): 120-124.

KOTLIŃSKA T., KANISZEWSKI S., KWIECIEŃ A. 2005. *Comparison of growing methods of bunching onion (Allium fistulosum* L.*).* Nowości Warzywnicze/Veget. Crops News, 40: 25-32. (in Polish)

KOTLIŃSKA T., KOJIMA O. 2000. *Japanese bunching onion (Allium fistulosum* L.*) as a valuable vegetable crop.* Rocz. AR. Pozn. CCCXXIII, Ogrodn., 31(2): 311-311. (in Polish)

LAZIĆ B., TODOROVIČ V., DARDIČ M. 2002. *Effect of production method on earliness and yield of Allium fistulosum.* L. Acta Hortic., 579: 359-362.

MARTINEZ L. E. 2005. *Introgression of Allium fistulosum* L. *into interspecific hybrid backcrosses between A. fistulosum* L. *and A. cepa.* L. Acta Hortic., 688: 109-115.

MYSIAK B., TENDAJ M. 2006. *Content of flavonoids in some Allium species grown for green bunching*. Veg. Crops. Res. Bull., 65: 105-110.

MYSIAK B., TENDAJ M. 2008. *Content of phenolic acids in edible parts of some Alliums species grown for the green bunching.* Acta Sci. Pol. Hort. Cult., 7(4): 57-62.

P*olish National List of Vegetable Plant Varieties.* 2010.

RABINOWITCH H.D. CURRAH L. 2002. *Allium crop science: Recent advances.* CAB Int. Wallingford UK.

RUBATZKY V.E., YAMAGUCHI M. 1997. *World vegetables. Principles, production and nutritive values*. Chapman & Hall. New York.

ŠTAJNER D., MILIĆ N., ČANADONOWIĆ-BRUNET J., KAPOR A., ŠTAJNER M., POPOVIĆ B.M. 2006. *Exploring Allium species as a source of potential medicinal agents*. Phytother. Res., 20: 581--584. DOI: 10.1002/ptr.1917

SU H., XU K., LIU W. 2007. *Cold tolerance and winter cultivation of Welsh onions*. Acta Hortic., 760: 335-340.

TENDAJ M., MYSIAK B. 2007a. *The yield of Japanese bunching onion (Allium fistulosum* L.*) depending on planting time of transplant and use of flat covers.* Ann. UMCS, 17(2): 5-10. (in Polish)

TENDAJ M., MYSIAK B. 2007b. *Usefulness of Japanese bunching onion (Allium fistulosum* L.*) for forcing in greenhouse*. Acta Agrobot., 60 (1): 143-146.

TENDAJ M., MYSIAK B. 2010. *Growth characteristic of Japanese bunching onion (Allium fistulosum* L.*) cultivated from seed sowing and seedling transplanting*. Mat. Konf. Siedlce 24-25 czerwca 2010 r., 163-165. (in Polish)

TENDAJ M., MYSIAK B. 2011. *Growth characteristic of Welsh onion (Allium fistulosum* L.*) grown from seeds and transplants.* Fol. Hort., 23(1): 3-8.

WANG D., GAO J., LIU G. 2005. *General situation of Allium crops in China*. Acta Hortic., 688: 327-332.

WARADE S.D., SHINDE K.G. 1998. *Handbook of vegetable science and technology: production, composition storage and processing.* Ed. by SALUNKHE D.K. and KADAM S.S., Marcel Dekker. Inc. New York.

WONNEBERGER CH., KELLER F., BAHNMÜLLER H., BŐTTHER H., GEYER B., MEYER J. 2004. Gemüsebau. Eugen Ulmer. Stuttgart.

YAMASAKI A., TANAKA K., NAKASIMA N. 2003. *Effect of photoperiod on the induction of devernalization by high day temperature in field-grown Japanese bunching onion (Allium fistulosum* L.*).* J. Japan Soc. Hort. Sci., 72 (1): 18-23.

ZHU W., LI S., GONG L., HUANG Z., ZHOU D. 1998. *Genetic diversity of nitrate accumulation in vegetable crops.* Acta Hortic., 467: 119-126.

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CONTENT OF SELECTED ESSENTIAL AND POTENTIALLY TOXIC TRACE ELEMENTS IN MILK OF COWS MAINTAINED IN EASTERN POLAND

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Abstract

Concentration of toxic metals in milk, especially in industrial regions, may serve as a direct bioindicator of the quality of milk and its products. But it can also function as an indirect indicator of contamination of the environment where milk is produced. The aim of the present study was to evaluate the content of some trace elements, i.e. Fe, Cu, Zn and Mn, as well as toxic elements (Cd and Pb) in milk from cows maintained on farms in three regions of Poland, including the production season as another aspect of the investigations. In total, 446 milk samples were analyzed. Significant influence of the season and production region on all the analyzed elements except Fe was noticed. Milk collected in the Biebrza and Bieszczady regions proved to be a richer source of Cu and Mn. Regardless of the production region, a higher content of Zn and Cu were observed in the summer, while more Mn was found in the winter milk. In none of the analyzed milk samples, Pb or Cd exceeded the permissible threshold.

Key words: bovine milk, trace elements, season, Eastern Poland.

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ZAWARTOŚĆ ISTOTNYCH I POTENCJALNIE TOKSYCZNYCH WYBRANYCH PIERWIASTKÓW ŚLADOWYCH W MLEKU KRÓW UTRZYMYWANYCH NA TERENIE WSCHODNIEJ POLSKI

Abstrakt

Stężenie metali ciężkich w mleku, przede wszystkim w rejonach uprzemysłowionych, jest bezpośrednim bioindykatorem jakości mleka i jego przetworów, a także wskaźnikiem pośrednim, wskazującym na stopień zanieczyszczenia środowiska, w którym produkuje się mleko. Celem pracy była ocena zawartości wybranych pierwiastków śladowych, tj. Fe, Cu, Zn i Mn, oraz toksycznych (Cd i Pb), w mleku krów utrzymywanych w gospodarstwach wschodniej Polski, z uwzględnieniem sezonu produkcji. Łącznie badaniami objęto 446 próbek. Stwierdzono istotny wpływ sezonu i regionu produkcji na zawartość analizowanych pierwiastków, z wyjątkiem Fe. Mleko pobierane w regionie Biebrzy i Bieszczad okazało się cennym źródłem Cu i Mn. Niezależnie od regionu produkcji, wyższą zawartość Zn i Cu stwierdzono w mleku pozyskiwanym w sezonie letnim, natomiast Mn w okresie zimowym. We wszystkich analizowanych próbkach mleka stężenie ołowiu i kadmu było stosunkowo niskie i mieściło się w dopuszczalnych granicach.

Słowa kluczowe: mleko krowie, pierwiastki śladowe, sezon, wschodnia Polska.

INTRODUCTION

Essential metals, beside basic nutrients, have a vital role in the functioning of a human organism. They participate in numerous metabolic processes and regulate biochemical reactions; they appear as activators or components of some specific enzymes. Fe, Cu, Zn and Mn play a crucial role in protection of a human body against the negative effect of toxic free radicals (CASHMAN 2006, SCHERZ, KIRCHOFF 2006). But bovine milk also contains heavy metals, which reduce its nutritional value and pose a hazard to the human health. Pb and Cd prove to be the most widespread environmental toxicants found in milk (LICATA et al. 2004).

The content of essential micronutrients and trace elements in milk depends mainly on external factors, i.e. given content of elements element content in soil, feed and water as well as its assimilability. To a lesser extent, concentration of mineral components is conditioned by factors related to secretion of milk, i.e. a cow's breed, stage of lactation, animal health (CASHMAN 2006, DOBRZAŃSKI et al. 2005, GABRYSZUK et al. 2010, MALBE et al. 2010, SOLA-LARRAÑAGA, NAVARRO-BLASCO 2009, VIDOVIC et al. 2005). Another important factor influencing the occurrence of trace elements, including toxic ones, is mainly of anthropogenic origin (DOBRZAŃSKI et al. 2005, LICATA et al. 2004). Some authors also point to the influence of a season and production region on the content of these elements (DOBRZAŃSKI et al. 2009, ELSAYED et al. 2011, LI-QIANG et al. 2009, PATRA et al. 2008, SOLA-LARRAÑAGA, NAVARRO-BLASCO 2009).

Milk and dairy products are staple components of a daily diet of contemporary consumers, especially children.Thus, it is crucial to monitor regularly milk quality, paying special attention toxic metals. Theirs concentration in milk, especially in industrial regions, may serve as a direct bioindicator of the quality milk and its products, but cal also be an indirect indicator of contamination in the environment where milk is produced (Licata et al. 2004, Vidovic et al. 2005).

The aim of the present study was to evaluate the concentration of some trace elements (essential and potentially toxic ones) in milk of cows maintained on farms located in three regions of Eastern Poland, i.e. the Biebrza River region (a buffer zone of Biebrza National Park), the Bieszczady Mountains and the Lublin region. Additionally, two production seasons were taken into consideration.

MATERIAL AND METHODS

Milk samples were collected individually from each cow (daily milk) during the summer (June-August) and winter (December-February). The research included a total of 446 milk samples (Biebrza – 134, Bieszczady – 163 and Lublin – 149) obtained from three areas in Poland (Figure 1). The Biebrza and Bieszczady regions are considered highly valuable nature areas and very attractive tourist destinations. They are unique in that the human interference with nature is almost negligible. Extensive milk production dominates in the farming practice in those regions, owing to large areas of available grasslands. In the Biebrza region the main cattle breeds are the Whiteback and Black-and-White cows, but in the Bieszczady Mountains the Simmental cows are reared. The nutrition of cows in spring relies mainly was on seasonal pasture forage comprising grasses and legumes supplemented with hay or straw. During the winter season, the animals were fed hay sillage, fodder beet with hay or straw additives. Milk samples from the Lublin region were obtained from intensive milk production farms maintaining the Simmental and Black and White cows. All year, the animals were given maize silage and hay silage, while in the summer their diet was supplemented with forage. On all the farms, dietary rations were enriched with nutritive fodder.

All analyses were performed in the Agroecological Central Laboratory of the University of Life Sciences in Lublin. All reagents were of analytical grade, with 65% HNO_3 and 70% $HClO_4$ of suprapure quality (POCh, Poland). The samples were wet-mineralized (Kjeldahl Flask Digestion) according to the official procedure AOAC 986.15 (AOAC 2000). The content of microelements (Cu, Fe, Zn, Mn) was determined with the flame atomic absorption spectrometry technique using a SOLAR 939 spectrometer (Uni-

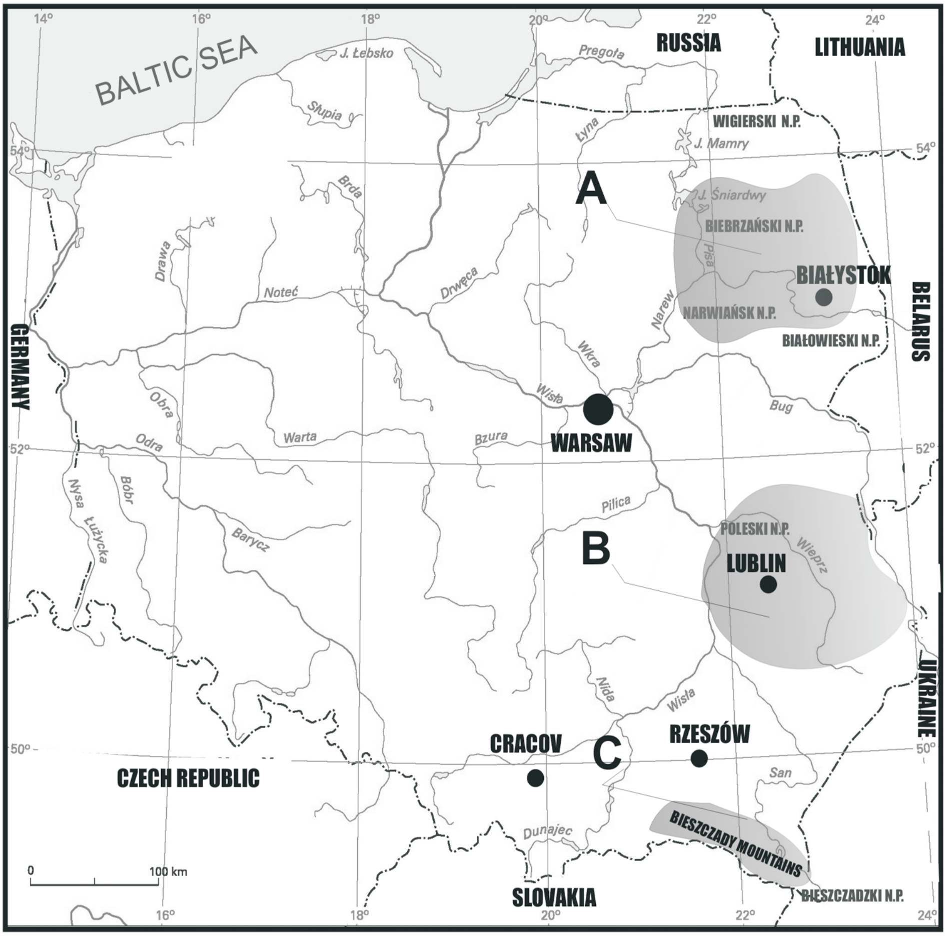

Fig. 1. Location of the research regions in Poland:
A – Biebrza River region, B – Lublin region, C – Bieszczady Mountains

cam). The content of Pb and Cd was established using the flameless atomic absorption spectrometry method in a SpectrAA 88OZ (Varian) spectrometer.

The results were analyzed statistically with the StatSoft Inc. Statistica ver.6 software (Statsoft INC. 2003) based on a two-factor analysis of variance, achieving mean values and standard deviation. Significance of differences between mean values was determined with Tukey's HSD test.

RESULTS AND DISCUSSION

The results indicate significant differences between the seasons in the content of Zn, Cu and Mn in milk collected in the three regions. Higher concentrations of Zn and Cu, regardless of the production region, were noticed in the summer, and the content of Mn was higher in the winter season (Table 1). Differences between the seasons (in favor of the summer) for the Zn content ranged from 0.426 mg L^{-1} to 0.753 mg L^{-1}, and for Cu – from 0.008 mg L^{-1} to 0.030 mg L^{-1}. Milk produced in the Bieszczady Mountains in the winter was characterized by twice as much Mn (0.132 mg L^{-1}) as in the summer season (0.066 mg L^{-1}). Seasonal differences in the Mn content did not occur in the other regions. Other authors also report seasonal variation in concentrations of the analyzed elements (ELSAYED et al. 2011, GUSTAFSON et al. 2007, RODRIGUEZ et al. 2001, SOLA-LARRAÑAGA, NAVARRO-BLASCO 2009). Like in our study, higher levels of the elements, especially Cu and Zn, were found in milk obtained in the summer, i.e. from cows fed mainly pasture forage. A contrary result, i.e. higher concentrations of Cu and Zn in milk in winter, was obtained GÓRSKA and OPRZĄDEK (2006). The level of Zn ranged from 3.2 (in summer) to 3.7 mg L^{-1} (in winter), and Cu – from 0.048 to 0.050 mg L^{-1}, respectively. In another study conducted in Silesia by DOBRZAŃSKI et al. (2005), milk of cows kept on pasture was characterized by more Cu (0.065-0.089 mg L^{-1}) and less Zn (3.085-3.164 mg L^{-1}) than in our investigations. The concentration of Mn, however, ranged from 0.051 mg L^{-1} in Upper Silesia to 0.102 mg L^{-1} in Lower Silesia.

In both analyzed seasons, a region influence on the content of mentioned microelements was also stated. In the winter season milk from Lublin region was characterized by the highest Zn content (4.764 mg L^{-1}) at the lowest Cu content (0.032 mg L^{-1}) and Mn (0.039 mg L^{-1}). However, milk collected in the Biebrza region contained over twice, and in the Bieszczady over three times more of Mn, respectively: 0.084 mg L^{-1} and 0.132 mg L^{-1}. In the case of Cu level, the differences between regions were much lower. In all regions in the summer season Zn and Cu content changes were similar to the changes in the winter. Concentration of Zn ranged from 4.312 mg L^{-1} (Biebrza region) to 5.341 mg L^{-1} (Lublin region), and Cu from 0.040 mg L^{-1} (Lublin region) to 0.078 mg L^{-1} (Biebrza region). The differences in concentration of these elements in milk mainly may be due to their diverse content in a feed (DOBRZAŃSKI et al. 2005, GABRYSZUK et al. 2010, MALBE et al. 2010, SCHERZ, KIRCHOFF 2006). MALBE et al. (2010) shows the effect of balancing a ration for animals kept in conventional and organic farms on the content of elements in milk. Low Cu concentration in the milk of cows kept in the Lublin region may indicate a high level of Zn in the ration. According to many authors (ARANCIBIA et al. 2006, LICATA et al. 2004), Zn taken from a feed, interacting with the copper absorption system, reduces the Cu bioavailability. HOSNEDLOVA et al. (2005) point at a gradual increase

Table 1

Content of trace elements ($\overline{x}$±SD) in milk of cows maintained in three regions of Eastern Poland

Microelement content (mg L^{-1})	Biebrza region		Bieszczady region		Lublin region		Factor		
	winter season	summer season	winter season	summer season	winter season	summer season	region	season	season x region interaction
No.	66	68	76	87	77	72			
Zn	3.559±1.785^{a}	4.312±1.896^{b}	4.416±1.612	4.842±1.822	4.764±1.864^{a}	5.341±2.413^{b}	*x*	*x*	*x*
Cu	0.048±0.013^{a}	0.078±0.028^{b}	0.039±0.007	0.048±0.019	0.032±0.011	0.040±0.017	*x*	*x*	*xx*
Fe	0.264±0.087	0.337±0.101	0.306±0.070	0.377±0.059	0.188±0.051	0.252±0.062	ns	ns	ns
Mn	0.084±0.029	0.085±0.052	0.132±0.056^{B}	0.066±0.028^{A}	0.039±0.013	0.030±0.015	*xx*	*xx*	*xx*

No. – number of cows
a, b, A, B – differences between seasons, *a, b* – differences significant at $P \leq 0.05$
A, B – differences significant at $P \leq 0.01$, *x* – differences significant at $P \leq 0.05$, *xx* – differences significant at $P \leq 0.01$, ns – not stated significant

of Zn concentration in milk, at a simultaneously Cu level decrease and an increase of cows' milk yield. In SIMSEK et al. (2000) opinion, a content of Cu is largely conditioned by an environment influence. This element high concentration (0.77-1.20 mg kg^{-1}), the authors noted in the milk from industrialized areas of Turkey. This is contrary to the results of own studies, in which showed higher levels of this element in the regions subjected to a small degree of human pressure, such as the Bieszczady and Biebrza regions.

The Fe content in milk was not found to have been affected by a region or production season, although the highest concentrations of this metal in milk were observed in the Bieszczady Mountains in summer (0.377 mg L^{-1}) and the lowest – in the Lublin region in winter (0.188 mg L^{-1}). Contradictory conclusions were presented by SOLA-LARRAÑAGA and NAVARRO-BLASCO (2009), who demonstrated the impact of a season on the Fe content in milk at a high value of Varimax factor (0.845). In another study conducted in seven regions of Egypt, with varying degrees of industrialization, the highest levels of Cu, Fe and Zn were recorded in the most heavily urbanized areas, although higher concentrations of Cu and Fe were noticed in summer months (May-June), and Zn – in winter (January-February) (ELSAYED et al. 2011). LI-QIANG et al. (2009), who evaluated milk from areas of China and Japan, stated that Zn and Fe can be transferred to milk from machinery and equipment used during collection and processing of raw milk.

The content of microelements recorded in Sweden (LINDMARK-MANSSON et al. 2003) was similar to our results obtained in the Biebrza and Bieszczady regions. The average concentrations of individual elements in cows' milk were as follows: Zn – 4.4 mg L^{-1}, Mn – 0.10 mg L^{-1}, and a higher content of Cu – 0.10 mg L^{-1}. In another study, conducted in Northern Sweden, milk was characterized by lower levels of these elements: Zn – 4.1 mg L^{-1}, Cu – 0.053 mg L^{-1} as well Mn – 0.024 mg L^{-1} (14). Very low concentrations of Zn (2.016 mg kg^{-1}) and Cu (0.002 mg kg^{-1}) were found by LICATA et al. (2004) in milk from various farms in Calabria, Italy. High levels of these components in milk were shown by RODRIGUEZ et al. (2001), who conducted their research on 8 farms located in the Canary Islands (Zn – 4.41 mg L^{-1} and Cu – 0.076 mg L^{-1}).

Nutritional evaluation of milk includes determination of the content of heavy metals such as Pb and Cd. It is important to emphasize that the mean Pb concentrations in the analyzed milk produced in three regions of Poland did not exceed the permissible limit, i.e. 0.020 mg L^{-1} (*Commission Regulation* 2006). In just two milk samples collected in the Lublin region (Table 2) the permissible level of heavy metals was surpassed. It was shown that regardless of the production season, milk collected in the Lublin region contained considerably more Pb than milk from the Bieszczady Mountains (winter – 4.18 µg L^{-1}, summer – 4.34 µg L^{-1}) and the Biebrza region (winter – 5.99 µg L^{-1}, summer – 6.93 µg L^{-1}). Presence of Cd was noticed in all

Table 2

Content of toxic elements in milk of cows maintained in three regions of Eastern Poland

Toxic elements content (µg L^{-1})		Biebrza region		Bieszczady region		Lublin region		Factor		
		winter season	summer season	winter season	summer season	winter season	summer season	region	season	season x region interaction
No.		66	68	76	87	77	72			
Cd	$\overline{x}$±SD min. max.	4.42±3.61 0.24 5.96	4.13±3.62 <0.02 6.51	4.74±5.45^{b} 0.12 6.80	2.22±2.13^{a} 0.14 6.32	7.10±2.91 0.83 8.17	7.34±4.75 0.32 8.94	*xx*	*x*	*xx*
Pb	$\overline{x}$±SD min. max.	6.44±5.42 0.15 13.90	6.21±4.02 0.50 14.52	8.25±6.22 0.53 10.51	8.80±3.81 3.93 13.43	12.43±5.60 3.40 15.88	13.14±4.52 3.84 20.20	*x*	ns	*xx*

No. – number of cows

a, b – differences between seasons within the region, significant at $P \leq 0.05$

x – differences significant at $P \leq 0.05$, *xx* – differences significant at $P \leq 0.01$, ns – not stated significant the analyzed milk samples, but its content did not exceed the permissible limit, i.e. 0.010 mg L^{-1} (*Commission Regulation* 2006). Milk from the Lublin region was characterized by the highest content of this element. Significant seasonal differences in the content of the analyzed heavy metals were observed only for Cd in milk of cows kept in the Bieszczady Mountains (summer – 2.22 µg L^{-1}, winter – 4.74 µg L^{-1}). Taking into consideration that animal feed, and consequently milk, is contaminated by dusts, fumes and gases emitted by industry and road traffic, the present results may indicate less heavy pollution in the Biebrza region and the Bieszczady Mountains than near Lublin. It should be added that the Lublin region is an area put to the strongest anthropopressure. The urban sprawl and increasing traffic lead to elevated emission of harmful substances. Intensive agricultural production in this region also contributes to pollution of the natural environment.

Significant differences in concentrations of trace elements between regions were also found by DOBRZAŃSKI et al. (2009). In milk produced on farms in Upper Silesia (contaminated region) significantly higher concentrations of Cu (0.659 mg L^{-1}), Cd (7.39 µg L^{-1}) and Pb (0.042 mg L^{-1}) were observed compared to from Bystrzyca (ecologically clean area), i.e. 0.228 mg L^{-1}, 3.88 µg L^{-1} and 0.021 mg L^{-1}, respectively. It should be noted that the limit for lead content in milk was exceeded in both regions. According to the authors, such a high concentration of this element in milk from Lower Silesia, a region considered as environmentally clean, may be due to inadequate milking hygiene and the impact of airborne pollution from industrial areas.

Studies of other authors (ASLAM et al. 2011, ELSAYED et al. 2011, LICATA et al. 2004, SOLA-LARRAŃAGA, NAVARRO-BLASCO 2009, VIDOVIC et al. 2005) also indicate that the location of farms has a significant impact on the heavy metal content in milk. LICATA et al. (2004), who evaluated bovine milk produced in various farms in Calabria (Italy), confirmed the Cd presence in only 3 out of 43 milk samples, where it equalled 1.14, 3.42 and 22.8 mg kg^{-1}. The Pb presence, however, was observed in all the samples but the recorded values, ranging from 0.1 to 9.92 mg kg^{-1}, did not surpass the permissible limit for this microelement. Low Cd concentration was also noticed in milk of cows maintained in the north of Serbia, where it oscillated from 0.001 up to 0.016 mg kg^{-1} (VIDOVIC et al. 2005). In Pakistan (Faisalabad), the heavy metal residues in milk exceeded several-fold the permissible limits according to the European standards. The average content of Cd was 0.147 mg L^{-1} and Pb – 19.972 mg L^{-1} (ASLAM et al. 2011). Such high values, considered to be potentially dangerous to the health of animals and humans, were probably caused by contamination of soil and water.

Higher concentrations of Pb and Cd in milk are mainly due to the location of farms along roads or near factories and power plants (BILAND•IĆ et al. 2011, ELSAYED et al. 2011, LI-QIANG et al. 2009, PATRA et al. 2008, SIMSEK et al.

2000). Several-fold higher Pb content was found Patra et al. (2008) in milk of cows kept near zinc smelter and aluminum processing plants (0.85 mg L^{-1}), compared to milk of cows kept in unpolluted areas (0.25 mg L^{-1}). Simsek et al. (2000) observed similar concentrations of Pb in an industrial area (0.049 mg kg^{-1}), and in areas with heavy traffic (0.032 mg kg^{-1}). In studies conducted in Lithuania, permissible limits for both analyzed elements were not exceeded, although, similarly to our study, a higher content was reported in the winter season (Valiukenaite et al. 2006). In the previous research of Król et al. (2006), similar values of the concentration of both microelements were determined. The average Cd level varied from 0.002 mg L^{-1} in milk from Simmental cows to 0.006 mg L^{-1} in milk from Polish Holstein-Friesian Black and White cows, while the Pb content varied from 0.011 to 0.016 mg L^{-1}, respectively. Higher concentration of both elements was observed in milk obtained in the summer. The results obtained by Elsayed et al. (2011) confirmed the significant influence of a season ($P \leq 0.05$) on the Pb content in milk. In the summer months, higher amounts of this element were found. There was no effect of a production season on the Cd content in milk.

CONCLUSIONS

In conclusion, it should be stated that the significant effect of a season and production region on the content of all the analyzed elements except Fe was noticed. Milk collected in the Biebrza and Bieszczady regions proved to be a richer source of Cu and Mn. Regardless of the production region, higher levels of Zn and Cu were observed in the summer season, and Mn was more abundant in the winter. The average concentration of Pb and Cd did not exceed the permissible limits. It should be underlined, however, that in all the analyzed milk samples the presence of both elements was observed, even in milk from the Biebrza and Bieszczady regions, which are commonly recognized as ecologically clean. Taking into consideration the fact that Pb and Cd accumulate in living organism, constant monitoring of these elements in milk and other food products seems advisable.

REFERENCES

AOAC, 2000. *Official Methods of Analysis of the AOAC* 986.15 Multi-element method. 17th Ed. Arlington – Virginia, USA.

Arancibia V., Peña C., Segura R. 2006. *Evaluation of powdered infant formula milk as chelating agent for copper under simulated gastric conditions of a baby's stomach.* Anal. Sci., 22(9): 1197-1200.

Aslam B., Javed I., Khan F.H., Rahman Z.U. 2011. *Uptake of heavy metal residues from sewerage sludge in the milk of goat and cattle during summer season.* Pak. Vet. J., 31(1): 75-77.

Bilandžić N., Dokić M., Sedak M., Solomun B., Varenina I., Kne•ević Z., Benić M. 2011. *Trace element levels in raw milk from northern and southern regions of Croatia.* Food Chem., 127: 63-66.

Cashman K.D. 2006. *Milk minerals (including trace elements) and bone heath.* Intern. Dairy J., 16: 1389-1398.

Commission Regulation (EC) *No 1881/2006) of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.* Official Journal of the European Union L364/32, 20/12/2006.

Dobrzański Z., Kołacz R., Górecka H., Chojnacka K., Bartkowiak A. 2005. *The content of microelements and trace elements in raw milk from cows in the Silesian region.* Pol. J. Environ. Stud., 14(5): 685-689.

Dobrzański Z., Skiba M., Brożyńska A., Kowalska-Góralska M. 2009. *Content of selected heavy metals in milk of ruminants (cows and goats) from industrial and ecologically clean areas.* Acta Sci. Pol., Med. Vet., 8(1): 3-14.

Elsayed E.M., Hamed A.M., Badran S.M., Mostafa A.A. 2011. *A survey of selected essential and toxic metals in milk in different regions of Egypt using ICP-AES.* Int. J. Dairy Sci., 6(2): 158-164.

Gabryszuk M., Słoniewski K., Metera E., Sakowski T. 2010. C*ontent of mineral elements in milk and hair of cows from organic farms.* J. Elementol., 15(2): 259-267.

Górska A., Oprządek K. 2006. *Levels of trace elements in cow milk from farms of Southern Podlasie Region.* Pol. J. Environ. Stud., 15(2a): 269-272.

Gustafson G.M., Salomon E., Jonsson S. 2007. *Barn balance calculations of Ca, Cu, K, Mg, Mn, N, P, S and Zn in conventional and organic dairy farm in Sweden.* Agric., Ecosyst. Environ., 119: 160-170.

Hosnedlova B., Travnicek J., Chrastny V. 2005. *Zinc and copper concentration in milk of dairy cows in the South Bohemia region.* ISAH, 1: 256-259.

Król J., Litwińczuk Z., Barłowska J., Kędzierska-Matysek M. 2006. *A macro-and microelements content in milk of cows Black and White and Simmental breed over the summer and winter feeding time.* Pol. J. Environ. Stud., 15(2a): 395-397.

Licata P., Trombetta D., Cristani M., Giofre F., Martino D., Calo M. 2004. *Levels of "toxic" and "essential" metals in samples of bovine milk from various dairy farms in Calabria, Italy.* Environ. Int., 30: 1-6.

Lindmark-Mansson H., Fonden R., Pettersson H.E. 2003. *Composition of Swedish dairy milk.* Int., Dairy J., 13: 409-425.

Li-Qiang Q., Xiao-Ping W., LI W., Tong X., Tong W. 2009. *The minerals and heavy metals in cow's milk from China and Japan.* J. Health Sci., 55: 300-305.

Malbe M., Otstavel T., Kodis I., Viitak A. 2010. *Content of selected micro and macroelements in dairy cows' milk in Estonia.* Agron. Res., 8: 323-326.

Patra R.C., Swarup D., Kumara P., Nandi D., Naresh R., ALI S.L. 2008. *Milk trace elements in lactating cows environmentally exposed to higher level of lead and cadmium around different industrial units.* Sci. Total Environ., 404: 36-44.

Rodriguez E.M., Sanz Alaejos M., Diaz Romero C. 2001. *Mineral concentrations in cows milk from the Canary Island.* J. Food Comp. Anal., 14: 419-430.

Scherz H., Kirchhoff E. 2006. *Trace elements in foods: Zinc contents of raw foods – A comparison of date originating from different geographical regions of the world.* J. Food Comp. Anal., 19: 420-433.

Simsek O., Gültekin R., Öksüz O., Kurultay S. 2000. *The effect of environmental pollution on the heavy metal content of row milk.* Nahrung, 44: 360-363.

SOLA-LARRAÑAGA C., NAVARRO-BLASCO I. 2009. *Chemometric analysis of minerals and trace elements in raw cow milk from the community of Navarra, Spain*. Food Chem., 112: 189-196.

VALIUKENAITE R., STANKEVICIENE M., STANKEVICIUS H., SKIBNIEWSKA K.A. 2006. *Lead and cadmium levels in raw cow's milk in Lithuania determined by inductively coupled plasma sector field mass spectrometry*. Pol. J. Food Nutr. Sci., 15: 243-246.

VIDOVIC M., SADIBASIC A., CUPIC S., LAUSEVIC M. 2005. *Cd and Zn in atmospheric deposit, soil, wheat and milk.* Environ. Res., 97: 26-31.

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ULTRACYTOCHEMICAL LOCALIZATION OF CALCIUM IONS IN THE PINEAL ORGAN OF THE DOMESTIC TURKEY

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Abstract

Calcium ions are involved in several processes occurring in the avian pineal, including the regulation of melatonin secretion. The aim of study was to investigate distribution of calcium ions in the pineal organ of domestic turkey at the level of electron microscopy. The study was performed on 12 female turkeys housed in a cycle of 12L : 12 D (light intensity 300 lux; 07:00 – 19:00), starting from the third week of life. At the age of 12 weeks, the birds were anesthetized with halothane and sacrificed by decapitation at 14:00 and at 02:00 (in darkness). The pineals were fixed using the potassium pyroantimonate method, which enables visualization of calcium ions in the form of electron dense precipitates.

Extremely numerous, large precipitates of calcium pyroantimonate were observed between collagen fibres of the connective tissue surrounding the pineal follicles. The number of precipitates in the follicles was much lower than in the neighbouring stroma. Precipitates were numerous in intercellular spaces between cells forming the follicular wall. In contrast, they were infrequently observed in the follicular lumen. In pinealocytes, large amounts of precipitates were present in the nucleus, mitochondria and short, wide cisterns of the smooth endoplasmic reticulum. Precipitates were sparse in the apical prolongations of rudimentary-receptor pinealocytes. The content of precipitates did not differ prominently between individual pinealocytes. Precipitates in the cytosol of both rudimentary-receptor pinealocytes and secretory pinealocytes were much more numerous in the organs taken *ex vivo* during nighttime than during daytime. Supporting cells contained much fewer precipitates than pinealocytes.

Key words: calcium, pineal organ, cytochemistry, pyroantimonate, turkey.

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ULTRACYTOCHEMICZNA LOKALIZACJA JONÓW WAPNIOWYCH W SZYSZYNCE INDYKA

Abstrakt

Jony wapniowe uczestniczą w wielu procesach zachodzących w szyszynce ptaków, w tym m.in. w regulacji wydzielania melatoniny. Celem badań było określenie rozmieszczenia jonów wapniowych w szyszynce indyka domowego na poziomie mikroskopu elektronowego. Badania przeprowadzono na 12 indyczkach utrzymywanych od 3. tygodnia życia w warunkach cyklu świetlnego 12 h światła : 12 h ciemności (światło o natężeniu 300 lx na poziomie podłogi; od 07:00 do 19:00). W wieku 12 tygodni ptaki poddano eutanazji o godzinie 14:00 oraz 02:00. Szyszynki utrwalono w mieszaninie zawierającej piroantymonian potasu, co umożliwiło wizualizację jonów wapniowych w postaci elektronowo gęstych precypitatów.

Szczególnie liczne duże precypitaty piroantymonianu wapnia występowały między włóknami kolagenowymi tkanki łącznej otaczającej pęcherzyki szyszynki, natomiast ich liczba w pęcherzykach była znacznie mniejsza. Liczne precypitaty występowały w przestrzeniach międzykomórkowych między komórkami budującymi ścianę pęcherzyków, zaś tylko pojedyncze obserwowano w świetle pęcherzyków. W pinealocytach bardzo liczne precypitaty były obecne w jądrze komórkowym, mitochondriach oraz krótkich, szerokich cysternach siateczki śródplazmatycznej gładkiej. W wypustkach wierzchołkowych pinealocytów szczątkowo-receptorowych występowała niewielka liczba precypitatów. Zawartość precypitatów nie różniła się wyraźnie w poszczególnych pinealocytach. Precypitaty występujące w cytozolu pinealocytów szczątkowo-receptorowych i pinealocytów wydzielniczych były znacznie liczniejsze w szyszynkach pobranych podczas nocy niż w trakcie dnia. Komórki podporowe zawierały znacznie mniej precypitatów niż pinealocyty.

Słowa kluczowe: wapń, szyszynka, cytochemia, piroantymonian, indyk.

INTRODUCTION

The avian pineal organ, probably due to its intermediate evolutionary position between the pineals of lower vertebrates and mammals, is characterized by a very intricate internal structure and complex functional organization (VOLLRATH 1981, PRUSIK, LEWCZUK 2008a, b). The pineal parenchyma in birds is composed of three cell populations: pinealocytes, supporting cells and neurons (VOLLRATH 1981, PRUSIK et al. 2006, PRUSIK, LEWCZUK 2008a). Fundamental differences in the ultrastructure of avian pinealocytes lead to their classification into receptor pinealocytes, rudimentary-receptor pinealocytes and secretory pinealocytes. Supporting cells are represented by ependymal-like cells and astrocyte-like cells. The histological structure of the avian pineal organ shows prominent interspecies differences, therefore three main forms of the organ are distinguished: saccular, tubulofollicular and solid (VOLLRATH 1981, PRUSIK, LEWCZUK 2008a). Recently, an intermediate solid-follicular type has been acknowledged (OHSHIMA, HIRAMATSU 1993, HALDAR, BISHNUPURI 2001).

Avian pinealocytes are directly photoreceptive due to the presence of two photopigments: pinopsin and melanopsin (OKANO et al. 1994, 1997, CHAURASIA et al. 2004, HOLTHUES et al. 2005). Moreover, they contain a circadian oscillator, composed by a set of clock genes, that is the genes responsible for generation of an endogenous circadian rhythm of pinealocyte activity (MURAKAMI et al. 1994, OKANO, FUKADA 2003, CSERNUS et al. 2005). Thus, pinealocytes in birds, unlike in mammals, are more or less autonomous (depending on the species and the stage of postembryonic development) in the creation and entrainment of the diurnal rhythm of melatonin secretion (BARRETT, UNDERWOOD 1992, PRUSIK 2005, PRUSIK, LEWCZUK 2008b). In the vast majority of species, this autonomy is partially limited by the sympathetic innervation, which belongs to the multisynaptic pathway connecting the pineal organ with the hypothalamus and the retina (CASSONE et al. 1990, PRUSIK, LEWCZUK 2008b). Norepinephrine released from the sympathetic nerve fibers, acting via α_2-adrenoceptors, inhibits melatonin secretion during the photophase (PRUSIK 2005, PRUSIK, LEWCZUK 2008b).

It is generally considered that calcium ions are involved in several processes occurring in avian pinealocytes, including photoreception, regulation of melatonin secretion and exocytosis of microvesicles; nevertheless, many aspects of the mechanisms of Ca^{2+} action in these cells are still poorly recognized (ZATZ 1989, 1992, ZATZ, MULLEN 1988b, D'-SOUZA, DRYER 1994, 1996, ZATZ, HEATH 1995, ALONSO-GOMEZ, IUVONE 1995, PABLOS et al. 1996, NIKAIDO, TAKAHASHI 1998, AGAPITO et al. 1998), for instance, subcelullar localization of Ca^{2+} in parenchymal cells has been not described in birds yet.

The present study was undertaken to investigate the distribution of calcium ions in the pineal organ of domestic turkey at the level of electron microscopy. The turkey pineal organ was chosen as the subject of our investigations because 1) it represents the tubulofollicular type of the pineal parenchyma organization, which is the most common in birds (PRZYBYLSKA-GORNOWICZ et al. 2005); 2) it contains well-developed rudimentary-receptor pinealocytes (with the prominent apical prolongations and regular, stratified distribution of organelles) and simultaneously numerous secretory pinealocytes (LEWCZUK 2000, PRUSIK, LEWCZUK 2008a); 3) turkey pinealocytes are photosensitive, possess an effective endogenous oscillator and are influenced by the sympathetic innervation (PRUSIK 2005, PRUSIK, LEWCZUK 2008b); 4) the diurnal rhythm of melatonin secretion by turkey pinealocytes is characterized by a high amplitude (PRUSIK 2005, ZAWILSKA et al. 2006, PRUSIK, LEWCZUK 2008b), which seems to be important in studies on day-night differences in Ca^{2+} distribution.

The study was performed using the potassium pyroantimonate ultracytochemical method, which enables visualization of calcium ions in the form of electron dense precipitates and is commonly used for ultracytochemical localization of calcium ions in biological samples (KLEIN et al. 1972, APPLETON, MORRIS 1979, KRSTIČ 1985, LEWCZUK et al. 1994, 2007, MENTRE, ESCAIG 1988, PIZARRO et al. 1989a,b, THERON et al. 1989, TUTTER et al. 1991, WICK, HEPLER 1982). Studies on chemical composition of pyroantimonate deposits have demonstrated that Ca is the predominant (beside small amounts of K, Na, Mg) or the only element associated with antimony (SUZUKI, SUGI 1989, MENTRE, ESCAIG 1988). The potassium pyroantimonate technique is the only one sensitive enough to demonstrate intracellular calcium ions at the level of electron microscopy in mammalian pinealocytes (TUTTER et al. 1991).

MATERIAL AND METHODS

The study was performed on 12 females of the domestic turkey (*Meleagris gallopavo*). The birds were housed in a cycle of 12 hours light : 12 hours dark, starting from the third week of life. During the photophase (between 07:00 and 19:00), fluorescent lamps provided light of the intensity of 300 lx at the floor level. The birds had free access to standard food and water. At the age of 12 weeks, turkeys were anesthetized with halothane and sacrificed by decapitation at 14:00 and at 02:00. All procedures (including euthanasia, tissue preparation and fixation) during the scotophase were made in darkness with the help of personal noctovisors. The experiment was performed in compliance with the Polish law.

The pineal organs were removed immediately after death, divided into small pieces and fixed in a solution containing 2% glutaraldehyde, 2% potassium pyroantimonate and 0.735% potassium acetate for 2 hours at 4°C (pH 7.5). After the first step of fixation, the pieces were rinsed three times (10 min each) with 0.735% potassium acetate in distilled water, incubated for 2 hours in 1% aqueous solution of osmium tetroxide containing 2% potassium pyroantimonate and 0.735% potassium acetate. Next, the pieces were washed in potassium acetate solution to remove any unreacted potassium pyroantimonate and to prevent nonspecific precipitation, dehydrated and embedded in Epon 812. Semithin sections were cut from each block of the tissue, stained with toluidine blue and examined under a light microscope in order to choose the sites for preparation of ultrathin sections. Both contrasted (with lead citrate and uranyl acetate) and uncontrasted ultrathin sections were examined in the Tecnai G2 Spirit BioTwin transmission electron microscope (FEI, USA) equipped with two digital cameras: Veleta (Olympus, Japan) and Eage 4k (FEI, USA).

For the control purposes, pieces of the pineal organs were prefixed for 30 minutes in a mixture of 2% glutaraldehyde and 0.735% potassium acetate, and then incubated (at 40°C) for 60 minutes in 0.735% potassium acetate solution with 2.5% EGTA or without EGTA, prior to the above described pyroantimonate procedure.

RESULTS

Ultrastructure of the turkey pineal organ

Parenchyma of the turkey pineal consisted of follicles containing a round or oval lumen, variable in size (Figure 1*a*). The follicles were surrounded by the thick basement membrane and separated from one another by the connective tissue septa, penetrating inside the organ from the capsule. The follicular wall was composed by rudimentary-receptor pinealocytes and ependymal-like supporting cells, which together created a single layer of elongated cells bordering the lumen, and by secretory pinealocytes and astrocyte-like supporting cells forming one or more layers of oval cells located at the periphery of the follicle (Figure 1*b*). Rudimentary-receptor pinealocytes possessed highly regular organization, with stratified distributions of organelles in the form of four zones: 1) perinuclear zone, 2) zone with the rough endoplasmic reticulum, 3) zone with the Golgi apparatus and 4) zone with mitochondria (Figure 1*b*). These cells created the apical prolongation extending into the follicular lumen and the basal process ending close to the basement membrane. Secretory pinealocytes were characterized by an irregular distri-

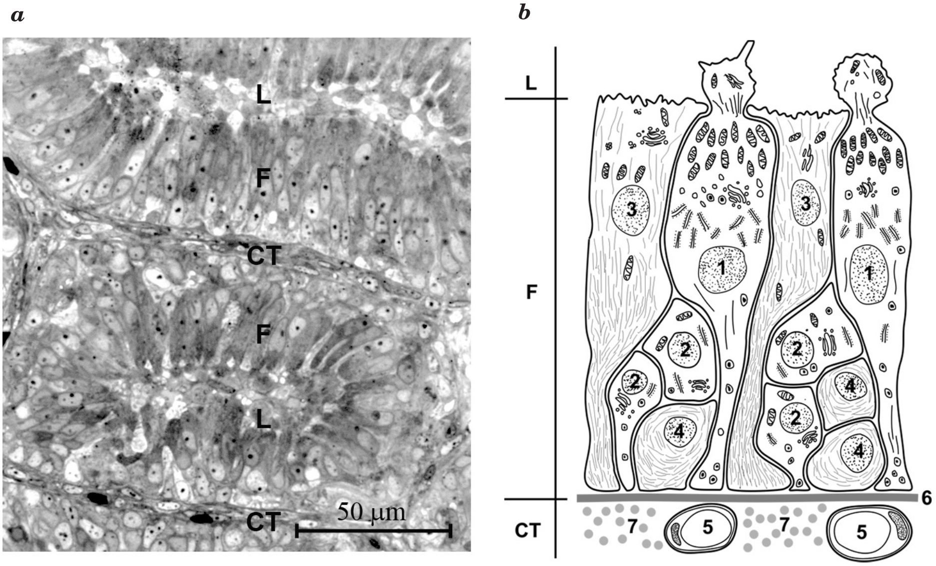

Fig. 1*a*. Fragment of the semithin section through the turkey pineal organ stained with toluidine blue showing the follicles (F) and the connective tissue stroma (CT). Note pseudo-stratified distribution of cells forming the follicular wall. L – follicular lumen; Fig. *b* – diagram illustrating the ultrastructure of the turkey pineal organ prepared on the basis of electron microscopic studies: L – follicular lumen, F – follicular wall, CT – connective tissue stroma, 1 – rudimentary-receptor pinealocytes, 2 – secretory pinealocytes, 3 – ependymal-like supporting cells, 4 – astrocyte-like supporting cell, 5 – capillary vessels, 6 – the basement membrane, 7 – collagen fibres

bution of organelles and possessed different numbers of cell processes (Figure 1*b*). Supporting cells contained numerous intermediate filament filling the basal part of ependymal-like cells and almost whole cytoplasm of astrocytes-like cells (Figure 1*b*).

Distribution of pyroantimonate precipitates in the pineal organs fixed during day-time

Extremely numerous, usually large, precipitates of calcium pyroantimonate were observed between collagen fibres of the connective tissue surrounding the pineal follicles (Figure 2*b, c*). The number of precipitates in the follicles was much lower than in the neighbouring stroma (Figure 2*a*). Precipitates were numerous in intercellular spaces between the cells forming the follicular wall (Figure 2*a*). In contrast, they were infrequently observed in the follicular lumen (Figure 2*a*, 3*b*). A clear difference in the amount of precipitates was observed between cells forming the wall of the follicle. Rudimentary-receptor pinealocytes (Figure 3*a–e*, 4*a–d*) and secretory pinealocytes (Figure 5) contained much more calcium precipitates than the supporting cells (Figure 6*a–e*).

In rudimentary-receptor pinealocytes, precipitates were present mainly in the nucleus (Figure 3*c*) and mitochondria (Figure 3*a,b,d*, 4*a*). Numerous precipitates were also found in short, wide cisterns located in the upper part of the cells (Figure 4*b*), whereas other components of the reticulum were sparse in precipitates (Figure 3*a,e*). Abundant precipitates were also observed in myelin-like structures surrounding the lipid droplets (Figure 4*c*). A few precipitates occurred in the endings of basal processes, where they were located close to a clear and granular vesicle as well as in the cytosol (Figure 4*d*). Precipitates were also sparse in apical prolongations extending into the follicular lumen, except for mitochondria, which – as usual – contained numerous precipitates (Figure 3*a,b*). There were no prominent differences in the amount of precipitates between individual pinealocytes of the rudimentary-receptor type.

In secretory pinealocytes, calcium pyroantimonate precipitates were accumulated mainly in the nucleus, mitochondria and some cisterns of smooth endoplasmic reticulum (Figure 5). Less numerous precipitates was found in cytosol. Amounts of precipitates did not differ markedly between individual secretory pinealocytes.

Calcium precipitates in ependymal-like cells were present mainly in the nucleus and in the upper part of cytoplasm, where they were localized mainly in mitochondria, cisterns of smooth endoplasmic reticulum and in the cytosol (Figure 6*a*). Precipitates were infrequently observed in the areas of cytoplasm filled with intermediate filaments (Figure 6*b,c*). Astrocyte-like supporting cells contained a small number of precipitates located mainly in the nucleus and mitochondria (Figure 6*d,e*).

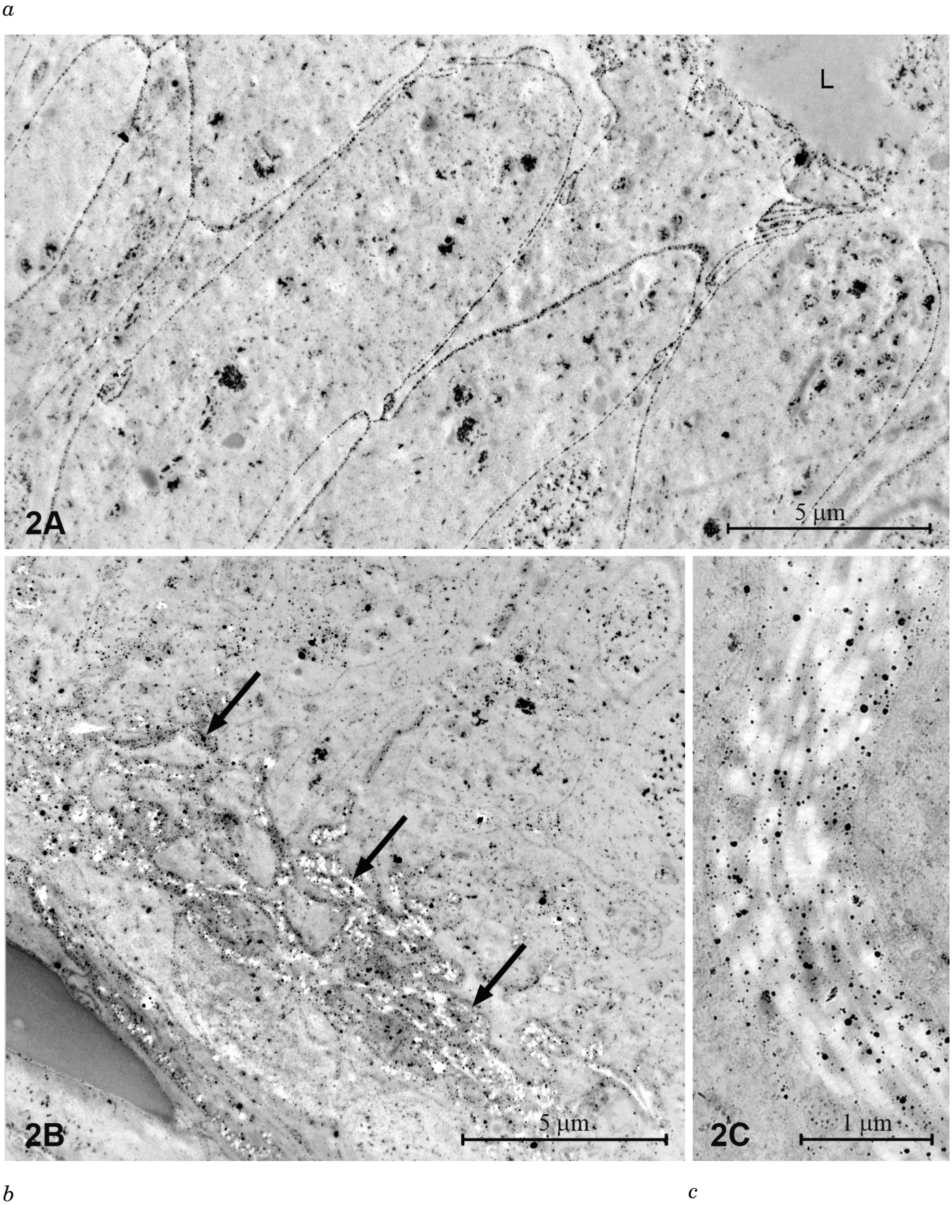

Fig. 2. Distribution of calcium pyroantimone precipitates in intercellular spaces between cells forming the follicular wall (Fig. *a*) and in the connective tissue surrounding the pineal follicle (Fig. *b, c*) in organs taken *ex vivo* during daytime. Intercellular spaces between the adjacent cells in the follicular wall are filled by precipitates (see Fig. *a*). Note the presence of numerous precipitates between collagen fibres (in Fig. *b* – arrows and Fig. *c*) and a few precipitates inside the follicular lumen (L in Fig. *a*).

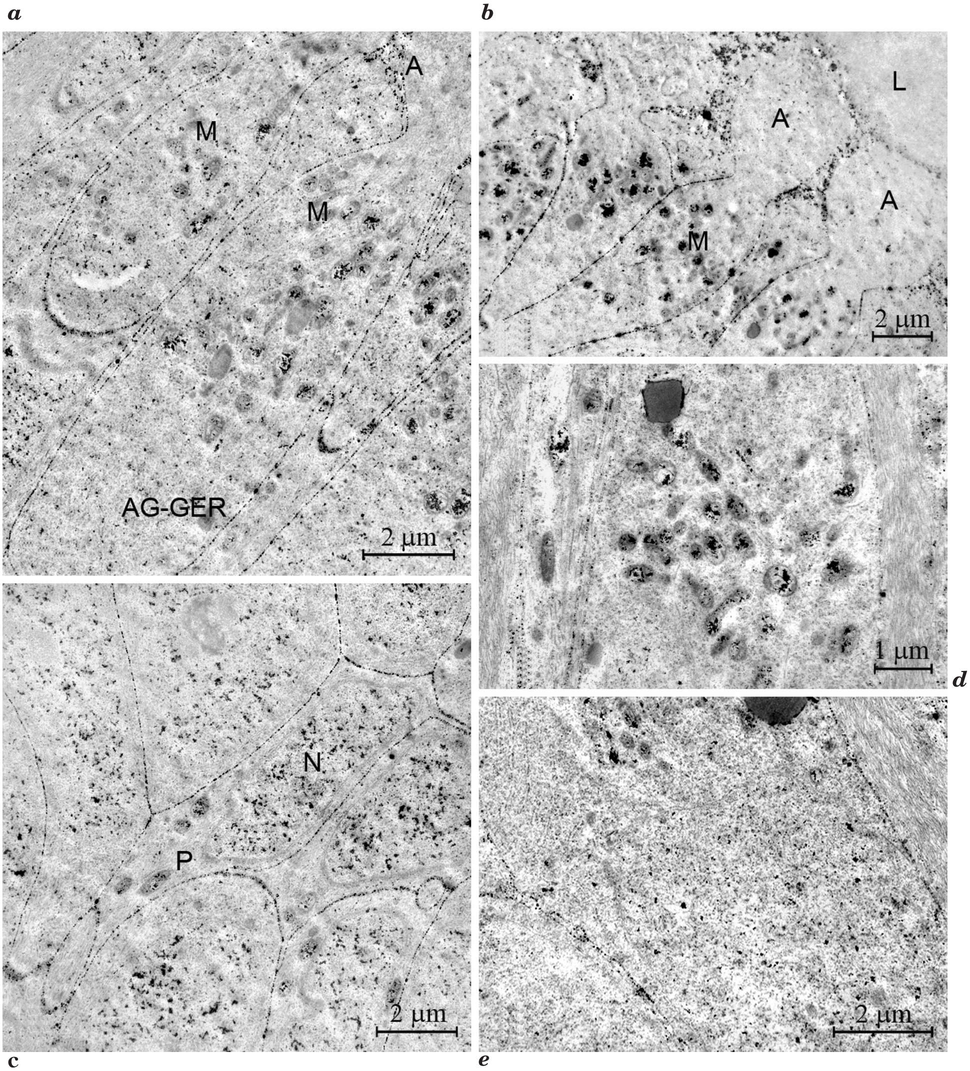

Fig. 3. Distribution of calcium pyroantimone precipitates in various parts of rudimentary-receptor pinealocytes in the pineal organs taken *ex vivo* during daytime: *a, b* – precipitates in the apical prolongations (A), the zones with mitochondria (M) and the zones containing the Golgi apparatus and granular endoplasmic reticulum (AG-GER), L – follicular lumen, *c* – precipitates in the cell nucleus (N) and the basal process (P), *d* – precipitates in the zone containing mitochondria, *e* – precipitates in the zones containing the Golgi apparatus and granular endoplasmic reticulum. Note the presence of numerous precipitates in mitochondria and cell nuclei as well as a low content of precipitates in the apical prolongations and the areas containing the granular endoplasmic reticulum

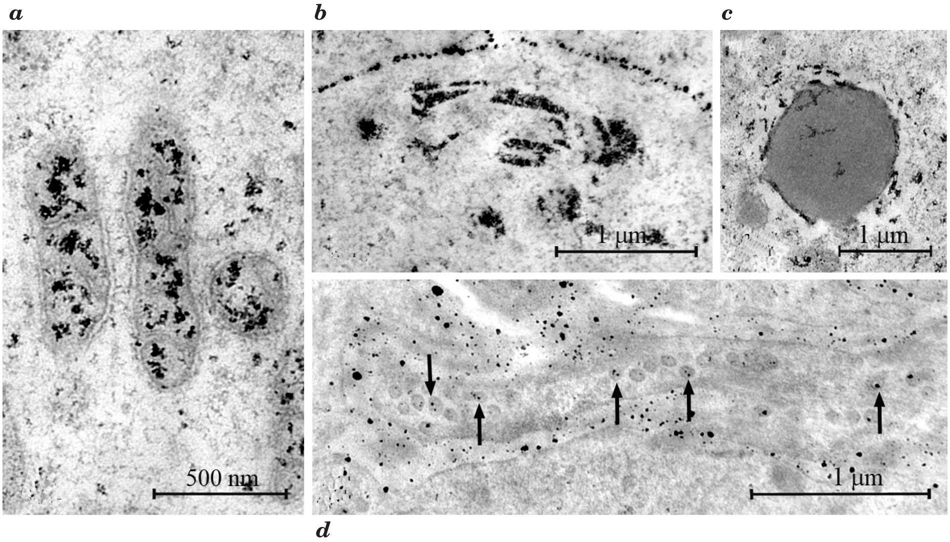

Fig. 4. Subcellular distribution of calcium pyroantimone precipitates in rudimentary-receptor pinealocytes in organs taken *ex vivo* during daytime: *a* – precipitates in the mitochondrial matrix, *b* – precipitates in wide cisterns of smooth endoplasmic reticulum, *c* – precipitates in myeloid-like structures surrounding lipid droplets, *d* – precipitates inside or close to clear vesicles (arrows)

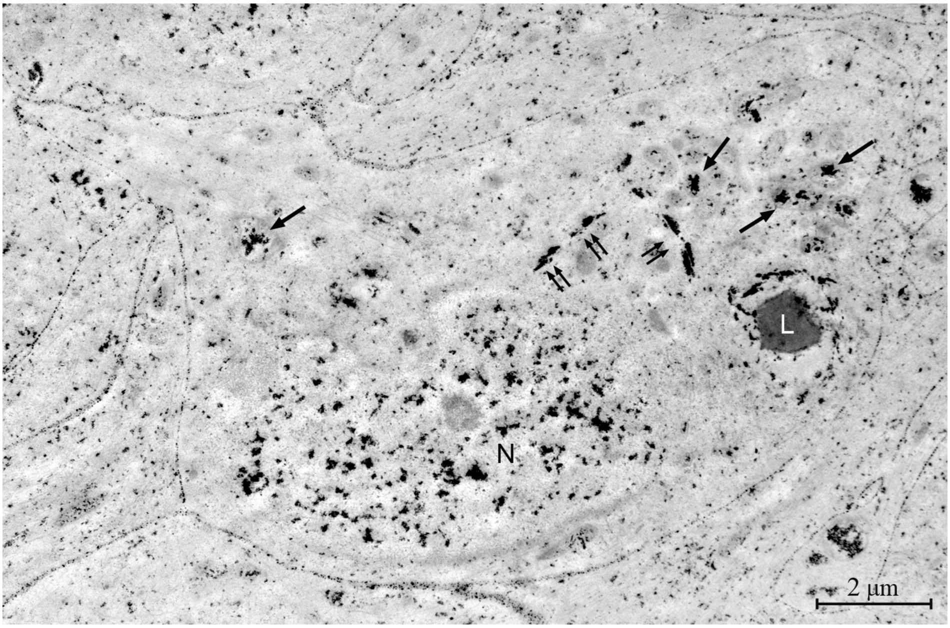

Fig. 5. Localization of calcium pyroantimone precipitates in secretory pinealocytes in organs taken *ex vivo* during daytime. Note the presence of numerous precipitates in the nucleus (N), mitochondria (arrows), wide cisterns of the smooth endoplasmic reticulum (double arrows) and around the lipid droplet (L)

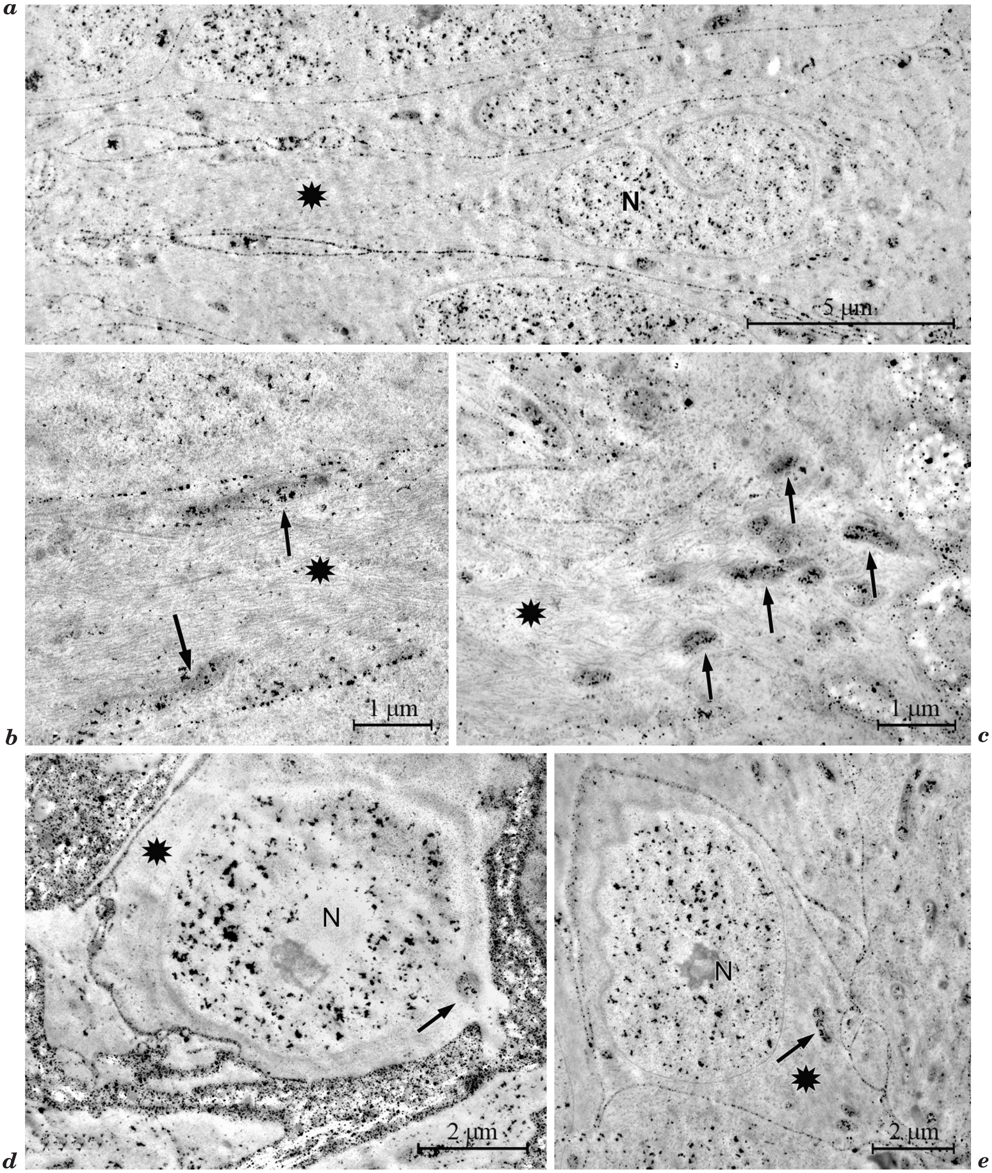

Fig. 6. Distribution of calcium pyroantimone precipitates in ependymal-like supporting cells (*a, b, c*) and astrocyte-like supporting cells (*d, e*) in organs taken *ex vivo* during daytime. Precipitates are present mainly in the nuclei (N) and in mitochondria (arrows). They are sparse in areas filled with filaments (asterisks)

Distribution of pyroantimonate precipitates in the pineal organs fixed during night-time

General patterns of calcium precipitate distribution in the pineals fixed during the scotophase were similar to those in the organs fixed during the photophase. The most important difference concerned the presence of much more numerous precipitates in the cytosol of rudimentary-receptor pinealocytes (Figure 7*a*) and secretory pinealocytes (Figure 7*b*) taken *ex vivo* during nighttime than during daytime.

Control

Pretreatment of tissues in a solution containing EGTA – divalent cations chelator – resulted in a complete lack of precipitates. Analogous incubation without the use of EGTA did not affect the presence of precipitates.

DISCUSSION

Calcium ions together with cAMP molecules play a crucial role in the regulation of melatonin secretion in avian pinealocytes, but the precise mechanisms of Ca^{2+} influence on the pineal hormone synthesis and the interactions between these two second messengers are poorly recognized (ZATZ, MULLEN 1988a, ZATZ 1989, 1992, ALONSO-GOMEZ, IUVONE 1995, PABLOS et al. 1996, AGAPITO et al. 1998). Two types of calcium channels have been described in the plasma membrane of chicken pinealocytes: voltage-dependent calcium channels of L-type (HARRISON, ZATZ 1989) and long-open-time cationic channels I_{LOT} (D'-SOUZA, DRYER 1996). Activator of the L-type channels (Bay K 8644) increases the nocturnal peak of melatonin secretion from chicken pinealocytes, whereas antagonists (nitrendipine and nifedipine) decrease the nocturnal rise in the pineal hormone level (ZATZ 1989, HARRISON, ZATZ 1989). None of these drugs influences the synchronization of the circadian rhythm of the pineal secretory activity with the light-dark cycle (ZATZ, MULLEN 1988b). Bay K 8644 and nitrendipine alter the cAMP level in avian pinealocytes (ZATZ 1992a). D'-SOUZA and DRYER (1996) investigating chick pinealocytes with the patch-clump technique demonstrated the presence of cationic channels with an unusually long open time and for that reason they were termed as I_{LOT}. These channels are not gated by voltage or soluble second messengers. They are cyclically synthesized and open for calcium ions at night and degraded during the day (D'-SOUZA, DRYER 1996). The rhythm of I_{LOT} channels activity persists in chicken pinealocytes kept in continuous darkness, therefore these channels are considered as being dependent on the endogenous oscillator function. Inhibitors of protein synthesis abolish the circadian rhythm in I_{LOT} channels activity. Summing up, the onset of nocturnal darkness in chick pinealocytes is associated with two phenomena: 1) depo-

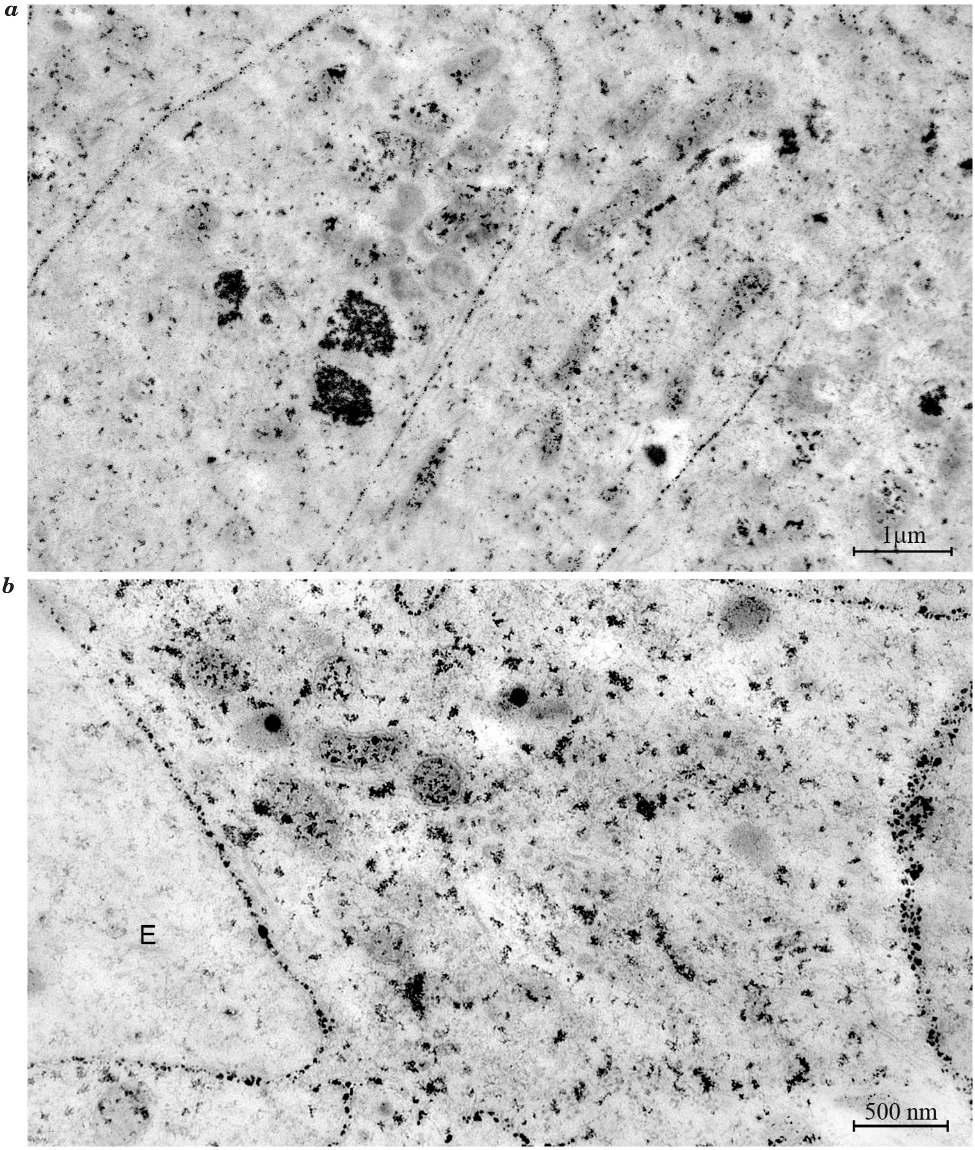

Fig. 7. Localization of calcium pyroantimone precipitates in rudimentary-receptor pinealocytes (*a*) and secretory pinealocytes (*b*) in the pineal organ taken *ex vivo* during night-time. Note the presence of numerous precipitates in cytosol of both types of pinealocytes. Only single precipitates are present in the cytoplasm of the ependymal-like supporting cell (E)

larization of plasma membrane and opening of voltage-dependent calcium channels, and 2) a dramatic increase in the number of I_{LOT} channels. These events are probably responsible for a large rise in intracellular free calcium in chicken pinealocytes at night. Elevation of Ca^{2+} influences the activity of calcium/calmodulin-stimulated adenylate cyclase and results in an increase (additional to the one caused by the biosynthesis of adenylate cyclase controlled by clock genes) in cAMP production in pinealocytes. Cyclic-AMP is the main factor controlling the level and activity of serotonin N-acetyltransferase (an enzyme limiting the melatonin secretion) in pinealocytes (NATESAN et al. 2002).

Substances affecting the calcium output from the intracellular stores, in contrast to membrane calcium-channels activators and antagonists, influenced the activity of the endogenous oscillator controlling the melatonin secretion in chick pinealocytes. Caffeine at high concentrations, increasing the calcium output from intracellular reserves, induced the phase shift in the rhythm of melatonin secretion similar to the effects evoked by light impulses (ZATZ, HEATH 1995). Cyclopiazonic acid, an inhibitor of calcium release from intracellular stores, synchronized the melatonin rhythm in the way resembling the effect of darkness (NATESAN et al. 2002). The cited data suggest that calcium ions released from the intracellular reserves participate in regulation of the chick pineal clock. It is worth noticing that spontaneous fluctuations of free calcium ions concentration were observed in about 10% of a population of chicken pinealocytes kept in monolayer culture (D'-SOUZA, DRYER 1994).

Until now, ultracytochemical localization of calcium ions has been investigated in the pineals of poikilothermic vertebrates (VIGH, VIGH-TEICHMANN 1989, VÍGH et al. 1998) and mammals (KRSTIČ 1985, THERON et al. 1989, PIZARRO et al. 1989a,b, TUTTER et al. 1991, LEWCZUK et al. 1994, 2007). As concerning the pineal organ in birds, the distribution of Ca^{2+} at the level of electron microscopy was described only in the areas of the formation of calcified concrements (VÍGH et al. 1998, PRZYBYLSKA-GORNOWICZ et al. 2009). Localization of calcium ions in pinealocytes and supporting cells of the avian pineal is reported for the first time in the present study.

Electron dense precipitates of calcium pyroantimonate were found in two components of the turkey pineal organ, separated from each other by the continuous basal membranes: the connective tissue stroma and the parenchyma formed by the pineal follicles. The amount of calcium ions differed markedly between these compartments. The connective tissue contained very numerous, frequently large calcium pyroantimonate precipitates and this observation well agrees with our previous studies concerning the formation of calcified concretions in the turkey pineal (PRZYBYLSKA-GORNOWICZ et al. 2009). In the follicles, precipitates were much less abundant comparing to the stroma. They were relatively numerous in the intercellular spaces separating the lateral domains of the adjoining cells, moderate in number in pinealocytes and supporting cells, and sparse inside the follicular lumen. These observations implicate that diffusion of calcium ions from the stroma to the extracellular spaces of the follicular wall and later to the follicular lumen is partially limited or undergoes some control mechanisms. This suggestion seems to be very interesting, although it could not be excluded that the low number of precipitates inside the follicular lumen may have its source in restricted penetration of pyroantimonate ions through the follicular wall. Explanation of this problem requires subsequent studies.

Both rudimentary-receptor and secretory pinealocytes contained much more precipitates than supporting cells. According to the results, the highest concentrations of calcium ions in pinealocytes occur in the nucleus, mitochondria and some cisterns of the smooth endoplasmic reticulum. High levels of Ca^{2+} in nuclei and mitochondria were reported in numerous cell types including pinealocytes (KRSTIČ 1985, PIZARRO et al. 1989a,b, WELSH 1984, LEWCZUK et al. 1994, 2007). It is reasonable to suspect that short cisterns of endoplasmic reticulum, present in both types of pinealocytes and distinguished in our study by the occurrence of numerous precipitates, are the main intracellular calcium storage sites related to the regulatory functions of this second messenger. Generally, distribution of calcium ions in turkey pinealocytes is similar to the one described in mammalian pinealocytes, where calcium pyroantimonate precipitates were found in nuclei, mitochondria, vesicles and cisterns of the smooth endoplasmic reticulum (including subsurface cisterns), the Golgi apparatus and cytosol (KRSTIČ 1985, PIZARRO et al. 1989a,b, WELSH 1984, LEWCZUK et al. 1994, 2007).

The results show that both rudimentary-receptor and secretory pinealocytes form homogenous subpopulations concerning the amount and distribution of calcium ions. Prominent differences in the presence and localization of Ca^{2+} between pinealocytes were reported in mammals, based on the investigations performed with the use of potassium pyroantimonate method, e.g. PIZARRO et al. (1989a,b) distinguished two kinds of rat pinealocytes: type I corresponding to the classic light pinealocytes with almost complete absence of calcium pyroantimonate precipitates and type II with the dense cyplasmic matrix and very abundant coarse precipitates inside the cytoplasm and the nucleus. Two types of pinealocytes, differing by the amount of calcium ions, were distinguished also in the domestic pig (LEWCZUK et al. 2007). The first type included cells containing a small or moderate amount of precipitates. These cells were usually characterized by low electron density of their cytoplasm. Pinealocytes classified as the second type were characterized by a large or very large content of precipitates both in the cell nucleus and the cytoplasm. They were characterized by the electron dense cytoplasm and the presence of numerous dense bodies – the structures specific for swine pinealocytes. Differences in the amount of precipitates were also observed between distinct forms of gerbil pinealocytes (KRSTIČ 1985, WELSH 1984).

Our study demonstrated a low concentration of calcium ions inside and around the apical prolongations of rudimentary-receptor pinealocytes. These structures contain a photopigment, pinopsin, thus they are considered as being involved in the light reception and analogous to the outer segments of classical photoreceptor cells (PRUSIK, LEWCZUK 2008a). It should be noted that numerous precipitates of calcium pyroantimonate were found in the outer segments of photoreceptor cells in the frog pineal gland (VIGH, VIGH-TEICHMANN 1989). Similarly to the pineal, precipitates of calcium pyroantimonate were numerous in the rod outer segments in the frog retina. In the cone outer segments, they were much less abundant (VIGH, VIGH-TEICHMANN 1989). Calcium pyroantimonate precipitates in frogs adopted to darkness were present on both cytoplasmic and intradiscal sides of the rod photoreceptor membranes, whereas in light adopted animals precipitates were concentrated inside the discs (VIGH et al. 1998). Numerous precipitates of calcium pyroantimonate were also reported in the outer segments of retinal rod photoreceptor cells in the rat (VAN REEMPTS et al. 1984) and the rabbit (VIGH et al. 1998). The above data show that there are important differences in amounts of calcium ions between the apical prolongations of avian pinealocytes and the outer segments of photoreceptor cells occurring in the pineals of poikilothermic vertebrates and in the retinas. Our results suggest that despite many similarities in the photoreception mechanisms between avian pinealocytes and classical photoreceptor cells, these are also important distinctions. It is worth remembering that the apical prolongations of rudimentary-receptor pinealocytes and the outer segments of photoreceptor cells show completely different structural organization. In secretory pinealocytes, which are also photosensitive, pinopsin is present in the processes penetrating between neighbouring cells (PRUSIK, LEWCZUK 2008a). In the present study, we did not find any calcium-rich processes outgrowing from or located in the vicinity of secretory pinealocytes. Summing up, the light-sensitive structures of turkey pinealocytes are characterized by a low content of calcium ions.

The study on day-night differences in the distribution of Ca^{2+} in the turkey pineal demonstrated that precipitates of calcium pyroantimonate in the cytosol of both rudimentary-receptor and secretory pinealocytes were more numerous in the organs taken *ex vivo* at 2.00 than at 14.00. Although in our opinion the potassium pyroantimonate fixation is not a valid method for quantitative comparisons, the differences in the occurrence of precipitates in the cytosol could be considered as reflecting the diurnal rhythm in the level of free calcium ions in cytosol of pinealocytes. The present data are in agreement with the results of physiological studies on chicken pinealocytes suggesting that the influx of calcium ions via two types of calcium channels occurs during scotophase (see the first part of discussion). We were not able to observe any day-night variations in the amount of calcium precipitates in short, wide cisterns of smooth endoplasmic reticulum. Two explanations of the lack of these differences should be taken into consideration. One is that visualization of changes was impossible because the level of Ca^{2+} in cisterns of the smooth endoplasmic reticulum was too high to notice the changes in the amount of precipitates related to the release of ions into the cytosol and their re-uptake. The second explanation is related to the discrepancy between the time when animals were sacrificed and occurrence of the nadir and peak in a putative diurnal rhythm of Ca^{2+} release from these stores. It should be underlined that in contrast to pinealocytes, no variations in the amount and distribution of precipitates were observed in ependymal-like and astrocyte-like supporting cells.

Until now, day-night differences in the localization of calcium ions have been reported in the pineal glands of mammals. Using the potassium pyroantimonate technique, THERON et al. (1989) described a prominent shift in Ca^{2+} distribution in baboon pinealocytes. During the dark phase, the location of calcium ions was predominantly intravesicular and intravacuolar, whereas during the photophase calcium ions were present mainly in cytosol. Contrary data were obtained in the gerbil pineal gland (TUTTER et al. 1991). The accumulation of precipitates occurred in all compartments of the endoplasmic reticulum including subsurface cisterns in pinealocytes of gerbils sacrificed during daytime but they were not present anywhere in the endoplasmic reticulum when the animals were euthanized at night. Analyzing the data obtained in mammals and birds, it is necessary to remember that the mechanisms regulating the level of calcium ions in pinealocytes of both groups of vertebrates are completely different.

CONCLUSIONS

1. The potassium pyroantimonate method enables visualization of the Ca^{2+} distribution at the level of electron microscopy in two compartments of the turkey pineal organ: the parenchyma formed by the follicles and the connective tissue stroma.

2. Concentration of calcium ions in the pineal follicles is markedly lower than in the surrounding extracellular matrix of the connective tissue. The follicular lumen is characterized by a low level of Ca^{2+}.

3. Rudimentary-receptor and secretory pinealocytes contain more calcium ions than the ependymal-like and astrocyte-like supporting cells.

4. The highest concentrations of free Ca^{2+} in both rudimentary-receptor and secretory pinealocytes are present in the nuclei, mitochondria and some cisterns of the smooth endoplasmic reticulum. Calcium ions are not accumulated in the apical prolongations of rudimentary-like pinealocytes and in the processes of secretory pinealocytes.

5. There are no differences in the amounts and distribution of Ca^{2+} between individual cells belonging to subpopulations of rudimentary-like pinealocytes and secretory pinealocytes.

6. Concentration of calcium ions in the cytosol of rudimentary-like pinealocytes and secretory pinealocytes is higher during the scotophase than during the photophase.

7. No day-night differences in the concentration of calcium ions in the cytosol occur in astrocyte-like and ependymal-like supporting cells.

REFERENCES

AGAPITO M.T., PABLOS M., REITER R.J., RECIO J.M., GUTIERREZ-BARAJA R. 1998. *Effects of ethylene glycol tetraacetic acid, A23187 and calmodulin, calcium activated neutral proteinase antagonists on melatonin secretion in perfused chick pineal gland.* Neurosci. Lett., 245(3): 143-146.

ALONSO-GOMEZ A.L., IUVONE P.M. 1995. *Melatonin biosynthesis in cultured chick retinal photoreceptor cells: calcium and cyclic AMP protect serotonin N-acetyltransferase from inactivation in cycloheximide-treated cells.* J. Neurochem., 65: 1054-1060.

APPLETON J., MORRIS D.C. 1979. *The use of the potassium pyroantimonate-osmium method as a means of identifying and localizing calcium at the ultrastructural level in the cells of calcifying systems.* J. Histochem. Cytochem., 27(2): 676-680.

BARRETT R.K., UNDERWOOD H. 1992. *The superior cervical ganglia are not necessary for entrainment or persistence of the pineal melatonin rhythm in Japanese quail.* Brain Res., 569(2): 249-254.

CASSONE V.M., FORSYTH A.M., WOODLEE G.L. 1990. *Hypothalamic regulation of circadian noradrenergic input to the chick pineal gland.* J. Comp. Physiol., A Sens. Neural Bahav. Physiol., 167(2): 187-192.

CHAURASIA S.S., PROVENCIO I., JIANG G., HAYES W.P., NATESAN A., ZATZ M., ROLLAG M.D., IUVONE P.M. 2004. *Differential circadian regulation of melanopsin mRNA expression in the avian retina and pineal gland.* Invest. Ophthalmol. Vis. Sci., 45: 46-48.

CSERNUS V., FALUHELYI N., NAGY A.D. 2005. *Features of the circadian clock in the avian pineal gland.* Ann. N. Y. Acad. Sci., 1040: 281-287.

D'-SOUZA T., DRYER S.E. 1994. *Intracellular free Ca++ in dissociated cells of chick pineal gland: regulation by membrane depolarization, second messengers and neuromodulators, and evidence for release of intracellular stores.* Brain Res., 656(1): 85-94.

D'-SOUZA T., DRYER S.E. 1996. *A cationic channel regulated by a vertebrate intrinsic circadian oscillator.* Nature, 382 (6587): 165-167.

FEJER Z., ROHLICH P., SZEL A., DAVID C., ZADORI A., MANZANO M.J., VIGH B. 2001. *Comparative ultrastructure and cytochemistry of the avian pineal organ.* Microscopy Res. Tech., 53: 12-24.

HALDAR C., BISHNUPURI K.S. 2001. *Comparative view of pineal gland morphology of nocturnal and diurnal birds of tropical origin.* Microsc. Res. Tech., 53(1): 25-32.

HARRISON N.L., ZATZ M. 1989. *Voltage-dependent calcium channels regulate melatonin output from cultured chick pineal cells.* J. Neurosci., 9(7): 2462-2467.

HO A.K., CHIK C.L., KLEIN D.C. 1988A. *Effects of protein kinase inhibitor 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H7) on protein kinase C activity and adrenergic stimulation of cAMP and cGMP in rat pinealocytes.* Biochem. Pharmacol., 37: 1015-1020.

HO A.K., THOMAS T.P., CHIK C.L., ANDERSON W., KLEIN D.C. 1988B. *Protein kinase C: subcellular redistribution by increased Ca2+ influx.* J. Biol. Chem., 263: 9292-9297.

HOLTHUES H., ENGEL L., SPESSERT R., VOLLRATH L. 2005. *Circadian gene expression patterns of melanopsin and pinopsin in the chick pineal gland.* Biochem. Biophys. Res. Commun., 326(1): 160-165.

KLEIN R.L., YEN S.-S., THURESON-KLEIN A. 1972. *Critique on the K-pyroantimonate method for semiquantitative estimation of cations in conjunction with electron microscopy.* J. Histochem. Cytochem., 20(1): 65-78.

KRSTIČ R. 1985. *Ultracytochemical localization of calcium in the superficial pineal gland of the Mongolian gerbil.* J. Pineal Res., 2(1): 21-37.

LEWCZUK B., BULC M., PRUSIK M., PRZYBYLSKA-GORNOWICZ B. 2007. *Calcium ions in the pig pineal gland – an ultracytochemical study.* J. Elementol., 12(4): 335-346.

LEWCZUK B., PRZYBYLSKA B., WYRZYKOWSKI Z. 1994. *Distribution of calcified concretions and calcium ions in the pig pineal gland.* Fol. Histochem. Cytobiol., 32(4): 243-249.

LEWCZUK B. 2000. *Ultrastructure of the pinealocytes of rudimentary receptor type in the pineal gland of the turkey.* Pol. J. Vet. Sci., 3(2): 26.

MENTRE P., ESCAIG F. 1988. *Localization of cations by pyroantimonate. I Influence of fixation on distribution of calcium and sodium. An approach by analytical ion microscopy.* J. Histochem. Cytochem., 36(1): 49-54.

MURAKAMI N., NAKAMURA H., NISHI R., MARUMOTO N., NASU T. 1994. *Comparison of circadian oscillation of melatonin release in pineal cells of house sparrow, pigeon and Japanese quail, using cell perfusion system.* Brain Res., 651(1-2): 209-214.

NATESAN A., GEETHA L., ZATZ M. 2002. *Rhythm and soul in the avian pineal.* Cell Tissue Res., 309 (1): 35-45.

NIKAIDO S.S., TAKAHASHI J.S. 1998. *Day/night differences in the stimulation of adenylate cyclase activity by calcium/calmodulin in chick pineal cell cultures: evidence for circadian regulation of cyclic AMP.* J. Biol. Rhythms, 13(6): 479-493.

OKANO T., FUKADA Y. 2003. *Chicktacking pineal clock.* J. Biochem., 134(6): 791-797.

OKANO T., TAKANAKA Y., NAKAMURA A., HIRUNAGI K., ADACHI A., EBIHARA S., FUKADA Y. 1997. *Immunocytochemical identification of pinopsin in the pineal glands of chicken and pigeon.* Brain Res. Mol. Brain Res., 50(1-2): 190-196.

OKANO T., YOSHIZAWA T., FUKADA Y. 1994. *Pinopsin is a chicken pineal photoreceptive molecule.* Nature, 372(6501): 94-97.

OHSHIMA K., HIRAMATSU K. 1993. *Ultrastructural study of post-hatching development in the pineal gland of the Japanese quail.* J. Vet. Med. Sci., 55(6): 945-950.

PABLOS M.I., AGAPITO M.T., GUTIERREZ-BARAJA R., REITER R.J., RECIO J.M. 1996. *Effect of calcium on melatonin secretion in chick pineal gland I.* Neurosci. Lett., 217(2-3): 161-164.

PIZARRO M.D.L., GIL J.A.L., VASALLO J.L., BARRAGAN L.M. 1989a. *Distribution of calcium in the pineal gland of normal rats.* In: *Advances in pineal research* Vol. 3. Eds. R.J. REITER & S.F. PANG. John Libbey & Co Ltd, pp. 39-41.

PIZARRO M.D.L., PASTOR F.E., GIL A.L., BARRAGAN L.M. 1989b. *Ultrastructural study of the distribution of calcium in the pineal gland of the rat subjected to manipulation of the photoperiod.* Histochem. Cell Biol., 92(2): 161-169.

PRUSIK 2005. *Mechanisms regulating melatonin secretion in the turkey pineal gland.* UWM Olsztyn, doctoral thesis. (in Polish)

PRUSIK M., LEWCZUK B., NOWICKI M., PRZYBYLSKA-GORNOWICZ B. 2006. *Histology and ultrastructure of the pineal gland of the domestic goose.* Histol. Histopathol., 21(10): 1075-1090.

PRUSIK M., LEWCZUK B. 2008a. *Structure of the avian pineal gland.* Med. Wet., 64(5): 737-848. (in Polish)

PRUSIK M., LEWCZUK B. 2008b. *Regulation of melatonin secretion in the avian pineal gland.* Med. Wet., 64(5): 617-736. (in Polish)

PRZYBYLSKA-GORNOWICZ B., LEWCZUK B., PRUSIK M., NOWICKI M. 2005. *Post-hatching development of the turkey pineal organ: histological and immunohistochemical studies.* Neuroendocrinol. Lett., 26 (4): 383-392.

PRZYBYLSKA-GORNOWICZ B., LEWCZUK B., PRUSIK M., BULC M. 2009. *Pineal concretions in turkey (Meleagris gallapavo) as a results of collagen mediated calcification.* Histol. Histopathol., 24 (4): 407-415.

SUZUKI S., SUGI H. 1989. *Evaluation of the pyroantimonate method for detecting intracellular calcium localization in smooth muscle fibers by the X-ray microanalysis of cryosections.* Histochemistry, 92 (2): 95-101.

THERON J.J., BIAGIO R.P., HENNING C.N. 1989. *Circadian changes in the ultracytochemical localization of calcium in the pinealocytes of the baboon (Papio ursinus).* In: *Advances in pineal research*. Vol 3. Eds R.J. REITER, S.F. PANG. John Libbey & Co Ltd, London, pp. 33-38.

TUTTER I., HEINZELLER T., SEITZ-TUTTER D. 1991. *Pinealocyte subsurface cisterns III: Storage of calcium ions and their probable role in cell stimulation.* J. Pineal Res., 10(2): 91-99.

VAN REEMPTS J.L., BORGERS M., DE NOLLIN S.R., GARREVOET T.C., JACOB W.A. 1984. *Identification of calcium in the retina by the combined use of ultrastructural cytochemistry and laser microprobe mass analysis.* J. Histochem. Cytochem., 32(7): 788-792.

VIGH B., VIGH-TEICHMANN I. 1989. *The pinealocyte forming receptor and effector endings: immunoelectron microscopy and calcium histochemistry*. Arch. Histol. Cytol. 52 (Suppl): 433-40.

VÍGH B., SZÉL A., DEBRECENI K., FEJÉR Z., MANZANO E SILVA M.J., VÍGH-TEICHMANN I. 1998. *Comparative histology of pineal calcification.* Histol. Histopathol., 13(3): 851-870.

VOLLRATH L. 1981. *The pineal gland.* In: *Handbuch der mikroskopischen Anatomie des Menschen.* Vol. VI/7, Springer Verlag, Berlin, Heidelberg.

WELSH M.G. 1984. *Cytochemical analysis of calcium distribution in the superficial pineal gland of the Mongolian gerbil.* J. Pineal Res., 1(4): 305-316.

WICK S.M., HEPLER P.K. 1982. *Selective localization of intracellular Ca2+ with potassium antimonate.* J. Histochem. Cytochem., 30(11): 1190-1204.

ZATZ M. 1989. *Relationship between light, calcium influx and cAMP in the acute regulation of melatonin production by cultured chick pineal cells.* Brain Res., 477(1-2): 14-18.

ZATZ M. 1992. *Agents that affect calcium influx can change cyclic nucleotide levels in cultured chick pineal cells.* Brain Res., 583(1-2): 304-307.

ZATZ M., HEATH J.R. 1995. *Calcium and photoentrainment in chick pineal cells revisited: effects of caffeine, thapsigargin, EGTA, and light on the melatonin rhythm.* J. Neurochem., 65(3): 1332-1341.

ZATZ M., MULLEN D.A. 1988a. *Photoendocrine transduction in cultured chick pineal cells. II. Effects of forskolin, 8-bromocyclic AMP, and 8-bromocyclic GMP on the melatonin rhythm.* Brain Res., 453(1-2): 51-62.

ZATZ M., MULLEN D.A. 1988b. *Does calcium influx regulate melatonin production through the circadian pacemaker in chick pineal cells? Effects of nitrendipine, Bay K 8644, Co^{2+}, Mn^{2+}, and low external Ca^{2+}*. Brain Res., 463(2): 305-316.

ZAWILSKA J.B., LORENC A, BEREZIŃSKA M, VIVIEN-ROELS B., PÉVET P, SKENE D.J. 2006. *Diurnal and circadian rhythms in melatonin synthesis in the turkey pineal gland and retina.* Gen. Comp. End., 145(2): 162-168.

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THE EFFECT OF MULTI-COMPONENT FERTILIZERS ON THE YIELD AND MINERAL COMPOSITION OF WINTER WHEAT AND MACRONUTRIENT UPTAKE

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Abstract

Multi-component fertilizers should be selected based on a thorough analysis of a variety of factors such as plant species, expected yield, nutrient availability, time and mode of application. Mixed fertilizers have to be applied in a rational manner, and their rates should be determined in view of crop yield, crop quality and fertilizer efficiency, so as to maximize the benefits of fertilizer use. The objective of this study was to determine the effect of multi-component fertilizers, Amofosmag 4 and Amofosmag 3, on winter wheat yield, and the content and uptake of macronutrients. A three-year field experiment (2008-2010) was carried out in a randomized block design at the Research and Experimental Station in Tomaszkowo, at the University of Warmia and Mazury in Olsztyn (NE Poland). The experiment comprised three fertilization treatments in four replications: control treatment (simple fertilizers) and two treatments with mixed multi-component fertilizers, Amofosmag 4 and Amofosmag 3. The tested crop was winter wheat cv. Bogatka.

Winter wheat grain yield was modified by fertilizers and weather conditions. The most beneficial effect was reported for Amofosmag 4, which increased the yield of wheat grain by 7% on average, compared with the remaining treatments. A significantly lower grain yield was noted in the second year of the study characterized by adverse weather conditions. The concentrations of the analyzed macronutrients in winter wheat grain and straw varied insignificantly between fertilization treatments. Simple and multi-component fertilizers exerted a comparable effect on the mineral composition of the test crop. The only exception was the potassium content of wheat straw, which was significantly higher after the application of multi-component fertilizers. Significant differences were observed in this

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respect between successive years of the study. The highest total uptake of nitrogen, phosphorus and magnesium by winter wheat plants was noted in the Amofosmag 4 treatment, and the highest total uptake of potassium and calcium was observed in the Amofosmag 3 treatment. Amofosmag 4 was found to be the most efficient fertilizer, which suggests that the nutrients contained in multi-component fertilizers are more readily available to plants.

Key words: winter wheat, yield, macronutrients, multi-component fertilizers, uptake.

WPŁYW NAWOZÓW WIELOSKŁADNIKOWYCH NA PLON, ZAWARTOŚĆ I POBRANIE MAKROELEMENTÓW PRZEZ PSZENICE OZIMĄ

Abstrakt

Wybór nawozu wieloskładnikowego musi być poprzedzony wieloetapową analizą czynników, takich jak: gatunek rośliny, oczekiwany plon, zasobność gleby w składniki pokarmowe oraz termin i technika stosowania nawozów. Stosowanie nawozów wieloskładnikowych powinno odbywać się w sposób racjonalny, z uwzględnieniem wysokich i dobrych jakościowo plonów oraz efektywności nawożenia z jednoczesnym wykorzystaniem zalet agrochemicznych nawozów. Celem pracy była ocena skuteczności nawozowej Amofosmagu 4 i Amofosmagu 3 na plon, zawartość i pobranie makroelementów przez pszenicę ozimą oraz efektywność rolniczą. Trzyletnie doświadczenie polowe (2008-2010) przeprowadzono w Ośrodku Dydaktyczno-Doświadczalnym w Tomaszkowie należącym do Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. Doświadczenie, założone metodą losowanych bloków, obejmowało trzy obiekty nawozowe w czterech powtórzeniach: obiekt kontrolny (nawozy jednoskładnikowe) oraz dwa obiekty nawożone nawozami wieloskładnikowymi (Amofosmag 4 i Amofosmag 3). Rośliną testowaną była pszenica ozima odmiany Bogatka. Z badań wynika, że nawożenie Amofosmagiem 4 miało istotny wpływ na zwiększenie plonu ziarna pszenicy ozimej. Zawartość badanych makroelementów w pszenicy w poszczególnych obiektach nawozowych była zbliżona, zastosowane nawozy działały równorzędnie. Wyjątek stanowiła zawartość potasu w słomie, w której istotnie większą koncentrację tego składnika uzyskano pod wpływem nawozów wieloskładnikowych. Zróżnicowanie w składzie mineralnym badanej rośliny wystąpiło między poszczególnymi latami badań. Największe łączne pobranie azotu, fosforu i magnezu przez pszenicę ozimą stwierdzono po zastosowaniu Amofosmagu 4, a potasu i wapnia w obiekcie z Amofosmagiem 3. Największą efektywność rolniczą uzyskano stosując Amofosmag 4. Świadczy to o lepszej przyswajalności składników pokarmowych z nawozów wieloskładnikowych niż jednoskładnikowych.

Słowa kluczowe: pszenica ozima, plon, makroelementy, pobranie, nawozy wieloskładnikowe.

INTRODUCTION

Fertilization is the main yield-forming factor, and one of the key indicators of agricultural production intensity and efficiency. Due to the growing demand for agricultural produce and commodities used to produce biofuels, fertilizer use will increase in the coming years (ZALEWSKI 2009). The major advantages of modern fertilizers include easy application and storage, solubility and complex composition. Since the 1990s, there has been a steady increase in the share of multi-component fertilizers in total mineral fertilizer consumption. Numerous fertilizer manufacturers offer a wide variety and range of mixed fertilizers, blends and compound fertilizers so as to meet the farmers' specific needs. In Poland, the driving force behind fertilizer market development is growing awareness among farmers of the importance and profitability of new technological solutions (Potarzycki, Lewicka 2002). A clear advantage of multi-component fertilizers over simple fertilizers is that the former supply a combination of nutrients at a time (Glabisz et al. 1992). The yield of cereals is determined by their nutritional status at the early stages of growth. The optimum nutrient supply during the critical growth stages enables crops to reach their full yield potential (Gaj 2010). According to Chwil (2000), rational fertilization of winter wheat is determined by the amounts of primary nutrients supplied, and their relative proportions.

The objective of this study was to determine the effect of mixed multi-component fertilizers, Amofosmag 4 and Amofosmag 3, on winter wheat yield, and the content and uptake of macronutrients, and fertilization efficiency.

MATERIAL AND METHODS

A three-year field experiment (2008-2010) was carried out in a randomized block design at the Research and Experimental Station in Tomaszkowo, at the University of Warmia and Mazury in Olsztyn. The experiment, which comprised three fertilization treatments in four replications: control treatment (simple fertilizers), Amofosmag 4 and Amofosmag 3, was established on proper brown soil developed from sandy loam, of quality class III b and very good rye complex. The physicochemical properties of soil in each year of the study are presented in Table 1. The tested crop was winter wheat (*Triticum aestivum* L. *emend)* cv. Bogatka. The preceding plant was winter triticale. Plot surface area was 10 m^2.

Based on the average levels of available phosphorus in the soil, 350 kg ha^{-1} Amofosmag 3 (NPK Mg 3:14:20:2 + 22% CaO + 9% SO_3: 10.5 kg N, 21.5 kg P, 58 kg K, 55 kg Ca, 4 kg Mg, 12.5 kg S on pure ingredient basis)

Table 1

Selected physicochemical properties of soil used in the experiment (mg kg^{-1})

Year	pH in 1 M KCl	Available forms		
		P	K	Mg
2008	6.2	72	207	28
2009	7.0	84	149	35
2010	5.7	70	244	36

and Amofosmag 4 (NPK Mg 4:15:15:2 + 24% CaO + 9% SO_3 : 12 kg N, 23 P, 43.5 kg K, 60 kg Ca, 4 kg Mg, 12.5 kg S on pure ingredient basis) were applied pre-sowing. The nitrogen rate of 80 kg per ha was supplemented with two doses of ammonium nitrate applied by top-dressing in all treatments, including control. In the control treatment, the following fertilizers were applied pre-sowing: 14 kg N in the form of urea, 23 kg P in the form of triple superphosphate and 43.5 kg K kg ha^{-1} in the form of potash salt 60%.

Samples of winter wheat were collected at the stage of full maturity. The grain and straw harvested in each plot was dried and weighed individually. Wet mineralized samples were assayed for the content of: total nitrogen – by the hypochlorite method, phosphorus – by the vanadium-molybdenum method, calcium and potassium – by atomic emission spectrometry (AES), and magnesium – by atomic absorption spectrometry (AAS). The results of chemical analyses were verified statistically by a two-factorial analysis of variance for a randomized block design. The experimental factors were as follows: *a* – fertilization, *b* – duration of the experiment. The least significant difference was assumed at $p = 0.05$.

RESULTS AND DISCUSSION

Air temperatures in 2007 and 2008 were generally higher that the long-term average (Table 2). October and November were colder and drier than the multiannual average. The winter dormancy period of wheat differed significantly from that observed in previous years, and it was characterized by considerably higher temperatures and precipitation levels. Precipitation total in May and June was substantially lower than the multiannual average,

Table 2

Weather conditions in 2008-2010 (data from the Meteorological Station in Tomaszkowo)

Month	Mean monthly temperature (°C)				Precipitation total (mm)			
	2007/ 2008	2008/ 2009	2009/ 2010	1970 - -2000	2007/ 2008	2008/ 2009	2009/ 2010	1970 - -2000
September	12.6	11.8	14.2	12.5	57.9	22.9	25.7	59.0
October	7.5	8.6	5.8	7.8	30.2	82.8	55.7	43.4
November	1.3	4.0	5.0	2.7	33.8	29.4	43.8	47.7
December	0.5	0.1	-1.9	-1.3	27.8	35.1	30.6	36.2
January	0.7	-3.2	-9.0	-2.9	66.2	24.7	19.4	28.8
February	2.5	-2.0	-3.0	-2.4	24.7	31.7	22.5	20.4
March	3.0	1.3	2.1	1.2	52.4	57.9	36.7	26.8
April	7.6	9.4	8.1	6.9	31.4	4.8	18.2	36.1
May	12.3	12.4	12.0	12.7	27.0	52.9	131.9	51.9
June	16.9	14.9	16.4	15.9	32.7	136.9	84.8	79.3
July	18.5	20.4	21.1	17.7	57.7	48.3	80.4	73.8
August	18.4	17.6	19.3	17.2	102.1	19.3	95.3	67.1

which could have adversely affected spike development. August was very wet, which retarded the harvest. In the second year of the study (2008/2009), the distribution of temperatures and rainfalls supported the growth and development of winter wheat. Considerable water deficiency was noted only in April, when rainfall accounted for only 13% of the long-term average, but high water supply in March (two-fold higher than the long period average) as well as high precipitation levels in May and June were sufficient to maintain adequate soil moisture content. July was warm and relatively wet, while August was dry, which contributed to even ripening and kernel plumpness. In 2009/2010, mean monthly temperatures and precipitation totals differed from the long-term averages. A warm and sufficiently wet fall was followed by a harsh and precipitation-deficient winter. Water deficiency in April (rainfall levels two-fold lower than the long-term average) was compensated for by rainfall surpluses in the subsequent months of the growing season, especially May when precipitation total was over 2.5-fold higher than the long period average. According to CHMIELEWSKI (1992), and CHMIELEWSKI and KÖHN (2000), a constant supply of water and moderate temperatures in winter and early spring support the growth and yield of winter cereals (high coefficients of productive tillering). Weather conditions could have affected the yield of winter wheat.

In the first year of the study (2008), the yield of winter wheat grain ranged from 6.54 to 7.23 t ha^{-1}, and it was significantly affected by the type of fertilizer (Table 3). The highest average yield of winter wheat was noted in the Amofosmag 4 treatment – it was by over 10% higher than in the control and Amofosmag 3 treatment. Wheat straw yield corresponded to grain yield, and it showed no significant changes. In an experiment with spring wheat conducted by NOGALSKA et al. (2010), multi-component fertilizers had a more desirable yield-forming effect than simple fertilizers. In the second

Table 3

Winter wheat yield after the application of Amofosmag 4 and Amofosmag 3 (t ha^{-1})

Treatment	Grain				Straw			
	2008	2009	2010	mean for *a*	2008	2009	2010	mean for *a*
NPK	6.56	5.30	6.80	6.22	6.69	6.40	7.63	6.91
Amofosmag 4	7.23	5.43	7.35	6.67	7.11	7.09	8.26	7.49
Amofosmag 3	6.54	5.51	7.01	6.33	6.48	7.06	7.87	7.14
Mean for *b*	6.76	5.41	7.05		6.76	6.85	7.92	
LSD$_{p=0.05}$ for *a* *b* *ab*	0.33 0.35 n.s.*				n.s.* 0.69 n.s.*			

Key: *a* – fertilization, *b* – duration of the experiment, *ab* – interaction, n.s. – non-significant difference year of the study (2009), the yield of winter wheat grain varied from 5.30 to 5.51 t ha^{-1}, and it was significantly lower than in 2008 and 2010. Dry April, followed by cold and wet May and June could have reduced the number and size of wheat ears. According to ALARU et al. (2003), cereal grains are highly sensitive to weather conditions. In a study by OLESEN et al. (2009), the average yield of winter cereals ranged from 3.2 to 5.1 t ha^{-1}. In the third year of the experiment (2010), similarly as in the first year, Amofosmag 4 had the most beneficial influence on wheat grain yield, which was found to increase significantly, by around 8% and 4.8%, compared with the control and Amofosmag 3 treatment. The applied fertilizers had no significant effect on wheat straw yield, whereas significant differences were observed in this respect between successive years of the study. Straw yield was significantly higher in 2010 than in 2008 and 2009, which resulted from the highest grain yield in 2010 and favorable weather conditions.

The results of the present study show that Amofosmag 4 caused an approximately 7% and 8% increase (on average) in the yield of wheat grain and straw, respectively, compared with simple fertilizers. In an experiment by CHWIL (2000), winter wheat yield ranged from 5.89 to 7.90 t ha^{-1}. An increase in the yield of different cereal species in response to the application of mixed fertilizers was also reported by ZAWARTKA and SKWIERAWSKA (2004 b), and NOGALSKA et al. (2010, 2011).

The results of chemical analyses of winter wheat grain and straw show that the concentrations of the analyzed macronutrients varied insignificantly between treatments (Table 4), while significant differences were found between the years of the study. In the second year, the average nitrogen content of winter wheat grain reached 10.63 g N kg^{-1} d.m., and it was twofold and 1.5-fold lower compared with the third and the first year, respectively. In 2008, the concentrations of phosphorus, potassium and calcium in wheat grain were significantly lower than in the next two years. The magnesium content of wheat grain remained stable throughout the experiment, reaching the highest level in 2010. Similar macronutrient concentrations in wheat grain were reported by RACHOŃ and SZUMIŁO (2009). The findings of other authors (FILIPEK 2001, KRZYWY et al. 2001, NOGALSKA et al. 2010, 2011) indicate that multi-component fertilizers have no significant effect on the mineral composition of cereal grain.

Macronutrient uptake was estimated based on the yield and macronutrient content of winter wheat grain and straw. Nitrogen uptake by wheat plants was highest in the third year of the study (186.01 kg N ha^{-1}), after the application of Amofosmag 3. High nitrogen uptake resulted from a high wheat yield and the highest nitrogen content of plants in 2010 (Table 5). Similar results were reported by FOSSATI et al. (1993). Phosphorus uptake levels were comparable in all treatments, and they tended to increase in response to Amofosmag 4. Phosphorus uptake varied considerably between years, reaching the highest level in 2010 (38.1 to 42.9 kg P ha^{-1}) when the

Table 4

Macronutrient content of winter wheat after the application of Amofosmag 4 and Amofosmag 3 (g kg^{-1} d.m.)

Macro-nutrient	Treatment	Grain				Straw			
		2008	2009	2010	mean for *a*	2008	2009	2010	mean for *a*
Nitrogen	NPK	14.00	10.63	20.42	15.01	3.24	3.38	5.27	3.96
	Amofosmag 4	16.90	11.95	20.19	16.34	3.34	3.07	4.52	3.64
	Amofosmag 3	18.45	9.32	20.66	16.14	3.83	2.75	5.26	3.94
Mean for *b*		16.45	10.63	20.42		3.47	3.06	5.01	
LSD$_{p=0.05}$ for	a b *ab*	n.s. 1.527 2.645				n.s. 0.563 n.s.			
Phosphorus	NPK	1.86	4.38	3.72	3.32	0.46	2.10	1.68	1.41
	Amofosmag 4	1.98	4.51	3.96	3.48	0.45	1.78	1.39	1.20
	Amofosmag 3	1.77	4.53	4.22	3.50	0.41	1.54	1.48	1.14
Mean for *b*		1.87	4.47	3.96		0.44	1.80	1.51	
LSD$_{p=0.05}$ for	a *b* *ab*	n.s. 0.312 n.s.				n.s. 0.240 n.s.			
Potassium	NPK	2.77	4.35	4.34	3.82	8.37	8.17	12.99	9.84
	Amofosmag 4	2.65	4.40	4.31	3.78	8.80	11.10	13.86	11.25
	Amofosmag 3	2.47	4.42	4.39	3.76	9.00	10.00	19.11	12.70
Mean for *b*		2.63	4.39	4.34		8.72	9.75	15.32	
LSD$_{p=0.05}$ for	*a* *b* *ab*	n.s. 0.264 n.s.				1.298 1.278 2.214			
Calcium	NPK	0.44	0.45	0.53	0.47	2.37	1.93	3.66	2.65
	Amofosmag 4	0.40	0.50	0.51	0.47	3.02	2.07	3.47	2.85
	Amofosmag 3	0.33	0.53	0.58	0.48	3.61	1.97	3.63	3.05
Mean for *b*		0.39	0.49	0.54		3.00	1.99	3.58	
LSD$_{p=0.05}$ for	*a* *b* *ab*	n.s. 0.081 n.s.				n.s. 0.456 n.s.			
Magnesium	NPK	0.91	0.80	0.91	0.87	0.40	0.54	0.55	0.49
	Amofosmag 4	0.88	0.79	1.02	0.89	0.42	0.55	0.62	0.53
	Amofosmag 3	0.80	0.81	0.97	0.85	0.50	0.51	0.57	0.52
Mean for *b*		0.86	0.80	0.96		0.44	0.53	0.58	
LSD$_{p=0.05}$ for	*a* *b* *ab*	n.s. 0.096 n.s.				n.s. 0.063 n.s.			

Explanations as under Table 3

Table 5

Nutrient uptake by winter wheat grain and straw (kg ha^{-1})

Treatment	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
2008					
NPK	113.51	15.2	74.16	18.73	8.63
Amofosmag 4	145.92	17.5	81.71	24.36	9.34
Amofosmag 3	144.91	14.2	74.39	25.53	8.44
2009					
NPK	77.96	36.6	76.77	14.73	7.69
Amofosmag 4	86.64	37.1	102.58	17.38	8.17
Amofosmag 3	70.67	35.8	94.91	16.81	8.05
2010					
NPK	179.06	38.1	128.62	31.52	10.37
Amofosmag 4	185.72	42.9	146.15	32.43	12.61
Amofosmag 3	186.01	41.2	181.12	32.62	11.27

highest yield of wheat grain and straw was obtained, and the lowest in 2008 (approximately two-fold lower than in 2009 and 2010) when wheat kernels were least abundant in phosphorus. The highest uptake of calcium (except in 2010) and magnesium was noted in the Amofosmag 4 treatment. Potassium uptake by winter wheat plants was highest in the third year of the experiment, in particular after the application of Amofosmag 3, mostly due to a high potassium content of wheat plants, especially straw. Such a trend was also observed with regard to total potassium uptake over three years (Figure 1). The highest total uptake of nitrogen, phosphorus and magnesium was observed in treatments with Amofosmag 4, which may suggest that the nutrients contained in multi-component fertilizers are more readily available to plants. Similar results were reported by NOGALSKA et al. (2010, 2011), who applied mixed fertilizers to spring wheat and spring barley.

Fertilization efficiency, expressed as an increase in wheat grain yield per kg NPK, ranged from 26.30 to 37.41 kg throughout the experiment (Table 6). Amofosmag 4 was found to be most efficient (27.63-37.41 kg wheat grain kg^{-1} NPK, depending on the year). Amofosmag 4 increased grain yield per kg NPK by 3.27 kg (10.7%) and 3.62 kg (11.9%) on average, in comparison to simple fertilizers and Amofosmag 3, respectively. The lowest fertilization efficiency was noted in the second year of the study – a drop by over 20% compared with the first and the third year. Considerable rainfall fluctuations (deficiency in April and surplus from June to September 2009) contributed to a decrease in winter wheat grain yield.

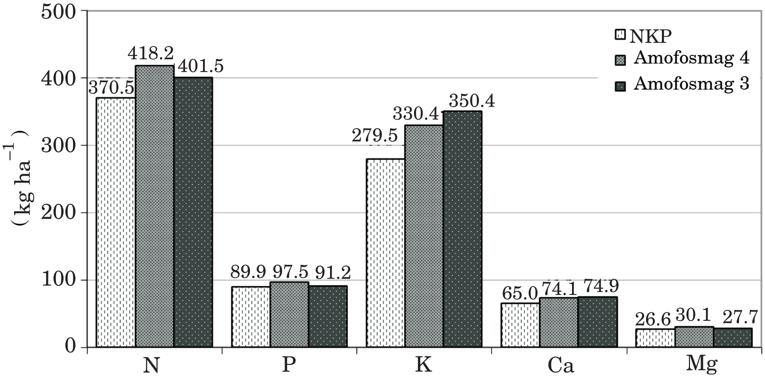

Fig. 1. Total macronutrient uptake by winter wheat over the three-year experiment

Table 6

Fertilization efficiency (kg grain kg^{-1} NPK)

Treatment	Grain			
	2008	2009	2010	mean for *a*
NPK	33.39	26.97	31.66	30.67
Amofosmag 4	36.80	27.63	37.41	33.94
Amofosmag 3	31.22	26.30	33.46	30.32
Mean for *b*	33.80	26.96	34.17	-

CONCLUSIONS

1. Winter wheat grain yield was modified by fertilizers and weather conditions. The most beneficial effect was reported for Amofosmag 4, which increased the yield of wheat grain by 7% on average, compared with the remaining treatments. A significantly lower grain yield was noted in the second year of the study characterized by adverse weather conditions.

2. The concentrations of the analyzed macronutrients in winter wheat grain and straw varied insignificantly between fertilization treatments. Simple and multi-component fertilizers exerted a comparable effect on the mineral composition of the test crop. The only exception was the potassium content of wheat straw, which was significantly higher after the application of multi-component fertilizers. Significant differences were observed in this respect between successive years of the study.

3. The highest total uptake of nitrogen, phosphorus and magnesium by winter wheat plants was noted in the Amofosmag 4 treatment, and the highest total uptake of potassium and calcium was observed in the Amofosmag 3 treatment. Amofosmag 4 was found to be the most efficient fertilizer, which suggests that the nutrients contained in multi-component fertilizers are more readily available to plants.

REFERENCES

ALARU M., LAUR Ü., JAAMA E. 2003. *Influence of nitrogen and weather conditions on the grain quality of winter triticale.* Agronomy Res., 1: 3-10.

CHMIELEWSKI F.M., KÖHN W. 2000. *Impact of weather on yield components of winter rye over 30 years.* Agr. Forest Meteorol., 102: 253-261.

CHMIELEWSKI, F.M. 1992. *The impact of climate changes on the crop yields of winter-rye in Halle during 1901–1980.* Clim. Res., 2: 23-33.

CHWIL S. 2000. *Influence of mineral fertilization intensity on winter wheat field and magnesium content in soil and plant.* Biul. Magnezol., 5(4): 278-283. (in Polish)

FILIPEK T. 2001. *The content nutrients and application of multicomponent fertilizers from KIZPS Siarkopol*. Fol. Univ. Agric. Stetin., Agricultura, 223(89): 41-46. (in Polish)

FOSSATI D., FOSSATI A., FEIL B. 1993.*Relationship between grain yield and grain nitrogen concentration in winter triticale.* Euphytica, 71: 115-123.

GAJ R. 2010. *Influence of different potassium fertilization level on nutritional status of winter wheat and on yield during critical growth stage*. J. Elementol., 15(4): 629-637.

GLABISZ U., KIC B., GRZMIL B. 1992. *Manufacture of low chloride multicomponent fertilizers based on conversion in aqueous solution.* J. Agric. Food Chem., 40: 1393-1397.

KRZYWY E., JAKUBOWSKI W., KRZYWY J. 2001. *The influence of multi- and one- component fertilizers on the amount and some qualitative properties of plant yields.* Part I. *The influence of multi- and one- component fertilizers on the yield of plants and the contents of nitrogen, phosphorous, potassium, calcium, magnesium and sulphur in the crop.* Fol. Univ. Agric. Stetin., Agricultura, 223(89): 99-106. (in Polish)

NOGALSKA A., CZAPLA J. SKWIERAWSKA M. 2010. *The effect of multi-component fertilizers on spring wheat yield, the content and uptake of macronutrients*. Pol. J. Natur. Sc., 25(4): 323-331.

NOGALSKA A., CZAPLA J. SKWIERAWSKA M. 2011. *The effect of multi-component fertilizers on spring barley yield, the content and uptake of macronutrients*. Pol. J. Natur. Sc., 26(2): 89-97.

OLESEN J. E., ASKEGAARD M., RASMUSSEN I. A. 2009. *Winter cereal yields as affected by animal manure and green manure in organic arable farming.* Eur. J. Agron., 30: 119-128.

POTARZYCKI J., LEWICKA L. 2002. *Yielding efficiency of multicomponent fertilizers in sugar beet cultivation.* Biul. IHAR, 222: 111-118. (in Polish)

RACHOŃ L., SZUMIŁO G. 2009. *Comparison of chemical composition of selected winter wheat species.* J. Elementol., 14(1):135-146.

ZALEWSKI A. 2009. *Situation on the market of mineral fertilizers in 2008.* J. Agribus. Rural Dev., 2(12): 279-286. (in Polish)

ZAWARTKA L., SKWIERAWSKA M. 2004. *Influence of multicomponent fertilisers on the crop field and phosphorus and other macroelements content in spring barley.* Pr. Nauk. AE Wrocław, Chemia, 1017: 149-157. (in Polish)

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THE INFLUENCE OF PRE-SOWING STIMULATION OF SEEDS ON CHANGES IN CHEMICAL COMPOSITION AND SUCROSE CONTENT IN SUGAR BEET

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Abstract

The changes of chemical composition and sucrose content in sugar beet beet after seeds stimulation were investigated. The seeds came from the energ'hill technology (Eh) and were subject to laser irradiation. The experiments were conducted in the laboratory and in field conditions in 2008-2010. In the experiment the researchers used the seed material of Tiziana variety (sugar type) in standard version (control) and stimulated: prepared in the energ'hill technology and irradiated. Irradiation with laser light was applied in the following doses: the 5-time multiplied (in the study marked as D5) and 7-time multiplied (in the study D7) of the basic dose 2.5 10^{-1}J cm^{-2}. The content of nitrogen, phosphorus, potassium, sodium, magnesium and calcium in leaves and roots samples collected in the first weeks of July, August, September and October. Pre-sowing stimulation with use of energ'hill technology and laser light irradiation resulted in an increase in leaf concentration of nitrogen, potassium, sodium and magnesium and the content of these elements positively correlated with sucrose content of mature roots. Pre-preparation (sprouted) clusters energ'hill technology and laser irradiation had a positive effect on sucrose content and its performance.

Key words: sugar beet, seeds stimulation, mineral macronutrients, sucrose.

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WPŁYW PRZEDSIEWNEJ STYMULACJI NASION NA ZMIANY SKŁADU CHEMICZNEGO I ZAWARTOŚĆ CUKRU W BURAKU CUKROWYM

Abstrakt

Zmiany składu chemicznego i zawartości sacharozy w buraku cukrowym analizowano pod wpływem przedsiewnej stymulacji nasion przygotowanych w technologii energ'hill i naświetlanych światłem lasera półprzewodnikowego. W badaniach polowych i laboratoryjnych przeprowadzonych w latach 2008 – 2010, z odmianą Tiziana, wysiewano kłębki standardowe (niestymulowane) i kondycjonowane w technologii energ'hill oraz naświetlane światłem lasera półprzewodnikowego w dawkach pięciokrotnej (D5) i siedmiokrotnej (D7) w stosunku do dawki podstawowej (2,5 10^{-1}J cm^{-2}). Zawartość azotu, fosforu, potasu, magnezu, sodu i wapnia w korzeniach i liściach analizowano w pierwszych tygodniach lipca, sierpnia, września i października. Przedsiewna stymulacja nasion w technologii energ'hill i naświetlanie światłem lasera powodowały w liściach wzrost koncentracji azotu, potasu, sodu oraz magnezu, a zawartość tych pierwiastków dodatnio korelowała z zawartością sacharozy w korzeniach dojrzałych. Przedsiewne przygotowanie (kondycjonowanie) kłębków w technologii energ'hill i naświetlanie światłem lasera wpłynęły pozytywnie na zawartość sacharozy i jej wydajność.

Słowa kluczowe: burak cukrowy, stymulacja przedsiewna, makroskładniki mineralne, sacharoza.

INTRODUCTION

In the process of sugar beet seeds improvement mechanical and chemical methods are commonly used. The former specify the calibration and shape of beet seed balls and the latter protect seedlings against pathogens in the early stages of their development. Researchers also analyze the possibility of pre-sowing stimulation of sugar beet by other methods, such as the influence of the electric field, magnetic field, microwave radiation, ionizing radiation, visible light, millimeter radiation and laser radiation (HERNANDEZ et al. 2010, KACHARAVA et al. 2009, KOPER et al. 1996, MARINKOVIĆ 2008, PIETRUSZEWSKI, WÓJCIK 2000a,b, PROŚBA et al. 2011, ROCHALSKA 2005, ROCHALSKA, ORZESZKO-RYWKA 2008, VASILEVSKI 2003, WÓJCIK 2006, WÓJCIK et al. 2004). The results of the experiments show the beneficial effects of these methods on yield and some features of the technological quality of roots. The study also highlights the beneficial effects of pre-sowing stimulation especially in stressful conditions or in conditions that are unfavorable for the emergence and the early development. The impact of pre-sowing stimulation of seeds according to researchers depends on the weather during the emergence, the vegetation conditions as well as on the genotype of the variety and fertilization.

In the studies on the effects of pre-sowing beet seeds stimulation on the content and efficiency of sugar the following features were analyzed: the changes in macro-mineral elements in roots and leaves during active accumulation of mass yield and in mature roots as well as the relationship between the content of these elements in roots and leaves and the sucrose concentration.

MATERIALS AND METHODS

In the field experiment, established by split–plot method in three replications and in the laboratory studies conducted in 2008–2010, the changes in concentration of mineral macronutrients and the sucrose content and yield under the influence of seeds laser stimulation and seeds improvement in the energ'hill technology (at work Eh) were analyzed. In the experiment the researchers used the seed material of the variety Tiziana (sugar type) in standard – unstimulated version and stimulated – prepared in Eh technology and irradiated. The energ'hill technology is based on priming that triggers a seed to initial phases of germination. As a result the enzymes synthesizing proteins are activated and the metabolism of spare materials begins. Irradiation with laser light was applied in the following doses: the 5-time multiplied (in the study marked as D5) and 7-time multiplied (in the study D7) of the basic dose 2.5 10^{-1} J cm^{-2}. Beet seeds balls irradiation with the use of semiconductor laser light (model CTL – 1106 MX) was carried out on the very day when the field experiment was being established and the irradiated surface was determined by the use of the scanner (model CTL 1202 S) co–operating with the laser.

The field experiment was carried on the soils classified as Haplic Luvisols (FAO WRB 2007) developed from sandy loam underlain by a sandy clay loam (soil texture – according to Polish classification of soil grain size distribution, PTG, 2008, based on USDA). Soils were slightly acidic in reaction (pH 5.9-6.4). The soil was characterized by a good abundance of assimilable phosphorus (77-99 mg P kg^{-1}), potassium (158-240 mg K kg^{-1}) and magnesium (57-67 mg Mg kg^{-1}).

Thermal conditions during the study did not impede the sugar beet vegetation and temperatures were close to average temperatures of the last 30 years (Figure 1). Precipitation distribution, however, was characterized by large fluctuations, especially in the years 2009 and 2010 during the rapid growth of the root mass and accumulation of sucrose. Rainfall exceeding twice the average monthly amount for the last 30 years occurred in July and August in 2009 and in August and September in 2010.

The content of nitrogen, phosphorus, potassium, sodium, magnesium and calcium in the dry-mineralized leaves and roots samples collected in the first weeks of July, August, September and October in all field reps was marked on the 210VGP Atomic Absorption Spectrophotometer. The sucrose content and other characteristics of the technological quality of mature roots were determined on the automatic line Venema.

The content of the analyzed elements (separately for each consecutive month) as well as the sucrose content and yield were analyzed statistically by the means of the analysis of variance. To compare the mean values Duncan's test was used and the correlation at the level of confidence $\alpha = 0.05$,

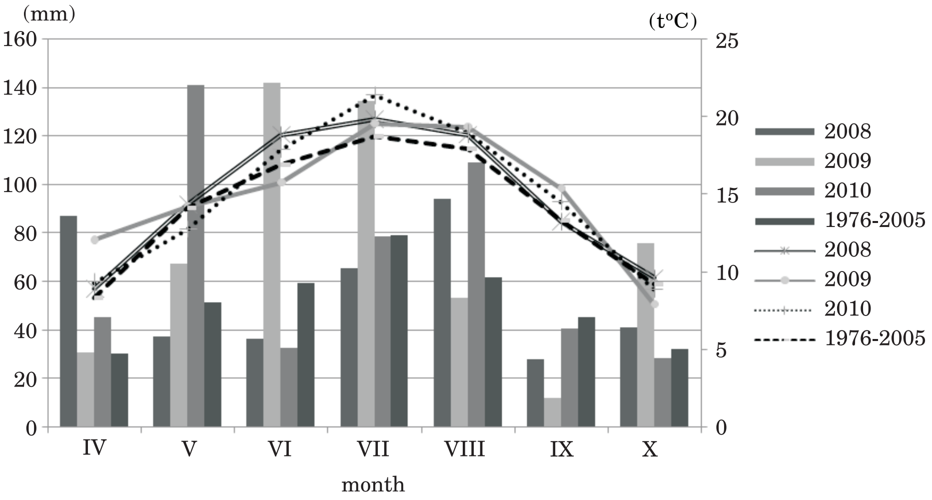

Fig. 1. Average monthly temperature and precipitation on the background of the multi-year average

$\alpha = 0.01$ and $\alpha = 0.001$ was calculated. The paper presents the content of macronutrients in the elemental form as average results for the years of research and for the objects of the experiment.

RESULTS AND DISCUSSION

The content of nitrogen and minerals in sugar beet has been the subject of numerous experiments in which their variability due to fertilizing, tillage, varieties and weather conditions was observed. The authors have demonstrated proven relationship between the level of concentration of mineral nitrogen and macronutrients and certain features of technological value of roots, especially sugar and molasses forming substances – α-amino nitrogen and sodium and potassium cations (ALLISON et al. 1997, SŁOWIŃSKI et al. 1997, BARŁÓG et al. 2002, BARŁÓG, GRZEBISZ 2004, DIATTA 2004, PROŚBA-BIAŁCZYK 2003, 2005a,b, PROŚBA-BIAŁCZYK, MYDLARSKI 2001).

In the study the content of nitrogen and mineral macroelements in plants – the roots and leaves – depending on the pre-sowing stimulation of seeds was analyzed (Table 1–6). The plants developed from Eh stimulated seeds and those developed from irradiated beet seed balls were characterized (in the leaves and roots) by higher concentrations of nitrogen than the plants emerged from standard seeds. The nitrogen content in leaves in the consecutive months – July, August, September and October – positively correlated with the sucrose content. Nitrogen in roots – from July to September – also positively affected the accumulation of sucrose, whereas in mature roots negative correlation between the content of nitrogen and sucrose was observed (Table 7).

Table 1

Effect of stimulation of seeds on the nitrogen content in dry mater in leaves and roots of sugar beet (g kg^{-1})

Method of stimulation	Designation term							
	leaves				roots			
	VII	VIII	IX	X	VII	VIII	IX	X
Control	31.05	23.05	19.55	18.83	12.44	8.28	7.70	6.70
Energ'hill	32.15	26.15	23.08	22.54	13.55	8.85	8.65	7.00
Laser D5	33.10	28.10	23.53	22.05	14.04	9.37	8.80	7.40
Laser D7	32.50	28.78	22.55	21.50	13.90	9.04	9.09	7.05
LSD	1.53	1.26	2.50	2.55	0.85	0.56	0.60	0.65

The phosphorus content in leaves showed no differences under the influence of seeds preparation in Eh technology and laser irradiation in comparison with unstimulated seeds. In roots, however, the influence of stimulation was observed. From August until the end of the growing season, the roots developed from the irradiated seeds were characterized by the higher amount of phosphorus (Table 2). Phosphorus accumulated in the leaves in July and August did not correlate with the content of sucrose. Yet, in September and October in leaves, and in all months in roots, a positive influence of phosphorus on sucrose content was noted (Table 7). Significant positive correlation between the content of phosphorus in the roots and the concentration of sucrose was also found in other studies (PROŚBA-BIAŁCZYK 2005b).

Table 2

Effect of stimulation of seeds on the phosphorus content in leaves and roots of sugar beet (g kg^{-1})

Method of stimulation	Designation term							
	leaves				roots			
	VII	VIII	IX	X	VII	VIII	IX	X
Control	1.74	1.88	2.05	1.90	0.90	1.75	1.75	1.80
Energ'hill	1.85	1.95	2.05	2.08	0.79	1.80	1.85	1.86
Laser D5	1.80	1.95	2.10	1.95	0.83	1.84	2.08	2.15
Laser D7	1.86	2.02	2.28	1.90	0.85	2.02	2.05	2.14
LSD	i. d.	i. d.	i. d.	i. d.	i. d.	0.25	0.25	0.25

Pre-sowing stimulation of seeds had impact on (from July to harvest) the concentration of potassium in leaves and roots (Table 3). The leaves of plants developed from Eh seeds and laser irradiated seeds contained more potassium than the leaves developed from unstimulated seeds. The content of this element in leaves significantly and positively correlated with sugar content in mature roots. In the roots from the irradiated seeds the concentration of potassium (in July, August and September) was higher than in the roots developed from unstimulated seeds. In mature roots, however, the lowest potassium level was observed when they developed from Eh seeds and laser irradiated at D_5 dose. Potassium in roots significantly and negatively affected the sucrose content (Table 7).

Table 3

Effect of stimulation of seeds on the potassium content in leaves and roots of sugar beet (g kg^{-1})

Method of stimulation	Designation term							
	leaves				roots			
	VII	VIII	IX	X	VII	VIII	IX	X
Control	32.28	34.16	27.47	25.85	12.56	8.55	8.54	8.55
Energ'hill	33.87	35.65	28.96	27.95	13.55	9.05	8.55	8.07
Laser D5	34.95	35.45	29.25	29.03	14.18	9.96	9.58	8.06
Laser D7	34.15	36.05	29.85	29.50	13.85	9.75	9.56	8.25
LSD	1.25	1.25	1.28	1.58	1.05	0.88	0.46	0.57

Table 4

Effect of stimulation of seeds on the sodium content in leaves and roots of sugar beet (g kg^{-1})

Method of stimulation	Designation term							
	leaves				roots			
	VII	VIII	IX	X	VII	VIII	IX	X
Control	5.85	9.66	9.86	6.95	0.86	1.00	0.73	0.50
Energ'hill	6.57	11.53	11.58	7.94	1.14	1.55	0.65	0.45
Laser D5	6.57	11.28	11.83	8.56	1.35	1.44	0.65	0.38
Laser D7	6.58	10.90	12.05	8.35	1.25	1.55	0.69	0.39
LSD	0.65	1.05	0.95	0.84	0.44	0.38	i. d.	i. d.

Preparation of seeds in Eh technology and laser stimulation of seeds modified the sodium content in plants. The leaves of plants from stimulated seeds were characterized by (from July to the end of vegetation) higher sodium content than the leaves of plants developed from standard seeds. In roots significant differences in sodium content under the influence of stimulation were noted in July and August during the period of intensive growth of root mass. In these months the roots of plants from stimulated seeds contained more sodium cations than the roots of plants developed from unstimulated seeds.The sodium content in leaves in the consecutive months – July, August, September and October – had positive influence on sucrose content. However, in roots a significant correlation between sodium and sucrose was observed in August and September. In mature roots, on the other hand, sodium negatively correlated with sucrose content. Therefore the study confirmed the significant effect of sodium concentration on the sucrose content (PROŚBA-BIAŁCZYK, MYDLARSKI 2001).

Pre-sowing seeds stimulation significantly modified the magnesium content (Table 5). The leaves and roots of plants developed from Eh seeds and from irradiated seeds from July to the end of the growing season were characterized by a higher concentration of magnesium than the leaves and roots developed from the standard seeds. Magnesium accumulated in leaves during the whole vegetation period significantly and positively affected the sucrose content while magnesium in roots did not correlate with the content of sucrose (Table 7). The increased amount of magnesium in the leaves of plants from stimulated seeds can be connected with the bigger amount of carotenoids and chlorophyll in the plants developed from stimulated seeds (PROŚBA-BIAŁCZYK et al. 201).

Table 5

Effect of stimulation of seeds on the magnesium content in leaves and roots of sugar beet (g kg^{-1})

Method of stimulation	Designation term							
	leaves				roots			
	VII	VIII	IX	X	VII	VIII	IX	X
Control	12.02	13.44	9.70	8.56	2.00	2.00	2.04	2.20
Energ'hill	12.95	14.35	10.40	9.36	2.65	2.55	2.55	2.48
Laser D5	13.58	14.82	10.55	9.45	2.75	2.74	2.75	2.40
Laser D7	13.72	14.54	10.45	9.28	2.85	2.50	2.50	2.55
LSD	0.44	0.80	0.42	0.45	0.36	0.45	0.47	0.36

The calcium content under the influence of pre–sowing seeds stimulation varied the leaves – in August, September and October. The leaves of plants developed from irradiated seeds were characterized by a lower level of calcium than the leaves of plants developed from Eh seeds and standard seeds (Table 6). Calcium accumulated in leaves had a positive impact on the sucrose content (Table 7).

Table 6

Effect of stimulation of seeds on the calcium content in leaves and roots of sugar beet (g kg^{-1})

Method of stimulation	Designation term							
	leaves				roots			
	VII	VIII	IX	X	VII	VIII	IX	X
Control	13.00	12.60	6.70	6.09	0.75	0.66	0.60	0.50
Energ'hill	13.30	12.70	6.55	6.20	0.70	0.65	0.50	0.53
Laser D5	13.15	11.85	5.87	5.13	0.85	0.40	0.58	0.44
Laser D7	13.38	12.04	5.30	5.15	0.88	0.44	0.48	0.48
LSD	i. d.	0.58	0.45	0.50	i. d.	i. d.	i. d.	i. d.

Table 7

Simple correlation coefficients between the content of macroelements in roots and leaves and sucrose content

Elements	Designation term							
	leaves				roots			
	VII	VIII	IX	X	VII	VIII	IX	X
Nitrogen	0.28**	0.30***	0.34***	0.28**	0.20*	0.28**	0.23**	- 0.15*
Phosphorus	0.13	0.13	0.26**	0.32**	0.15*	0.33***	0.44***	0.45***
Potassium	0.30***	0.44***	0.49***	0.55***	-0.20*	-0.34***	- 0.56***	- 0.49***
Sodium	0.44***	0.37***	0.32***	0.44***	0.10	0.30***	0.28**	- 0.23**
Magnesium	0.49***	0.46***	0.44***	0.15*	0.10	0.15*	0.10	0.10
Calcium	0.32**	0.45***	0.55***	0.32**	-0.12	0.09	0.09	0.13

* $\alpha = 0.05$
** $\alpha = 0.01$
***$\alpha = 0.001$

Pre-sowing seed stimulation resulted in significant changes in the sucrose content and yield (Table 8). The plants developed from seeds prepared in Eh technology and laser-irradiated were characterized by higher content and higher yield of sucrose than the plants developed from un-stimulated seeds. The results confirm the positive impact of laser irradiation on the yield and sugar content as also shown in earlier studies of beet seed stimulation (Koper et al. 1996, Siddiqui et al. 2006, Hernandez et al. 2010, Prośba-Białczyk et al. 2011). Moreover, in our research the positive influence of Eh technology was observed. The leaves of plants emerged from the Eh beet seed balls were characterized by (in the period of root growth and the accumulation of sucrose) higher nitrogen, potassium, sodium and magnesium content which in turn had a positive impact on sacharose content.

Table 8

Influence of stimulation of seeds on sugar content and yield

Method of stimulation	The sugar content	Biological productivity of sugar	Technological sugar yield
Control	16.70	9.82	8.65
Energ'hill	16.13	11.18	10.35
Laser D5	16.30	11.05	10.05
Laseer D7	16.49	11.03	9.95
LSD	0.18	1.05	1.18

CONCLUSIONS

1. Pre-sowing stimulation in the energ'hill technology and semiconductor laser irradiation in the period of intensive growth of plants modified their chemical composition and sucrose content.
2. Stimulating seeds in the energ'hill technology and laser irradiation resulted in an increased concentration of nitrogen, potassium, sodium and magnesium in leaves and the content of these elements positively correlated with the sucrose content of mature roots.
3. Pre-preparation (priming) of beet seed balls with the use of the energ'hill technology and laser irradiation had a positive effect on sucrose content and its yield.

REFERENCES

Allison M. N., Chapman J. L., Garat C. E., Todd A. D. 1997. *The potassium, sodium, magnesium, calcium and phosphate nutrition of sugar beet (Beta vulgaris) grown on soils containing incorporated straw.* J. Sci. Food Agric., 74(2): 220-261.

Barłóg P., Grzebisz W. 2004. *Yield-forming and diagnostic evaluation of fertilization of sugar beets with potassium, sodium and magnesium.* Part III. *Predicting yield and quality.* Biul. IHAR, Radzików, 234: 73-82.

Barłóg P., Grzebisz W., Górski D., Gaj R. 2002. *Response of high-yield cultivars of sugar beet to potassium, sodium and magnesium fertilization.* Biul. IHAR, Radzików, 222: 135-142.

FAO WRB, 2007. *World Reference Base for Soil Resources.* 2006, update 2007. FAO, ISRIC, ISSS. Rome.

Hernandez A. C., Dominguez P. A., Cruz O. A., Ivanov R., Caballo C. A., Zepeda B. R. 2010. *Laser in agriculture.* Int. Agroph., 24: 407-422.

Kacharava N., Chanishvili S., Badridze G., Chkhubianshvili E., Janukashvili N. 2009. *Effect of seed irradiation on the content of antioxidants in leaves of Kidney bean, cabbage and beet cultivars.* Australian J. Crop Sci., 3(3): 137-145.

KOPER R., WÓJCIK S., KORNAS-CZUCZWAR B., BOJARSKA U. 1996. *Effect of the laser exposure of seeds on the field and chemic composition of sugar beet roots.* Int. Agroph., 10: 103-108.

MARINKOVIĆ B., GRUJIĆ M., MARINKOVIĆ D., CRNOBARAC J., MARINKOVIĆ J., JAĆIMOVIĆ G., MIRCOV D. V. 2008. *Use of biophysical methods to improve yields and quality of agricultural products.* J. Agric. Sci., 53(3): 235-241.

PIETRUSZEWSKI S., WÓJCIK S. 2000a. *Effect of magnetic field on yield and chemical composition of sugar beet roots.* Int. Agroph., 14: 89-92.

PIETRUSZEWSKI S., WÓJCIK S. 2000b. *Effect of magnetic field on yield of sugar beet cultivar Kalwia and Polko.* Inż. Rol., 5(16): 207-210. (in Polish)

PROŚBA-BIAŁCZYK U. 2003. *Effect of fertilization on the content of sodium and productivity of sugar beet.* J. Elementol., 8(1): 5-14.

PROŚBA-BIAŁCZYK U. 2005a. *Influence of fertilization on content and intake of potassium and productivity of sugar beet (Beta vulgaris).* Part 1. J. Elementol., 10(3): 571-584.

PROŚBA-BIAŁCZYK U. 2005b. *Effect of fertilization on concentration and uptake of phosphorus as well as productivity of sugar beet.* J. Elementol., 10(1): 99-112.

PROŚBA-BIAŁCZYK U., MYDLARSKI M. 2001. *Effect of sodium chloride in foliar application on sugar beet productivity and technological value.* Biul. IHAR, 222: 215-222.

PROŚBA-BIAŁCZYK U., SZAJSNER H., GRZYŚ E., DEMCZUK A., SACAŁA E. 2011. *Effect of seeds stimulation on sugar beet productivity. Listy cukrovarnicke a reparske,* 127: 344-347. (in Czech)

ROCHALSKA M., ORZESZKO-RYWKA A. 2008. *Influence of alternating magnetic field on respiration of sugar beet seeds.* Int. Agroph., 22: 255-259.

SIDDIQUI M.H., KHAN M.M.A., KHAN M.N., MOHAMMAD F., NAEEM M. 2006. *Hill reaction, photosynthesis and chlorophyll content in non-sugar-producing (Turnip, Brassica rapa* L.*) and sugar-producing (Sugar beet, Beta vulgaris* L.*) root crop plants.* Turkish J. Biol., 30: 153-155.

SŁOWIŃSKI H., PROŚBA-BIAŁCZYK U., PYTLARZ-KOZICKA M., NOWAK W. 1997. *Effect of organic and mineral fertilization on content of macronutrients and sugar in sugar beet.* Biul. IHAR, 202: 149-157.

VASILEVSKI G. 2003. *Perspectives of the application of biophysical methods in sustainable agriculture.* Bulg. J. Plant Physiol., Special Issue, 179-186.

WÓJCIK S. 2006. *Yields and technological quality of sugar beet roots in response to seed stimulation.* Inż. Rol., 6: 383-388.

WÓJCIK S., DZIAMBA M., PIETRUSZEWSKI S. 2004. *Effect of microwave radiation on the field and technological quality of sugar beet roots.* Acta Agroph., 3(3): 623-63.

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CONTENT OF AVAILABLE Cu, Zn AND Mn IN SOIL AMENDED WITH MUNICIPAL SEWAGE SLUDGE

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Abstract

Municipal sewage sludge is an unavoidable byproduct of the contemporary live and business activities of people. Proper handling and utilization of sewage sludge continue to create serious problems in Poland although this waste product is a source of both organic carbon and macronutrients. The present study has been carried out in order to assess the influence of municipal sewage sludge on the content of available forms of Cu, Zn and Mn in soil. A microplot experiment set up according to the random sub-block method was conducted in 2005-2008. The experiment was established on anthropogenic, humic urban soil developed from loamy sand rich in phosphorus and magnesium, but poor in potassium, and alkaline in reaction. The design of the trials comprised 5 doses of municipal sewage sludge from the Łyna Municipal Wastewater Treatment Plant in Olsztyn: 0, 70, 140, 210 and 280 Mg ha^{-1} of fresh matter. The tests have demonstrated that a dose of sewage sludge had a significant effect on the content of available forms of Cu, Zn and Mn in soil. In alkaline soil, however, the observed increase in the content of available forms of Cu, Zn and Mn was not hazardous to the environment, but could improve the plant nutrition with these elements. It is highly probable that the availability of Cu may increase in the second and third year after the application of sewage sludge. The accumulation of soluble Zn in soil started to decrease in the second year, but did not become significantly limited until four years after sewage sludge application. Sewage sludge raised the amount of soluble manganese in soil during the first three years, but in the final year of the experiment the quantity of soluble Mn in soil did not undergo any significant fluctuations.

Key words: sewage sludge, available forms of Cu, Zn and Mn.

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ZAWARTOŚĆ PRZYSWAJALNYCH FORM Cu, Zn I Mn W GLEBIE UŻYŹNIONEJ KOMUNALNYM OSADEM ŚCIEKOWYM

Komunalne osady ściekowe są nieodzownym produktem działalności bytowo-gospodarczej współczesnego człowieka, a ich zagospodarowanie to wciąż poważny problem w Polsce. Odpad ten jest nie tylko źródłem węgla organicznego i makroelementów, ale także mikroelementów. Celem badań była ocena wpływu komunalnego osadu ściekowego na zawartość przyswajalnych form Cu, Zn i Mn w glebie. Mikropoletkowe doświadczenie prowadzono w latach 2005-2008 metodą losowanych podbloków na glebie antropogenicznej urbanoziemnej próchnicznej wytworzonej z piasku gliniastego o wysokiej zawartości fosforu i magnezu oraz niskiej potasu i o odczynie zasadowym. Schemat doświadczenia obejmował 5 dawek komunalnego osadu ściekowego z Miejskiej Oczyszczalni Ścieków „Łyna" w Olsztynie: 0, 70, 140, 210 i 280 Mg ha^{-1} świeżej masy. W badaniach udowodniono istotny wpływ dawki osadu ściekowego na zawartość przyswajalnych form miedzi, cynku i manganu w glebie. W warunkach gleby zasadowej wzrost zawartości przyswajalnych form Cu, Zn i Mn w glebie nie stwarzał zagrożenia dla środowiska, a jedynie mógł poprawić odżywienie roślin tymi mikroelementami. Z dużym prawdopodobieństwem można oczekiwać wzrostu dostępności Cu przyswajalnej w glebie w 2. i 3. roku po zastosowaniu osadu ściekowego. Nagromadzenie rozpuszczalnego Zn w glebie zmniejszało się od 2. roku, ale istotne ograniczenie wystąpiło dopiero w 4. roku po zastosowaniu osadu ściekowego. Osad ściekowy miał wpływ na zwiększenie ilości rozpuszczalnego manganu w glebie w pierwszych trzech latach, a w ostatnim roku ilość rozpuszczalnego manganu w glebie nie uległa już znaczącym zmianom.

Słowa kluczowe: osad ściekowy, przyswajalne formy Cu, Zn i Mn.

INTRODUCTION

Waste containing organic matter and biogenic elements should be managed – as long as possible – so as to return it to the environment (SŘREN et al. 2003, SPEIR et al. 2003, CASADO-VELA et al. 2006, SPYCHAJ-FABISIAK et al. 2007, CZEKAŁA 2009, MAZUR, SIENKIEWICZ 2009). In the contemporary world, municipal sewage sludge is an unavoidable byproduct of our live and business activities, but its proper handling and utilization continue to create problems in Poland. It is the organic carbon contained in sewage sludge that draws most attention as an element which should be reintroduced into the soil-plant cycle. The positive effect of sewage sludge on soil properties has been evidenced in numerous papers by Polish and foreign researchers (STĘPIEŃ et al. 2000, JAKUBUS 2006, SĄDEJ et al. 2007, DELIBACAK ET AL. 2009, SINGH and AGRAWAL 2008, KLASA et al. 2007). Municipal sewage sludge is also a source of micronutrients (KALEMBASA et al. 1999, SPIAK, KULCZYCKI 2004, IŻEWSKA et al. 2006). However, special care should be taken with respect to micronutrients so as not to introduce excessive amounts of these elements, which could have an adverse effect on the environment, especially when soil is acidic (MERCIK et al. 2003, PASCUAL et al. 2004, DELIBACAK et al. 2009). The purpose of this work has been to evaluate the effect of municipal sewage sludge on the content of available forms of Cu, Zn and Mn in soil.

METHODS

A microplot experiment (1m x 1m) was carried out in 2005-2008. The experiment was set up on anthropogenic, humic urban soil developed from loamy sand in a random sub-block design. Prior to the experiment, the soil had a high content of phosphorus and magnesium, a low level of potassium and alkaline reaction. The design of the experiment comprised 5 doses of municipal sewage sludge from the Łyna Municipal Wastewater Treatment Plant in Olsztyn: 0, 70, 140, 210 and 280 Mg ha^{-1} of fresh matter. Municipal sewage sludge was applied once. The chemical properties of the sludge are presented in Table 1.

Sewage sludge was mixed with soil to the depth of about 10 cm. Once fertilized with municipal sewage sludge, the soil was sown with mixes of lawn grasses. The available forms of Cu, Zn and Mn after extraction with 1 mol HCl dm^{-3} were determined with the atomic absorption spectrophotometric method. The results were submitted to statistical analysis using the software programme Statistica (version 9.0) by SatSoft, Inc. 2009.

Table 1

Chemical properties of sewage sludge

Determination		Unit	Value
pH in H_2O		-	8.43
Corg.		g kg^{-1} d.m.	186.53
Total elements	Nog.		33.30
	P		14.40
	K		1.20
	Mg		5.00
Micronutrients	Ca	mg kg^{-1} d.m.	27.90
	Cu		251.00
	Zn		1340.00
	Mn		320.00

RESULTS

The content of available copper in soil ranged from 4.65 to 5.89 mg kg^{-1}, which should be regarded as rather small variation. The tested doses of sewage sludge were evidenced to have had a significant effect on the content of copper in soil (Table 2). This effect was also demonstrated by the

Table 2

Content of available copper in soil (mg kg^{-1})

Year	Dose of sewage sludge (Mg ha^{-1})					Mean
	0	70	140	210	280	
2005	4.65	5.41	5.58	5.71	5.89	5.45
2006	4.70	5.60	5.60	5.82	5.78	5.50
2007	4.67	5.55	5.64	5.78	5.82	5.49
2008	4.73	5.46	5.63	5.68	5.77	5.45
Mean	4.69	5.51	5.61	5.75	5.82	

$LSD_{0.05}$ year – n.s., dose – 0.09, year × dose – n.s.

analysis of correlation and regression (Figure 1). It is worth noticing that the soil pH in 1 mol HCl dm^{-3} varied within a narrow range (6.50-7.14). Under such condition (no acidification), the solubility of Cu was low. Analogously to our study, Delibacak et al. (2009) found out an increase in the content of soluble copper in soil caused by increasing doses of sewage sludge introduced to soil. In contrast, Pascual et al. (2004) showed depressed concentrations of available forms of Cu in soil under the influence of a higher dose of sewage sludge (140 Mg ha^{-1}). Such discrepancies, reported by different authors, in the effect of sewage sludge on the content of Cu in soil may be substantiated, for example, by different concentrations of this metal in sewage sludge. On the other hand, sorptive properties of soil, and especially the content of organic matter, can affect the availability of copper in soil.

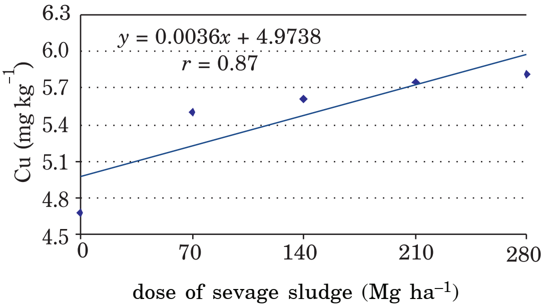

Fig. 1. Changes in the content of available copper in soil depending on the dose of sewage sludge

It is highly probable that the accessibility of available copper in soil can increase in the second and third year after the application of sewage sludge. This tendency can be explained by a larger influx of copper originating from mineralization of organic matter added to soil together with sewage sludge (Figure 2).

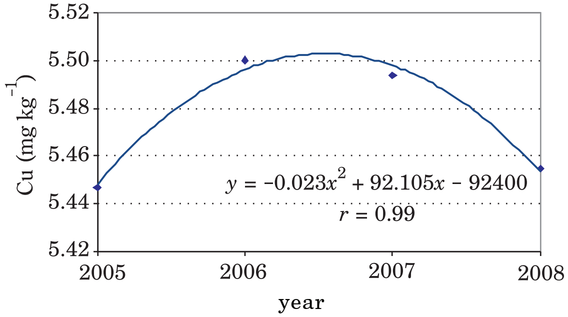

Fig. 2. Changes in the content of available copper in soil in the following years of the experiment

The content of available zinc in soil changed significantly in the subsequent years of the research (Table 3). A non-significant increase occurred in the second year compared to the first one. In the subsequent years, the accumulation of available Zn in soil tended to decrease but its significant limitation did not occur until 2008 (Figure 3). Contrary results were obtained by Iżewska et al. (2006), who found a significant rise in the content of soluble zinc in soil three years after the soil amendment with sewage sludge. In turn, Patorczyk-Pytlik (2004) reported that even six years after application of sewage sludge to soil labile forms of zinc constituted 29% of the total content of zinc in soil.

Table 3

Content of available zinc in soil (mg kg^{-1})

Year	Dose of sewage sludge (Mg ha^{-1})					Mean
	0	70	140	210	280	
2005	67.31	76.33	83.45	86.37	95.01	81.69
2006	66.65	77.55	86.05	90.38	91.85	82.50
2007	68.57	74.83	82.94	88.64	92.16	81.43
2008	68.70	72.28	80.29	87.97	92.03	80.25
Mean	67.81	75.25	83.18	88.34	94.90	67.81

$LSD_{0.05}$ year – n.s., dose – 1.44, year x dose – 2.88

The zinc introduced to soil with sewage sludge was relatively quickly converted into more easily assimilable forms but then, due to the processes occurring in soil, it underwent retardation. The conditions present in our experiment, i.e. a high content of organic matter, high soil abundance in available phosphorus and alkaline soil reaction, did not favour the appearance of excessive amounts of Zn available forms in soil. Similar results were achieved by Patorczyk-Pytlik (2004), who noticed a considerable loss of the available forms of zinc in each year of a three-year pot experiment.

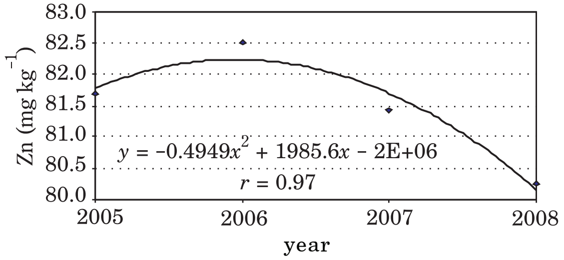

Fig. 3. Changes in the content of available zinc in soil in the following years of the experiment

As the dose of sewage sludge added to soil increased, so did the content of soluble zinc in soil (Table 3, Figure 4). The highest dose of sewage sludge (280 Mg ha^{-1}) caused an over 36% increase in the concentration of this element in soil compared to the control soil.

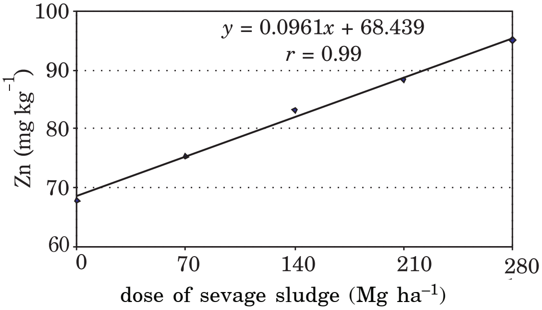

Fig. 4. Changes in the content of available zinc in soil depending on the dose of sewage sludge

The results congruent with ours were reported by Delibacaka et al. (2009), who also demonstrated higher concentrations of zinc in soil induced by higher doses of sewage sludge in the initial period of the experiment. However, the results obtained by Pascual et al. (2004) are contrary as the quantities of available zinc in soil decreased under the effect of incremental doses of sewage sludge.

The content of available Mn in soil was strongly affected by the doses of sewage sludge introduced to soil (Table 4). Each increase in the dose of this waste by 70 Mg ha^{-1} caused a significant increase in the concentration of easily soluble forms of manganese in soil. Such strong influence of the doses of sewage sludge on the content of available Mn in soil was confirmed by the regression and correlation analysis (Figure 5). Higher accumulation of available manganese in soil under the influence of sewage sludge has also been demonstrated by Iżewska et al. (2006). In contrast, Pascual et al. (2004) inform that the content of this metal decreased under the effect of higher

Table 4

Content of available manganese in soil (mg kg^{-1})

Year	Dose of sewage sludge (Mg ha^{-1})					Mean
	0	70	140	210	280	
2005	120.68	129.05	137.39	142.63	148.48	135.65
2006	122.05	125.18	137.80	144.32	148.07	135.49
2007	121.96	134.05	137.31	142.73	153.20	137.85
2008	121.16	131.63	138.24	142.51	151.43	136.99
Mean	121.46	129.98	137.68	143.05	150.29	

$LSD_{0.05}$ year – n.s., dose – 1.65, year x dose – n.s.

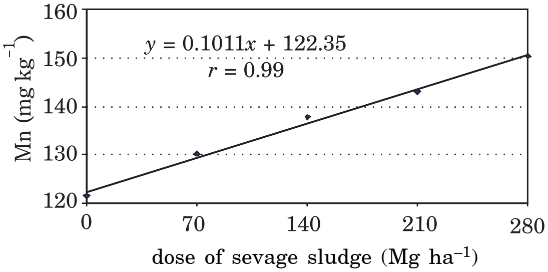

Fig. 5. Changes in the content of available manganese in soil depending on the dose of sewage sludge

doses of sewage sludge. Based on the calculations performed in this study, somewhat weaker increase in the concentration of available Mn in soil was also shown in the following years (Figure 6). The course of the regression curve reveals that the effect of sewage sludge was more evident in the first three years. In the last year of the experiment, the amount of soluble manganese in soil did not undergo any considerable changes.

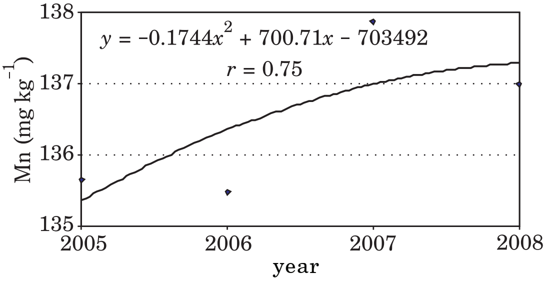

Fig. 6. Changes in the content of available manganese in soil in the following years

Manganese is a very reactive element in the environment; it easily changes its oxidation degree. In the present experiment, however, the soil pH did not change much and the soil was not waterlogged, which otherwise may have induced reactive conditions.

CONCLUSIONS

An increase in available Cu in soil in response to the introduced sewage sludge was most evident in the second and third year after the application of sewage sludge.

The content of soluble Zn in soil began to decrease in the second year after the application of sewage sludge, but its significant limitation occurred in the fourth year.

Sewage sludge significantly increased the content of soluble manganese in soil in the first three years.

Based on the present experiment, we can recommend the tested sewage sludge for non-agricultural use (lawns, slopes, rehabilitation of degraded areas).

REFERENCES

Casado-Vela J., Sellés S., Navarro J., Bustamante M. A., Mataix J., Guerrero C., Gomez I. 2006. *Evaluation of composted sewage sludge as nutritional source for horticultural soils.* Waste Manag., 26: 946-952.

Czekała J. 2009. *Sewage sludge – fertilizer or waste?* Wodociągi – Kanalizacja, 1: 30. (in Polish)

Delibacak S., Okur B., Ongun A. R. 2009. *Influence of treated sewage sludge applications on temporal variations of plant nutrients and heavy metals in a Typic Xerofluvent soil.* Nutr. Cycl. Agroecosyst., 83: 249-257.

Iżewska A., Krzywy E., Wołoszyk Cz., Balcer K. 2006. *Content of heavy metals in light soil in the third year after application of sewage sludge and composts made from sewage sludge.* Zesz. Probl. Post. Nauk Rol., 512: 173-181. (in Polish)

Jakubus M. 2006. *Influence of long-term application of sewage sludge on chamges in the selected chemical proeprties of soil.* Zesz. Probl. Post. Nauk Rol., 512.1: 209-219. (in Polish)

Kalembasa S. Pakuła K., Becher M. 1999. *Content of macro- and micronutrients in sewage sludge produced at selected WTPs near Siedlce.* Fol. Univ. Agric. Stet. Agric., 77: 125--128. (in Polish)

Klasa A., Gotkiewicz W., Czapla J. 2007. *Modifications of physico-chemical soil properties following application of sewage sludge as soil amendment.* J. Elementol., 12(4): 287--302.

Mazur Z., Sienkiewicz S. 2009. *Effect of urea applied with composts on concentration of Cu, Zn and Mn in corn fresh matter.* J. Elementol., 14(2): 323-330.

Mercik S., Stępień W., Gębski M. 2003. *Uptake by plants and solubility of Cu,Zn,Pb and Cd in different extraction solutions depending on soil acidity*. Zesz. Probl. Post. Nauk Rol., 493: 913-921.

Pascual I., Antolín M. C., García C., Polo A., Sánchez-Díaz M. 2004. *Plant availability of heavy metals in a soil amended with a high dose of sewage sludge under drought conditions.* Biol. Fertil. Soils, 40: 291-299.

Patorczyk-Pytlik B. 2004. *Chemical forms of zinc in soil two, four and six years after application of sewage sludge and vermicompost*. Zesz. Probl. Post. Nauk Rol., 505 (2): 945--952. (in Polish)

Sądej W., Bowszys T., Wierzbowska J. 2007. *Modifications of the physicochemical properties of grey soil fertilized with sewage sludge*. Zesz. Probl. Post. Nauk Rol., 520: 363-369. (in Polish)

Singh R. P., Agrawal M. 2008. *Potential benefits and risks of land application of sewage sludge.* Waste Manage., 28: 347-358.

Sřren O. P., Petersen J., Rubćk G. H. 2003. *Dynamics and plant uptake of nitrogen and phosphorous in soil amended with sewage sludge*. Appl. Soil Ecol., 24: 187-195.

Speir T. W., van Schaik A. P., Lloyd-Jones A. R., Kettles H. A. 2003. *Temporal response of soil biochemical properties in a pastoral soil after cultivation following high application rates of undigested sewage sludge*. Biol. Fertil. Soils, 38: 377-385.

Spiak Z., Kulczycki G. 2004. *Content of micronutrients in sewage sludge from selected WTPs in the Province of Lower Silesia*. Zesz. Probl. Post. Nauk Rol., 505: 973-978. (in Polish)

Spychaj-Fabisiak E., Kozera W., Majcherczak E., Ralcewicz M., Knapowski T. 2007. *Effect of organic waste and farmyard manure on fertility of light soil*. Acta Sci. Pol., Agric., 6(3): 69-76. (in Polish)

Stępień W., Szulc W., Mercik S. 2000. *Estimation of the fertilization value of raw and amended sewage sludge*. Fol. Univ. Agric. Stetin., Agric., 84: 465-470. (in Polish)

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CONCENTRATION OF ZINC IN WATER AND BOTTOM SEDIMENTS IN SMALL WATER RESERVOIRS LOCATED IN RURAL AREAS

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Abstract

Depending on its concentration in the environment, zinc can be either an important bio-element for development of living organisms, or a toxic heavy metal. Small water reservoirs are especially sensitive to contamination, as because of their location in the lowest point of the catchment they tend to receive contaminants from its whole area. The purpose of this work was to assess changes in concentration of zinc in surface, supra-bottom and interstitial water, and in the upper layer of sediment, in five small water reservoirs located in rural areas. The reservoirs G1 and G2 were located in a golf course, water body SW was in a village, very close to farmyards, water body P1 was in the middle of a crop field, and water body P2 was on fallow land. For this research, samples were collected once a month, during three vegetation seasons. The water reservoirs contained an elevated average concentration of zinc in water from 0.026 to 0.063 mg dm^{-3}). The average concentration of zinc in the sediments (from 25.8 to 118.2 mg kg^{-1}) classified the examined reservoirs into the geochemical purity class 1. The highest content of zinc was in the village pond. Highly significant correlations were determined between the content of organic matter and zinc in the bottom sediment in the investigated reservoirs. At the same organic matter content, the zinc concentration in the bottom sediments of the rural pond was find-fold higher than in the other reservoirs. The concentration of zinc in water and the factor of its accumulation in the sediment showed a clear seasonal character of changes. In the spring season, concentration of zinc in interstitial water was lower than in the water depth, whereas in the vegetation season, the zinc concentration was observed to rise in all layers, up to over ten-fold in the interstitial water. In the sediment, the zinc accumulation factor changed in a reverse manner, i.e. it reached thee highest values during spring but fell the lowest in autumn.

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Key words: zinc, interstitial and surface water, bottom sediment, small water reservoirs.

ZAWARTOŚĆ CYNKU W WODZIE I OSADACH DENNYCH MAŁYCH ZBIORNIKÓW WODNYCH ZLOKALIZOWANYCH NA OBSZARACH WIEJSKICH

Abstrakt

Cynk, w zależności od jego zawartości w składnikach ekosystemów, może być ważnym w rozwoju organizmów żywych biopierwiastkiem lub toksycznym metalem ciężkim. Szczególnie wrażliwe na zanieczyszczenia są małe zbiorniki wodne, które ze względu na swoje położenie w najniższym miejscu zlewni odbierają zanieczyszczenia z całego jej obszaru. Celem pracy była ocena zmian zawartości cynku w wodzie: powierzchniowej, przydennej i międzyosadowej oraz w powierzchniowej warstwie osadu w pięciu małych zbiornikach wodnych zlokalizowanych na terenach wiejskich. Zbiorniki G1, G2 były umiejscowione na terenie pola golfowego, zbiornik SW w centrum wsi, zbiornik P1 na polu uprawnym, a zlewnię zbiornika P2 stanowił zadarniony odłóg. Próby do badań pobrano 1 raz w miesiącu w ciągu trzech sezonów wegetacyjnych. W badanych zbiornikach stwierdzono podwyższoną koncentrację cynku w wodzie (od 0,026 do 0,063 mg dm^{-3}). Średnia zawartość cynku w osadach (od 25,8 do 118,2 mg kg^{-1}) klasyfikowała badane zbiorniki do I geochemicznej klasy czystości. Największą zawartość cynku stwierdzono w stawie wiejskim. Obliczono wysoce istotne korelacje między zawartością materii organicznej i cynku w osadzie dennym. Przy tej samej zawartości substancji organicznej, zawartość cynku w osadzie dennym stawu wiejskiego była pięciokrotnie większa niż w pozostałych zbiornikach wodnych. Stężenie cynku w wodzie oraz współczynnik jego kumulacji w osadzie wykazywały wyraźną sezonowość zmian. W sezonie wiosennym stężenie cynku w wodzie międzyosadowej było mniejsze niż w toni wodnej, natomiast w trakcie sezonu wegetacyjnego stwierdzono wzrost jego stężenia we wszystkich warstwach wody, w wodzie międzyosadowej nawet o kilkanaście razy. Odwrotnie zmieniał się współczynnik kumulacji cynku w osadzie, przyjmując największe wartości w sezonie wiosennym, a najmniejsze w sezonie jesiennym.

Słowa kluczowe: cynk, woda interstycjalna i powierzchniowa, osad denny, małe zbiorniki wodne.

INTRODUCTION

Zinc is a heavy metal and, like some other heavy metals e.g. iron, copper, nickel, and chrome, it is essential for proper functioning of living organisms and only after exceeding a certain level it becomes harmful. Although zinc is one of the least toxic heavy metals, concentration of this element has an effect on the quality of water in a reservoir. Zinc levels above 5 mg dm^{-3} give water a bitter and metallic taste and in an alkaline environment they cause turbidity of water. Zinc does not bio-accumulate in organisms very well, which limits its migration in the trophic chain (Brooks, Mahnken 2003, Dean et al. 2006). Concentrations of zinc in water substantially affect biotic processes occurring in it. Zinc is incorporated into enzymes and takes part in the bio-synthesis of nucleic acids and polypeptides. This influence is most frequently observed in biocenoses that overgrow underwater subgrades (Paulsson et al. 2000, 2001, Zhou et al. 2007). Based on micro-system laboratory tests, Paulsson et al. (2001) stated that as the concentration of mobile zinc in water increases, the amount of phosphorus in the peryphiton and the biomass of organisms decrease, while the activity of phosphohydrolytic enzymes increases. Growing activity of these enzymes on the residue surface associated with a rise in the amount of zinc in interstitial water was also recorded by Zhou et al. (2007). A high concentration of zinc results in its accumulation in the radicular system, leading to some disturbance of photosynthesis, chlorosis and metabolism disorders. If the concentration of zinc exceeds 0.100 mg dm^{-3}, ions of this metal slow down the process of water self-purification.

The thin line between zinc deficit and toxicity is very easy to cross. Among the contributing factors are: increasing use of chemicals in agriculture, industrialization and using some waste materials for agricultural purposes. Small water reservoirs are especially pollution sensitive, as their location is a bio-geo-chemical barrier for metals, where they are captured mainly in bottom sediments (Szyperek 2005, Senze et al. 2010). Like all other heavy metals, zinc has a distinct ability to accumulate in various elements of aquatic environments, especially in bottom sediments. Thus, bottom sediments constitute an element of the water environment which acts as a moderator, influencing chemical qualities of the water which remains in contact with these sediments. This is particularly true about heavy metals that are arrested in bottom sediments by precipitation, sedimentation or sorption, but in the course of chemical and bio-chemical decomposition, dissolution and desorption, they can pollute the water environment again.

The content of zinc is usually tested in reservoir waters and bottom sediments in drainage basins which have been polluted by industrial sewage (Nocoń 2006). There are no comprehensive studies on the content of zinc in reservoirs located in agricultural areas. The aim of this research is to evaluate the seasonal character of fluctuations in the content of zinc during a plant growing season in small water reservoirs located in agricultural areas. Changes in the zinc content were tested in the upper, benthic and interstitial waters and in the top layer of bottom sediments.

MATERIAL AND METHODS

The research involved five, small, drainless, pond-like water reservoirs with the permanent water table, located in Szczecin Landscape Park. These reservoirs are characterized by differently developed direct drainage basins. Two (G1, G2) are located within the premises of a golf club in Binowo. Their drainage basins are dominated by brown ground soils (mainly fawn), produced from light clay, sanded on top (*Soil-agricultural map* 1973). Two other reservoirs are located on fields in the village Kolowo. During the study, the drainage basin of reservoir P1 was a farm field and the one of reservoir P2 was turfed fallow land. The fifth reservoir (SW) is a typical rural pond, located in the nearest vicinity of the farmyards in Kolowo. The drainage basin of this reservoir covers areas of brown, leached soil, formed from light clay sand that transforms into light clay at the depth of 50-100 cm, and of typical brown soil made from light clay. (*Soil-agricultural map* 1973).

Samples of water and sediments for specific tests were taken twice during the spring (March-May), summer (June-August) and autumn (September-November) in three consecutive years: 2005-2007. The samples were collected from the central parts of the water reservoirs. Firstly, surface layer water was sampled with a scoop from the depth of 30 cm. Secondly, samples of benthic water and of ceiling sediment with an undisturbed structure were taken with the use of a sediment core sampler (produced by KC- Denmark). A 10-centimeter layer of benthic water was probed by the means of a sampling pipe with a semi-automatic pipette Swiftpet. From the remaining sample, a 4-centimeter surface layer was extracted and separated with a special set consisting of a piston and a tray for samples. Each time, extraction of samples was repeated four times. When delivered to the laboratory, the samples were centrifuged in a lab centrifuge for ten minutes at 3000 rpm. Water collected above the centrifuged sediment was decanted and treated as inter-sedimentary. After each sampling of water and sediment, averaged aggregated samples from the surface layer of the sediment and from each layer of water (surface, benthic and interstitial) were analyzed chemically.

In the examined samples of water and sediment, after wet mineralization of the sedeiments in concentrated nitric acid in a microwave oven, total content of zinc was determined with atomic absorption spectrometry, where zinc detectability limit was 2.5 mg kg^{-1}. Determination of zinc was conducted in a spectrometer Solaar S by ThermoElemental. Additionally, in the centrifuged sediments, organic carbon was determined with Orlow and Grindel's method. The determinations were conducted in a double-beam spectrophotometer UV/VIS 8500 by Techcomp. The content of organic carbon was converted into an organic substance quantity by multiplying it by the modulus 1.724.

RESULTS AND DISCUSSION

Our comparison of the concentrations of zinc in the examined reservoirs to the threshold boundary values, given in the guidelines for classification of surface waters (*Regulation...* 2008), showed that all the examined water reservoirs were in good ecological condition as far as the content of zinc was concerned. In the surface stratum, the mean concentration of that element calculated for the whole vegetative season remained in the range of 0.026 mg dm^{-3} in reservoir G2 at the golf club and 0.063 in reservoir P2, whose drainage basin was a fallow field (Table 1). In each reservoir, the mean concentration of zinc in water tended to increase towards the bottom, with the difference between zinc levels in interstitial water and those in the water column being relatively small. It was only in reservoir G1 that the concentration of zinc in interstitial water was three-fold higher than in the benthic layer, while in the other reservoirs it was only 30% higher.

The mean content of zinc in the sediments of the examined water reservoirs was within 25.8 mg kg^{-1} (in reservoir G2 at the golf club) and

Table 1

Concentration of zinc in water and sediment, n=18

Examined layer	Reservoir	Statistical parameter					
		average	min.	max.	geometric mean	median	SD
Surface water (mg dm^{-3})	G1	0.057	0.010	0.493	0.060	0.046	0.044
	G2	0.026	0.006	0.034	0.018	0.021	0.029
	SW	0.032	0.006	0.178	0.021	0.026	0.048
	P1	0.046	0.004	0.165	0.041	0.044	0.047
	P2	0.063	0.004	0.268	0.039	0.037	0.076
Bottom water (mg dm^{-3})	G1	0.028	0.010	0.100	0.027	0.027	0.020
	G2	0.077	0.001	0.619	0.020	0.022	0.173
	SW	0.079	0.007	0.502	0.031	0.030	0.145
	P1	0.053	0.010	0.183	0.041	0.038	0.051
	P2	0.063	0.018	0.165	0.057	0.059	0.048
Interstitial water (mg dm^{-3})	G1	0.087	0.001	0.443	0.046	0.100	0.131
	G2	0.103	0.005	0.253	0.050	0.060	0.111
	SW	0.089	0.004	0.719	0.038	0.071	0.197
	P1	0.078	0.002	0.303	0.051	0.072	0.091
	P2	0.078	0.015	0.357	0.060	0.037	0.116
Sediment (mg kg^{-1} d.m.)	G1	111.1	20.8	195.9	101.5	118.7	30.2
	G2	25.8	17.7	151.1	28.8	24.7	7.9
	SW	118.2	28.9	167.3	105.2	117.8	45.0
	P1	34.4	21.1	48.1	28.5	26.0	11.4
	P2	40.4	15.9	51.6	36.5	38.7	26.8

G1, G2 – reservoirs located in a golf course
SW – a village pond
P1, P2 – reservoirs located on arable land

118.2 mg kg^{-1} of the dry sediment mass (in the village pond). It was lower than the threshold value (200.0 mg kg^{-1}), thus, in respect of this parameter, the examined reservoirs can be qualified as class 1 of geochemical purity. Reservoirs G1 and SW were an exception, because their zinc levels exceeded the geochemical background, which equals 48.0 mg kg^{-1} (BOJAKOWSKA, SOKOŁOWSKA 1998). Lower or similar values of this parameter were observed in other rural water reservoirs, e.g. in Owiesno pond it was 14.9 mg kg^{-1} (SENZE et al. 2010) and in Psurow pond it was 90.7 mg kg^{-1} (GAŁKA, WIATKOWSKI 2010). No significant correlations between the concentration of zinc in the examined layers of water and its content in the sediment were found. Significant positive correlations were observed between the zinc level and the content of organic matter in the sediment (Figure 1). The determination coefficient for the linear regression function for the samples collected in the village pond was 0.77, and for the samples taken from the other reservoirs it was 0.94. With respect to this dependence, some significant correlations were also found by BASARAN et al. (2009), who studied sediments in the Gulf of Gulluk, Turkey. Comparison of the regression coefficients of linear functions describing the relationship between the content of zinc and the content of organic matter in sediments in some water reservoirs indicated that, at the same content of organic matter, the level of zinc in the rural pond sediments was five-fold higher than in the other water reservoirs. This difference could be attributed to different sources of pollution, as the rural pond was mainly polluted with household sewage and run-offs from the farmyard, while the other reservoirs were fed by run-offs from their drainage basins.

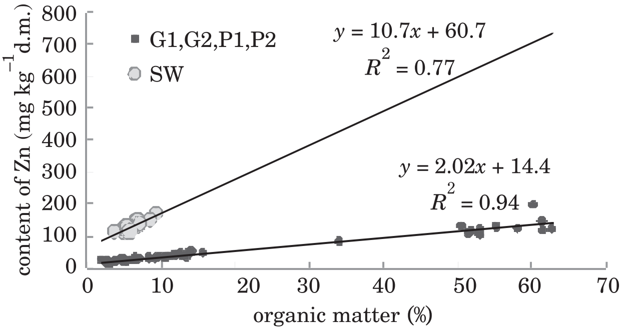

Fig. 1. Relationships between the content of organic matter and zinc in bottom sediment of the reservoirs

Concentrations of zinc in water collected from the analyzed reservoirs underwent some distinct seasonal changes (Figure 2). In the springtime, the concentration of this element in interstitial water was lower than in the

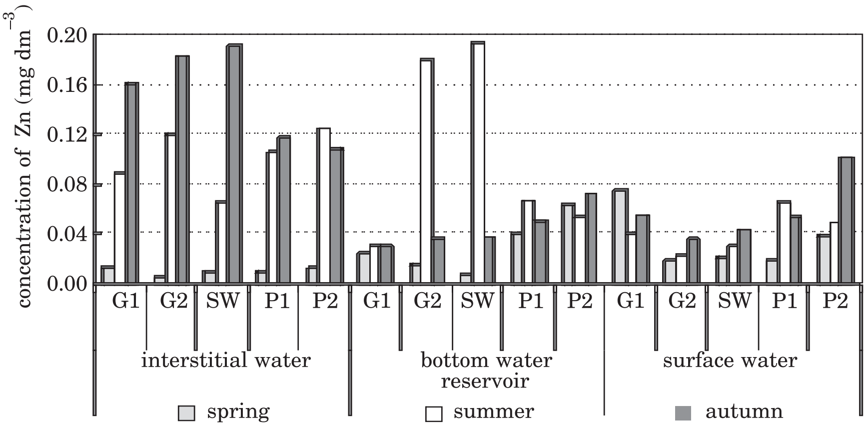

Fig. 2. Mean seasonal concentrations of zinc in investigated layers of water, *n=6*

water column, while during the vegetation season it increased even more than a dozen times. This indicates that in spring, the gradient of zinc concentration was shifted toward the water column, while during summer and autumn, it moved towards the interstitial water. In the examined reservoirs, the average concentration of zinc in summer and autumn was higher than the threshold for this element (0.0025 mg dm^{-3}), at which development of phytoplankton biomas is depressed (Paulsson et al. 2000, 2001). The highest levels of zinc observed in the interstitial water in summer and autumn show that the seasonal variations in the concentration of zinc in the studied reservoirs might be related to some biotic processes, especially the biocenoses growing on submerged surfaces (Paulsson et al. 2000, 2001, Zhou et al. 2007). Seasonal fluctuations of zinc levels in the examined water reservoirs confirm that in spring zinc is permanently bound in the sediment. Then, in summer and autumn, like phosphorus and other heavy metals, it can be released in processes of organic matter mineralization and photochemical degradation, which are more intensive during the vegetation season in poly-mictic reservoirs (Górniak 1996).

Seasonal changes in concentrations of zinc in water were not reflected by a change in the zinc content in the surface layer of the sediment (Figure 3), which indicates low mobility of zinc in sediments during the vegetation season. Alkaline pH of the examined sediments may prove a potentially low risk of releasing zinc should the chemical balance in the examined reservoirs change. In the sediments, about 40% of zinc is bound to Fe-Mn oxides. This is confirmed by numerous studies, such as speciation analysis of zinc in sediments of lakes in Wielkopolski National Park (Sobczyński, Siepak 2001) or in some smaller reservoirs (Madeyski et al. 2009). Zinc may be reinstated from this fraction into the bio-circulation if strong reducing conditions occur in the near-bottom zone.

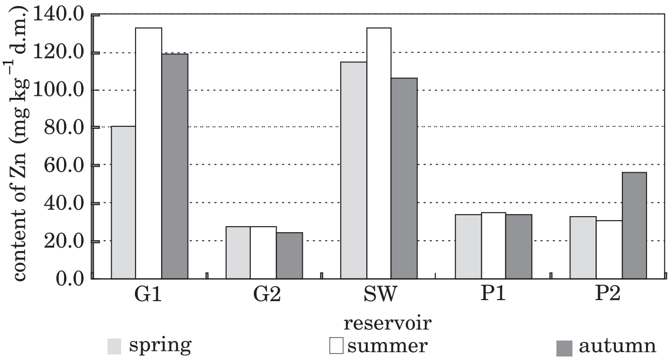

Fig. 3. Mean seasonal concentration of zinc in bottom sediments of the water reservoirs, *n=6*

CONCLUSIONS

1. With the determined concentrations of zinc, the small reservoirs examined proved to be in good ecological condition and belonged to class 1 of geochemical purity. The highest level of zinc was found in the village pond.

2. In all the reservoirs, highly significant correlations between the content of organic matter and zinc in sediments were found. At the same content of organic matter, the zinc level in the village pond sediments was fivefold higher than in the sediments of in the other reservoirs.

3. The zinc concentrations in water collected from the studied reservoirs was subject to significant seasonal changes. In spring, the gradient of concentrations of Zn ions shifted towards the water column, while during summer and autumn it moved towards interstitial water.

REFERENCES

Basaran A.K., Aksu M., Egemen O. 2009. *Impacts of the fish farms on the water column nutrient concentrations and accumulation of heavy metals in the sediments in the eastern Aegean Sea (Turkey).* Environ. Monit. Assess., 162: 439-451.

Bojakowska I., Sokołowska G. 1998. *Geochemical classes of water and sediment purity*. Prz. Geol., 46(1): 49-54. (in Polish)

Brooks K.M., Mahnken C.V.W. 2003. *Interactions of Atlantic salmon in the Pacific Northwest environment* III. *Accumulation of zinc and copper*. Fish. Res., 62: 295-305.

Dean R.J., Shimmield T.M., Black K.D., 2006. *Copper, zinc and cadmium in marine cage fish farm sediment: An extensive survey*. Environ. Pollut., 145: 84-95.

Gałka B., Wiatkowski M. 2010. *Heavy metals in water and bottom sediments of a small water reservoir called Psurów.* Ochr. Środ. Zas. Natur., 42: 225-232. (in Polish)

GÓRNIAK A. 1996: *Humic substances and their role in the functioning of freshwater water bodies*. Dissertationes Universitatis Varsoviensis 448, Białystok, 113-114. (in Polish)

Soil – agricultural map. 1973. District Stare Czarnowo, 1:5000. Wojewódzkie Biuro Geodezji i Urzadzeń Rolnych w Szczecinie.

MADEYSKI M., TARNOWSKI M., JASIEWICZ, C. BARAN A. 2009. *Fractionation of chosen heavy metals in bottom sediments of small water reservoirs*. Arch. Environ. Protect., 35(3): 47-57.

NOCOŃ W. 2006. *Content of heavy metals in the bottom sediments of the Kłodnica River*. J. Elementol., 11(4): 457-466. (in Polish)

PAULSSON M., MÍNSSON V., BLANCK H. 2002. *Effect of zinc on the phosphorus availability to preyiphyton communities from te river Göta Alv*. Aquatic Toxicol., 56: 103-113.

PAULSSON M., NYSTRÖM B., BLANCK H., 2000. *Long-term toxicity of zinc to bacteria and algae in preyiphyton communities from the river Göta Alv, based on a microcosm study*. Aquatic Toxicol., 47: 243-257.

Regulation of the Minister of Environment 2008. Classification of the condition of uniform sections of surface waters. Dz.U. 2008, nr 162 poz. 1008. (in Polish)

SENZE M., KOWALSKA-GÓRALSKA M., ZIĘBA E. 2010. *Bioaccumulation of copper, nickel, cadmium, lead and zinc in bottom sediments of the reservoir called Owiesno*. Zesz. Nauk. UP Wroc., Biol. Hod. Zwierz., 60(577): 125-134. (in Polish)

SOBCZYŃSKI T., SIEPAK J. 2001. *Studies on accumulation of biogenic compounds and speciation of metals in bottom sediments of lakes in Wielkopolski National Park*. Zesz. Nauk. Wydz. Bud. i Inż. Środ. Politechniki Koszalińskiej, Inż. Środ., 20: 265-290. (in Polish)

SZYPEREK U. 2005. *Role of water ponds as a bio-geo-chemical barrier for runoffs of calcium and magnesium from agricultural environments*. J. Elementol., 10(4): 1083-1090. (in Polish)

ZHOU Y., CAO X., SONG C., LI J., CHEN J., PENG L. 2007. *Variations in kinetics of alkaline phosphatse in sediments of eutrophic, shallow, Chinese lakes*. Hydrobiology, 581: 109-116.

J. Elem. s. 669–688 DOI: 10.5601/jelem.2012.17.4.10

EFFECT OF DIFFERENTIATED FERTILIZING SYSTEMS ON NITROGEN ACCUMULATION PATTERNS DURING THE GROWING SEASON – SUGAR BEET AS AN EXAMPLE

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Abstract

The rate of nitrogen uptake by sugar beet canopy during the growing season is a driving factor in both dry matter production and its distribution between leaves and the storage root. It has been hypothesized that nitrogen accumulation in both parts of the beet is significantly affected by the regime of P and K supply to plants. This assumption has been verified with the data obtained from a field static experiment, conducted in 2001-2003, with eight fertilizing variants: without nitrogen (absolute control, PK), without one of the main nutrients (KN, PN), with a reduced amount of phosphorus and potassium (N + 25% PK, N + 50% PK) and with the recommended amounts of basic nutrients (NPK, NP^*K, P^* – P in the form of PAPR). Amounts of in-season accumulated nitrogen in sugar beet parts were measured on eight consecutive sampling dates, in two- to three-week intervals. The general pattern of N accumulation in leaves is best described by a quadratic equation, but follows a linear function in storage roots. The maximum rate of nitrogen accumulation depended on years and fertilizing variants. Limited supply of nutrients to beet plants, caused by the course of the weather or the applied fertilizers (less than 50% of the recommended N rate and without K), was the main reason for a lower rate of nitrogen accumulation in storage roots in the first part of the growing season. The course of absolute and relative nitrogen uptake rates shows that in the second part of the season the sugar beet could compensate the uptake of N from its soil resources. However, the effect of compensatory N uptake on yield of storage roots was inconsistent. When water and nutrients were in ample supply, e.g. in 2001, the additionally absorbed nitrogen could be used as an indicator of the yield potential of sugar beet. Under other growth conditions, it is used

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mainly for restoration of leaf growth, with a different effect on the final yield of storage roots. The quadratic trend of N accumulation in beet canopy during the growing season reflects the crop's N saturation status, a prerequisite of high yields of storage roots, as in 2001. The linear model, manifesting itself in years with pronounced drought, represents a sub-optimal status of N management in sugar beet canopy, resulting in much lower yields.

Key words: sugar beet, fertilizing variants, N accumulation patterns.

WPŁYW ZRÓŻNICOWANYCH SYSTEMÓW NAWOŻENIA NA WZORCE AKUMULACJI AZOTU W OKRESIE WEGETACJI – NA PRZYKŁADZIE BURAKA CUKROWEGO

Abstrakt

Szybkość pobierania azotu przez plantację buraki cukrowe w okresie wegetacji jest czynnikiem decydującym zarówno dla produkcji, jak i rozdziału suchej masy między liście i korzeń spichrzowy. W związku z tym postawiono tezę, że akumulacja azotu w obu częściach rośliny istotnie zależy od reżimu zaopatrzenia rośliny w P i K. Postawioną tezę zweryfikowano w doświadczeniu polowym statycznym, prowadzonym w latach 2001-2003, w którym zastosowano 8 wariantów nawozowych: bez azotu (kontrola absolutna, PK), bez jednego głównego makroskładnika (NK, NP), ze zredukowana dawką P i K (N + 25% PK; N + 50% PK) oraz z zalecaną dawką składników (NPK, NP^*K, P^* – P w nawozie fosforowym, tzw. wzbogaconym). Ilość azotu akumulowanego przez buraki w okresie wegetacji mierzono w 8 kolejnych terminach w cyklach dwu- do trzytygodniowych. Ogólny model akumulacji azotu w liściach buraków najlepiej opisuje równanie kwadratowe, a w korzeniach – liniowy. Maksymalna szybkość akumulacji azotu zależała istotnie od lat i wariantu nawozowego. Ograniczone zaopatrzenie roślin w skladniki pokarmowe, wynikające zarówno z przebiegu pogody w sezonie wegetacyjnym, jak i wariantu nawozowego (dawka P i K poniżej 50% dawki zalecanej), było główną przyczyną mniejszej szybkości akumulacji azotu przez korzenie buraków w pierwszym okresie wegetacji. Przebieg krzywych bezwzględnej i względnej akumulacji azotu w okresie wegetacji wskazuje na zdolność buraka cukrowego do kompensacji pobierania tego składnika z jego rezerw w glebie w drugiej części okresu wegetacji. Efekty plonotwórcze kompensacyjnego pobierania azotu nie są jednoznaczne. W warunkach optymalnego zaopatrzenia roślin w wodę i skladniki pokarmowe, t.j. w 2001 r, dodatkowo pobrany azot jest wskaźnikiem realizacji potencjału plonotwórczego buraków. W innych warunkach wegetacji dodatkowo pobrany azot zostaje zużyty na odtworzenie liści, z różnym skutkiem dla końcowego plonu korzeni. Model kwadratowy akumulacji azotu w okresie wegetacji odzwierciedla stan wysycenia roślin azotem, warunkując duży plon korzeni, jak w 2001 roku. Model liniowy, ujawniający się w latach z wyraźnie zaznaczoną suszą, przedstawia suboptymalny stan gospodarki azotem na plantacji buraków, prowadząc jednakże do zmniejszenia plonu korzeni.

Słowa kluczowe: burak cukrowy, warianty nawożenia, dynamika akumulacji azotu.

INTRODUCTION

The main aim of farmers producing sugar crops may seem self-contradictory because of the reciprocal relationships occurring between a high storage roots yield and its technological quality. Adequate nitrogen management is needed in order to obtain both goals.

On the one hand, high yields of storage roots are significantly related to the amount of nitrogen taken up by plants during the growing season. On the other hand, excess nitrogen in beet plant at harvest results in a high content of soluble nitrogen compounds, which deteriorate the quality of storage roots (Burba 1996, Hoffmann 2005).

God nitrogen management in sugar beet farming, a driving factor in sugar production, is difficult due to many factors. Supply of nitrogen to plants throughout the growing season depends on both soil N resources and externally applied nitrogen fertilizers. In addition, the distribution of assimilates between the storage root and leaves is strongly influenced by the soil nitrogen dynamics. The size of this N pool significantly depends on the weather course during the season. The weather is the primary factor shaping the soil N rate mineralization, and it is highly unpredictable. Consequently, the relationship between N accumulation in beets and applied rates of fertilizer nitrogen is typically a weak one (Malnou et al. 2008, Werker et al. 1999).

The yield potential of sugar beet in most areas of Europe can be considerably impaired due to the shortage of water during both early and later stages of the crop's growth. It is strongly affected by the weather variability, especially in summer months (Kenter et al. 2006). However, the impact of the weather can be modified by a soil nutrient regime, which can at least partly control water shortages (Grzebisz et al. 2002). The degree to which production resources can be substituted depends on the nitrogen management, as pointed out by Freckleton et al. (1999). Therefore, the main problem for achieving efficient N management in sugar beet is to understand the degree of its impact on the dynamics of leaf and storage root growth throughout a growing season.

The objective of this study was to determine patterns of N accumulation in sugar beet parts, using the growth analysis procedure. In other words, we analyzed to what extends a fertilizing regime affects the dynamics of nitrogen accumulation during the course of a growing season.

MATERIAL AND METHODS

The study was based on sets of data obtained from a static field experiment, which was carried out on a private farm at Wieszczyczyn (52°02'N17°05'E) during three consecutive growing seasons 2001, 2002, 2003. The soil originating from sandy loam underlined by loam is classified according to the Polish system as class IV a, good rye complex, and in the agronomic categories as light soil. The field trial, arranged in a one-factor design replicated four times, consisted of eight treatments:

1. Control – absolute control, i.e. no applied fertilizers (acronym Control);
2. PK – only phosphorus and potassium (VPK, Variant PK);
3. NK – only nitrogen and potassium (VNK);

4. NP – only nitrogen and phosphorus (VNP);
5. NPK – basic set of nutrients, but P, K rates limited to 25% of adjusted quantity (V25);
6. NPK – basic set of nutrients, but P, K rates limited to 50% of adjusted quantity (V50);
7. NPK – basic set of nutrients, full rate of adjusted quantity of nutrients (V100);
8. $NP^{*}K$ – basic set of nutrients, as in the W100 variant, but P was applied as partially acidulated phosphoric rock (V100P).

The preceding crop for sugar beet (variety *Kassandra*) was winter wheat. The main rates of phosphorus and potassium were calculated annually based on the expected yield of storage roots (60 t ha^{-1}) and the current soil P and K fertility for the NPK treatment. The actually applied rates of both nutrients followed the experimental design. The rate of fertilizer nitrogen was also calculated annually taking into account three components; i) content of soil mineral nitrogen in the layer 0.9 m, ii) the expected yield, and iii) unit nitrogen accumulation of four kg N t^{-1} (taproots + respective amount of tops). It amounted to 150 kg ha^{-1} in 2001 and 2003 and 120 kg ha^{-1}. All basic fertilizers and the first rate of nitrogen equal 80 kg N ha^{-1} were applied in spring before seedbed preparation. The remaining nitrogen rate was top-dressed at the stage of 3(5) leaf.

For purposes of this particular study, eight plants were sampled (1 m^2) on eight consecutive dates during the sugar beet growth, counting days after sowing (DAS): 40, 55, 77, 92, 113, 134, 155, 175. On each date, every plant sample was divided into sub-samples of leaves and the storage root, and then dried (65°C). The results were expressed on the dry matter (DM) basis. Nitrogen concentration in plant parts was determined by standard macro-Kjeldahl procedure. Nitrogen accumulation (yield) at each sampling date was calculated based on its concentration and dry matter yield of particular parts of sugar beet plants.

The growth analysis procedure was applied to determine the dynamics of nitrogen accumulation during the growing season. Two parameters were applied, determined separately for leaves and taproots and also for the total N uptake by sugar beet canopy. The first parameter, termed as Crop Nitrogen Uptake Rate (CNUR), was calculated from the formula:

$$\mathrm{CNUR} = \frac{N_{a2} - N_{a1}}{T_2 - T_1} \cdot$$

The second growth parameter, the Relative Rate of Nitrogen Uptake, was calculated using the formula:

$$\mathrm{RRNU} = \frac{\mathrm{Ln}N_aW_2 - \mathrm{Ln}N_aW_1}{T_2 - T_1},$$

where:

N_{a2}, N_{a1} – yield of accumulated nitrogen in two consecutive samplings; kg ha^{-1};

T_2, T_1 – two consecutive sampling dates, days after sowing (DAS).

The experimentally obtained sets of data were subjected to conventional analysis of variance, using the computer program Statistica 7. The least significant differences (LSD at $P = 0.05$) were calculated to establish the significance of means for each factor or their interactions. Simple regression was applied to estimate the strength of relationships between some plant characteristics.

RESULTS AND DISCUSSION

Leaves

Nitrogen accumulation (N_a) in leaves significantly depended on the tested fertilizing variants, but at the same time showed significant year-to-year variability (Table 1). The highest effect of the annual weather fluctuation occurred at early stages of the growth. At the stages BBCH 17 and 43, coefficients of variation were 83% and 43%, respectively. These two stages

Table 1

Statistical evaluation of main factors affecting nitrogen accumulation in sugar beet leaves during the growing season (kg ha^{-1} d.m.)

Factors	Level of factor	Days after sowing, DAS							
		40	57	77	92	113	134	155	175
Experimental variants (V)	control	2.79	8.04	19.36	53.9	57.76	73.94	76.7	78.29
	PK	3.63	11.18	30.13	64.2	65.08	69.45	69.3	79.03
	KN	6.99	30.80	64.88	127.4	133.6	105.4	109.1	132.0
	PN	7.10	25.63	59.57	99.7	112.4	114.8	117.5	118.9
	W25	6.10	31.41	73.07	124.2	123.6	110.5	121.1	135.3
	W50	7.61	32.38	67.94	128.9	115.8	103.2	106.9	123.0
	W100	8.72	32.33	63.70	112.8	135.0	125.9	104.4	117.9
	W100P	8.41	32.10	70.31	138.6	118.1	130.4	105.4	130.0
$LSD_{0.05}$		0.75	4.30	8.25	15.3	14.55	13.9	12.05	16.25
Years (Y)	2001	8.47	18.29	51.25	134.7	144.2	127.6	111.9	119.5
	2002	6.65	49.25	82.49	98.3	90.1	86.6	99.6	131.1
	2003	4.14	8.91	34.62	85.6	88.7	98.4	92.4	92.4
F-factor for years		88.3***	252.6***	91.6***	29.5***	50.3***	24.4***	7.2**	77.1***
F-factor for years and variants		5.43***	7.68***	4.55***	2.26*	1.67 n.s	2.53**	2.71**	1.85*

*, **, *** – probability levels of 0.05; 0.01; 0.001; n.s. – non significant

are critical for sugar beet plant development and yielding, as they are responsible for efficiency of solar-energy use (MALNOU et al. 2006). The calculated coefficients clearly indicate differences in the nitrogen uptake rate by growing plants. The general trend of nitrogen accumulation in leaves during the growing season was progressive, following the quadratic regression model:

1. 2001: $N_a = -0.014\ DAS^2 + 3.97\ DAS - 142.7$ for $R^2 = 0.84$ and $N_{aop} = 142$ DAS
2. 2002: $N_a = -0.007\ DAS^2 + 2.12\ DAS - 53.76$ for $R^2 = 0.85$ and $N_{aop} = 151$ DAS
3. 2003: $N_a = -0.009\ DAS^2 + 2.61\ DAS - 98.23$ for $R^2 = 0.92$ and $N_{aop} = 145$ DAS

The day of the maximum N accumulation (N_{amax}) in leaves did not show high year-to-year differences. In 2001 and 2003, it occurred in the second decade of September, but in 2002, it took place one week later. However, the N_{amax} showed substantial seasonal differences, amounting to *ca* 138, 100 and 107 kg N ha^{-1}, respectively.

The effect of the tested fertilizing variants on N_a in sugar beet leaves was significant at each stage of plant sampling. At stage BBCH 14, four homogenous groups of variants are distinguishable, presented below in the decreasing order:

V100, V100P > V50, PN = KN, V25 > PK, control

This order was transient and changed considerably from the stage of BBCH 17 onwards, when the whole population of variants can be regrouped as follows:

V100, V100P, V50, V25, KN > PN > PK, control.

These groups dominated to the mid-season, characterized by the highest absolute rate of total biomass growth (GRZEBISZ et al. 2012). One of the most important observations about the sugar beet response to nutrient application appeared in the PN variant, i.e., in which potassium was omitted from the fertilizing program. Plants grown without fresh supply of potassium gathered significantly much more nitrogen in leaves in comparison to treatments without nitrogen, but at the same time far less than those fertilized with potassium. This phenomenon recurred at the final phase of sugar beet growth. The difference indirectly underlines the importance of potassium in the processes of nitrogen uptake (MARSCHNER et al. 1995). However, the stimulating effect of potassium, as in the NK case, on the yield-forming effect of nitrogen taken up by beet plants is a controversial matter. High accumulation of nitrogen in leaves at harvest is related to its high concentration in the storage root, which depresses its technological quality (HOFFMANN 2006).

The quadratic pattern of N accumulation in sugar beet leaves is typical, but the observed differences among the fertilizing variants can be explained better by the growth analysis procedure. The first parameter, the crop nitrogen uptake rate (CNUR), informs us about the absolute rate of nitrogen accumulation in sugar beet canopy during the growing season. In order to present typical trends, three out of the eight tested variants were selected, i.e., VPK, V25 and V100P. The first one, representing the treatment without nitrogen, showed the highest (except the absolute control) year-to-year variability of harvested yields of storage roots (average yield of 54.4 Mg ha^{-1} and CV = 34.4%). The second one, characterized by the application of 25% of the recommended rate of P and K, showed both the highest average yield of beets (71.8 Mg ha^{-1}) and the lowest CV (9.9%). The third variant, comprising full amounts of recommended nutrients, showed a high yield of storage roots (72.4 Mg ha^{-1}) but almost a three-fold higher CV (+ 29%) than the V25 variant (not all data are not reported in the paper, but details are available from the authors).

Patterns of the in-season CNUR courses were much more dependent on the year-to-year weather variability than on the fertilizing variant (Figure 1a,b,c). In 2001 and 2003, patterns of the nitrogen accumulation rate in sugar beet leaves were very similar, irrespective of the treatment (Figure 1a and c). In the first part of the season, the CNUR increased exponentially, reaching the top at 92 DAS. Since 92 DAS onwards, a sudden, exponential drop was observed, demonstrating however a secondary, nutrient-specific increase. In the second season, in 2002, the elevation of the N rate uptake occurred much earlier in the nitrogen fertilized variants, i.e., at the stage of BBCH 17. The effect of the weather on the seasonal CNUR course is best presented for the V100P variant. The highest nitrogen uptake rate, irrespective of the stage of sugar beet growth, was *ca* 8, 6, and 4 kg N ha^{-1} d^{-1} in 2001, 2003, 2002, respectively (Figure 1c). The recorded differences reflect, albeit only partly, the importance of CNUR$_{max}$ for the

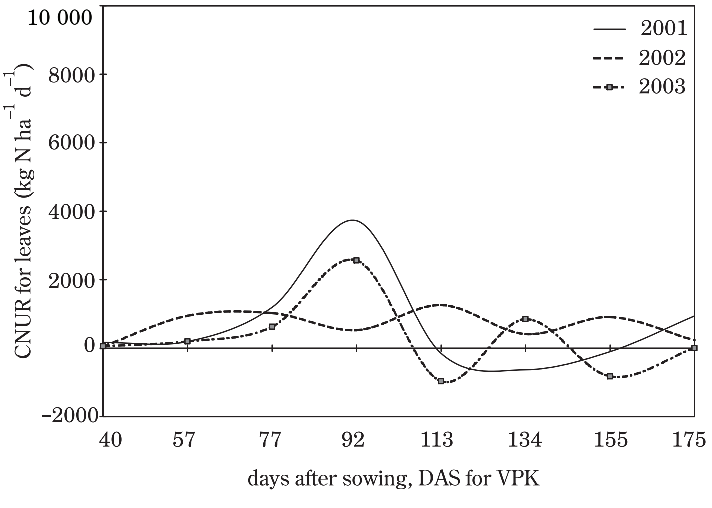

Fig. 1a. Effect of fertilizing variants on the dynamics of absolute nitrogen accumulation rate in sugar beet leaves, PK treatment

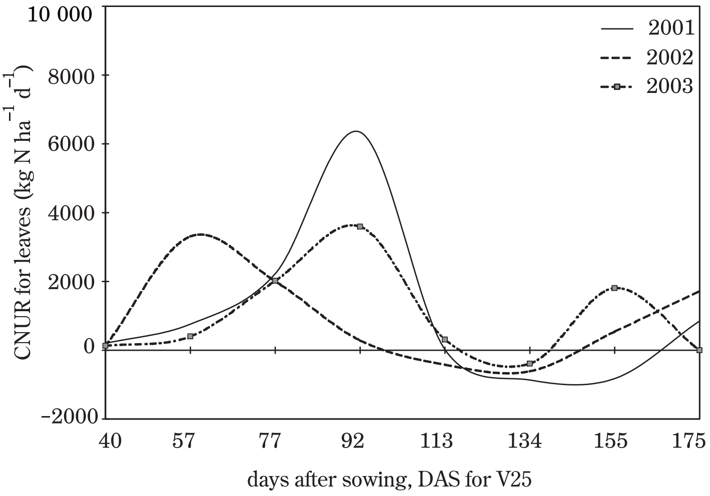

Fig. 1b. Effect of fertilizing variants on the dynamics of absolute nitrogen accumulation rate in sugar beet leaves, V25 treatment

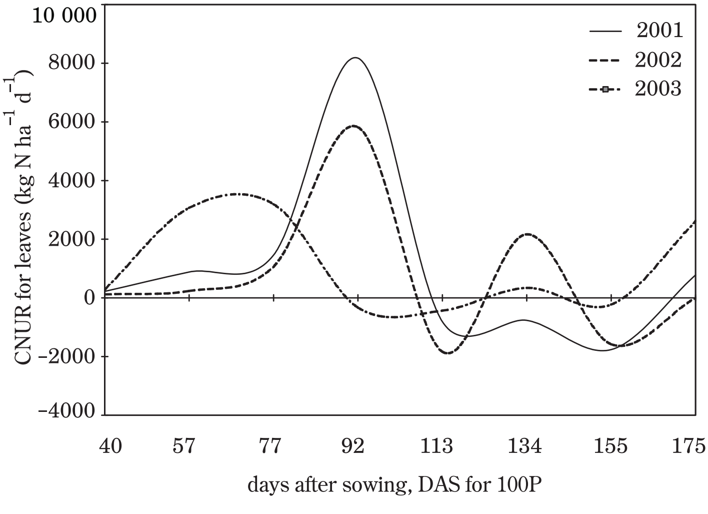

Fig. 1c. Effect of fertilizing variants on the dynamics of absolute nitrogen accumulation rate in sugar beet leaves, V100P treatment

final yield of storage roots. They also indicate that sugar beet plants under water stress are able to compensate the rate of storage root growth.

The second-growth parameter taken into account in this study is the relative rate of nitrogen uptake (RRNU). The course of the developed curves can be described by some attributes such as i) the average value for the whole season, ii) the maximum RRNU value, the DAS of $\mathrm{RRNU}_{\max}$ elevation (Table 2). The general pattern of this growth parameter was very similar in the first part of the season. In 2001 and 2003, the minor peak of the RRNU intake appeared at early stages of sugar beet growth, i.e., at the stage of BBCH 17. The second, major one, took place at 92 DAS (Figure 2). The effect of the fertilizing variants on the average RRNU values was relatively low, achieving the highest values in 2002. For all the fertilizing variants and years, the RRNU showed high in-season variability, as described by coefficients of variation. This was, nevertheless, an attribute of the second part of the season and treatments with nitrogen. The reported recovery of the N uptake corroborates the thesis of Freckleton et al. (1999) of the importance of N fertilization in controlling water shortage. The main reason of certain inconsistency of the coefficient of variation was the weather variability in August and September, in turn affecting the uptake of fertili-

Table 2

Statistical overview of relative rate of nitrogen uptake (RRNU)
by sugar beet during the growing season

Statistical parameters		Leaves			Storage roots			Total		
		VPK	V25	V100P	VPK	V25	V100P	VPK	V25	V100P
2001										
Max	kg kg^{-1} d^{-1}	0.32	0.24	0.34	0.27	0.28	0.28	0.32	0.34	0.29
DAS$_{max}$		92	92	65	92	92	92	57	92	92
Average		0.13	0.11	0.14	0.19	0.20	0.20	0.21	0.17	0.17
SD*		0.12	0.11	0.13	0.06	0.06	0.06	0.06	0.12	0.12
CV**	%	94	93	95	34	30	31	30	72	70
2002										
Max	kg kg^{-1} d^{-1}	0.26	0.28	0.28	0.27	0.29	0.29	0.32	0.33	0.34
DAS$_{max}$		57	57	57	92	92	92	92	92	92
Average		0.20	0.16	0.13	0.16	0.17	0.17	0.19	0.20	0.17
SD		0.05	0.11	0.12	0.09	0.09	0.09	0.6	0.10	0.12
CV	%	27	71	92	55	54	54	30	53	73
2003										
Max	kg kg^{-1} d^{-1}	0.31	0.31	0.33	0.29	0.31	0.30	0.32	0.33	0.34
DAS$_{max}$		92	92	92	92	92	92	92	92	92
Average		0.13	0.16	0.13	0.16	0.15	0.20	0.16	0.17	0.17
SD		0.12	0.12	0.13	0.09	0.12	0.07	0.12	0.12	0.12
CV	%	95	73	95	57	76	34	73	72	73

*standard deviation, **coefficient of variation

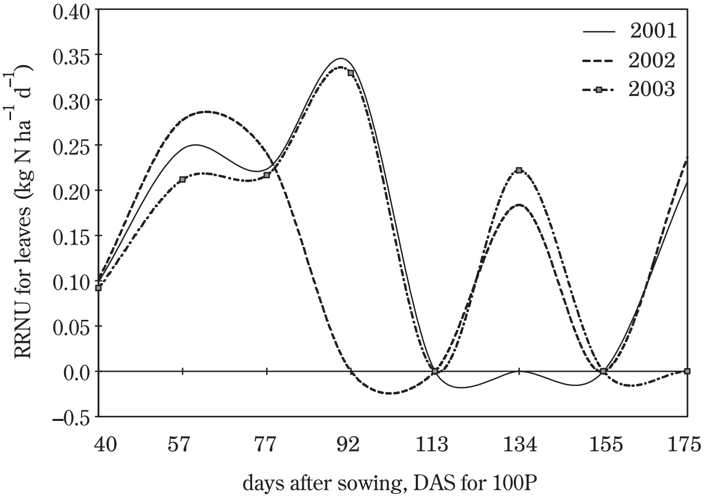

Fig. 2. Effect of the full sugar beet fertilizing on the dynamics of relative nitrogen accumulation rate in sugar beet leaves, V100P treatment

zer nitrogen. This outcome is in agreement with Kenter et al. (2006), who stressed the importance of the course of weather in these two months for the final yield of storage root yields.

Storage roots

The effect of the fertilizing variants on nitrogen accumulation in storage roots was significant, but at the same time year-dependent for the most stages in the growing seasons (Table 3). As in the case of leaves, the course of these attributes during the growing season can be described by year-specific regression models:

1. 2001: $N_a = 0.967$ DAS – 45.56 for $R^2 = 0.99$;
2. 2002: $N_a = -0.00013$ DAS^3 + 0.04 DAS^2 - 3.12 DAS + 66.00 for $R^2 = 0.998$;
3. 2003: $N_a = -1.017$ DAS – 52.49 for $R^2 = 0.96$.

In the first and third growing season, as results from the developed linear models, nitrogen accumulation in storage roots was continuously progressing. This pattern of N in-season accumulation indirectly underlines an ample supply of nitrogen to beet plants from both soil and fertilizer resources until the end of the growing season. This uptake scenario is possible because the storage root can continue its growth for more than 175 days, as occurred in 2001 (Maelander et al. 2003). The linear model of N accumulation can be therefore considered as positive, provided that it results in a simultaneous increase in yield of storage root. This happened in 2001 but not in 2003. For example, in 2001, the yield of storage roots for the 100 P variant slightly exceeded 95 t ha^{-1}, whereas in 2003 amounted only to 60 t ha^{-1}.

Table 3

Statistical evaluation of main factors affecting nitrogen accumulation in sugar beet storage roots during the growing season (kg ha^{-1} d.m)

Factors	Level of factor	Days after sowing, DAS							
		40	57	77	92	113	134	155	175
Experimental variants (V)	control	0.08	1.27	6.75	20.06	37.70	54.02	60.02	76.0
	PK	0.12	2.21	8.68	23.58	38.31	62.52	69.13	80.6
	KN	0.37	5.19	18.07	46.40	75.13	86.28	107.41	114.0
	PN	0.34	3.49	16.51	36.40	66.54	97.10	104.73	113.6
	W25	0.37	5.56	18.68	44.49	78.02	79.46	120.19	114.8
	W50	0.34	5.66	17.55	46.77	75.88	86.12	99.84	148.4
	W100	0.40	6.09	15.21	43.97	77.40	92.91	109.78	136.3
	W100P	0.38	5.20	21.10	47.49	62.54	98.04	112.89	142.9
$LSD_{0.05}$		0.06	0.81	2.73	5.34	7.79	8.60	13.10	15.70
Years (Y)	2001	0.26		24.32	46.50	58.76	81.69	101.4	130.6
	2002	0.38	1.95	13.44	32.41	60.93	78.41	93.9	86.2
	2003	0.27	4.31	8.20	37.03	72.13	86.05	98.7	130.1
F-factor for years		14.9***	92.7***	96.4***	19.2***	9.00***	2.1 n.s	0.88	28.3***
F-factor for years and variants		0.68	4.7***	6.9***	2.8**	1.9*	1.9*	2,5**	1.2 n.s

*, **, *** – probability levels of 0.05; 0.01; 0.001; n.s. – non significant

Quite a different pattern of nitrogen accumulation was recorded in 2002. The developed regression model indicates occurrence of some factors limiting the N uptake rate during the season. Calculating the first derivative, the transition day of nitrogen uptake rate can be fixed. In this case, it occurred at 103 DAS, i.e. it took place at the end of the third decade of July. The main cause of the decreased rate of N accumulation in storage roots was water shortage, which reduced the rate of nitrogen uptake. This pattern of N accumulation can be considered as positive provided that yield of storage roots follows the same course or increases.

The effect of the fertilizing variants on the nitrogen accumulation course showed the highest variability and dependence on the actual weather conditions, but limited to the first part of the season. Again, the most interesting is the NP variant. Plants grown without fertilizer potassium accumulated far less until 113 DAS than in the other N fertilized variants. At harvest, the nitrogen status in storage roots can provide much useful information. All the fertilizing variants, according to LSD, can be divided into three distinctive groups based on the quantity of accumulated N:

V50, V100, V100P > V25, VKN, VPN > VPK, control.

The order of the variants is simply related to rates of applied P and K fertilizers. Therefore, it can be concluded that rates of both nutrients below

50% of the recommended rate do not guarantee to cover sugar beet requirements for nitrogen. This conclusion is true, assuming that the growth conditions allowing full exploitation of the sugar beet yielding potential. Its achievement depends, however, on the weather course during the middle and second part of the season (Figure 3a, b). Sugar beet plants could compensate the rate of nitrogen accumulation in storage roots during the second part of the season, in turn stimulating the rate of both canopy and storage root growth (Grzebisz et al. 2012). This finding is contrary to the observation reported for sugar beets grown in Britain and made by Malnou et al. (2008). These authors did not find any significant effect of late-summer, compensatory supply of N on beet on sugar yield.

The conducted growth analysis showed high, but inconsistent variability of the nitrogen accumulation rate in storage roots (Figure 3a, b). The general pattern of CNUR showed a sinusoidal-like course. It inconsistency was both year- and nutrient-specific. Plants grown on plots fertilized with 25% of the recommended P and K rates, showed high recurring peaks, which took place at 92 and 155 DAS in 2003 and at 113 and 155 DAS in 2002. For comparison, in 2001 plants grown on the 100P plot showed a constant rate of N accumulation from the stage of BBCH 43 onwards. This pattern of growth was a prerequisite of a significantly high yield of storage roots. However, as presented in the Figure 3b, the in-season variable pattern of N accumulation can be considered as an attribute of a high-yielding sugar beet plantation. The fully fertilized variants showed a multi-elevation pattern of the CNUR course. The highest yield of storage roots can be related to a very fast rate of N accumulation in the early stages of growth, followed by secondary and even tertiary elevations. Any factor delaying the rate of N accu-

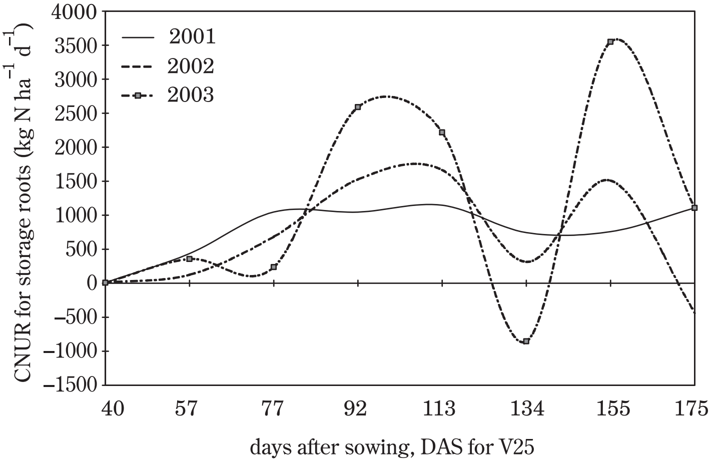

Fig. 3a. Effect of fertilizing variants on the dynamics of absolute nitrogen accumulation rate in storage roots, V25 treatment

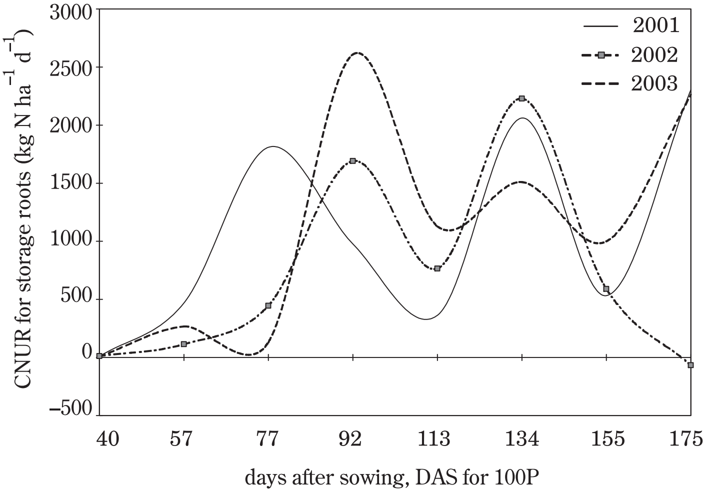

Fig. 3b. Effect of fertilizing variants on the dynamics of absolute nitrogen accumulation rate in storage roots, V100P treatment

mulation in the storage root in the early stages of growth negatively affects its final yield, as occurred in 2002. The pattern of N accumulation dynamics, as described for the 100P variant in 2001, fully corroborates the thesis presented by Boiffin et al. (1992), who assumed that any factor disturbing the rate of canopy growth at the early stages of sugar beet growth negatively affected the interception of solar radiation by the sugar beet canopy, thus slowing down the rate of plant growth due to inferior utilization of solar energy. It can be therefore concluded that any factor retarding the N uptake accumulation in the storage root during the early stages of sugar beet growth negatively affects its yield (Malnou et al. 2006). It has been documented that nitrogen accumulated in the young storage root is important for the development of parenchyma rings, a prerequisite of its potential storage size for sugar accumulation (Bell et al. 1996).

The general pattern of the relative rate of nitrogen accumulation (RRNA) in the storage root was highly consistent in years and fertilizing variants (Table 2, Figure 4). S a rule, plants achieved $RRNA_{max}$, which ranged from 0.27 to 0.31 kg N kg^{-1} d^{-1}, at 92 DAS. The average RRNA, however, was slightly affected by the annual weather course, being much lower in 2002 and 2003. The main reason for high in-season RRNA variability, as indicated by the coefficient of variation, was its inconsistency in the second part of the season. It was both nutrient- and year-specific.

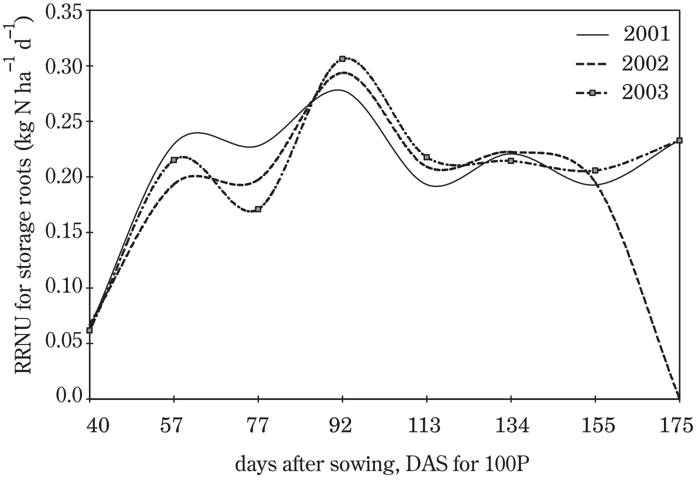

Fig. 4. Effect of the full sugar beet fertilizing on the dynamics of relative nitrogen accumulation rate in storage roots, V100P treatment

Total nitrogen uptake

The total nitrogen accumulation in sugar beet canopy during the growing season showed a significant response to the tested fertilizing variants. However, it was distinctly modified by the course of weather in each stage of plant growth except at harvest (Table 4). The strongest effect of the weather was manifested in the early stages of sugar beet development. The general pattern of total N accumulation in beets during the growing season can be described by two regression models. In 2001, it followed a quadratic function but in 2002 and 2003, it was described by a linear one, as presented below:

1. 2001: $N_{at} = -0.014\,DAS^2 + 4.741DAS - 177.8$ for $R^2 = 0.93$ and $DAS_{op} = 169$;
2. 2002: $N_{at} = -\,1.469\,DAS - 28.37$ for $R^2 = 0.95$;
3. 2003: $N_{at} = 1.757\,DAS - 67.34$ for $R^2 = 0.93$.

The models of N accumulation during the growing season indirectly indicate completely different growing conditions in 2001 in comparison to the other years. In 2001, sugar beet plants could fully exploit soil and fertilizer N resources, reaching the *nitrogen saturation* status. This is verified by the maximum N uptake of 223.6 kg N ha^{-1}, achieved at 169 DAS. The presented model coincides with the maximum yield of storage roots. Therefore, the quadratic model of N accumulation by sugar beet plants throughout the season can be considered as optimal for the maximum N productivity. In the other two years, the linear model of in-season N accumulation dominated, in turn underlying the *unsaturated status* of nitrogen management by beet plants. The term simply means that plants could accumulate high amounts of nitrogen, but did not convert them into a respective biomass of

Table 4

Statistical evaluation of main factors affecting total nitrogen accumulation in sugar beet during the growing season (kg ha^{-1} d.m.)

Factors	Level of factor	Days after sowing, DAS							
		40	57	77	92	113	134	155	175
Experimental variants (V)	control	2.87	9.30	26.11	73.96	95.5	128.0	136.7	154.3
	PK	3.75	13.39	38.81	87.76	103.4	132.0	138.5	159.7
	KN	7.36	35.99	82.95	173.9	208.7	191.7	216.5	246.0
	PN	7.45	29.12	76.07	136.1	178.9	211.9	222.2	232.5
	W25	6.48	36.97	91.75	168.7	201.6	190.0	241.3	250.0
	W50	7.95	38.03	85.49	175.7	191.7	189.3	206.7	271.3
	W100	9.12	38.42	78.91	156.8	212.4	218.8	214.2	254.3
	W100P	8.79	37.31	91.41	186.1	180.7	228.4	218.3	272.9
$LSD_{0.05}$		0.77	4.84	9.70	17.95	17.94	18.83	20.48	20.25
Years (Y)	2001	6.91	55.99	106.8	144.8	148.9	168.3	200.9	250.0
	2002	8.85	20.24	64.7	167.1	205.1	206.0	205.9	217.3
	2003	4.40	13.21	42.8	122.6	160.8	184.5	191.1	223.0
F-factor for years		88.2***	238***	112.7***	16.3***	28.9***	10.7***	1.4 n.s	45.4***
F-factor for years and variants		5.2***	7.0***	5.9***	2.5**	1.9*	2.5**	3.2***	1.4 n.s.

*, **, *** – probability levels of 0.05; 0.01; 0.001; n.s. – non significant

beets. As a result, the harvested yields of storage roots were much lower than in 2001. Therefore, this model presents a sub-optimal model of N management by sugar beet canopy.

Nitrogen accumulation (N_a) during the course of a growing season was stage-to-stage variable. Its seasonal fluctuation, induced by the weather course, occurs mainly between the treatments fertilized with nitrogen. The applied index, determined at sugar beet harvest, known as nitrogen use efficiency (NUE), confirms high year-to-year N_a variability (Figure 5). The average NUE values were 72%, 48%, 62% for 2001, 2002, and 2003, respectively. What is even more interesting is the nutrient-induced variability of NUE, as described by the coefficient of variation (CV, %):

VPN (9.9) < VKN (15.1) < V25 (20.9) < V100 (22.8) < V50 (31.5) < V100P (37.3).

The most stabile value of NUE, but at the same time the lowest among the fertilizing variants, is attributed to the PN treatment (55.5%), i.e., without potassium. The importance of this particular nutrient for N uptake is supported by the fact that efficiency of N fertilizer, as found for the KN variant, was 10% higher (65.1%). The third group of variants consists of two treatments, i.e., V25 and V100. For both, the NUE increased slightly above 70%, but at the same time its seasonal variability exceeded 20%. The fourth group, characterized by the highest variability of both indices, i.e., the NUE (> 81%) and CV (above 30%), comprises V50 and V100P variants. The impact

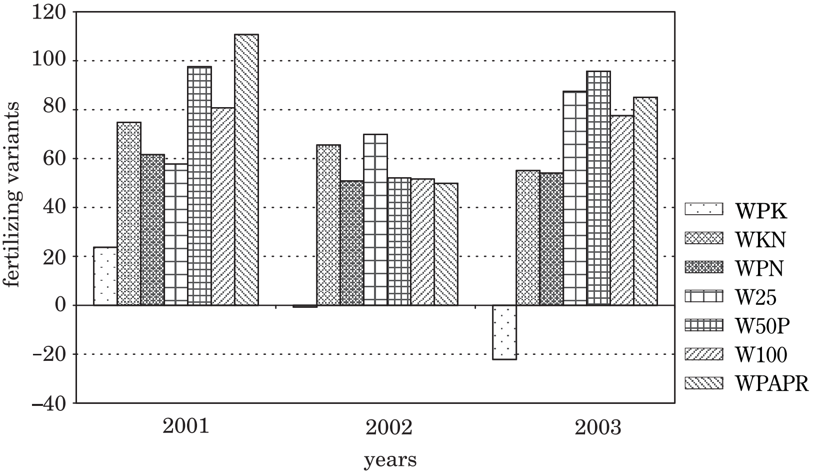

Fig. 5. Effect of differentiated fertilizing variants on nitrogen use efficiency in three consecutive growing seasons

of full supply of nutrients on nitrogen recovery was extremely high, both in the wet year 2001, when it approached 100% and in the dry year 2003.

The above variability of NUE indirectly reflects high dynamics of nitrogen accumulation in sugar beet plants throughout the season. The in-season course of the crop nitrogen uptake rate (CNUR) for total N showed high variability in response to both years and applied nutrients. The effect of variable weather conditions allows us to distinguish two main patterns of N uptake, as presented in Figure 6a-c. In 2001 and 2003, sugar beet canopy

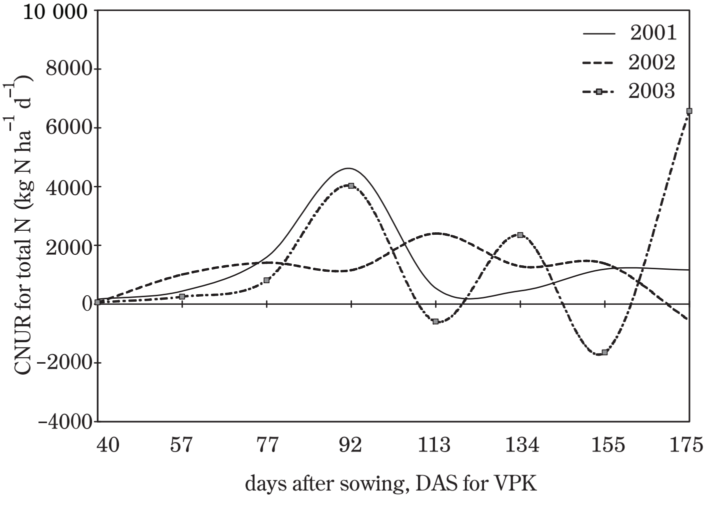

Fig. 6a. Effect of fertilizing variants on the dynamics of absolute nitrogen accumulation rate in sugar beet canopy, PK treatment

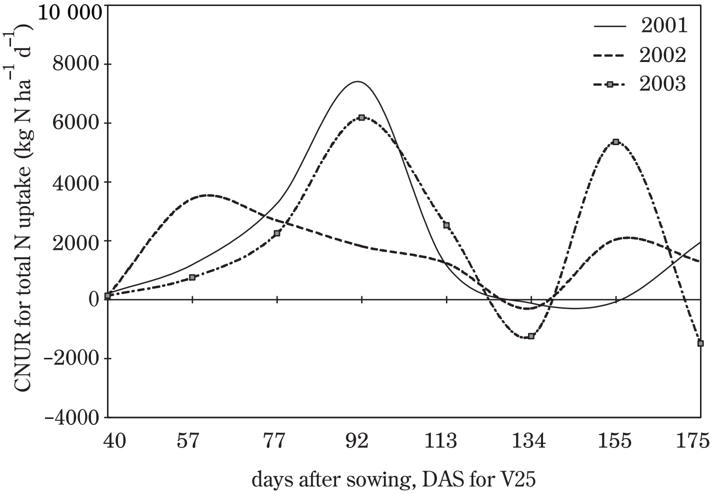

Fig. 6b. Effect of fertilizing variants on the dynamics of absolute nitrogen accumulation rate in sugar beet canopy, V25 treatment

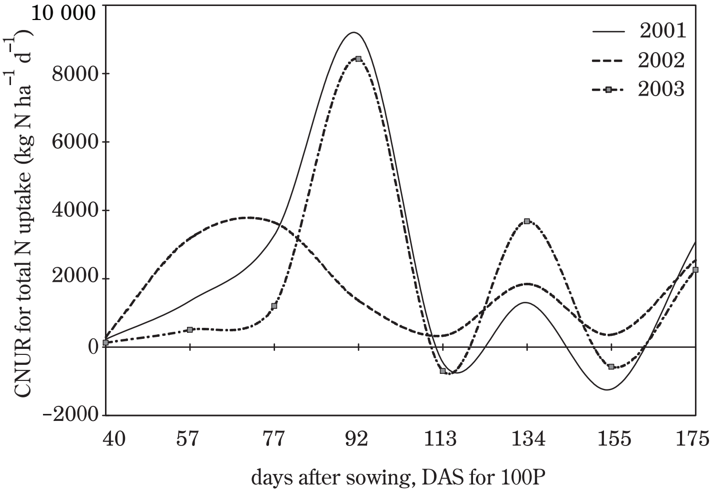

Fig. 6c. Effect of fertilizing variants on the dynamics of absolute nitrogen accumulation rate in sugar beet canopy, V100P treatment

achieved the maximum CNUR at 92 DAS, but in 2002 it happened five weeks earlier, i.e. at BBCH 17. This year-induced difference in the nitrogen accumulation rate was combined with a significant effect of the fertilizing variants. Plants fertilized with 25% of the recommended rate of PK but with a full rate of N accumulated N at the maximum rate of 6 kg N ha^{-1} d^{-1}, but those fertilized with a full PK rate could take up $^1/_3$ more nitrogen on a daily basis, i.e., 8 kg N ha^{-1} d^{-1}. For comparison, beet plants grown in 2002 did not exceed $CNUR_{max}$ of 4 kg N ha^{-1} d^{-1}. Therefore, it can be concluded that in 2002 sugar beet plants experienced nitrogen stress immediately before the period of the highest natural rate of canopy growth. An insufficient supply of nitrate-nitrogen to plants in the early stages of their growth is to a large extent related to an insufficient supply of soil potassium, as was confirmed by the in-season N accumulation in beets grown in the PN treatment (Table 4). As pointed out by MARSCHNER et al. (1996), deficient supply of potassium to growing plants negatively affects the rate of CO_2 fixation, in turn decreasing the size of beets leaves. We should be aware that this nitrate nitrogen temporarily lost is taken up by plants during the later stages of sugar beet growth, in turn disturbing the partitioning of assimilates and deteriorating the technological quality of storage roots (HOFFMANN 2005, POCOCK et al. 1990, WOJCIECHOWSKI et al. 2002).

Despite our expectations, the second-growth parameter, the relative rate of nitrogen uptake (RRNU), did not show high variability in response to the studied factors (Table 2, Figure 7). Sugar beet plants achieved the maximum RRNU generally at 92 DAS, i.e. covering the highest absolute rate of both leaves and taproot growth. As presented in Fig. 7, sugar beet canopy showed a secondary in-growth rate of nitrogen uptake, which for the PK limited treatments in years with drought (2002, 2003) took place three weeks earlier than in wet 2001 (Figure 7a). For the variants with a full supply on both nutrients, the RRNU curves showed the same course, irrespective of

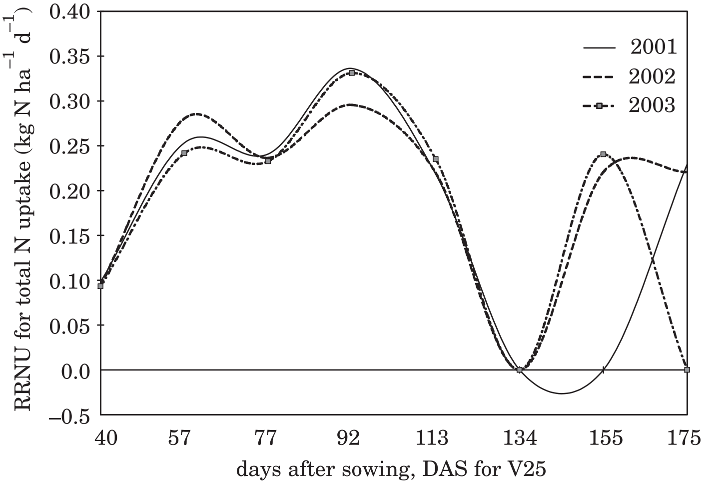

Fig. 7a. Effect of fertilizing variants on the dynamics of relative nitrogen accumulation rate in sugar beet canopy, V25 treatment

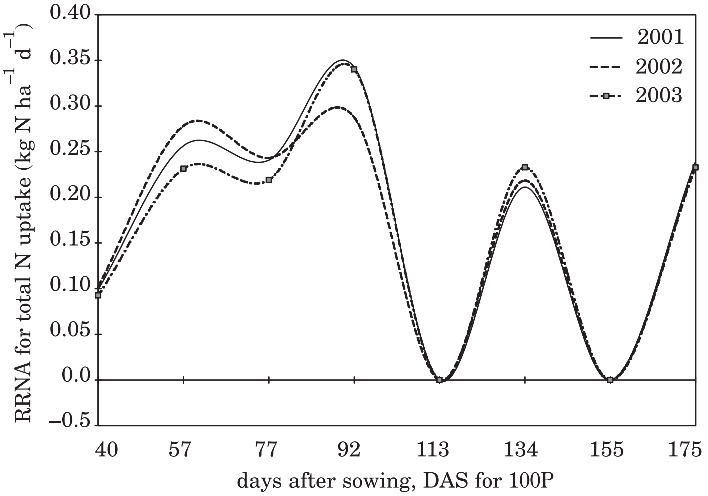

Fig. 7b. Effect of fertilizing variants on the dynamics of relative nitrogen accumulation rate in sugar beet canopy, V100P treatment

the weather conditions (Figure 7b). However, in the first year of the experiment, plants' response focused on storage root yield increase, whereas in the years with water stress, the secondary in-growth of leaves was more strongly affected.

CONCLUSIONS

1. The general pattern of nitrogen accumulation in leaves during the course of the growing season is well described by the quadratic regression model. The course of the weather significantly affects the maximum of N accumulation.

2. The crop nitrogen uptake rate for leaves is slightly variable in the first part of the sugar beet growing season, but, but becomes significantly varied in the second one.

3. The $CNUR_{max}$ for leaves reflects fairly well the yielding potential of sugar as affected by the weather course and level of applied nutrients. Phosphorus and potassium rates below 50% of the recommended rate do not ensure that the storage root requirements for nitrogen are covered.

4. As a rule, the nitrogen accumulation in leaves and the storage root was lower in plants without fertilizer potassium.

5. The developed patterns of absolute and relative uptake rate of nitrogen shows compensatory uptake of N from its soil resources in the second

part of the season. However, its effect on the yield of storage roots was inconsistent. Under ample water and nutrient supplies, it could be considered as a factor allowing complete exploitation of the yielding potential of sugar beets.

6. The quadratic model of the total nitrogen accumulation in sugar beet canopy throughout the

season indicates high efficiency of both soil and fertilizer nitrogen taken up by plants.

REFERENCES

Bell Ch., Milford G., Leigh R-A. 1996. *Sugar beet*. In: *Photoassimilate distribution in plants and crops.* Eds. Zamski E., Schaffer A. Nowy Jork, Marcel Dekker Inc., 691-707.

Boiffin J., Durr C., Fleury A., Marin-Lafleche A., Maillet I. 1992. *Analysis of the variability of sugar beet (Beta vulgaris* L*) growth during the early stages*. I. *Influence of various conditions on crop establishment.* Agronomie, 12: 515:525.

Burba M. 1996. *Der Schädliche Stickstoff als Kriterium der Rübenqualität*. Zuckerind, 121(3): 165-173.

Freckleton R., Watkinson A., Weeb D., Thomas T. 1999. *Yield of sugar in relation to weather and nutrients*. Agric. Forest Meteorol., 93: 39-51.

Grzebisz W., Musolf R., Barłog P., Potarzycki J. 2002. *Potassium fertilization, water shortages during vegetation and crop yielding variability, the case of sugar beet.* Biul. IHAR, 222: 19-30.

Grzebisz W., Szczepaniak W., Pepliński K., Barłóg P., Cyna K. 2012. *Impact of nitrogen concentration in sugar beet plant parts throughout the growing season on dry matter accumulation patterns.* J. Elementol. (submitted)

Hoffmann C. 2005. *Changes in N composition of sugar beet varieties in response to increasing N supply*. J. Agron. Crop Sci., 191: 138-145.

Kenter Ch., Hoffmann C., Maerlaender B. 2006. *Effects of weather variables on sugar beet yield development* (*Beta vulgaris* L.). Europ. J. Agron., 24: 62-69.

Malnou C., Jaggard K., Sparkes D. 2006. *A canopy approach to nitrogen fertilizer recommendation for the sugar beet.* Europ. J. Agron., 25: 254-263.

Malnou C., Jaggard K., Sparkes D. 2008. *Nitrogen fertilizer an the efficiency of the sugar beet crop in late summer*. Europ. J. Agron., 28: 47-56.

Marlander B., Hoffmann C., Koch H., Ladewig E., Merkers R., Petersen J., Stockfish N. 2003. *Environmental situation and yield performance of sugar beet crop in Germany: heading for sustainable development.* J. Agron. Crop Sci., 189: 201-226.

Marschner H., Kirkby E.A., Cakmak I. 1996. *Effect of mineral nutritional status on shoot-root partitioning of photo-assimilates and cycling of mineral nutrients.* J. Exp. Bot., 1255-1263.

Pocock T.O., Milford G., Armstrong M. 1990. *Storage roots quality in relation to nitrogen uptake*. J. Agric. Sci. Cambridge, 115: 335-362.

Werker R., Jaggard K., Allison M. 1999. *Modelling partitioning between structure and storage in sugar beet: effects of drought and soil nitrogen.* Plant Soil, 207: 97-106.

Wojciechowski A., Szczepaniak W., Grzebisz W. 2002. *Effect of potassium fertilizer on yield and technological quality of storage roots.* Part IV. *Soil and fertilizer nitrogen and yield of recoverable sugar*. Biul. IHAR, 222: 77-82. (in Polish)

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CHANGES IN THE CONTENT OF SELECTED HEAVY METALS IN GROUNDWATER EXPOSED TO THE IMPACT OF A MUNICIPAL LANDFILL SITE*

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Abstract

Landfilling is the most popular method of waste treatment in Poland. Old and usually uninsulated landfills constitute a major source of contaminants, which are leached by precipitation waters. As rainwater percolates through a landfill, suspended and dissolved components, waste decomposition products and various microorganisms are leached. Given the lack of any protection against leachate contacting groundwater, contaminants spread over a distance ranging from dozens of meters to several kilometers.

The purpose of this paper was to determine the degree of contamination of leachate and groundwater with heavy metals (manganese, iron, nickel, chromium, zinc, copper, lead and cadmium) in the surroundings of the Maślice municipal landfill site in Wrocław. The landfill was created without prior insulation of its base, and it has accumulated *ca* 2.4 mln m^3 of waste in over thirty years of its use. Studies on the groundwater were carried out from 1995 to 2008, and the examination of the leachate was concluded in 2004.

The analysis of the results shows that the groundwater flowing to the research object is already contaminated. While water percolates through the uninsulated base of the landfill, its quality continues to deteriorate. A large quantity of accumulated waste contributes to the severe contamination of the groundwater.

Leachate from the insulated part of the landfill displayed properties that correspond to "old" landfills. Inside such landfills, the processes occurring during the methanogenic phase of waste decomposition are abundant. Concentrations of heavy metals found during

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the research (except some characteristic values of iron and chromium) were within the limits that allow discharge to sewage facilities, surface waters or soil.

Changes in the content of analyzed heavy metals in the leachate and groundwater give a reason to suppose that in the years to come the amount of leached contamination may grow. Thus, it is necessary to continue the research – even in a scope broader than required by regulations concerning the monitoring of landfills.

Key words: municipal waste, landfilling, groundwater, leachate, contamination, heavy metals.

ZMIANY ZAWARTOŚCI WYBRANYCH METALI CIĘŻKICH W WODACH PODZIEMNYCH NARAŻONYCH NA ODDZIAŁYWANIE SKŁADOWISKA ODPADÓW KOMUNALNYCH

Abstrakt

Składowanie jest najbardziej rozpowszechnioną formą unieszkodliwiania odpadów w Polsce. Stare, często nieuszczelnione składowiska stanowią poważne źródło zanieczyszczeń, wymywanych przez wody opadowe. Przesiąkanie wody przez złoże odpadów powoduje wymywanie do wód gruntowych zawieszonych i rozpuszczonych składników, substancji powstających w trakcie rozkładu odpadów oraz rozmaitych mikroorganizmów. Brak zabezpieczeń uniemożliwiających kontakt wód odciekowych z podziemnymi powoduje rozprzestrzenianie się zanieczyszczeń, których zasięg może wynosić od kilkudziesięciu metrów do kilku kilometrów.

Celem pracy było określenie zanieczyszczenia wybranymi metalami ciężkimi (mangan, żelazo, nikiel, chrom, cynk, miedź, ołów i kadm) wód odciekowych i podziemnych na terenach otaczających składowisko odpadów komunalnych „Maślice” we Wrocławiu. Powstało ono bez wcześniejszego uszczelnienia podłoża, a w ciągu ponad trzydziestu lat eksploatacji zgromadzono na nim ok. 2,4 mln m^3 odpadów. Badania wód podziemnych prowadzono w latach 1995-2008, badania odcieków zakończono w 2004 roku.

W badaniach wykazano, że już wody podziemne dopływające do składowiska są zanieczyszczone. Podczas przepływu przez nieuszczelnione podłoże składowiska następuje dodatkowe pogorszenie ich jakości. Duża ilość nagromadzonych odpadów jest przyczyną poważnego zanieczyszczenia wód podziemnych.

Wody odciekowe z uszczelnionej części składowiska wykazywały właściwości odpowiadające „starym” składowiskom, w których wnętrzu dominują procesy związane z fazą metanogenną rozkładu odpadów. Stwierdzone w trakcie badań stężenia metali ciężkich (oprócz niektórych wartości charakterystycznych dla żelaza i chromu) mieściły się w granicach pozwalających zarówno na odprowadzanie do kanalizacji, jak i do wód lub do ziemi.

Zmiany zawartości analizowanych metali ciężkich w wodach podziemnych i odciekowych pozwalają przypuszczać, że w kolejnych latach ilość wymywanych zanieczyszczeń może się zwiększać. W związku z tym konieczne jest kontynuowanie badań, nawet w zakresie szerszym od wymaganego przez przepisy dotyczące monitoringu składowisk.

Słowa kluczowe: odpady komunalne, składowanie, wody podziemne, wody odciekowe, zanieczyszczenie, metale ciężkie.

INTRODUCTION

Landfilling is the most popular method of waste treatment in Poland. In 2009 alone, the amount of municipal waste dumped grew by 7,859,000 tons (Environment 2010), whereas its aggregate quantity in landfills is difficult to estimate. Old and usually uninsulated landfills constitute a major source of contaminants, which are leached by precipitation waters. As rainwater percolates through a landfill, suspended and dissolved components, waste decomposition products and various microorganisms are leached. The composition of produced leachate depends on the diversity and composition of the waste, degree of its decomposition, water content, type of environmental management, etc. Depending on the amount of precipitation and the method of waste concentration, the resultant leachate can equal 10% up to 60% of the annual precipitation (SZYC 2003). The time elapsing between rainfall and production of leachate can be 1 to 2 months (BENDZ et al. 1997). Leachate generated in the early phase of waste decomposition (acetogenesis) is characterized by a high content of organic acids, which increases the solubility of heavy metals. In the later phase (methanogenesis), leachate has neutral to alkaline pH (organic acids are decomposed to methane and carbon dioxide), which limits the solubility and migration of metals to leachate. Apart from the above properties, leachate can contain various other constituents, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and chloroorganic compounds (WILLIAMS 2002, ÖMAN, JUNESTEDT 2008, KULIKOWSKA 2009). When unused or past sell-by date medications are disposed to municipal waste containers, they are released into the environment. Studies have shown the presence of steroids, vitamins and sulfonamides in leachate from municipal landfills. Such substances as ibuprofen, naproxen, carbamazepine penetrate into groundwater (EGGEN et al. 2010). It has not been verified whether there is an indirect relationship between the content of such metals as nickel or cadmium and the toxicity of leachate. However, recurrence of organic contamination has contributed to its elevated toxicity. In order to remove the metaloorganic bonds of heavy metals, it is necessary to apply complex physicochemical purification methods (ÇEÇEN, GÜRSOY 2000, OLIVERO-VERBEL et al. 2008). These methods are recommended particularly in the case of leachate from long-exploited landfills, while leachate from "young" objects may be purified by biological methods (WILLIAMS 2002, KOC-JURCZYK, JURCZYK 2007, RENOU et al. 2008, KULIKOWSKA 2009).

The risk of heavy metal migration is linked to some other factors apart from the pH of leachate. At landfills in the vicinity of Prague (one operating and one closed), only arsenium, selenium and rubidium were found in the dissolved and mobile form of leachate (MATURA et al. 2010). The other analyzed metals (iron, aluminum, nickel, chromium, zinc, copper and lead) partly created inorganic colloidal bonds with carbonates or components of clay. Between 20% to almost 100% of iron, aluminum and lead were bound in this form. The occurrence of colloidal heavy metal bonds restricts their migration to groundwater. Research conducted at three uninsulated and long exploited landfills (Augsburg, Munich, Gallenbach) has shown that a change in hydrochemical conditions (on the border between the bed of the landfill and the area where groundwater is found) constitutes an effective barrier which stops metals in a colloidal form and reduces their leaching from a landfill (BAUMANN et al. 2006). Similar effects have been observed when leachate contacts surface waters. In oxidating conditions, metals were immobilized in carbonate and organic complexes (ETTLER et al. 2006). Given the lack of any protection against leachate contacting groundwater, contaminants spread over a distance ranging from dozens of meters to several kilometers (WILLIAMS 2002).

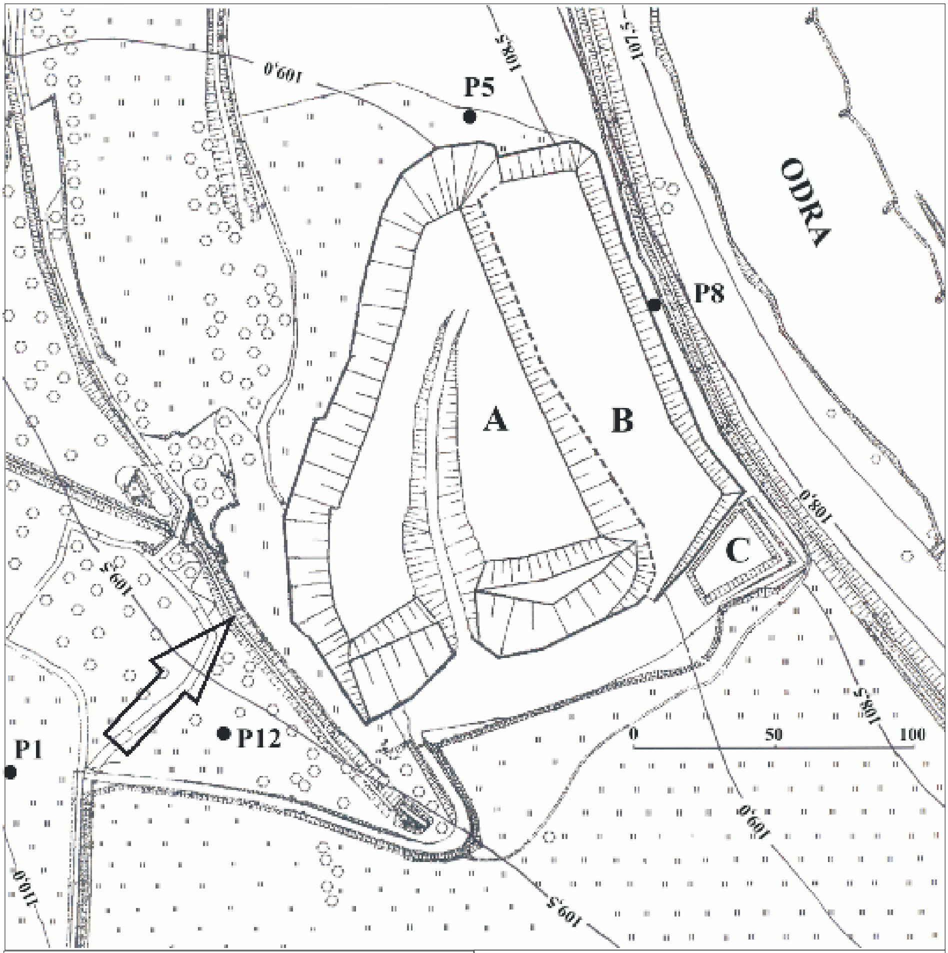
The rules for monitoring the environment in the surroundings of different types of landfills were specified in the Regulations of The Minister of Environment of 9 December 2002, concerning the range, time, method and conditions of monitoring landfill sites. The act defines the obligatory range of assays for every element of the environment (groundwater, surface water, leachate and landfill gas). Monitoring must take place from the moment the location is selected (the pre-exploitation phase), throughout the whole period of exploitation and for 30 years after the decision to close it down.

The purpose of this paper was to determine the contamination of leachate and groundwater with heavy metals (manganese, iron, nickel, chromium, zinc, copper, lead and cadmium) in the surroundings of the Maślice municipal landfill site in Wrocław.

MATERIAL AND METHODS

The object of this research was the municipal landfill site in Maślice, located in Kozia Street in the north-western part of Wrocław (Figure 1). It was built in the late 1960s to fill up an excavation pit remaining after sand deposits had been removed. In the first phase of its use, the waste was deposited into the excavation pit without any prior insulation.

After the excavation area had been filled up, an aboveground part of the landfill started to form. In the 1990s, the facility was expanded by building a new, insulated section and a ground reservoir (from which leachate was channeled) equipped with a monitoring system for the groundwater (a network of piezometers) and a pair of car scales. The area of the “old” section was *ca* 7 ha, while the area of the “new” section was *ca* 2 ha. The use of the latter part began in 1994 and all dumped waste was directed there for several years. When a deposit almost as high as the layer of waste in the old section had been formed, the dumping of waste continued throughout the whole available area, both in the insulated and uninsulated sections.



A – the old section of the landfill
B – the new section of the landfill
C – leachate reservoir

P1, P12, P8, P5 – piezometers
– 109,5 –, – 109,0 – hydroisohipsis
→ the direction of groundwater flow

Figure 1. The location of sample collection sites

The landfill functioned in this manner until the turn of 1999 and 2000. Eventually, the heap of waste reached a height of 50 meters containing nearly 2.4 million m^3 of waste. Rehabilitation works included for example stabilization of the waste body, installation of an outer cover and a screen isolating groundwater, as well as a system for removal of gas from the landfill. In 2004, the reservoir for leachate was bulldozed.

The location of the landfill is advantageous, mainly because it is isolated from adjacent terrains. To the west, partly north and south, it is surrounded by a fairly wide and ecologically varied stretch of woodland, which runs over to arable fields and meadows. Housing estates are about 800-1000 meters away from the facility. To the south of the landfill, the area is covered by allotments and industrial plants. Apart from these objects, the south-eastern side of the landfill site is bordered by meadows with narrow belts of shrubs, bushes and single trees. The Odra River flows 50-100 meters to the east of the site.

In the base of the landfill there are tertiary and quaternary (Pleistocene and Holocene) deposits. Tertiary deposits (clays, dusty clays, compact silty clays; local dust and sandy dust) lie at an average depth of 4.0-6.0 meters and they are 100-120 meters thick. Above them, there are quaternary deposits, which originate from the river (gravels, clay sand-gravel mixes and sands) with silty sand lenses. The total thickness of quaternary deposits ranges from 3.5 to 9.5 meters. One permanent aquifer has been found in the deposits. Its whose water table near the landfill is at a depth of 3-5 meters. Groundwater flows away towards the Odra riverbed, where the nature of drainage is in question (Figure 1).

The paper presents the results of analyses of the content of selected heavy metals (manganese, iron, nickel, chromium, zinc, copper, lead and cadmium) in groundwater (flowing to and away from the landfill) as well as in leachate. The studies of the chemical composition of the groundwater were carried out from 1995 to 2008; the studies of the leachate were finished in 2004, when the reservoir was bulldozed.

Research samples were taken 3 or 4 times a year. Leachate samples were collected from the reservoir, and groundwater samples came from four piezometers located on the sides of the inflow (2) and outflow (2) below the landfill (Figure 1). Before groundwater samples were taken, the stagnant water in the piezometer well was pumped out twice. The content of manganese, iron, nickel, chromium, zinc, copper, lead and cadmium in the samples was determined by means of atomic emission spectrometry, with excitation in inductively coupled plasma (ICP-AES). All the determinations were made at the Centre for Environmental Quality Sciences, the Institute of Soil Science and Environmental Protection at the Wrocław University of Environmental and Life Sciences. The evaluation of significance of the differences between groundwater and leachate from the landfill was made on the basis of a one-way analysis of variance (test F) at the significance level of p=0.05. Statistical analysis of the research results was carried out with Statistica 9 software.

RESULTS AND DISCUSSION

Table 1 shows characteristic values (the average, standard deviation and coefficient of variation) of concentrations of analyzed heavy metals in the groundwater flowing to the landfill. The results show that from 1995 to

Table 1

Characteristic values (average, standard deviation, coefficient of variation) of heavy metal concentrations in groundwater flowing to the landfill

Specification	n	Average	SD	V
Mn (mg dm^{-3})	106	1.909	2.315	121.3
Fe (mg dm^{-3})	106	41.09	54.87	133.5
Ni (mg dm^{-3})	98	0.235	0.212	90.20
Cr (mg dm^{-3})	98	0.092	0.181	196.3
Zn (mg dm^{-3})	98	0.688	1.218	177.0
Cu (mg dm^{-3})	98	0.046	0.032	70.02
Pb (mg dm^{-3})	98	0.021	0.026	123.9
Cd (mg dm^{-3})	98	0.002	0.002	144.2

SD – standard deviation, V – coefficient of variation, n – sample dimension

2008, groundwater flowing into the landfill could not be classified as natural. Average values often exceeded the ranges acknowledged as geochemical background, which proved the existence of a source (or sources) of contamination already flowing out from the landfill. Although the scope of research did not provide explicit identification of the cause of this phenomenon, the low pH of the analyzed waters and high content of sulphates (SZYMAŃSKA-PULIKOWSKA 2009) in conjunction with higher than normal concentrations of heavy metals may lead to a conclusion that biochemical oxidation of metal sulfides occurs in this area. This results in a considerable decrease of the pH and an increase in the heavy metal content of groundwater. Values deemed natural were most significantly exceeded for the concentrations of iron, manganese and nickel. The situation looked slightly better for lead and cadmium. Compared to the levels specified in the Regulation of the Minister of Environment of 23 July 2008 (on the criteria and methods of assessment of groundwater conditions), the values shown in Table 1 indicate severe contamination as well as high variability in the properties of water flowing to the landfill at Maślice.

Table 2 shows characteristic values (average, standard deviation, coefficient of variation) of the concentrations of selected heavy metals in water leaching from the Maślice municipal landfill (landfill leachate). The average contents of the analyzed constituents in leachate from the Maślice landfill were relatively low. Compared to concentrations of a typical methanogenic phase, the average values for the analyzed water were lower in the case of iron, copper, zinc, cadmium and lead and similar for nickel. It was only the average content of chromium that was markedly higher than the value reported in the literature (WILLIAMS 2002). All the average values determined for the leachate from the Maślice landfill exceeded corresponding values ob-

Table 2

Characteristic values (average, standard deviation, coefficient of variation) of heavy metal concentrations in landfill leachate

Specification	*n*	Average	SD	*V*
Mn (mg dm^{-3})	33	1.842	3.670	199.2
Fe (mg dm^{-3})	33	18.38	36.98	201.1
Ni (mg dm^{-3})	33	0.176	0.079	44.58
Cr (mg dm^{-3})	33	0.254	0.133	52.23
Zn (mg dm^{-3})	33	0.382	0.334	87.30
Cu (mg dm^{-3})	33	0.093	0.075	80.79
Pb (mg dm^{-3})	33	0.037	0.028	76.79
Cd (mg dm^{-3})	33	0.003	0.003	127.7

SD – standard deviation, *V* – coefficient of variation, *n* – sample dimension

tained during analyses of twelve Swedish landfills exploited for a period of several to 50 years (ÖMAN, JUNESTEDT 2008).

When compared with the results obtained for Polish landfills, the samples taken in Wrocław contained from 3- to 4-fold more chromium and copper and about 30% more zinc. Cadmium was the only metal whose concentration was three-fold lower than in the leachate from the Wysieka landfill, which has been exploited for a few years (KULIKOWSKA, KLIMIUK 2008). Analyses of leachate from old sections of another municipal landfill (MELLER et al. 2001) also revealed twice as much lead. The concentrations of zinc and cadmium were similar. Leachate from Maślice, on the other hand, contained markedly more manganese and iron (nine- and two-fold, respectively) and approximately 25% more nickel.

The extreme variability of the results reported in the literature enable us to conclude that inside the landfill at Maślice the dominant phase is the methanogenic one, when heavy metal concentrations in leachate decline. Similar changes were also observed during research conducted at other landfills, where most contaminants leached from "old" waste were organic and inorganic compounds. This poses a grave threat to the environment and yet is often neglected while planning the scope of monitoring studies (KULIKOWSKA, KLIMIUK 2008, ÖMAN, JUNESTEDT 2008, RENOU et al. 2008, SZYMAŃSKA-PULIKOWSKA 2008, 2008a).

The scale of heavy metal contamination of the analyzed leachate can also be determined through a comparison of their concentration with the levels detected in sewage discharged to sewage facilities or to surface water or soil. Among standardized constituents, the average concentrations of nickel, chromium, zinc, copper, lead and cadmium fall into the range of values that allow their channeling to both sewage facilities and surface water or soil. Iron was an exception because its content exceeded the level acceptable for discharging untreated leachate to the environment.

Table 3 shows characteristic values (average, standard deviation, coefficient of variation) of concentrations of selected heavy metals in waters flowing away below the Maślice municipal landfill.

Table 3

Characteristic values (average, standard deviation, coefficient of variation) of heavy metal concentrations in groundwater flowing away below the landfill

Specification	*n*	Average	SD	*V*
Mn (mg dm^{-3})	103	3.366	6.789	201.7
Fe (mg dm^{-3})	106	31.33	26.25	83.79
Ni (mg dm^{-3})	96	0.362	0.568	157.1
Cr (mg dm^{-3})	96	0.955	1.251	131.1
Zn (mg dm^{-3})	95	0.406	0.503	123.9
Cu (mg dm^{-3})	95	0.175	0.246	140.9
Pb (mg dm^{-3})	96	0.049	0.080	164.3
Cd (mg dm^{-3})	96	0.004	0.008	179.0

SD – standard deviation, *V* – coefficient of variation, *n* – sample dimension

The average heavy metal concentrations in water exposed to contact with the uninsulated base of the landfill differed from the corresponding values in water flowing to the facility. In most cases (manganese, chromium, nickel, copper, lead and cadmium), the average concentrations were significantly higher (even several-fold) due to the inflowing contaminants. The increase is noticeable despite the presence of reducing conditions in the landfill base, which encourage the precipitation of insoluble forms of the analyzed elements (SZYMAŃSKA-PULIKOWSKA 2009). Compared to the water flowing to the landfill, the water flowing away from the landfill contained on average less of just two elements: iron and zinc. The data presented in Table 3 also shows greater variability in the content of metals in outflowing water than in water flowing to the landfill (except iron, chromium and zinc). The water flowing away from the landfill must be classified as poor quality, mainly due to high concentrations of manganese, iron, nickel and chromium. The average heavy metal content in groundwater was even higher than the corresponding values in leachate, which proves continuous inflow from the uninsulated part of the landfill. Large accumulation of deposited waste creates a risk of emitting large quantities of contaminants. However, research conducted within the surroundings of small municipal landfills has not revealed such bad contamination of groundwater. The values considered natural can be exceeded only in isolated cases or for certain metals (STRUK-SOKOŁOWSKA et al. 2005).

Table 4 presents a range of concentrations (non-outlier values) of heavy metals marked in groundwater flowing away below the landfill and in leachate. Evaluation of the significance of differences between the average values (presented in Tables 2 and 3) was made by means of one-dimensional analysis of variance (test F) at the level of significance p=0.05. The measures of positions presented in the chart illustrate a greater range of fluctuations of the content of nearly all the analyzed constituents (except zinc) in the water flowing away below the landfill. The values characterizing the leachate were more balanced. Significant differences between the average concentrations of metals in the leachate and groundwater were only found for iron and chromium. The research revealed maximum values of concentrations of selected heavy metals (except for zinc and copper) in groundwater exposed to the influence of the landfill, thus leaving no other option but to classify the groundwater as the worst quality grade of ordinary groundwater. In the case of leachate, maximum concentrations of iron and chromium prohibited its discharge to the environment, whereas no values found in the research have made it impossible to discharge it to sewage facilities. The ranges of cadmium, lead and copper shown in Table 4 were similar to those found during the studies on large, uninsulated landfills in Germany (Augsburg, Munich, Gallenbach). Concentrations of chromium, iron, manganese and nickel in the surroundings of the landfill at Maślice were higher, and only the amounts of zinc were lower than in analogous German facilities (BAUMANN et al. 2006).

Table 4

The range of concentrations and significance of differences between average contents of heavy metals in groundwater flowing away below the landfill and in leachate

Specification	Range*		Test F
	groundwater flowing away below the landfill	leachate	
Mn (mg dm^{-3})	0.7-5.75	0.04-2.47	1.516
Fe (mg dm^{-3})	21.16-41.1	0.8-30.9	4.973**
Ni (mg dm^{-3})	0.135-0.589	0.142-0.223	3.474
Cr (mg dm^{-3})	0.462-1.28	0.17-0.3	10.26**
Zn (mg dm^{-3})	0.207-0.606	0.153-0.582	0.064
Cu (mg dm^{-3})	0.085-0.27	0.086-0.137	3.488
Pb (mg dm^{-3})	0.017-0.077	0.018-0.056	0.703
Cd (mg dm^{-3})	0.0013-0.007	0.0004-0.0035	1.477

* non-outlier values

** the marked differences are significant at level of p=0.05

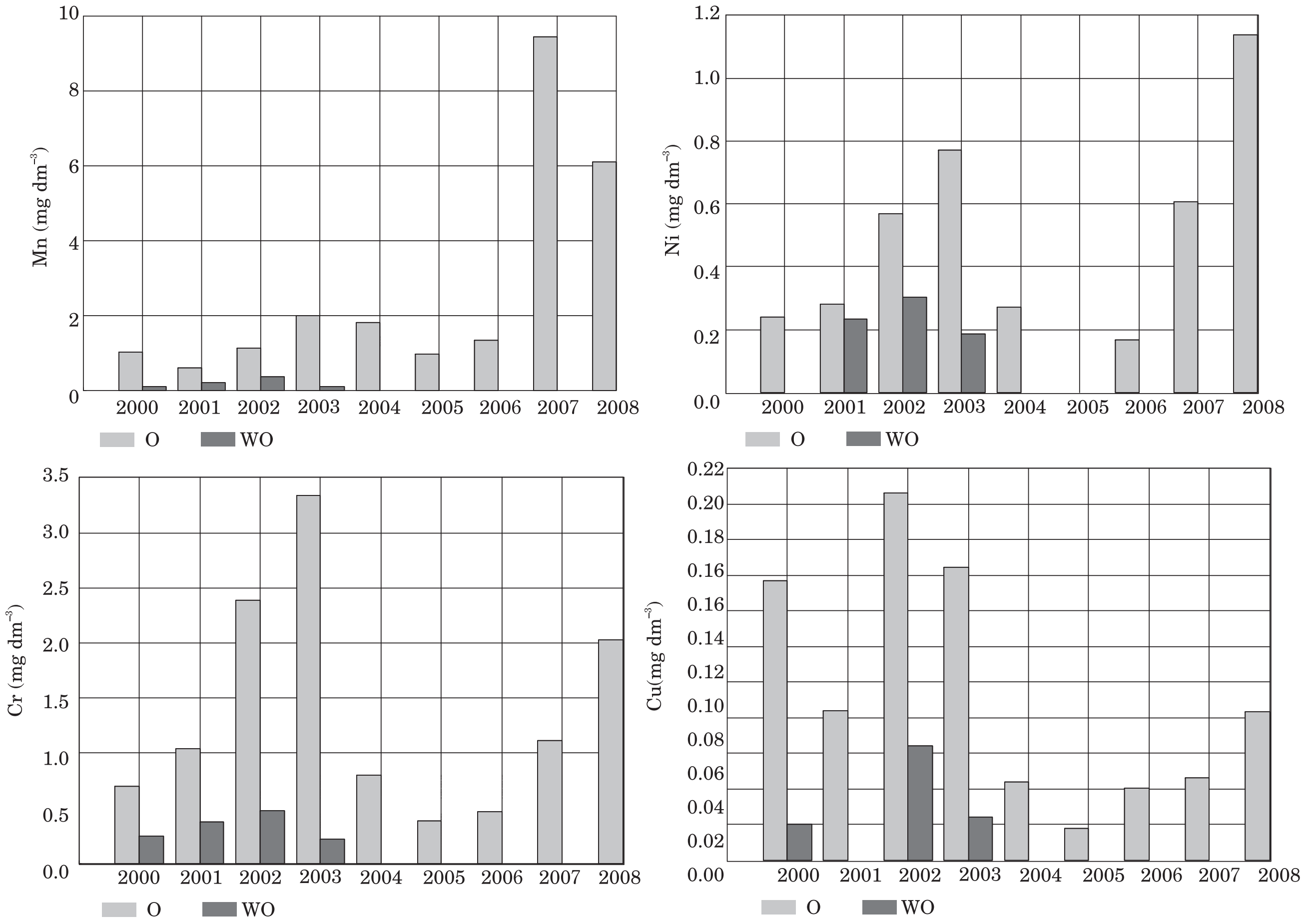


Fig. 2. The average concentrations of manganese, nickel, chromium and copper in leachate (WO) and groundwater flowing away below the landfill (O) in successive years of research

Figure 2 shows a comparison of average annual concentrations of manganese, nickel, chromium and copper that occur in leachate and groundwater flowing away below the landfill, after its closure. From the year 2000 to 2003 (when it was possible to calculate the average values) leachate was characterized by significantly lower contents of the above constituents. Both in leachate and in the water flowing away below the landfill until 2003-2004, a rise in the concentration of manganese, nickel and chromium was observable as well as a persistently high copper content. Afterwards, the concentrations of these metals declined, although in the successive years (2006-2008) their content in groundwater rose again, which was probably caused by the leaching of metals from the waste accumulated in the landfill in the final years of exploitation.

The picture of changes in the properties of leachate occurring in time is not always clear. Research conducted in the first eight years of exploitation of the municipal landfill in Sianów has shown an increasing concentration of heavy metals in leachate in the first 3 to 4 years of exploitation (Szymański et al. 2007). Particularly high concentrations were found for zinc, iron, manganese, copper, lead and nickel. The concentrations of chromium and cadmium were considerably lower. During twelve years of research of leachate from Dyer Boulevard Landfill (Florida, USA), it was observed that with time the content of chromium and nickel was decreasing, while the concentrations of iron and manganese were increasing. In other cases, many research results indicated concentrations below detection levels (Statom et al. 2004). The occurrence of higher concentrations of some contaminants in groundwater under the impact of the Maślice landfill, after a short-lasting decline, is a signal that the area requires constant monitoring, and the emission of contamination may last for many years to come

CONCLUSIONS

1. The groundwater in the whole area surrounding the Maślice municipal landfill is of poor chemical quality. The persistent inflow of contaminants from the uninsulated section adds to its evident deterioration.

2. The analyzed leachate showed properties corresponding to “old” landfills, in which processes related to the methanogenic phase of waste decomposition dominate. Concentrations of heavy metals found during the research (except some values characteristic of iron and chromium) were within the limits that allow discharging leachate to sewage facilities, surface water or soil.

3. Changes in the concentration of the analyzed constituents in groundwater and leachate which occur after a landfill is closed down suggest that

the amount of leached contaminants will grow in the following years. That is why it is necessary to continue research, even in a wider scope than required by existing regulations concerning the monitoring of landfills:

REFERENCES

BAUMANN T., FRUHSTORFER P., KLEIN T., NIESSNER R. 2006. *Colloid and heavy metal transport at landfill sites in direct contact with groundwater.* Water Res., 40: 2776-2786. DOI:10.1016/j.watres.2006.04.049.

BENDZ D., SINGH V.P., ĹKESSON M. 1997. *Accumulation of water and generation of leachate in a young landfill*. J. Hydrol., 203: 1-10. DOI:10.1016/S0022-1694(97)00080-2.

ÇEÇEN F., GÜRSOY G. 2000. *Characterization of landfill leachates and studies on heavy metal removal.* J. Environ. Monitor., 2: 436-442. DOI:10.1039/B004729P.

EGGEN T., MOEDER M., ARUKWE A. 2010. *Municipal landfill leachates: A significant source for new and emerging pollutants.* Sci. Total Environ., 408: 5147-5157. DOI:10.1016/j.scitotenv.2010.07.049.

ETTLER V., MATURA M., MIHALJEVIČ M., BEZDIČKA P. 2006a. *Metal speciation and attenuation in stream waters and sediments contaminated by landfill leachate*. Environ. Geol., 49(4): 610-619. DOI:10.1007/s00254-005-0110-3.

KOC-JURCZYK J., JURCZYK Ł. 2007. *Efficiency of removal of heavy metals from municipal landfill leachate*. J. Elementol., (12)4: 327-334.

KULIKOWSKA D. 2009. *Characteristics and methods of inorganic contamination removal from leachate generated in stabilized municipal landfills.* Ecol. Chem. Eng. 16(3): 389-402. (in Polish)

KULIKOWSKA D., KLIMIUK E. 2008. *The effect of landfill age on municipal leachate composition*. Biores. Technol., 99: 5981-5985. DOI:10.1016/j.biortech. 2007.10.015.

MATURA M., ETTLER V., JE•EK J., MIHALJEVIČ M., ŠEBEK O., SÝKORA V., KLEMENTOVÁ M. 2010. *Association of trace elements with colloidal fractions in leachates from closed and active municipal solid waste landfills.* J. Hazard. Mater., 183: 541-548. DOI:10.1016/j.hazmat.2010.07.058.

MELLER E., NIED•WIECKI E., WOJCIESZCZUK T. 2001. *Concentration of macro and trace elements in leachate generated in three sections of municipal landfill in Sieraków*. Zesz. Probl. Post. Nauk Rol., 477: 405-410. (in Polish)

Environment 2010. Statistical information and elaborations. Central Statistical Office, Warsaw 2010, s. 609.

OLIVERO-VERBEL J., PADILLA-BOTTET C., DE LA ROSA O. 2008. *Relationship between physicochemical parameters and the toxicity of leachates from a municipal solid waste landfill.* Ecotox. Environ. Safe., 70: 294-299. DOI:10.1016/j.ecoenv.2007.05.016.

ÖMAN C.B., JUNESTEDT C. 2008. *Chemical characterization of landfill leachates – 400 parameters and compounds*. Waste Manage., 28: 1876-1891. DOI:10.1016/j.wasman.2007.06.018.

RENOU S., GIVAUDAN J.G., POULAIN S., DIRASSOUYAN F., MOULIN P. 2008. *Landfill leachate treatment: Review and opportunity*. J. Hazard. Mater., 150: 468-493. DOI:10.1016/j.hazmat.2007.09.077.

STATOM R.A., THYNE G.D., MCCRAY J.E. 2004. *Temporal changes in leachate chemistry of a municipal solid waste landfill cell in Florida, USA*. Environ. Geol., 7(45): 982-991. DOI:10.1007/s00254-003-0957-0.

STRUK-SOKOŁOWSKA J., ŻEBRANOWICZ E., WIATER J. 2005. *The impact of municipal landfills on the quality of groundwater.* J. Elementol., 10(3): 821-828. (in Polish)

SZYC J. 2003. *Leachate from municipal landfills*. Inst. Ochr. Środ., Warszawa, s. 93. (in Polish)

SZYMAŃSKA-PULIKOWSKA A. 2008. *Calcium and magnesium in underground waters around a communal waste dump*. J. Elementol., 13(3): 391-399.

SZYMAŃSKA-PULIKOWSKA A. 2008a: *Sodium and potassium in the groundwater in areas near the Maślice municipal refuse dump in Wrocław*. J. Elementol., 13(4): 665-673.

SZYMAŃSKA-PULIKOWSKA A. 2009. *The quality of groundwater in the area of potential impact of municipal landfills*. Wyd. Uniw. Przyr. we Wrocławiu, 150. (in Polish)

SZYMAŃSKI K., SIDEŁKO R., JANOWSKA B., SIEBIELSKA I. 2007. *The monitoring of municipal landfills.* Mater. from VIII Nat. Sc. Conf. on “Comprehensive and detailed problems of environmental engineering”, Politechnika Koszalińska, 75-133. (in Polish)

WILLIAMS P.T. 2002. *Emissions from solid waste management activities*. Issues in Environ. Sci. Technol., 18: 141-170.

J. Elem. s. 703–712 DOI: 10.5601/jelem.2012.17.4.12

FLUORIDE CONTENT AND BIOLOGICAL VALUE OF FLOWERS OF SOME CHAMOMILE (*MATRICARIA RECUTITA* L.) CULTIVARS

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Abstract

Chamomile (*Matricaria recutita* L.) is one of the most important medicinal plant in Europe. Phytotherapeutically useful are inflorescences – *Anthodium chamomillae*. Their main biologically active compound is an essential oil. Among other active agents there are polyphenols, carotenoids and ascorbic acid. The aim of the experiment was to compare fluoride content and biological value of four Polish chamomile cultivars: Promyk (2*n*), Mastar (2*n*), Złoty Łan (4*n*) and Dukat (4*n*). The experiment was carried out in 2010 in Poland. The chemical analyses included determination of the content of dry matter, total ash, total nitrogen, total protein, crude fibre, nitrates V, total and reducing sugars, saccharose, total chlorophyll, chlorophyll a and b, L-ascorbic acid, total carotenoids, total polyphenols, total flavonoids, essential oil, titratable acidity, antioxidant activity and fluoride concentration. It was proved that among the tested cultivars, the diploid cultivars of chamomile Promyk and Mastar were characterized by high biological value. They contain high amounts of total polyphenols, total flavonoids, dry matter, total ash, total nitrogen, total protein, crude fibre, total and reducing sugars and saccharose. However, the highest content of essential oil and fluoride content was determined in flower anthodia of the tetraploid cultivar Dukat.

Key words: chamomile cultivars, essential oil, polyphenols, flavonoids, antioxidant activity, fluoride.

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ZAWARTOŚĆ FLUORKÓW I WARTOŚĆ BIOLOGICZNA KOSZYCZKÓW WYBRANYCH ODMIAN RUMIANKU POSPOLITEGO (*MATRICARIA RECUTITA* L.)

Abstrakt

Rumianek pospolity (*Matricaria recutita* L.) jest jedną z najważniejszych roślin leczniczych w Europie, której głównym surowcem farmakologicznym są kwiatostany – koszyczki (*Anthodium chamomillae*). Ich głównym biologicznie czynnym składnikiem jest olejek eteryczny, a ponadto polifenole, karotenoidy i kwas askorbinowy. Celem badań było porównanie wartości biologicznej oraz zawartości fluorków w koszyczkach czterech polskich odmian rumianku: Promyk (2*n*), Mastar (2*n*), Złoty Łan (4*n*) i Dukat (4*n*). Analizy chemiczne obejmowały oznaczenie zawartości suchej masy, popiołu ogólnego, azotu ogólnego, białka ogółem, błonnika surowego, azotanów V, cukrów ogółem i redukujących, sacharozy, chlorofili ogółem, chlorofilu a i b, kwasu L-askorbinowego, karotenoidów ogółem, polifenoli ogółem, flawonoidów ogółem, olejku eterycznego. Oznaczono także kwasowość ogólną, aktywność antyoksydacyjną oraz koncentrację fluorków. Stwierdzono, że spośród badanych w doświadczeniu odmian wysoką wartością biologiczną odznaczały się diploidalne odmiany rumianku: Promyk i Mastar. Wykazano w nich dużą zawartość polifenoli ogółem, flawonoidów ogółem, a także suchej masy, popiołu ogólnego, azotu ogólnego, białka ogółem, błonnika surowego, cukrów ogółem i redukujących oraz sacharozy. Natomiast istotnie największą zawartość olejku eterycznego oraz fluorków stwierdzono w koszyczkach tetraploidalnej odmiany Dukat.

Słowa kluczowe: odmiany rumianku, olejek eteryczny, polifenole, flawonoidy, aktywność antyoksydacyjna, fluorki.

INTRODUCTION

Chamomile (*Matricaria recutita* L., syn. *Matricaria chamomilla* L. or *Chamomilla recutita* (L.) Rauschert, family *Asteraceae*) is one of the most important medicinal plant native to southern and eastern Europe, and adjoining Asian countries (SINGH et al. 2011). It is cultivated in Poland on 650--750 ha (SEIDLER-ŁOŻYKOWSKA 2007)

The medicinal part of the plant is the flower (*Matricariae flos*) and the medicinal substance is an essential oil (*Aetheroleum chamomillae*) (FP VIII, 2008). Over 120 constituents have been identified in chamomile (MCKAY, BLUMBERG 2006). Among its active agents we can find volatile and non-volatile components (GOSZTOLA et al. 2006, SALAMON 2009). The flowers contain 0.24-1.9% essential oil, of which the main components are a-bisabolol, bisabolol oxides, (E)-β-farnesene, chamazulene, and en-yn-dicycloethers (ORAV et al. 2001, RAAL et al. 2003, SALAMON 2009). Its blue colour is due to chamazulene, a secondary product of hydrodistillation and it is a result of proazulene (matricin) transformation (SVÁBNÉ 2000).

Among non-volatile components of chamomile flowers the most important are flavonoids, coumarins and pectine-like mucilages (GOSZTOLA et al. 2006). Flavonoids exhibit strong antioxidant and free radical scavenging activity, which also plays an important role in their anticarcinogenic effect

(Švehlíková, Repčák 2000). The most abundant chamomile flavonoid is apigenin, localized only in the white ligulate florets of the anthodium (Carle et al. 1993). Besides flavonoids and other phenolic compounds, there are other plant antioxidants, for example liposoluble carotenoids and tocopherols and water soluble vitamin C (Ivanova et al. 2005, Chrpová et al. 2010). Also, chlorophylls exhibit significant antioxidant activity. Antioxidant activity of main carotenoids and chlorophylls present in herbs decrease in the order: chlorophyll a > lutein > pheophytin a > chlorophyll b > β-carotene > pheophytin b (Suzuki, Shioi 2003).

Over the last twenty years, much information has accumulated on the role of fluoride in cellular respiratory processes and associated free radical reactions (Rzeuski et al. 1998). Fluoride is also known to be an inhibitor/activator of numerous enzymes. Although the relationship in human fluorosis between free radical generation, lipid peoxidation, and antioxidant systems has been investigated extensively, various studies have produced conflicting results (Chlubek 2003)

Chamomile is used as a component of herbal teas, single ingredient herbal tea or tisane as well as a valuable ingredient of many galenic preparations (tinctures, extracts) (Böttcher et al. 2001, Mckay, Blumberg 2006). Both the flowers and the essential oil have been reported to possess anti-inflammatory, antiseptic, antispasmodic, sedative and antiulcer properties (Mericli 1990, Sashidhara et al. 2006, Salamon 2009). They are used in the pharmaceutical, beverage and cosmetics industries (Reda et al. 2010).

The aim of the experiment was to compare fluoride content and biological value of four cultivars of chamomile flowers.

MATERIAL AND METHODS

The experiment was carried out in 2010 at the Horticultural Experiment Station in Dołuje, which belongs to the Department of Horticulture of West Pomeranian University of Technology in Szczecin. The laboratory part of the experiment was conducted in the Department of Biochemistry and in the Laboratory of Storage and Processing (Department of Horticulture) of West Pomeranian University of Technology in Szczecin. The research material consisted of fresh flowers of chamomile (*Matricaria recutita* L.) cultivars. Two diploid cultivars: Promyk and Mastar, and two tetraploid cultivars: Złoty Łan and Dukat were tested.

The experiment was established in randomized blocks with four replications. The experimental plot area was 1.44 m^2 (1.2 m × 1.2 m). The field was prepared with agronomic techniques proper for chamomile cultivation (Mordalski 2010). Mineral fertilization was quantified according to the results of chemical analysis of the soil. The seeds of chamomile were sown on

6th April, at a distance of 40 cm between rows. During the growing season, the crop management included mainly irrigation, weeding and soil cultivation.

The flower harvest was done at full-bloom stage, on 5th July. The chemical analyses of raw plant material included determination of the content of dry matter (drying at 105°C to constant weight), total ash (incineration of samples in 500°C), total protein (using factor 6.25 for amount of nitrogen determined by the method of Kjeldahl), crude fibre (KLEPACKA 1996), nitrates V (by the colorimetric method, ZALEWSKI 1971), total and reducing sugars, and saccharose (by the method of Luff-Schoorl), total chlorophyll, chlorophyll a and b (LICHTENTHALER, WELLBURN 1983), vitamin C as L-ascorbic acid (by the method of Tillmans), titratable acidity (KREŁOWSKA-KUŁAS 1993), total carotenoids (LICHTENTHALER, WELLBURN 1983), total polyphenols – in a spectrophotometer, using gallic acid as the reference and Folin-Ciocalteu reagent (SINGLETON, ROSSI 1965). Scavening effect of chamomile flowers on the DPPH-radical was determined according to the method of YEN and CHEN (1995). Raw homogenised material was diluted 400 times in 100% methanol. DPPH percent inhibition was calculated according to ROSSI et al. (2003). Some of the raw plant material was dried in a through-flow laboratory dryer set at 35°C. The concentration of flavonoid compounds (using the spectrophotometric method – FP VI, 2002) and essential oil content (with use of distillation of the sample together with water in Deryngs apparatus, FP VI, 2002) were determined in the dried and pulverized material.

Fluoride content was assayed according to the method of SZYMCZAK and GRAJETA (1982). Fluoride concentration was determined in the presence of TISAB III buffer using the potentiometric method with an Orion Research ion-selective electrode.

The results were statistically evaluated by the analysis of variance. Significance of the differences was tested by Tukey's test at p=0.05.

RESULTS AND DISCUSSION

Measurements of the content of chemical compounds of chamomile flowers showed significant variation between the tested cultivars.

The highest content of dry matter was determined for cultivar Promyk (23.80%), lower for Mastar (21.53%) and the least for Dukat and Złoty Łan (lower on average by 4.28% in comparison with Promyk and by 2.01% comparing to Mastar) – Table 1.

According to BÖTTCHER et al. (2001), the dry matter content of chamomile flowers varies between 18.5 and 23.5% according to the sowing date.

In the present experiment, the content of crude fibre, total ash, total nitrogen and total protein were also determined (Table 1). The highest con-

Table 1

Content of dry matter, crude fibre, total ash, total nitrogen and total protein of chamomile flower anthodia according to the cultivar

Cultivar	Dry matter (%)	Crude fibre (% f.w.)	Total ash (% f.w.)	Total nitrogen (% f.w.)	Total protein (% f.w.)
Złoty Łan	19.40*c*	1.93*b*	1.81*bc*	0.58*c*	3.60*c*
Promyk	23.80*a*	1.87*b*	2.23*a*	0.71*a*	4.44*a*
Dukat	19.64*c*	1.84*b*	1.76*c*	0.67*b*	4.16*b*
Mastar	21.53*b*	2.29*a*	2.00*b*	0.60*c*	3.72*c*
Mean	21.09	1.98	1.95	0.64	3.98

tent of crude fibre was noted for cv. Mastar, and lower for Złoty Łan, Promyk and Dukat (on average by 0.41% in comparison with Mastar).

The cultivar Promyk was characterized by the highest content of total ash, total nitrogen and total protein. Less total nitrogen and total protein occurred in cv. Dukat and the lowest amounts of these nutrients were in cv. Mastar and Złoty Łan. The cultivars Mastar and Złoty Łan were characterized by a lower total ash content than Promyk. However, there were no significant differences between Złoty Łan and Dukat, which had the lowest total ash content.

The highest content of total sugars, reducing sugars and saccharose was determined for cv. Mastar (Table 2). The cultivar Dukat was characterized by a significantly lower content of total sugars and reducing sugars than cv. Mastar (0.39 and 0.12% less, respectively). The lowest content of total sugars was found in cv. Złoty Łan, and reducing sugars – in the cultivars Promyk and Złoty Łan. In comparison with cv. Mastar, the cultivars Promyk, Dukat and Złoty Łan were characterized by a significantly lower content of saccharose.

Table 2

Content of total and reducing sugars, saccharose, nitrates V and titratable acidity of chamomile flower anthodia according to the cultivar

Cultivar	Sugars (% f.w.)			Titratable acidity (% citric acid f.w.)	Nitrates V (mg $NaNO_3$ kg^{-1} f.w.)
	total	reducing	sacchrose		
Złoty Łan	1.15*d*	0.94*c*	0.20*bc*	0.035*a*	364.54*a*
Promyk	1.33*c*	1.01*c*	0.31*b*	0.040*a*	210.16*a*
Dukat	1.46*b*	1.21*b*	0.25*b*	0.030*a*	359.44*a*
Mastar	1.85*a*	1.33*a*	0.50*a*	0.035*a*	475.55*a*
Mean	1.45	1.12	0.31	0.035	352.42

The yield of essential oil of *Matricaria recutita* depends on the plant genotype as well as the environmental conditions under which the plants are grown (LUTOMSKI, CZABAJSKA 1993, RAAL et al. 2003, SASHIDHARA et al. 2006, SALAMON 2007). GRGESINA et al. (1995) compared the essential oil content in different parts of chamomile plants (a diploid cultivar). The highest concentration of this compound was determined for yellow florets (0.49%), lower for flower heads (0.43%) and petals (0.28%) and the lowest for stems and leaves (0.08%). In the opinion of BÖTTCHER et al. (2001), the essential oil content of chamomile ranges from 0.56 up to 0.86 cm^3 per 100 g dry drug subject to the sowing date.

In the research by GOSZTOLA et al. (2006), the essential oil content differed significantly between examined chamomile populations, ranging from 0.2% (a wild type) up to 0.93% (a tetraploid cultivar). In our work, the highest content of essential oil (2.69 cm^3 100 g^{-1} d.m.) was determined for the tetraploid cultivar Dukat (Table 3). Less of this compound was determined in cv. Złoty Łan (4*n*) and Promyk (2*n*). However, there were no significant differences found between the cultivars Promyk and Mastar (2*n*), which contained the least essential oil.

Table 3

Content of essential oil, total polyphenols, total flavonoids and antioxidant activity of chamomile flower anthodia according to the cultivar

Cultivar	Essential oil (cm^3 100 g^{-1} d.m.)	Total polyphenols (mg 100 g^{-1} f.w.)	Total flavonoids (% d.m.)	Antioxidant activity (% DPPH)
Złoty Łan	1.36*b*	316.64*b*	0.50*a*	8.14*a*
Promyk	0.93b*c*	420.35*a*	0.48*a*	7.66*a*
Dukat	2.69*a*	308.78*b*	0.37*b*	6.97*a*
Mastar	0.78*c*	354.10*ab*	0.49*a*	8.62*a*
Mean	1.44	349.97	0.46	7.85

Significantly more polyphenols were determined in the cultivars Promyk and Mastar (Table 3). However, there were no significant differences found between the cultivars Mastar, Złoty Łan and Dukat.

The content of total flavonoids determined in the experiment varied from 0.37 to 0.50% d.m. The cultivars Złoty Łan, Mastar and Promyk were characterized by a significantly higher content of flavonoids. The lowest content of flavonoids was determined in cv. Dukat. GRGESINA et al. (1995) found on average 0.93% of flavonoids in chamomile flowers.

Several laboratories have examined the antioxidant potential of chamomile. In the study of REKKA et al. (1996), chamazulene poorly interacted with 1.1-diphenyl-2-picrylhydrazyl (DPPH). BUŘIČOVA and RÉBLOVÁ (2008) determined the antioxidant activity of water and ethanol extracts of 17 medicinal plants using the DPPH radical. Chamomile flowers had low antioxidant activity comparing to the other species, lower than for example sweet balm, thyme, oregano and mint herb, elder and nettle flowers. Contrary to this, Moraes-de-Souza et al. (2008), Yoo et al. (2008), Al-Ismail and Aburjai (2004), classified chamomile flowers as a plant with high or medium antioxidant activity. In our study, the antioxidant activity of chamomile flowers reached on average 7.85% DPPH (moderate antioxidant activity) and there were no significant differences between the tested cultivars. The antioxidant activity of herbs and other plants mostly depends on the content of flavonoids, polyphenols, L-ascorbic acid and carotenoids.

Moreover, the chamomile cultivars compared in the experiment did not differ significantly in the content of L-ascorbic acid, total carotenoids, total chlorophylls, chlorophyll a, chlorophyll b and titratable acidity, whose average values for the tested cultivars were, respectively: 39.72 mg 100 g^{-1} f.w., 143.53 mg kg^{-1} f.w., 210.36 mg kg^{-1} f.w., 139.69 mg kg^{-1} f.w., 50.33 mg kg^{-1} f.w. and 0.035% citric acid f.w. (Tables 2, 4).

Table 4

Content of dry matter, crude fibre, total ash, total nitrogen and total protein of chamomile flower anthodia according to the cultivar

Cultivar	L-ascorbic acid (mg 100 g^{-1} f.w.)	Total carotenoids (mg kg^{-1} f.w.)	Chlorophyll (mg kg^{-1} f.w.)		
			total	a	b
Złoty Łan	37.92*a*	134.76*a*	212.00*a*	145.78*a*	48.50*a*
Promyk	38.88*a*	162.25*a*	223.26*a*	153.79*a*	47.84*a*
Dukat	41.76*a*	138.73*s*	192.19*a*	120.84*a*	48.99*a*
Mastar	40.32*a*	138.37*a*	213.98*a*	138.35*a*	55.97*a*
Mean	39.72	143.53	210.36	139.69	50.33

Beside medicinal components, herbs also contain nitrates and nitrites, which are well extractable and easily pass to water solutions (Özcan, Akbulut 2007, Grzeszczuk, Jadczak 2008).

According to Figura and Pluta (2006), the content of nitrates (V) in chamomile flowers is on average 598.06 mg $NaNO_3$ kg^{-1} d.m. However, Özcan and Akbulut (2007) did not detect any nitrates (III, V) in chamomile flowers. The content of nitrates V determined in our experiment did not differ significantly between the cultivars and reached on average 352.42 mg $NaNO_3$ kg^{-1} f.w. (Table 2).

The highest fluoride content was determined in the cultivar Dukat, where it equalled 5.39 mg F kg^{-1} d.m. In comparison with cv. Dukat, the cultivars Promyk, Mastar and Złoty Łan were characterized by significantly less fluoride, ranging from 2.29 to 2.84 mg F kg^{-1} d.m. (Table 5).

Table 5

Fluoride content in chamomile flower anthodia according to the cultivar

Cultivar	Fluoride content (mg F kg^{-1} d.m.)
Złoty Łan	2.29*c*
Promyk	2.26*c*
Dukat	5.39*a*
Mastar	2.84*b*
Mean	3.19

There are few studies on the fluoride content in herbal plants, but EKMELI-ALTURFAN et. al. (2009) showed that the fluoride content in infusions from chamomile and another herbal plants was 0.03 mg F dm^{-3}. Similar findings are reported by CHAN and KOH (1996)

CONCLUSIONS

1. Among chamomile cultivars compared in the experiment, cv. Promyk and Mastar were characterized by higher biological value. Flower anthodia of cv. Promyk contained significantly more dry matter, total ash, total nitrogen and total protein, while those of cv. Mastar were richer in crude fibre, total and reducing sugars, and saccharose. Moreover, both cultivars were characterized by a high content of total polyphenols and total flavonoids.

2. The highest content of the essential oil and fluoride was determined in flower-heads of the cultivar Dukat.

REFERENCES

AL-ISMAIL K.M., ABURJAI T. 2004. *Antioxidant activity of water and alcohol extracts of chamomile flowers, anise seeds and dill seeds*. J. Sci. Food Agric., 84: 173-178.

BÖTTCHER H., GÜNTHER I., FRANKE R., WARNSTORFF K. 2001. *Physiological postharvest responses of Matricaria (Matricaria recutita* L.*) flowers*. Postharvest Biol. Technol., 22: 39-51.

BUŘIČOVA L., RÉBLOVÁ Z. 2008. *Czech medicinal plants as possible sources of antioxidants*. Czech J. Food Sci., 26: 132-138.

CARLE R., DÖLLE B., MÜLLER W., BAUMEISTER U. 1993. *Thermospray liquid chromatography/ mass spectrometry (TSP LC/MS): Analysis of acetylated apigenin-7-glucosides from Chamomilla recutita*. Pharmazie, 48: 304-306.

CHAN J.T., KOH S.H. 1996. *Fluoride content in coffeinated, decoffeinated and herbal teas.* Caries Res., 30(1): 88-92.

CHLUBEK D. 2003. *Fluoride and oxidative stress*. Fluoride, 36(4): 217-228.

Chrpová D., Kouřimská L., Gordon M.H., Heřmanová V., Roubíčková I., Pánek J. 2010. *Antioxidant activity of selected phenols and herbs used in diets for medicinal conditions*. Czech J. Food Sci., 28(4): 317-325.

Determination of flavonoids. 2002. Farmakopea Polska VI. (in Polish)

Emekli-Alturfan E., Yarat A. Akyuz S. 2009. *Fluoride levels in various black tea, herbal and fruit infusions consumer in Turkey.* Food Chem. Toxicol., 47: 1495-1498.

Figura B., Pluta J. 2006. *The effect of III and V nitrates level in tap water on contamination of herbal infusions*. J. Elementol., 11(3): 271-281. (in Polish)

Gosztola B., Németh É., Sárosi Sz., Szabó K., Kozak A. 2006. *Comparative evaluation of chamomile (Matricaria recutita L.) populations from different origin*. Int. J. Hort. Sci., 12(1): 91-95.

Grgesina D., Mandić M.L., Karuza L., Klapec T., Bočkinac D. 1995. *Chemical composition of different parts of Matricaria chamomilla*. Prehrombeno-technol. Biotechnol. Rev., 33(2-3): 111-113.

Grzeszczuk M., Jadczak D. 2008. *Estimation of biological value and suitability for freezing of some species of spice herbs*. J. Elementol., 13(2): 211-219.

Ivanova D., Gerova D., Chervenkov T., Yankova T. 2005. *Polyphenols and antioxidant capacity of Bulgarian medicinal plants*. J. Ethnopharmacol., 96: 145-150.

Klepacka M. 1996. *Food analysis*. Fundacja Rozwój SGGW, Warszawa. (in Polish)

Krełowska-Kułas M. 1993. *Determmination of total acidity by the potentiometric titration method*. In: *Quality assessment of food products'.* PWE, Warszawa. (in Polish)

Lichtenthaler H.K., Wellburn A.R., 1983. *Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents*. Biochem. Soc. Trans., 603: 591-592.

Lutomski J., Czabajska W. 1993. *Polish variety of common camomile*. Acta Hort., 344: 352-360.

Matricariae flos. Chamomile flower. 2008. Farmakopea Polska VIII, 2: 2254-2256. (in Polish)

Mericli A.H. 1990. *The lipophilic compounds of a Turkish Matricaria chamomilla variety with no chamazuline in the volatile oil*. Int. J. Crude Drug Res., 28: 145-147.

McKay D.L., Blumberg J.B. 2006. *A review of the bioactivity and potential health benefits of chamomile tea (Matricaria recutita* L.*).* Phytother. Res., 20: 519-530.

Moraes-de-Souza R.A., Oldoni T.L.C., Regitano-dArce M.A.B., Alencar S.M. 2008. *Antioxidant activity and phenolic composition of herbal infusions consumed in Brazil*. Cienc. Technol. Aliment., 6(1): 41-47.

Mordalski R. 2010. *Chamomilla recutita (*L.*) Rauschert*. In: *Herb plants* (ed. B. Kołodziej). PWRiL, Poznań, pp. 377-381.

Orav A., Kailas T., Ivask K. 2001. *Volatile constituents of Matricaria recutita* L. *from Estonia*. Proc. Estonian Acad. Sci. Chem., 50(1): 39-45.

Özcan M.M., Akbulut M. 2007. *Estimation of minerals, nitrate and nitrite contents of medicinal and aromatic plants used as spices, condiments and herbal tea*. Food Chem., 106: 852-858.

Raal A., Arak E., Orav A., Ivask K. 2003. *Comparison of essential oil content of Matricaria recutita* L. *from different origins*. Ars Pharmaceutica, 44(2): 159-165.

Reda F., Abd El-Wahed M.S.A., Gamal El Din K.M. 2010. *Effect of indole acetic acid, gibberellic acid and kinetin on vegetative growth, flowering, essential oil pattern of chamomile plant (Chamomile recutita* L. *Rausch).* World J. Agric. Sci., 6(5): 595-600.

Rekka E.A., Kourounakis A.P., Kourounakis P.N. 1996. *Investigation of the effect of chamazulene on lipid peroxidation and free radical processes*. Res. Commun. Mol. Pathol. Pharmacol., 92: 361-364.

Rossi M., Giussani E., Morelli R., Scalzo R., Nani R.C., Torreggiani D. 2003. *Effect of fruit blanching on phenolics and radical scavenging activity of highbush blueberry juice*. Food Res. Int., 36: 999-1005.

Rzeuski R., Chlubek D., Machoy Z. 1998. *Interaction between fluoride and biochemical free radical reactions*. Fluoride, 31: 43-45.

Salamon I. 2007. *Effect of the internal and external factors on yield and qualitative-quantitative characteristics of chamomile essential oil*. Acta Hort., 749: 45-65.

Salamon I. 2009. *Chamomile biodiversity of the essential oil qualitative-quantitative characteristics*. In: *Innovations in chemical biology.* Ed. B. Sener. Springer Science + Business Media B.V., pp. 83-90.

Sashidhara K.V., Verma R.S., Ram P. 2006. *Essential oil composition of Matricaria recutita* L. *from the lower region of the Himalayas*. Flav. Fragr. J., 21: 274-276.

Seidler-Łożykowska K. 2007. *Chamomile cultivars and their cultivation in Poland.* Acta Hort., 749: 111-115.

Singh O., Khanam Z., Misra N., Srivastava M.K. 2011. *Chamomile (Matricaria chamomilla* L.*): An overview*. Phcog. Rev., 5: 82-95.

Singleton V.L., Rossi J.A. Jr. 1965. *Colorymetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents*. Am. J. Enol. Viticult., 16: 144-158.

Suzuki Y., Shioi Y. 2003. *Identification of chlorophylls and carotenoids in major teas by high-performance liquid chromatography with photodiode array detection*. J. Agric. Food Chem., 51: 5307-5314.

Svábné J. 2000. *Matricaria recutita*. In: Bernáth J. Ed.: Gyógy- és aromanövények. Mezőgazda Press, Budapest, 413-421.

Švehlíková V., Repčák M. 2000. *Variation of apigenin quantity in diploid and tetraploid Chamomilla recutita (*L.*) Rauschert*. Plant Biol., 2: 403-407.

Szymczak J., Grajeta H. 1982. *Content of fluoride in plant products from industrial areas*. Bromat. Chem. Toksykol., 15(1-2): 47-51. (in Polish)

Yen G.C., Chen H.Y. 1995. *Antioxidant activity of various tea extracts in relation to their antimutagenicity*. J. Agric. Food Chem., 43: 27-32.

Yoo K.M., Lee C.H., Lee H., Moon B., Lee C.Y. 2008. *Relative antioxidant and cytoprotective activities of common herbs*. Food Chem., 106: 929-936.

Zalewski W. 1971. *The question of occurrence of different forms of nitrogen in vegetables subject to nitrogen fertilization. I determination method*. Bromat. Chem. Toksykol., 4(2): 147-154. (in Polish)

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DOES CHROMIUM (+3) DECREASE THE GLUCOSE CONCENTRATION IN EPILEPTIC CHILDREN?

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Abstract

Epilepsy is a common neurological disorder. It affects 50 million people worldwide, over half of which are children. Many children who suffer from epilepsy are also affected by metabolic disorders, characterized by obesity as well as intolerance and deficient transport of glucose. Recently, many researchers have indicated chromium (+3) as an essential trace mineral which probably plays an important role in metabolism of glucose and insulin. There are no data about chromium alterations in the body of epileptic patients. The purpose of this study was to find possible correlation between chromium (+3) concentration and the glucose level in children with epilepsy. Material and methods: Twenty-three untreated epileptic children with idiopathic generalized tonic-clonic seizures (9 girls and 14 boys) aged 13.4±2.7 years and 25 healthy children (sex-age-matched) served as a control were recruited to this study. The chromium blood and serum concentrations were determined as well as serum glucose level. Results: There were no statistically significant differences between epileptic and healthy children in the mean chromium (+3) blood concentration as well as according to sex in both analyzed groups of children. The mean serum chromium concentration in epileptic children was significantly lower than in healthy subjects generally ($p<0.001$), as well as in boys group ($p<0.001$) and girls ($p<0.001$). Although, the negative statistically significant correlation between serum glucose and chromium concentrations was found in epileptic children ($p<0.01$), we have not found so associations in whole blood and in healthy children. Conclusions: Our research has shown that during epilepsy the concentration of serum chromium was lower than in healthy subjects what was associated

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with glucose abnormalities. These preliminary results suggest that the detail research on the chromium in epilepsy are necessary.

Key words: glucose, chromium, epilepsy, children.

CZY CHROM OBNIŻA POZIOM GLUKOZY WE KRWI DZIECI Z EPILEPSJĄ?

Abstrakt

Epilepsja stanowi jedną z najczęstszych chorób neurologicznych. Ocenia się, że na całym świecie choroba ta dotyka ponad 50 mln ludzi, z czego ponad połowa to dzieci. Wielu dzieciom z epilepsją, w czasie ich rozwoju, towarzyszy także zespół metaboliczny, którego charakterystyczną cechą są otyłość i zaburzony metabolizm glukozy. W ostatnich latach wielu badaczy zwróciło uwagę na chrom (+3) jako niezbędny pierwiastek, który prawdopodobnie odpowiada za transport glukozy do tkanek przez stabilizowanie jej połączeń z insuliną. Rola chromu w tym aspekcie jest jednak wciąż niejasna. Brak jest również danych dotyczących zawartości chromu w organizmach dzieci z padaczką. W związku z powyższym celem pracy była ocena zawartości chromu (+3) w surowicy i pełnej krwi dzieci z padaczką oraz próba skorelowania jego zawartości z poziomem glukozy we krwi tych dzieci w odniesieniu do grupy kontrolnej. Badaniem objęto 23 dzieci ze świeżo rozpoznaną padaczką (9 dziewcząt i 14 chłopców) w wieku 13,4 ± 2,7 lat oraz 25 dzieci zdrowych odpowiadających grupie badanej strukturą wieku i płci. U wszystkich dzieci zmierzono stężenie chromu w surowicy krwi i pełnej krwi oraz ocenie poddano stężenie glukozy we krwi. Wykazano brak różnic w zawartości chromu (+3) w pełnej krwi, a także glukozy we krwi między grupą dzieci z padaczką a dziećmi zdrowymi, podobnie brak różnic w ocenie tych parametrów stwierdzono w odniesieniu do płci dzieci niezależnie od występowania choroby. Wykazano istotnie statystycznie niższy poziom chromu w surowicy krwi dzieci z padaczką w porównaniu z grupą kontrolną (p<0,001), jak też w surowicy chłopców i dziewcząt z padaczką w porównaniu z chłopcami i dziewczętami zdrowymi (p<0,001). Równocześnie zanotowano istotną ujemną zależność między stężeniem chromu w surowicy krwi dzieci z padaczką a stężeniem glukozy we krwi (p<0,01), przy czym nie zauważono podobnej zależności u dzieci zdrowych. Badania sugerują, iż istnieje możliwość związku między poziomem chromu (+3) u dzieci z padaczką a notowanymi w literaturze zaburzeniami transportu glukozy. Wydaje się uzasadnione rozwijanie tego tematu badań w dokładniejszych pracach, które mogłyby się przyczynić do głębszego zrozumienia padaczki.

Słowa kluczowe: glukoza, chrom, padaczka, dzieci.

INTRODUCTION

Epilepsy is a common neurological disorder with the heterogeneous nature, which affects 50 million people worldwide, over half of which are children (Volpe et al. 2007). In about 25% patients with epilepsy the number of seizures is not greatly reduced even when two or three anti-epileptic drugs are used. As suggested by some authors (Volpe et al. 2007, Bertoli et al. 2006, Vianna et al. 2006), about 20 to 25% of patients administered the highest tolerated doses fail to control seizures.

More than 50% of epileptics are children. Metabolic disorders have frequently been demonstrated in children with epilepsy together with growth retardation (Volpe et al. 2007) and intolerance and deficient transport of glucose (Vianna et al. 2006).

In the last years, it has been suggested that serum trace element concentrations in epileptic patients can show some abnormalities (Hirate et al. 2002, Verrotti et al. 2002, Johnson 2001, Ashraf et al. 1995). Balance of certain minerals is crucial for a healthy nervous system and neuronal susceptibility to excitability (Hamed et al. 2004). Several reports suggested that electrolytes (sodium, potassium, calcium) and the level of some trace elements (zinc, copper, iron) in the body play an important role in development of seizure condition (Hamed et al. 2004, Dudek 2001, Ikeda 2001, Pisacane et al. 1996, Smith, Bone 1982).

As suggested by Wallach (1985), the essential role of chromium in animal and human nutrition is now well accepted. Animal studies have shown that chromium deficiency occurs in diabetic-like states, impaired growth, elevated blood lipids, etc. In people, chromium deficiency has been demonstrated clearly only in just one clinical situation: patients on total parenteral nutrition without added Cr have been observed to have impaired glucose tolerance, hyperglycemia, relative insulin resistance, peripheral neuropathy and metabolic encephalopathy (Wallach 1985, Jeejeebhoy et al. 1977). The latest research indicates that Cr supplementation can improve glucose metabolism in glucose intolerance individuals, and Cr deficiency may be important in diabetes mellitus for stabilization of the insulin/glucose complex (Ali et al 2011). There are no data about chromium in epilepsy.

To sum up, the aim of this preliminary study was to assess chromium concentrations in blood and serum, and the glucose serum level in epileptic and healthy children.

MATERIAL AND METHODS

Twenty-three untreated epileptic children with idiopathic generalized tonic--clonic seizures (9 girls and 14 boys) aged 13.4±2.7 years and 25 healthy children (sex-age-matched) as the control were recruited to this study. The height and weight of all subjects (epileptic and healthy children) were within the normal range of the percentile growth charts for age and sex of children (between 50-75%, no overweight and obesity) (Kuczmarski et al. 2002). The characteristics of both populations are presented in Table 1. The study was approved by the Ethical Committee at the Poznan University of Medical Sciences. The parents of the patients and the healthy subjects were under the care of the Department and Clinic of Children Neurology at the Poznan University of Medical Sciences, and were informed about the study in details. The blood samples used in this research were obtained from the blood drawn for normal analytical procedures in the clinic. From each sub-

Table 1

The characteristics of the analyzed populations

Characteristic of subjects	Mean ± SD (range)
Age (years)	
Epileptic	13.4 ± 2.7 (9-17)
Girl	14.1 ± 2.4 (9-16)
Boys	13.0 ± 2.8 (9-17)
Control	14.2 ± 2.6 (9-16)
Girl	13.9 ± 2.8 (9-16)
Boys	14.7 ± 2.1 (9-16)
Sex (girls/boys)	
Epileptic	9/14
Control	10/15
Duration of illness (months)	
Epileptic	2.1 ± 1.7 (0.4-6)

jects, after an overnight fast, 5 ml venous blood was collected to 2 PP tubes; one tube from each subject was allowed to stay at room temperature and was centrifuged at 5000 rpm for 10 minutes, afterwards the serum was stored frozen at –20°C until chromium analyses. The second tube with a blood sample, mixed with heparin, was stored at –20°C until the chromium analyses. The glucose concentration was measured immediately by the colorimetric method using an Accu-Check Active glucometer (Roche).

The serum and blood concentrations of chromium were determined by the Atomic Absorption Spectrometry method with a graphite furnace spectrometer (AAS-5, Zeiss), having diluted the samples with de-ionized water as required. The accuracy of the method was assessed by comparison with the certified serum control (Human Serum, Merck).

There are no reference values for serum chromium concentrations.

The data are presented as arithmetic means ± standard deviation (SD) and statistically significant differences were determined by t-test and Wilcoxon test using Excel 2007 and Statistica ver. 7.0. Statistical significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

Childhood epilepsy is the most common neurological disorder in children. More than 50% of the 50 millions of epileptic patients are children. The metabolic syndrome is an aggregate of metabolic and cardiovascular abnormalities including obesity, impaired glucose tolerance, dyslipidemia, and cardiovascular morbidity (Grundy et al. 2005). As suggested Daniels et al. (2009), children with epilepsy usually have a high rate of obesity at initial presentation, as a consequence of metabolic abnormalities. In our study, however, we did not observe body mass abnormalities in the children with epilepsy, or different glucose concentrations (Table 2) compared to healthy subjects (epileptic – 87 mg dL^{-1}; healthy – 93 mg dL^{-1}). On the other hand, animal (Lee et al. 2011) as well as human studies (Vianna et al. 2006) show that glucose intolerance is common during epilepsy, especially in children, and can be associated with the metabolic syndrome, which can result in increased hippocampal pathology and more freuqnet seizures. In the last years, it has been suggested that serum trace element concentrations in epileptic patients can show some abnormalities (Hirate et al. 2002, Verrotti et al. 2002, Johnson 2001, Ashraf et al. 1995). Balance of certain minerals is crucial for a healthy nervous system and neuronal susceptibility to excitabil-

Table 2

The mean serum and blood levels of chromium and blood glucose level in control and epileptic children

Total	Control, *n*=25 mean ± SD (range)	Epileptic, *n*=25 mean ± SD (range)	
Blood Cr ($ng\ dL^{-1}$)	261.6 ± 38.2 (123.0-345.2)	254.1 ± 44.2 (153.0-328.3)	ns
Serum Cr ($ng\ dL^{-1}$)	181.3 ± 23.4 (64.6-243.2)	113.0 ± 28.0 (41.0-151.8)	*P*=0.000
Blood glucose ($mg\ dL^{-1}$)	87.0 ± 18.2 (65.0-117.0)	92.8 ± 17.1 (70.0-134.0)	ns
Girls	control, *n*=10 mean ± SD (range)	epileptic, *n*=9 mean ± SD (range)	
Blood Cr ($ng\ dL^{-1}$)	254.2 ± 46.3 (123.0-321.9)	259.1 ± 29.8 (217.9-301.2)	ns
Serum Cr ($ng\ dL^{-1}$)	182.4 ± 19.9 (64.6-223.4)	116.2 ± 18.3 (93.7-151.2)	*P*=0.000
Blood glucose ($mg\ dL^{-1}$)	97.4 ± 21.3 (74.0-117.0)	89.0 ± 11.8 (76.0-112.0)	ns
Boys	control, *n*=15 mean ± SD (range)	epileptic, *n*=14 mean ± SD (range)	
Blood Cr ($ng\ dL^{-1}$)	267.7 ± 67.2 (132.7-345.2)	250.9 ± 52.3 (153.0-328.3)	ns
Serum Cr ($ng\ dL^{-1}$)	191.2 ± 34.6 (71.3-243.2)	111.0 ± 33.2 (41.0-151.8)	*P*=0.000
Blood glucose ($mg\ dL^{-1}$)	78.4 ± 19.2 (65.0-99.0)	95.3 ± 19.8 (70.0-134.0)	ns

ns – non-significant

ity (Hamed et al. 2004). The micronutrient chromium is of interest as it can potentially improve glucose tolerance by reducing insulin resistance (Ali et al. 2011, Krejpcio et al. 2007). There are no data about the role and concentration of chromium in epilepsy. Table 2 shows the determined concentrations of chromium in the blood and serum of epileptic and healthy children. However, we did not observe significant differences between epileptic and healthy children in the blood chromium concentration (~260 ng dL^{-1}), whereas the serum chromium level was significantly lower ($p<0.001$) in epileptic total (~113 ng dL^{-1}), girls (~116 ng dL^{-1}), and boys (~111 ng dL^{-1}) than in healthy total (~181 ng dL^{-1}), girls (~182 ng dL^{-1}) and boys (~191 ng dL^{-1}). A significantly higher level of chromium in hair of epileptic male and female patients in Pakistan compared to healthy subjects was described earlier by Ashraf et al. (1995). In Nigeria, Nsonwu et al. (2005) obtained similar levels of chromium (~250 ng dL^{-1} vs. ~265 ng dL^{-1}, respectively) in type 2 diabetics and non-diabetics.

The association between chromium and glucose in epileptic patients can prove significant correlation between the glucose level and chromium concentration in epileptic children (Figure 1), although not correlated in healthy subjects. It has been suggested that chromium plays an important role not only in diabetes mellitus but also in epilepsy. In conclusion, once the role of chromium and other metals in epilepsy is clearly explained, it should help us combat this disease more successfully and perhaps stop neural deficits caused by the metabolic syndrome and other epilepsy associated dysfunctions.

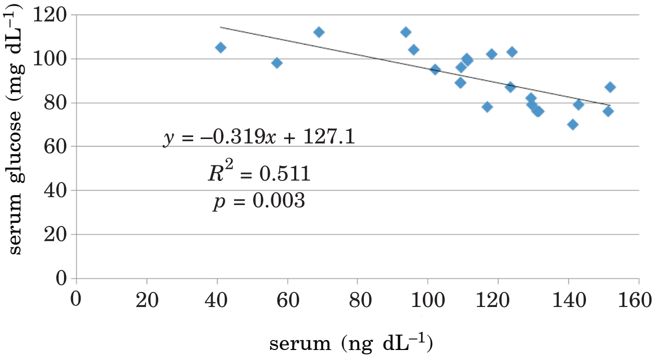

Fig. 1. Correlation between the serum chromium level and blood glucose concentration in epileptic children

REFERENCES

Ali A., Ma Y., Reynolds J., Pierce Wise J. Sr., Inzucchi S.E., Katz D.L. 2011. *Chromium effects on glucose tolerance and insulin sensitivity in persons at risk for diabetes mellitus*. Endocr. Pract., 17: 16-25.

Ashraf W., Jaffar M., Mohammed D., Iqbal J. 1995. *Utilization of scalp hair for evaluating epilepsy in male and female groups of the Pakistan population*. Sci. Total Environ., 164: 69-73.

BERTOLI S., CARDINALI S., VEGGIOTTI P., TRENTANI C., TESTOLIN G., TAGLIABUE A. 2006. *Evaluation of nutritional status in children with reflactory epilepsy.* Nutr. J., 5: 14-23.

DANIELS Z.S., NICK T.G., LIU C., CASSEDY A., GLAUSER T.A. 2009. *Obesity is a common comorbidity for pediatric patients with untreated, newly diagnosed epilepsy.* Neurology, 73: 658-664.

DUDEK F. E. 2001. *Zinc and epileptogenesis.* Epilepsy Curr., 1: 66-70.

GRUNDY S.M., CLEEMAN J.I., DANIELS S.R., DONATO K.A., ECKEL R.H., FRANKLIN B.A., GORDON D.J., KRAUSS R.M., SAVAGE P.J., SMITH S.C. JR., SPERTUS J.A., COSTA F. 2005. *Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement.* Circulation, 112: 2735-2752.

HAMED S.A., ABDELLAH M.M., EL-MELEGY N. 2004. *Blood levels of trace elements, electrolytes, and oxidative stress/antioxidant systems in epileptic patients.* J. Pharmacol. Sci., 96: 465-473.

HIRATE M., TAKEDA A., TAMANO H., ENOMOTO S., OKU N. 2002. *Distribution of trace elements in the brain of EL (epilepsy) mice.* Epilepsy Res., 51: 109-116.

IKEDA M. 2001. *Iron overload without the C282Y mutation in patients with epilepsy.* J. Neurol. Neurosurg. Psychiatry, 70: 551-553.

JEEJEEBHOY K.N., CHU R.C., MARLISS E.B., GREENBERG G.R., BRUCE-ROBERTSON A. 1977. *Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition.* Am. J. Clin. Nutr., 30: 531-536.

JOHNSON S. 2001. *Micronutrient accumulation and depletion in schizophrenia, epilepsy, autism and Parkinson's disease?* Med. Hypoth., 56: 641-645.

KREJPCIO Z., KURYL T., DEMBSKI B., WOJCIAK R.W. 2007. *Effect of dietary supplementation with fructans and chromium (III) on blood glucose and insulin and beta-oxidation in lymphocytes of type 1 diabetic rats.* Med. Wet., 63: 1494-1496.

KUCZMARSKI R.J., OGDEN C.L., GUO S.S. *CDC growth charts for the United States: Methods and development.* National Center for Health Statistics. Vital Health Stat, 11: 1-189.

LEE E.B., WARMAN G., DHIR R., AHIMA R.S. 2011. *Metabolic dysfunction associated with adiponectin deficiency enhances kainic acid-induced seizure severity*. J. Neurosci., 31: 14361--14366.

NSONWU A.C., USORO C.A. O., ETUKUDO M.H., USORO I.N. 2005. *Serum and urine levels of chromium and magnesium in type 2 diabetics in Calabar, Nigeria.* Mal. J. Nutr., 11: 133-142.

PISACANE A., SANSONE R., IMPAGLIAZZO N., COPPOLA A., ROLANDO P., D'APUZZO A., TREGROSSI C. 1996. *Iron deficiency anaemia and febrile convulsion: case-control study in children under 2 years.* B.M.J., 313: 343-344.

SMITH W. G., BONE I. 1982. *Copper, zinc and magnesium plasma levels in epilepsy.* J. Neurol. Neurosurg. Psychiatry, 45: 1072-1073.

VERROTTI A., BASCIANI F., TROTTA D., POMILIO M.P., MORGESE G., CHIARELLI F. 2002. *Serum copper, zinc, selenium, glutathione peroxidase and superoxidase levels in epileptic children before and after 1 year of sodium valproate and carbamazepine therapy.* Epilepsy Res., 48: 71-75.

VIANNA J.B.M., ATALLAH A.N., PRADO G.F., VALENTE O., DUARTE-BARROS M.L., VIANNA E.C.S., MELLO L.E.A.M. 2006. *The oral glucose tolerance test is frequently abnormal in patients with uncontrolled epilepsy.* Epilepsy Behav., 9: 140-144.

VOLPE S.L., SCHALL J.I., GALLAGHER P.R., STALLINGS V.A., BERGQVIST A.G.C. 2007. *Nutrient intake of children with intractable epilepsy compared with healthy children.* J. Am. Diet. Assoc., 107: 1014-1018.

WALLACH S. 1985. *Clinical and biochemical aspects of chromium deficiency.* J. Am. Coll. Nutr., 4: 107-120.

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COMPOSITION OF ESSENTIAL OILS AND CONTENT OF MACRONUTRIENTS IN HERBAGE OF TARRAGON (*ARTEMISIA DRACUNCULUS* L.) GROWN IN SOUTH-EASTERN POLAND

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Abstract

Tarragon is a spice but also used for medicinal purposes. In Poland, tarragon plants are grown on plantations to produce herbage used as raw material. The aim of the study was to evaluate the yield of tarragon grown from seedlings in south-eastern Poland and to assess the content of minerals and essential oils in raw material. The experiment was established using seedlings produced in a greenhouse and planted at 30 x 40 cm spacing. The essential oil content in ground herbs was determined in a Deryng apparatus according to a method defined in Pharmacopoeia. Qualitative and quantitative composition of essential oils was characterized by means of gas chromatography coupled with the mass spectrometry (GC/MS) technique. Ground herb underwent determinations of total nitrogen, ammonia, nitrates, phosphorus, potassium, calcium, magnesium and sulfur. The level of atmospheric precipitation considerably affected the height of tarragon plants and fresh herb yields. Total nitrogen and potassium were dominant elements in tarragon herb (32.0 g kg^{-1} d.m. and 28.8 g kg^{-1} d.m., respectively). The content of essential oils in herb ranged from 0.75 to 0.95%. Presence of 35 compounds, including two unidentifiable ones, was detected in the essential oil. Most of the identified compounds were monoterpenes. The following substances dominated in the essential oil: elemicin (56.0%), sabinene (20.9%), methyleugenol (6.2%), and E-asarone (6.2%). These compounds belong to different chemical classes.

Key words: tarragon, yield, oil, elemicine, sabinene, macroelements.

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SKŁAD OLEJKU ETERYCZNEGO ORAZ ZAWARTOŚĆ MAKROSKŁADNIKÓW W ZIELU BYLICY ESTRAGON (*ARTEMISIA DRACUNCULUS* L.) UPRAWIANEJ W POŁUDNIOWO-WSCHODNIEJ POLSCE

Abstrakt

Bylica estragon jest rośliną przyprawową oraz wykorzystywaną w celach leczniczych. Surowcem jest ziele pozyskiwane w naszym kraju z upraw. Celem badań była ocena plonowania bylicy estragon uprawianej z rozsady w południowo-wschodniej Polsce oraz ocena zawartości składników mineralnych i zawartości olejku w surowcu. Doświadczenie założono z rozsady wyprodukowanej w szklarni, którą posadzono w rozstawie 30 x 40 cm. Zawartość olejku eterycznego w zielu otartym oznaczono w aparacie Derynga wg metody farmakopealnej. Skład jakościowy i ilościowy olejku eterycznego wyznaczono metodą chromatografii gazowej i spektrometrii masowej (GC/MS). W zielu otartym oznaczono zawartość azotu ogółem, azotu amonowego, azotanów, fosforu, potasu, wapnia, magnezu i siarki. Wielkość opadów atmosferycznych miała istotny wpływ na wysokość roślin bylicy estragon oraz plon świeżego ziela. W zielu estragonu stwierdzono największą zawartość azotu ogółem (3.20% d.m.) oraz potasu (2.88% d.m.). Zawartość olejku eterycznego w zielu wahała się od 0.75 do 0.95%. Stwierdzono obecność 35 związków w olejku eterycznym, z których dwa nie zostały zidentyfikowane. Większa liczba związków to monoterpeny. Głównymi związkami olejku estragonowego były: elemicin (56.0%), sabinen (20.9%), methyleugenol (6.2%) i E-asarone (6.2%). Związki te należą do różnych grup chemicznych.

Słowa kluczowe: bylica estragon, plon, olejek, elemicin, sabinen, makroelementy.

INTRODUCTION

Over 800 plant species worldwide belong to *Artemisia* sp. genus (JUDŢENTIENĖ, BUZELYTĖ 2006). Tarragon (*Artemisia dracunculus* L.) can be found in natural habitats in central and northern Russia, in Siberia and North America (LAMER-ZARAWSKA et al. 2007, BALCEREK, MODNICKI 2008). Tarragon (*Dracunculi herba*) herbage is raw material. Owing to its aroma and taste, the herb is a popular spice (BALCEREK, MODNICKI 2008). JADCZAK and GRZESZCZUK (2008) recommend tarragon for direct consumption as an abundant source of minerals. Tarragon herbage can be also frozen (GRZESZCZUK, JADCZAK 2008). Medicinal properties of tarragon are well-recognized. Tarragon stimulates bile secretion, intensifies gastric acid production and stimulates digestion. Therefore, tarragon is recommended to patients, particularly children and elderly persons, who suffer from gastritis (LAMER-ZARAWSKA et al. 2007). Tarragon is grown on many continents, including Europe (LAMER-ZARAWSKA et al. 2007, BALCEREK, MODNICKI 2008).

Yields of herbal plants as well as the content of biologically active substances and minerals depend on many factors. The aim of the study was to assess the yielding of tarragon grown from seedlings in south-eastern Poland and to determine the content of minerals and essential oils in raw material. The qualitative and quantitative composition of essential oils from tarragon herbage was also determined.

MATERIALS AND METHODS

The experiments on tarragon were carried out in 2005-2006 at the Experimental Farm of the University of Life Sciences in Lublin (51°14'N 22°34'E). Seeds were purchased from the PNOS, Ożarów Mazowiecki. The seedlings were produced in a greenhouse. Seeds were sown in mid-March into boxes filled with peat substrate. After two weeks, seedlings were transferred into multi-cell pots. At the end of May, they were transplanted onto a field at 30 x 40 cm spacing. The plot area was 2.4 m^2. The experiment was set up in a randomized block design with replicates. During the vegetative season, the plantation was manually weeded twice and soil in the interspaces was loosened. The height of plants was determined before herbage harvest on 20 randomly selected plants. Herbage was cut at the beginning of flowering, i.e. at the end of August. Fresh herbage was weighed after the harvest, while the weight of air-dry and ground herbage was determined after drying under natural conditions. The content of essential oils was determined in dried material according to a method specified in the Pharmacopoeia (Polish Pharmacopoeia VI 2002).

The qualitative and quantitative composition of essential oils was characterized by means of gas chromatography coupled with the mass spectrometry (GC/MS) technique. Analyses were performed applying an ITS-40 device (GC/ITMS system by Finnigan MAT, USA) with a DB-5 column (J&W, USA) of 30 m length, 025 mm diameter, and 0.25 mm stationary phase film thickness. The injector temperature was 280°C, whereas the temperature gradient was 35°C for 2 minutes, afterwards raised by 4°C up to 280°C.

The qualitative analysis was made on the basis of MS spectra by comparing them with the NIST library (62 thousand spectra) and LIBR terpene library (TR) provided by Finnigan MAT. Identities of the recorded compounds were confirmed by retention indices from literature (ADAMS 2001).

Chemical analyses of ground tarragon herbage were performed in 2% acetic acid extracts by means of a versatile method according to NOWOSIELSKI (1988). Mineral nitrogen was determined by Bremner's method with Starck's modifications; total nitrogen was determined by Kjeldahl's method; phosphorus was assessed using ammonium metavanadate; sulfur was tested colorimetrically (Nicolet Evolution 300 spectrophotometer) using $BaCl_2$. Potassium, calcium and magnesium were determined by the AAS technique (Analyst 300 Perkin Elmer) after digesting the herbage at 550°C and dissolving the ash in diluted hydrochloric acid (1:2, v/v).

The results were statistically processed applying variance analysis for single classification at the significance level of α=0.05.

RESULTS AND DISCUSSION

The data presented in Table 1 indicate that thermal conditions in 2005-2006 favored the growth and development of tarragon plants. The mean air temperatures in May, June and August were similar to the average multi-annual ones. In July, the mean air temperature was slightly higher than the multi-year average.

Table 1

Mean air temperature and total precipitation in 2005 and 2006 against the background of multi-annual averages at the Felin Experimental Farm (Lublin)*

Specification	Month	2005				2006				1951-2005
		decade			mean	decade			mean	
		I	II	III		I	II	III		
Temperature (°C)	May	10.8	10.5	18.0	13.1	13.5	14.6	12.8	13.6	13.0
	June	13.4	17.2	17.4	16.0	11.6	17.9	21.1	16.9	16.5
	July	18.9	19.9	20.4	19.7	21.2	20.8	23.5	21.9	17.8
	Aug	16.5	16.4	17.8	16.9	18.4	18.3	15.6	17.3	17.1
Precipitation (mm)	month	decade			Σ	decade			Σ	1951-2005
		I	II	III		I	II	III		
	May	32.8	65.0	0.2	98.0	9.0	18.4	32.1	59.5	57.7
	June	47.1	7.4	1.4	55.9	28.4	0.0	9.5	37.9	65.7
	July	0.0	22.4	87.4	109.8	0.0	6.8	0.0	6.8	83.5
	Aug	103.9	3.2	1.6	108.7	73.0	79.7	45.6	198.3	68.6

*according to the Laboratory of Agro-meteorology at the University of Life Science in Lublin

In 2005, total precipitation from May until July was higher than in 2006 and considerably different from the multi-year average. Lack of rainfalls in mid-June and at the beginning and in mid-July 2006 could have affected the height of tarragon harvested at the end of August. The study revealed that the plants were significantly higher in 2005, when more intensive rainfalls were recorded (Table 2). The mean tarragon height reached 86.69 cm, which was similar to heights reported in literature (Martyniak-Przybyszewska, Wojciechowski 2004, Martyniak-Przybyszewska 2005).

Substantially higher yield of raw tarragon herbage was recorded in 2005 (Table 2), owing to higher precipitation from May to July in 2005 than in 2006 (Table 1). The yield of air-dry herbage was also remarkably higher in 2005 (Table 2). However, no significant differences in ground tarragon herbage yield were found.

The share of ground tarragon herbage in air-dry herbage was 29.27%, i.e. stems that are discarded while grinding the material made up over 70% (Table 2). A lower share of ground in air-dry herbage was recorded in 2005 (26.20%) than in 2006 (32.34%).

Table 2

Height of plants, yield and essential oil content in the herbage of *Artemisia dracunculus* L.

Year	Height of plant (cm)	Yield of fresh herbage (kg m^{-2})	Yield of air dry herbage (kg m^{-2})	Yield of herbage without stems (kg m^{-2})	Share of herbage without stems in dry herb (%)	Essential oil (%)
2005	92.79	1.515	0.580	0.152	26.20	0.750
2006	80.60	1.260	0.470	0.152	32.34	0.950
Mean	86.69	1.387	0.525	0.152	29.27	0.850
$LSD_{0.05}$	0.050	0.025	0.029	n.s.	-	0.036

n.s. – non-significant differences

Significant differences in the content of essential oils in tarragon plants between the years were found (Table 2). More oils were determined in ground tarragon herb in 2006 than in 2005 (0.95% and 0.75%, respectively). LOPEZ-LUTZ et al. (2008) determined half of that amount of essential oils, i.e. 0.4%.

Thirty-five compounds, including two unidentifiable ones, were found in tarragon essential oils (Table 3). Substances from the phenylpropanoid class made up 62.2%. Studies by LOPEZ-LUTZ et al. (2008) revealed that the percentage of phenylpropanoid compounds was slightly lower, i.e. 52.2%. Terpenes dominated in oils of other species from *Artemisia* sp. (AKROUT et al. 2003, BARAZANDEH 2003, CHALCHAT et al. 2003, HAIDER et al. 2003, SHAFI et al. 2004, MORTEZA-SEMNANI, AKBARZADEH 2005, VILJOEN et al. 2005, JUDŢENTIENË, BUZELYTË 2006, KAZEMI, AKHAVANI 2009). The following substances prevailed in the tarragon essential oil: elemicine (56.0%), sabinene (20.9%), methyleugenol (6.2%) and E-asarone (6.2%). A similar content of elemicine (53.0%) was found by PINO et al. (1996). According to KOWALSKI et al. (2007), the elemicine concentration was lower (48.78%) and so was that of sabinene (18.88%). ARABHOSSEINI et al. (2006) reported sabinene, elemicine and methyleugonol to be the main components of tarragon essential oils. According to these authors, the sabinene and methyleugenol content was higher than in our experiment, i.e. 39.4% and 14.7%, respectively, although the level of elemicine was lower (16.0%). Methyleugenol also dominated in tarragon essential oils studied by LOPEZ-LUTZ et al. (2008), and its content was 35.8%.

Our analysis of the chemical composition revealed that the mean content of total nitrogen in tarragon herbage was 32.0 g kg^{-1} d.m. (Table 4). The average level of ammonia nitrogen in the same material was 1.41 g kg^{-1} d.m. The tarragon herbage harvested in 2006 contained more total nitrogen but less ammonia. Nitrates were absent in tarragon herbs.

Table 3

Chemical composition, retention indices and percentage composition of the *Artemisia dracunculus* L. essential oil (2005-2006)

Compounds	Retention indice (RI)	(%)
1	2	3
α-Thujene	929	0.3
α-Pinene	936	0.2
Sabinene	975	20.9
β-Pinene	979	0.4
β-Myrcene	991	0.8
α-Terpinene	1017	0.4
p-Cymene	1025	0.2
Limonene	1029	0.2
(Z)-β-Ocimene	1037	0.8
(E)-β-Ocimene	1051	0.5
γ-Terpinene	1062	0.6
cis-Sabinene hydrate	1070	0.3
Terpinolene	1089	0.2
trans-Sabinene hydrate	1098	0.3
allo-Ocimene	1132	0.1
Terpinen-4-ol	1177	1.1
α-Terpineol	1189	0.1
Thymol	1290	0.5
Carvacrol	1299	0.3
Elemene	1338	0.1
Citronellylacetate	1353	0.8
Geranyl acetate	1381	0.4
Methyl eugenol	1404	6.2
β-Caryophyllene	1418	0.2
Germacrene D	1485	0.2
β-Ionone	1489	0.1
Bicyclogermacrene	1500	0.1
δ-Cadinene	1523	0.1
Elemicin	1557	56.0
n.i.	1574	0.1

cont. Table 3

1	2	3
Spathulenol	1578	0.7
Caryophyllene oxide	1583	0.1
n.i.	1617	0.1
T-Muurolol	1651	0.1
E-Asarone	1676	6.2
Total		99.7
Groupe components:		
Monoterpene		35.7
Sesquiterpene		1.6
Phenylopropanoid		62.2
Other		0.2

n.i. – not identified

Table 4

The content of macroelements in the herbage of *Artemisia dracunculus* L. (g kg^{-1} d.m.)

Year	N-total	N-NH$_4$	N-NO$_3$	P	K	Ca	Mg	S
2005 2006	28.25 35.80	1.53 1.30	- -	2.65 4.30	29.60 28.05	11.75 11.20	1.30 0.90	2.35 2.00
Mean	32.02	1.41	-	3.47	28.82	11.47	1.10	2.17
LSD$_{0.05}$	0.319	0.013	-	0.077	0.064	n.s.	n.s.	n.s.

n.s. – non-significant differences

The mean concentration of phosphorus in tarragon herbage was 3.47 g kg^{-1} d.m. Significantly more phosphorus was determined in 2006 (4.3 g kg^{-1} d.m.), a year characterized by less precipitation than 2005 (2.65 g kg^{-1} d.m.).

Tarragon herbage accumulated large amounts of potassium. The level of this element was considerably different in particular years of the experiment. More potassium was recorded in 2005 (29.6 g kg^{-1} d.m.) than in 2006 (28.1 g kg^{-1} d.m.).

No substantial differences in calcium, magnesium and sulfur concentrations in tarragon herbage between the particular years were reported. The mean content of calcium was 11.47 g kg^{-1} d.m., while that of magnesium was 1.1 g kg^{-1} d.m. The sulfur concentration in tarragon herbage reached 0.21% d.m. JADCZAK and GRZESZCZUK (2008) reported high contents of minerals in tarragon grown for direct consumption.

Other authors (Özcan 2004, Özcan, Akbulut 2007) report that concentrations of potassium and calcium in herbal plant species were on similar levels as in the present experiment. The levels of magnesium and phosphorus found in our experiment were lower than those recorded by the above authors.

CONCLUSIONS

1. Atmospheric precipitation had a significant influence on the height of tarragon plants. In 2005, tarragon plants were higher. Also, fresh herbage and air-dry yields were superior owing to more rainfall.

2. Significantly more essential oils, total nitrogen and phosphorus were determined in tarragon herbage in 2006.

3. The phenylpropanoid substances prevailed in the essential oil. Elemicin, sabinene, methyleugenol and E-asarone dominated in tarragon essential oil.

REFERENCES

Adams R.P. 2001. *Identification of essential oil compounds by gas chromatography/quadruple mass spectroscopy.* Allured: Carol Stream, IL.

Akrout A.A., Chemli R., Simmonds M., Kite G., Hammami M., Chreif I. 2003. *Seasonal Variation of the Essential Oil of Artemisia campestris* L. J. Essent. Oil Res., 15: 333-336.

Arabhosseini A., Padhye S., van Beek T.A., van Boxtel A.JB., Huisman W., Posthumus M. A., Müller J. 2006. *Loss of essential oil of tarragon (Artemisia dracunculus* L.*) due to drying.* J. Sci. Food Agric., 86: 2543-2550.

Balcerek M., Modnicki D. 2008. *Tarragon rediscovered.* Panacea 1(22): 9-11. (in Polish)

Barazandeh M.M. 2003. *Essential oil composition of Artemisia fragrans Willd. from Iran.* J. Essent. Oil Res., 15: 414-415.

Chalchat J-C., Cabassu P., Petrovic S.D., Maksimovic Z.A., Gorunovic M.S. 2003. *Composition of essential oil of Artemisia campestris* L. *from Serbia.* J. Essent. Oil Res., 15: 251-253.

Grzeszczuk M., Jadczak D. 2008. *Estimation of biological value and suitability for freezing of some species of spice herbs.* J. Elementol., 13(2): 211-220.

Haider F., Dwivedi P.D., Naqvi A.A., Bagchi G.D. 2003. *Essential oil composition of Artemisia vulgaris harvested at different growth periods under Indo-Gangetic plain conditions.* J. Essent. Oil Res., 15: 376-378.

Jadczak D., Grzeszczuk M. 2008. *Effect of a sowing data on the quantity and quality of the yield of tarragon (Artemisia dracunculus* L.*) grown for a bunch harvest.* J. Elementol., 13(2): 221-226.

Judžentienė A., Buzelytė J. 2006. *Chemical composition of essential oils of Artemisia vulgaris* L. *(mugwort) from North Lithuania.* Chemija, 17(1): 12-15.

Kazemi M., Akhavani S. 2009. *Constituents of Artemisia tournefortiana Rchb. Essential oil from Iran.* JACR, 3 (10), www.SID.ir.

Kowalski R., Wawrzykowski J., Zawiślak G. 2007. *Analysis of essentials oils and extracts from Artemisia abrotanum* L. *and Artemisia dracunculus* L. Herba Pol., 53(3): 246-253.

Lamer-Zarawska E., Kowal-Gierczak B., Niedworok J. 2007. *Phytotherapy and plant medicines.* Wyd. Lek., PZWL, Warszawa. (in Polish)

Lopez-Lutz D., Alviano D.S., Alviano C., Kolodziejczyk P.P. 2008. *Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils.* Phytochemistry, 69: 1732-1738.

Martyniak-Przybyszewska B. 2005. *Growth and yield of some spice plants.* Zesz. Nauk. Akad. Rol. Wroc., Rol., 515: 347-351. (in Polish)

Martyniak-Przybyszewska B., Wojciechowski T. 2004. *Yields of some spice plant species grown near Olsztyn.* Fol. Univ. Agric. Stetin., Agricultura, 239(95): 245-248. (in Polish)

Morteza-Semnani K., Akbarzadeh M. 2005. *Essential oils composition of Iranian Artemisia absinthium* L. *and Artemisia scoparia Waldst. et Kit.* J. Essent. Oil Res., 17: 321-322.

Nowosielski O. 1988. *Guidelines for elaborating fertilization recommendations in horticulture.* PWRiL, Warszawa. (in Polish)

Özcan M. 2004. *Mineral contents of some plants used as condiments in Turkey*. Food Chem., 84: 437-440.

Özcan M., Akbulut M. 2007. *Estimation of minerals, nitrate and nitrite contents of medicinal and aromatic plants used as spices, condiments and herbal tea.* Food Chem., 106: 852-858.

Pino J.A., Rosado A., Correa M.T., Fuentes V. 1996. *Chemical composition of essential oil of Artemisia dracunculus* L. *from Cuba*. J. Essent. Oil Res., 8: 563-564.

Polish Pharmacopoeia VI. 2002. Polskie Towarzystwo Farmaceutyczne, Warszawa.

Shafi P.M., Nambiar M.K.G., Clery R.A., Sarma Y.R., Veena S.S. 2004. *Composition and antifungal activity of the oil of Artemisia nilagirica (Clarke) Pamp.* J. Essent. Oil Res., 16: 377-379.

Viljoen A.M., Vuuren S.F., Gwebu T., Demirci B., Başer K.H.C. 2005. *The geographical variation and antimicrobial activity of African Wormwood (Artemisia afra Jacq.) essential oil.* J. Essent. Oil Res., 18: 19-25.

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