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AVAILABLE FORMS OF NUTRIENTS IN SOIL FERTILIZED WITH LIQUID MANURE AND NPK

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Abstract

The aim of the experiment, conducted from 1973 to 1999, was to determine the effect of long-term fertilization with liquid manure (annual and biennial) and with mineral fertilizers on selected physicochemical and chemical properties of soil, i.e., the content of phosphorus, potassium, magnesium, sulphate sulphur, iron, manganese, pH_{KCl} and organic carbon. Fertilization with liquid manure was observed to have resulted in a significant reduction in the content of soil-available phosphorus in comparison to fertilization with NPK. Fertilization with both liquid manure and NPK led to a significant increase in the content of available potassium and magnesium in the soil profile. The highest amounts of S-SO$_4$ were reported from the topsoil layer, irrespective of the type of fertilizer applied. Fertilization with liquid manure and NPK caused an irregular rise in the content of iron soluble in 1 M HCl in the soil, whereas the content of manganese increased slightly and irregularly in response to the fertilization applied. A negligible rise in pH_{KCl} of the topsoil layer was observed at annual application of increasing doses of liquid manure. It was reported that fertilization with liquid manure led to a steady rise in the organic carbon content in the soil profile.

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

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PRZYSWAJALNE FORMY SKŁADNIKÓW POKARMOWYCH
W GLEBIE NAWOŻONEJ GNOJOWICĄ I NPK

Abstrakt

Celem eksperymentu prowadzonego w latach 1973-1999 było określenie wpływu wieloletniego nawożenia gnojowicą, co rok i co dwa lata, oraz nawozami mineralnymi na wybrane właściwości fizykochemiczne i chemiczne gleby, tj. zawartość fosforu, potasu, magnezu, siarki siarczanowej, żelaza, manganu, pH_{KCl} i węgla organicznego. Stwierdzono, że nawożenie gnojowicą, szczególnie stosowaną co rok, spowodowało istotne zmniejszenie zawartości fosforu przyswajalnego w glebie, w porównaniu z nawożeniem NPK. Natomiast zastosowanie nawożenia gnojowicą, a także nawożenia mineralnego, przyczyniło się do istotnego przyrostu zawartości potasu, o największej koncentracji K w dwóch najgłębszych badanych warstwach. Zastosowanie zarówno nawożenia gnojowicą, jak i NPK przyczyniło się do istotnego przyrostu zawartości magnezu przyswajalnego w profilu glebowym, szczególnie w dwóch (NPK) lub trzech (gnojowica) warstwach. Stosunkowo najwięcej S-SO$_4$ było w warstwie ornej, niezależnie od rodzaju nawożenia. Nawożenie gnojowicą i NPK spowodowało nieregularny przyrost zawartości żelaza rozpuszczalnego w 1 mol HCl w glebie, począwszy od warstwy 26-50 cm, zaś zawartość manganu przyrastała nieznacznie i nieregularnie pod wpływem zastosowanego nawożenia. Zaobserwowano nieznaczny wzrost pH_{KCl} warstwy ornej gleby pod wpływem corocznego stosowania wzrastających dawek gnojowicy. Stwierdzono, że nawożenie gnojowicą spowodowało systematyczne zwiększenie zawartości węgla organicznego w profilu glebowym, szczególnie w dwóch warstwach: 0-25 cm i 26--50 cm.

Słowa kluczowe: gnojowica, nawożenie naturalne, właściwości gleby, długoletnie nawożenie.

INTRODUCTION

Liquid manure is a natural fertilizer composed of faeces and urine of livestock, usually with addition of a small amount of water. It is produced in litter-free breeding houses. Its fertilizing value and chemical composition depend on the animal species and age, feeding system, direction of use and production of animals, dilution with water and the storage system (BOROWIEC 1986, MAĆKOWIAK 2000, MAZUR et al. 1998, MURZYŃSKI 1998, POTARZYCKI 2000). An annual dose of liquid manure (and other fertilizers) should not exceed the amount corresponding to 170 kg ha^{-1} N. Fertilization with liquid manure should ensure optimal use of components that would be adjusted to nutrient demands of plants in order to prevent contamination of the environment, particularly with nitrogen and phosphorus. The best results are obtained by application of liquid manure and mineral fertilizers in combination (BOROWIEC, MAGIERSKI 1980/1981, MAZUR, SĄDEJ 2002, MAZUR et al. 1998). Locally, fertilization with liquid manure may pose certain problems related to its effect on the environment; therefore, it is essential to recognise the influence of this important element of the environment on soil properties on the basis of long-term experiments (MAĆKOWIAK 2000, MURZYŃSKI 1998).

The aim of the study was to determine the effect of long-term fertilization with liquid manure and mineral fertilizers on some physicochemical and chemical soil properties.

MATERIAL AND METHODS

The present report is based on the results of chemical analyses of soil samples obtained in the experiment conducted by MAĆKOWIAK (2000) in spring 2002 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 the level of 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. In the 0-25 cm layer only, the soil was mixed with low clay, humic, sandy loam (the 2:1 ratio) sampled from the topsoil of an arable field at the Experimental Station of the IUNG in Sadłowice, Poland. The concentrations of the available components in the topsoil were 2.09 mg kg^{-1} P and 6.47 mg kg^{-1} K; pH$_{KCl}$ – 5.6; the content of humus was 1.06%.

Liquid cattle manure was applied annually at the doses of 25, 50, 100 and 200 m^3 ha^{-1} and biennially at double doses. Liquid manure in the above doses was applied in 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 1995, 31.4 kg ha^{-1} of phosphorus fertilizers were applied to the treatments with 25, 50 and 100 m^3 doses of liquid manure. Also, all the treatments with liquid manure were supplemented with 99.6 kg ha^{-1} of potassium fertilizers. In 1998 and 1999, mineral fertilization was applied in the whole experiment at equal doses of 120 kg ha^{-1} N, 31.4 kg ha^{-1} P and 99.6 kg ha^{-1} K.

In order to compare the liquid manure and mineral fertilizers, the experiment included only four treatments fertilized with NPK in the form of mineral fertilizers at a dose approximately corresponding to half the dose of each of these components introduced into the soil in the respective treatments where liquid manure was applied annually. The doses were: 50, 100, 200 and 400 kg ha^{-1} N; 10.9; 21.8; 43.6 and 87.2 kg ha^{-1} P; and 41.5; 83; 166 and 332 kg ha^{-1} K. Nitrogen was applied as ammonium nitrate, phosphorus as granular single superphosphate, and potassium as 60% potassium salt. In 1973-1989, the 1NPK treatment was supplemented with 850 kg N, 185.3 kg P and 705.5 kg K, which was equivalent to the dose of 25 m^3 ha^{-1}

of liquid manure. In 1987, the designed nitrogen and potassium doses were reduced by 50% in treatments 3NPK and 4NPK, and the phosphorus dose was decreased by 50% in the 4NPK treatment. In the two successive years, the original nitrogen and phosphorus doses in treatment 3NPK were reduced by 25% and the dose of potassium by 50%; in treatment 4NPK, the doses of nitrogen and phosphorus were lowered by 50%, and that of potassium by 70%. This change was necessitated by the lower crop yield resulting from poorer germination rates and plant density per unit area. Signs of potassium and, to a lesser extent, phosphorus excess were observed on the growing plants. Mineral fertilizers in the re-adjusted doses were applied throughout the experimental period until 1999. In 1990 and 1991, the doses were as follows: N – 50, 100, 150 and 200 kg ha^{-1}; P – 10.9; 21.8; 32.7 and 43.6 kg; and K – 41.5; 62.3; 83 and 103.8 kg ha^{-1}. In 1993-1994 and in 1997, the application of phosphorus and potassium fertilizers was discontinued due to the observed symptoms of excess of the two elements.

The chemical analyses were performed in an accredited laboratory at the Regional Chemical and Agricultural Station, Lublin, Poland, with the following methods: available P and K – with Egner-Riehm method; available Mg – according to Schatschabel method after extraction of 0.0125 mol dm^{-3} CaCl$_2$ from the soil; the content of humus – according to Tiurin method; Fe and Mn soluble in 1 M HCl dm^{-3} – with the ASA method; S-SO$_4$ – with the nephelometric method according to Bardsley-Lancaster; and pH was assessed in 1 mol KCl dm^{-3} (*A catalogue of methods...* 2007). Statistical analyses were employed such as variance analysis with Tukey's confidence semi-intervals (α=0.05).

RESULTS AND DISCUSSION

The pH$_{KCl}$ of the topsoil layer (0-25 cm) increased slightly in response to the annual application of increasing doses of liquid manure from 5.2 (25 m^3 ha^{-1}) to 5.7 (200 m^3 ha^{-1}) – Table 1. This increase was more pronounced in the deeper soil layers, particularly at 26-50 and 51-75 cm. The biennial application of double doses of this fertilizer did not change the pH of the topsoil, which remained at the level 5.4-5.5. In deeper layers, particularly at 51-75 and 76-100 cm, there was a remarkable increase in pH. Application of liquid manure changed the soil reaction from acid (0-25 cm) to slightly acid (26-50 cm) and neutral in the deeper layers (or even alkaline in the 51--75 cm layer of the soil fertilized biennially with liquid manure). Application of increasing NPK doses contributed to slight acidification of the soil, particularly in the 0-25 and 26-50 cm layers (Table 1). Irrespective of the type of fertilization – organic or mineral – an increase in the pH$_{KCl}$ in the soil profile was reported, which was particularly evident to the depth of 51-75 cm.

It was apparently caused by leaching of alkaline compounds (mainly calcium and magnesium), primarily from the 0-25 cm layer. Borowiec (1986) observed an increase in pH in the successive layers of the soil profile induced by increasing doses of liquid manure as well as by mineral fertilization. Potarzycki (2000) and Mazur et al. (1998) found a slight rise in pH_{KCl} caused by increasing doses of liquid manure; a similar finding was reported by Murzyński (1998). An increase in pH resulting from manure application was also found by Candemira and Glsera (2011), whereas Silva et al. (2009) showed a pH increase upon application of liquid dairy manure and a fall in pH following mineral fertilization.

It was found that fertilization (annual and biennial) with liquid manure led to a steady increase in the content of organic carbon in the soil profile, particularly in the 0-25 cm layer, and a slightly smaller rise in the 26-50 cm layer (Table 1). Mineral fertilization did not act as clearly as liquid manure, but also in this case the highest $C_{org.}$ content was observed in the two top layers of the soil (0-25 and 26-50 cm; Table 1). A similar phenomenon was reported in other investigations (Borowiec 1986, Mazur, Sądej 2002, Mazur et al. 1998, Murzyński 1998, Potarzycki 2000). The decline in the content of organic C after 20 years of the NPK treatment amounted to approximately 10%, whereas the increase in the organic C concentration after 20 years of the application of cattle manure at the rate of 320 Mg ha^{-1} y^{-1} reached 30% (Aoyama, Kumakura 2001). The soil total organic carbon measured in black soil by Han et al. (2006) decreased by 6.5% over 18 years in control and by 5.6% in recycled organic manure treatment.

The annual and biennial fertilization with liquid manure and mineral compounds resulted in a significant increase in the content of available phosphorus, predominantly in the 0-25 and 26-50 cm layers (Table 2). It was found that fertilization with liquid manure, particularly applied every year, produced a significant decline in the phosphorus content in the soil profile; a reverse phenomenon was observed as a result of the NPK fertilization (particularly at the depth of 26-100 cm). In long-term fertilization with liquid manure and chemical fertilizers, the content of available P increased in the 0 to 20 cm soil layer (Yanga et al. 2011). Application of high doses of liquid manure, particularly to acid and light soils, did not prove to contribute to the transfer (leaching) of phosphorus down into the soil profile. Ssimilar observations were reported by Borowiec (1986), Murzyński (1998) and Potarzycki (2000). The study of Ebeling et al. (2003) concerning the impact of various sources of phosphorus on its availability in soil demonstrated that P availability changed depending on the type of organic-mineral fertilization applied.

Application of increasing doses of liquid manure and NPK resulted in a significant rise in the content of available potassium (Table 2). Simultaneously, regardless of the type of the fertilization used – organic or mineral - the highest K concentration was observed in the 51-75 cm soil layer, being

Table 1

Soil pHKCl and the content of organic carbon in soil upon fertilization with liquid manure and NPK

pH$_{KCl}$

| Soil layer (cm) | Liquid manure (m³ ha⁻¹) | | | | | | | | | | NPK (kg ha⁻¹) | | | | | Mean |
| | annually | | | | | biennially | | | | | | | | | | |
	25	50	100	200	x	50	100	200	400	x	1	2	3	4	x	
0-25	5.2	5.6	5.4	5.7	5.5	5.5	5.4	5.5	5.4	5.4	5.4	5.2	5.2	5.1	5.2	5.4
26-50	5.3	5.8	5.9	6.5	5.9	5.5	5.7	5.5	5.7	5.6	5.5	5.4	5.2	5.1	5.3	5.6
51-75	6.7	7.0	7.5	7.3	7.1	7.2	7.2	7.2	7.4	7.3	6.6	7.2	7.1	7.2	7.0	7.1
76-100	6.7	6.4	6.6	7.1	6.7	6.9	6.6	6.8	6.9	6.8	6.5	6.8	6.9	6.7	6.7	6.7
Mean	6.0	6.2	6.3	6.6	6.3	6.3	6.2	6.3	6.4	6.3	6.0	6.1	6.1	6.0	6.1	6.2

HSD (0.05) (fertilization type) – 0.3; (depth×fertilization rate) – 0.6; (fertilization type×fertilization rate) – 1.2; (depth×fertilization type×fertilization rate) – 1.7

C (%)

| Soil layer (cm) | annually | | | | | biennially | | | | | NPK | | | | | Mean |
	25	50	100	200	x	50	100	200	400	x	1	2	3	4	x	
0-25	0.48	0.55	0.81	1.02	0.71	0.55	0.59	0.58	0.89	0.65	0.47	0.44	0.51	0.49	0.48	0.61
26-50	0.19	0.21	0.25	0.36	0.25	0.27	0.20	0.22	0.30	0.25	0.24	0.20	0.23	0.21	0.22	0.24
51-75	0.09	0.07	0.11	0.11	0.10	0.06	0.07	0.07	0.09	0.07	0.10	0.12	0.07	0.10	0.10	0.09
76-100	0.09	0.09	0.13	0.14	0.11	0.05	0.05	0.09	0.13	0.08	0.10	0.09	0.08	0.10	0.09	0.10
Mean	0.21	0.23	0.33	0.41	0.29	0.23	0.23	0.24	0.35	0.26	0.23	0.21	0.22	0.23	0.22	0.26

HSD (0.05) (fertilization type) – 0.03; (depth×fertilization type) – 0.10; (fertilisation type×fertilization rate) – 0.15; (depth×fertilization type×fertilization rate) – 0.20

Table 2

The content of available phosphorus and potassium in the soil upon fertilization with liquid manure and NPK

Soil layer (cm)	Liquid manure (m³ ha⁻¹)										NPK (kg ha⁻¹)					Mean
	annually					biennially										
	25	50	100	200	x	50	100	200	400	x	1	2	3	4	x	
mg P (kg⁻¹ d.m.)																
0-25	2.36	2.88	3.65	7.88	4.19	2.27	2.73	3.45	6.65	3.77	2.24	3.08	4.19	6.55	4.01	3.99
26-50	1.56	1.76	2.09	4.57	2.49	1.13	1.51	1.93	3.46	2.01	1.41	1.88	1.98	2.27	1.88	2.13
51-75	1.86	2.15	2.63	2.41	2.27	2.17	2.21	2.09	2.50	2.24	1.73	2.82	2.62	2.81	2.49	2.33
76-100	2.31	1.60	1.59	2.02	1.88	1.66	1.69	2.47	2.37	2.05	4.93	2.50	2.47	2.55	3.11	2.35
Mean	2.02	2.10	2.49	4.22	2.71	1.81	2.04	2.49	3.75	2.52	2.58	2.57	2.81	3.54	2.88	2.70

HSD (0.05) (fertilization type) – 0.63; (depth×fertilization type) – 2.01; (fertilisation type×fertilization rate) – 2.08; (depth×fertilization type×fertilization rate) – 3.70

Soil layer (cm)	25	50	100	200	x	50	100	200	400	x	1	2	3	4	x	Mean
mg K (kg⁻¹ d.m.)																
0-25	7.11	7.59	8.11	11.9		8.19	7.64	8.94	10.30		6.72	7.94	7.28	11.0	8.22	
26-50	4.79	5.73	8.39	14.2	8.28	5.09	5.62	8.03			4.62	4.70	6.36		5.82	
51-75	9.96	10.70	19.20	29.3	17.30	13.00	15.10	15.50	34.90	19.60	9.22	11.6	12.2	15.9	12.2	16.4
76-100	10.30	8.22	9.77	17.8	11.50	8.11	10.10	10.40	25.90	13.60	9.77	10.2	13.2	10.4	10.9	12.0
Mean	8.03	8.06	11.40	18.3	11.40	8.60	9.61	10.70	20.20	12.30	7.58	8.61	9.76	11.2	9.29	11.0

HSD (0.05) (fertilization type) – 1.92; (depth×fertilization type) – 7.04; (fertilisation type×fertilization rate) – 7.14; (depth×fertilization type×fertilization rate) – 8.99

slightly lower in the 76-100 cm layer, which implies relatively easy leaching of this element from the topsoil, particularly in light and acid soils. Such behaviour of K was not clearly confirmed by Borowiec (1986) or Murzyński (1998), who claim that the content of available potassium forms in soil of the control plot and in the plot that had been steadily fertilized with liquid manure for 20 years did not differ significantly, and that the content was the highest in the 0-20 cm layer.

Annual or biennial fertilization with liquid manure as well as mineral fertilizer application contributed to a significant rise in the content of available magnesium in the soil profile, particularly in two (NPK) or three (liquid manure) layers (Table 3). This implies that Mg easily travels downward through this soil profile. In his study, Borowiec (1986) did not observe this phenomenon, and Murzyński (1998) reported that the highest increase in the content of available magnesium occurred in the 0-20 cm layer. The treatment with liquid dairy manure led to an increase in exchangeable Mg^{2+} levels to the depth of 30 cm, but the mineral fertilizer caused an increase from 30 cm downwards (Silva et al. 2009).

The impact of fertilization with liquid manure and NPK on the content of sulphate sulphur in soil does not demonstrate such a clear tendency as in the case of the elements discussed above (Table 3). The topsoil layer contained the highest amounts of this S form, whose concentration tended to rise as this element leached into the deeper soil profile levels.

Application of liquid manure and mineral fertilizers also resulted in an increase (although irregular) in the content of iron soluble in 1 M HCl in soil (Table 4). A significant increase in the amount of this element was observed in the profile from the 26-50 cm layer downwards. Ye et al. (2006) showed that Fe availability might be increased by allowing manure to decompose in soil.

The content of manganese soluble in 1 M HCl in the soil profile increased slightly and irregularly as a result of the fertilization used (Table 4). The highest content of this element was observed in the topsoil layer. A similar regularity was reported by Murzyński (1998), who found the highest content of this Mn form in the 0-10, 10-20 and 20-30 cm layers, as well as Mazur et al. (1998), particularly upon application of liquid cattle manure. Borowiec and Magierski (1980/1981), however, did not observe such dependencies in light soils fertilized with liquid manure.

Selected chemical or physicochemical soil properties were also presented in the papers by Debska et al. (2010) and Pypers et al. (2011).

Table 3

The content of available magnesium and S-SO$_4$ in soil upon fertilization with liquid manure and NPK

Soil layer (cm)	Liquid manure (m³ ha⁻¹)										NPK (kg ha⁻¹)					Mean
	annually					biennially										
	25	50	100	200	x	50	100	200	400	x	1	2	3	4	x	
	mg Mg (kg⁻¹ d.m.)															
0-25	14.0	17.7	21.7	31.7	21.3	13.3	14.7	20.0	22.7	17.7	20.3	22.3	25.7	33.7	25.5	21.5
26-50	32.7	35.7	48.7	58.7	43.9	27.3	29.3	38.3	38.3	33.3	33.3	32.3	37.7	43.3	36.7	38.0
51-75	62.0	62.0	66.7	74.0	66.2	49.7	54.3	60.0	68.7	58.2	62.0	53.3	48.3	38.7	50.6	58.3
76-100	70.0	57.3	77.3	58.7	65.8	62.7	60.0	56.7	52.7	58.0	66.0	75.3	58.7	52.0	63.0	62.3
Mean	44.7	43.2	53.6	55.8	49.3	38.3	39.6	43.8	45.6	41.8	45.4	45.8	42.6	41.9	44.0	45.0

HSD (0.05) (fertilization type) – 4.3; (depth×fertilization type) – 11.9; (fertilisation type×fertilization rate) – 20.5; (depth×fertilization type×fertilization rate) – 26.0

Soil layer (cm)	25	50	100	200	x	50	100	200	400	x	1	2	3	4	x	Mean
	mg S-SO$_4$ (kg⁻¹ d.m.)															
0-25	4.93	6.83	29.00	12.80	13.40	7.28	9.73	5.35	5.43	6.95	3.90	20.9	10.0	14.6	11.6	10.6
26-50	4.79	3.07	9.45	5.03	5.58	10.10	7.07	3.62	1.17	5.49	9.02	10.0		12.4	6.82	6.82
51-75	2.93	4.47	6.15	3.73	4.32	8.15	7.17	4.92	6.55	6.70	13.6	10.6	12.3	14.3	12.7	7.91
76-100	3.32	4.47	6.57	6.43	5.22	8.87	8.25	6.62	7.70	7.75	10.2	11.6	12.6	14.5	12.2	8.40
Mean	3.99	4.73	12.80	7.00	7.13	8.60	8.05	5.01	5.21	6.72	9.18	13.3	10.3	14.0	11.5	8.43

HSD (0.05) (fertilization type) – 1.63; (depth×fertilization type) – 4.36; (fertilization type×fertilization rate) – 4.26; (depth×fertilization type×fertilization rate) – 6.25

Table 4

The content of iron and manganese soluble in 1 M HCl dm^{-3} in the soil upon fertilization with liquid manure and NPK

Soil layer (cm)	Liquid manure (m³ ha⁻¹)										NPK (kg ha⁻¹)					Mean
	annually					biennially										
	25	50	100	200	x	50	100	200	400	x	1	2	3	4	x	
	mg Fe (kg⁻¹ d.m.)															
0-25	773.3	799	796	814	796	729	711	797	889	781	726	830	760	852	792	790
26-50	745.0	734	635	664	694	653	719	697	669	685	709	750	726	732	729	703
51-75	919.7	928	846	679	843	841	953	833	764	847	943	1027	1051	971	998	896
76-100	1009.3	937	935	800	920	920	958	966	838	921	978	1091	1130	1084	1071	971
Mean	861.8	850	803	739	813	786	835	823	790	809	839	925	917	910	898	840

HSD (0.05) (fertilization type) – 32; (depth×fertilization type) – 86; (fertilization type×fertilization rate) – 130; (depth×fertilization type×fertilization rate) – 171

Soil layer (cm)	annually					biennially					NPK					Mean
	25	50	100	200	x	50	100	200	400	x	1	2	3	4	x	
	mg Mn (kg⁻¹ d.m.)															
0-25	93.6	96.8	87.7	95.9	93.5	74.3	79.0	89.0	101.4	85.9	87.7	88.8	89.7	79.2	86.3	88.6
26-50	55.1	59.6	51.3	65.2	57.8	49.7	56.2	58.7	67.0	57.9	58.0	58.5	64.6	81.0	65.5	60.4
51-75	66.2	64.4	58.6	61.5	62.7	58.2	68.3	68.5	75.4	67.6	64.8	81.0	73.2	72.8	72.9	67.7
76-100	59.4	54.2	54.4	53.6	55.4	55.8	60.6	63.4	69.6	62.4	62.0	73.3	75.4	66.9	69.3	62.4
Mean	68.6	68.8	63.0	69.1	67.4	59.5	66.0	69.9	78.4	68.5	68.1	75.4	75.7	75.0	73.5	69.8

HSD (0.05) (fertilization type) – 3.6; (depth×fertilization type) – 10.1; (fertilization type×fertilization rate) – 14.5; (depth×fertilization type×fertilization rate) – 21.7

CONCLUSIONS

1. Fertilization with liquid manure applied annually and biennially (double doses) and with mineral fertilizers resulted in a steady, significant increase in the content of nutrients (particularly in the topsoil layer), including available phosphorus, potassium, and magnesium, and in pH_{KCl} (only in the case of liquid manure) and organic carbon.

2. The highest concentrations of available phosphorus, soluble manganese and S-SO$_4$ were found in the 0-25 cm layer, irrespective of the type of fertilization applied; the content of the available forms of potassium and magnesium increased along with the depth of the profile, especially downwards to 75 cm.

3. Annual and biennial (double dose) application of liquid manure and of equivalent amounts of mineral fertilizers resulted in a similar increase in the nutrient content (the phenomenon was not always confirmed by statistical calculations) and in pH_{KCl} and C$_{org.}$

REFERENCES

A catalogue of methods for conducting agrichemical tests in agricultural and chemical stations. 2007 OSCH-R Lublin, pp. 1-19. (in Polish)

AOYAMA M., KUMAKURA N. 2001. *Quantitative and qualitative changes of organic matter in an ando soil induced by mineral fertilizer and cattle manure applications for 20 years.* Soil Sci. Plant Nutr., 47: 241-252.

BOROWIEC J. 1986. *Comparison of the effects of slurry fertilization on mineral and peat soils.* Ann. UMCS, sect. E, 41: 217-227. (in Polish)

BOROWIEC J., MAGIERSKI J. 1980/1981. *Effect of slurry fertilization on the content of trace elements in light soil.* Ann. UMCS, sect. E, 35/36: 275-284. (in Polish)

CANDEMIRA F., GLSERA C. 2011. *Effects of different agricultural wastes on some soil quality Indexes in clay and loamy sand fields.* Commun. Soil Sci. Plant Anal., 42: 13-28.

DEBSKA B., BANACH-SZOTT M., DZIAMSKI A., GONET S.S. 2010. *Chromatographic characteristics (HPLC, HPSEC) of humic acids of soil fertilised with various organic fertilisers.* Chem. Ecol. Supp., 26: 49-57.

EBELING A.M., COOPERBAND L.R., BUNDY L.G. 2003. *Phosphorus source effects on soil test phosphorus and forms of phosphorus in soil.* Commun. Soil Sci. Plant Anal., 34: 1897-1917.

HAN X., WANG S., VENEMAN P.L. M., XING B. 2006. *Change of organic carbon content and its fractions in black soil under long-term application of chemical fertilizers and recycled organic manure.* Commun. Soil Sci. Plant Anal., 37: 1127-1137.

MAĆKOWIAK C. 2000. *Comparison of the fertilizing effect of slurry and mineral fertilizers.* Nawozy i Nawożenie, 4: 110-118. (in Polish)

MAZUR T., SĄDEJ W. 2002. *Comparison of the acidification of soils caused by long-term fertilization with slurry, FYM and mineral fertilizers.* Zesz. Probl. Post. Nauk Rol., 482: 375-383. (in Polish)

MAZUR T., WOJTAS A., MAZUR Z., SĄDEJ W. 1998. *Comparison of the effect of organic and mineral fertilization on the reaction and acidity of soil.* Zesz. Probl. Post. Nauk Rol., 456: 251-255. (in Polish)

MURZYŃSKI J. 1998. *Content of phosphorus and other elements in the 0 to 50 cm soil layers after 20-year-long steady application of swine slurry fertilization.* Pr. Nauk. Akad. Ekon. we Wrocławiu, Chemia, Nieorganiczne związki fosforu, 677: 42-51. (in Polish)

POTARZYCKI J. 2000. *Chemical properties of soils after twenty years of bovine slurry and NPK fertilization.* 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.

SILVA J.C.P.M., MOTTA A.C.V., PAULETTI V., FAVARETTO N., BARCELLOS M., OLIVEIRA A.S., VELOSO C.M., SILVA L.F.C. 2009. *Dairy cattle liquid manure combined with mineral fertilization on chemical properties of a Brown Oxisol.* Rev. Bras. Cienc. Solo, 32: 2563-2572.

YANGA L., LIB T., LIC F., LEMCOFFD J.H., 2011. *Long-term fertilization effect on fraction and distribution of soil phosphorus in a plastic-film house in China.* Commun. Soil Sci. Plant Anal., 42: 1-12.

YE Z., JIANG P., XU Q. 2006. *Use of composting manure to improve plant iron and zinc,* Commun. Soil Sci. Plant Anal., 37: 2577-2593.

CONTENT OF SELECTED NUTRIENTS IN WHEAT, BARLEY AND OAT GRAIN FROM ORGANIC AND CONVENTIONAL FARMING

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Abstract

The success of organic farming results from the need to produce top-quality food and, additionally, to protect the natural environment by applying eco-friendly agricultural practices and abandoning synthetic fertilizers and chemical pesticides. The aim of this study was to assess the impact of organic and conventional production systems on the content of minerals and fatty acids in grain of oat, wheat and hulled and naked barley.

Wheat grain from organic farming was characterized by a higher content of Mn and by significantly higher content of Fe, Zn, Ca and Mg when compared to grain originating from conventional farming. The increased availability of potassium in soil, caused by the applied potassium salt fertilization, was reflected in a higher content of this macronutrient in grain of all cereals from conventional cultivation. The tillage system was found not to exert the same effect on the content of Ca and Mg in all the cereals examined. Lower content of iron and zinc was determined in organic grains of barley and oat. Grain from the conventional cropping system (except naked barley) proved to be richer in lipids. Crude oil of organic cereals was richer in the most valuable polyunsaturated fatty acids than that of the conventionally-grown crops The study demonstrated a stronger dependency between the level of the elements examined and cereal species than between the level of elements and cultivation system.

Key words: organic farming, conventional farming, mineral elements, fatty acids, cereal.

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ZAWARTOŚĆ WYBRANYCH SKŁADNIKÓW ODŻYWCZYCH W ZIARNIE PSZENICY, JĘCZMIENIA I OWSA Z UPRAWY EKOLOGICZNEJ I KONWENCJONALNEJ

Abstrakt

Popularność rolnictwa ekologicznego wynika z poszukiwania żywności o najwyżej jakości oraz z potrzeby ochrony środowiska naturalnego, przez stosowanie zabiegów agrotechnicznych przyjaznych ekosystemowi. Celem pracy była ocena wpływu ekologicznego i konwencjonalnego systemu uprawy na zawartość składników mineralnych i kwasów tłuszczowych w ziarnie owsa, pszenicy oraz jęczmienia oplewionego i nagiego.

Ziarniaki pszenicy z uprawy ekologicznej zawierały więcej Mn oraz istotnie więcej Fe, Zn, Ca i Mg w porównaniu z pochodzącymi z uprawy konwencjonalnej. Zwiększona dostępność potasu w glebie, spowodowana zastosowanym nawożeniem w postaci soli potasowej, odzwierciedla się wyższą zawartością tego makroskładnika w ziarniakach wszystkich zbóż z uprawy konwencjonalnej. Nie stwierdzono jednokierunkowego wpływu systemu uprawy na zawartość Ca i Mg w przypadku wszystkich badanych zbóż, natomiast niższą zawartość żelaza i cynku stwierdzono w ziarniakach jęczmienia i owsa z upraw ekologicznych. Ziarniaki z uprawy konwencjonalnej były zasobniejsze w tłuszcz (wyjątek stanowił jęczmień nagi). Tłuszcz zbóż ekologicznych był bogatszy w większość cennych kwasów wielonienasyconych w porównaniu ze zbożami konwencjonalnymi. Obserwowano silniejszą zależność zawartości badanych składników od gatunku zboża niż od systemu uprawy.

Słowa kluczowe: uprawa ekologiczna, uprawa konwencjonalna, składniki mineralne, kwasy tłuszczowe, zboże.

INTRODUCTION

Both in Poland and in the world, agricultural plant production relies on crop cultivation for food and feed. Cereals are obviously at the top of the food pyramid. As important sources of carbohydrates, proteins and mineral compounds, they are characterized by a species-specific combination of nutritive properties. The absorption and accumulation of nutrients by plants are affected by numerous factors, e.g. the concentration and form of ions available in soil as well as pH of soil solution (ZÖRB et al. 2009). More and more food products are made from wholemeal flour and provide valuable nutrients, including mineral compounds, which so far have been removed along with the seed coat. Daily consumption of food products containing 200 g of wholemeal flour may satisfy over 70% of the recommended daily intake of Cu, Fe, Mg, Zn, Mn, etc. (HUSSAIN et al. 2010)

The growing success of organic farming results from the need to produce top-quality food and, additionally, to protect the natural environment by applying ecosystem-friendly agricultural practices and abandoning synthetic fertilizers and chemical pesticides. Investigations conducted globally have focused mainly on the effect of organic crop tillage on parameters of soil or crop yield (RÖHLING, ENGEL 2010). Recently, however, researchers have been paying more attention to assessment of nutrients in organic food prod-

ucts. The aim of this study has been to assess the impact of organic and conventional production systems on the content of mineral elements and fatty acids in grain of oat, wheat and hulled and naked barley.

MATERIAL AND METHODS

The experiment was conducted with grain of cereals cultivated in the organic and conventional systems. Grain of winter wheat (cultivar Legenda), spring barley (gymnospermous cv. Rastik and hulled cv. Skarb), and oat (cv. Borowiak) originated from a plot experiment conducted by the Department of Herbology and Crop Cultivation Technology at the Experimental Farm Czesławice – harvest of 2009. The experiment was established on 2nd complex soil (quality class II), characterized by the humus content of 1.2-1.6%, high to very high content of potassium, magnesium and phosphorus, and acid to slightly acid pH.

The experiment included two, five-field cereal rotations:

– organic crop: sugar beet ? hulled/naked spring barley + additional red clover → red clover → winter wheat + tansy phacelia/white mustard (stubble intercrop) → oat + broad bean and pea (stubble intercrop);

– conventional crop: sugar beet → hulled/naked spring barley + additional red clover → red clover → winter wheat → oat.

In the organic system, the cereals were cultivated without mineral fertilization. Their needs for nutrients and fertilizers were met by plowing the intercrops and by increased fertilization with manure, whereas weed control was assured by stubble intercrops and harrowing.

Fertilizers and pesticides applied to the cereals are specified in Table 1.

The grains were analyzed for the content of mineral elements: Ca, K, Mg, Cu, Mn, Fe, and Zn with atomic absorption spectrometry (AAS) after incineration in a muffle furnace. Crude lipids were determined with the extraction method by Soxhlet (PN-64/A-74039) in a Soxtec System HT 1043 apparatus. Fatty acids were separated with the gas chromatography method using a UNICAM 610 apparatus with flame-ionization detector (FID).

Table 1

Elements of agrotechnology of cereals in the conventional cultivation system

Specification		Oat	Wheat	Barley
Mineral fertilization	N	70 kg ha^{-1} ammonium nitrate (34%) 60% before sowing 40% tillering phase	140 kg ha^{-1} ammonium nitrate (34%): 50% before moving vegetation 35% the stage of shooting 20% the stage of earing	90 kg ha^{-1} ammonium nitrate (34%) 60% before sowing 40% tillering phase
	P$_2$O$_5$	70 kg ha^{-1} triple superfosfate, granulated (46%) 100% before sowing	60 kg ha^{-1} triple superfosfate, granulated (46%) 100% before sowing	70 kg ha^{-1} triple superfosfate, granulated (46%) 100% before sowing
	K$_2$O	100 kg ha^{-1} potassium salt (60%) 100% before sowing	80 kg ha^{-1} potassium salt (60%) 100% before sowing	90 kg ha^{-1} potassium salt (60%) 100% before sowing
Seed dressing		Vitawax 200 SF	Divident 030FS	Vitawax 200 SF
Fungicides		not applicable	Alert 375 SC dose 1 dm^3 ha^{-1} Tilt Plus 400 SC dose 1 dm^3 ha^{-1}	Alert 375 SC dose 1 dm^3 ha^{-1} Tilt Plus 400 SC dose 1 dm^3 ha^{-1}
Herbicides		Chwastoksem Turbo 340 SL dose 2 dm^3 ha^{-1}	Apyros 75 WG dose 26.5 g ha^{-1} Tomigan 250 EC dose 0.7 dm^3 ha^{-1} applied together	Basagran 600 SL dose 2 dm^3 ha^{-1}
Insecticides		Karate 025 E^C dose 0.15 dm^3 ha^{-1}	Karate 025 EC dose 0.15 dm^3 ha^{-1}	Karate 025 EC dose 0.15 dm^3 ha^{-1}
Retardants		Antywylegacz plynny 675 SL dose 2 dm^3 ha^{-1}	Stabilan 750 SL dose 1 dm^3 ha^{-1}	Celefon 465 SL dose 1.5 dm^3 ha^{-1}

RESULTS AND DISCUSSION

Mineral compounds in plants serve a variety of functions, e.g. they are structure-forming elements (macronutrients) and participate in the regulation of biochemical processes (micronutrients) (BARCZAK et al. 2006). They are also essential for the proper growth and development of humans and animals. Their accumulation in cereal grain is influenced by multiple factors, including species and soil conditions: organic matter content, pH, and fertilization applied (WIŚNIOWSKA-KIELIAN, KLIMA 2007). Most of the research addressing the issue of mineral content in organic grain has focused on wheat (HUSSAIN et al. 2010, ZÖRB et al. 2009).

In the reported experiment, grain of oat, wheat as well as hulled and naked barley was analyzed for the content of 3 macronutrients, whose level was decreasing in the following order: K, Mg, Ca (Table 2). Oat grain proved to be the poorest in these cations (except for potassium). A higher content of these macronutrients in oat, than in barley had been determined earlier by MAKARSKA et al. (2006). In turn, the lowest content of Mg and Ca was assayed in wheat grain.

The highest content of potassium was found in the grain of hulled barley (E=6.87 mg kg^{-1}, C=7.03 mg kg^{-1}). A tendency for its considerably higher content (significantly higher in the case of wheat) was observed when

Table 2

Content of mineral elements in grain of oat, wheat and barley from organic (E) and conventional (C) cultivation

Specification	K	Ca	Mg	Cu	Mn	Fe	Zn
	g kg^{-1}			mg kg^{-1}			
Oat E	4,159a	0.411a	0.871a	3.386a	30.39a	44.47a	28.90a
Oat C	4.437a	0.475b	0.837b	3.182a	28.25a	45.76a	30.71a
LSD $_{p\leq0.05}$	0.328	0.008	0.015	1.715	3.020	19.83	15.61
Wheat E	3.984a	0.295a	0.692a	2.091a	29.20a	27.59a	29.05a
Wheat C	5.666b	0.245b	0.630b	2.548a	25.64a	22.91b	21.57b
LSD $_{p\leq0.05}$	0.332	0.0019	0.0122	1.000	13.27	3.64	2.45
Hulled barley E	6.868a	0.365a	0.802a	3.702a	11.99a	30.19a	30.56a
Hulled barley C	7.028a	0.393a	0.848b	3.675a	10.45b	36.08a	32.18a
LSD $_{p\leq0.05}$	1.225	0.0031	0.0167	1.891	0.629	6.096	12.04
Naked barley E	4.912a	0.330a	0.827a	3.208a	9.40a	30.40a	28.58a
Naked barley C	4.030a	0.306b	0.871b	3.576b	9.46a	38.12b	32.51a
LSD $_{p\leq0.05}$	2.619	0.0206	0.037	0.288	0.331	2.490	6.461

a,b – mean values marked with the same letter do not differ statistically at $p\leq0.05$

compared to the organic grain, which is presumably caused by potassium salt fertilization.

The content of magnesium was significantly higher in the grain of oat and wheat from organic cultivation than in conventionally grown crops. In turn, the level of this element in both cultivars of barley turned out to be higher in the grain from conventional crop. Hence, the farming methods were not confirmed to exert the same effect on all cereals. ZÖRB et al. (2009) attributed a slightly lower magnesium content in conventional wheat grain (analogous dependency was also observed in our study) to the better filling and higher volume of kernels.

Grains of wheat and naked barley from organic crops were significantly richer in calcium than those from the conventional cultivation system. A reverse dependence was observed in the case of oat. In hulled barley, the lower content of that element in organic grain was not statistically significant. ZÖRB et al. (2009) did not find any significant differences in the calcium content between wheat grain from organic and conventional cultivation, although grain from the organic biodynamic system was characterized by its higher content. Despite a few significant system-based differences, no tendency towards higher or lower levels of mineral elements in any farming system was suggested by MÄDER et al. (2007).

The level of such cations as iron, manganese, and copper in grain plays a very significant role. The mean content of micronutrients in grain of the analyzed cereals is presented in Table 2. The lowest content of Cu and Fe (analogously to macronutrients) appeared in wheat. The lowest level of Mn was found in both cultivars of barley. The lowest content of Zn was found in grain of conventional wheat and organic naked barley.

In turn, the highest content of copper was determined in grain of hulled barley (E=3.70 mg kg^{-1}, C=3.67 mg kg^{-1}). Differences in the copper content between grain from organic and conventional cultivation were statistically significant only in the case of naked barley cv. Rastik, with a higher content of copper noted in the conventionally-cultivated crop (E=3.21 mg kg^{-1}, C=3.58 mg kg^{-1}). The same tendency was observed in wheat grain (E=2.09 mg kg^{-1}, C=2.55 mg kg^{-1}). Among the analyzed cereals, the highest content of Mn was determined in oat (E=30.39 mg kg^{-1}, C=28.25 mg kg^{-1}) and wheat grain (E=29.20 mg kg^{-1}, C=25.64 mg kg^{-1}). More Mn was found in grain from the organic cultivation system, except naked barley, in which the Mn content was identical in both organic and conventional grain.

Further analyses demonstrated the highest content of iron in oat grain (E=44.47 mg kg^{-1}, C= 45.76 mg kg^{-1}), and the highest content of zinc in hulled and naked barley from conventional cultivation (32.18 and 32.18 mg kg^{-1}, respectively). In the grain of oat and both barley cultivars, the content of iron and zinc was higher in the crops with mineral fertilization and chemical protection. In contrast, statistically significantly higher content of Fe and Zn was determined in the organic grain of wheat. RYAN et

al. (2004) attributed a higher content of zinc in organic grain to improved colonization of the crops by *Arbuscular mycorrhizal* fungi, which improved absorption of mineral elements (including Ca and Zn).

The content of crude lipids in grain was higher in conventionally grown crops, except naked barley (Table 3). The richest source of crude lipids was oat grain (E=4.36% and C=4.49%), whereas hulled barley from organic cultivation contained the smallest amounts of these compounds. The results of our determinations of the lipid content in grain of oat and barley from the organic cultivation system are similar to the findings reported by Bobko et al. (2009). In contrast, Szumiło and Rachoń (2006), who analyzed lipids in naked and hulled oat, found differences due to the level of pesticides applied, namely stronger pest control meant a lower content of lipids in naked oat but more lipids in hulled grain.

The ratio of fatty acids in grain lipids of the analyzed cereals is shown in Table 3. A higher level of saturated fatty acids (SFA) was found in lipids of cereals from the conventional cropping system, whereas grain from organic farming was richer in unsaturated fatty acids (UFA). In both cases, it was only wheat grain that responded differently. A tendency for enhanced synthesis of monounsaturated fatty acids (MUFA) was observed in the conventional crops except oat. This relationship was evident in the case of crude lipids of wheat monoens. Cereal grain is an excellent source of essential polyunsaturated fatty acids, especially linoleic acid. A higher percentage of these acids was demonstrated in crude lipids of grain from the organic system. When comparing the analyzed cereals, the highest content of linoleic acid (18/2) was determined in lipids of organic wheat grain, whereas the lowest one – in oat grain from both cropping system. A lower percentage of polyunsaturated fatty acids in oat grain is compensated for by a very high content of crude lipids in grains of that cereal and an exceptionally high level of ecosadienoic acid (22/2), compared to other cereals. In addition to linoleic acid, grain oil is rich in both oleic (18/1) and palmitic acids (16/0). Conventional grain (except for oat) appeared to be richer in this monoenic acid. However, lipids of organic kernels of wheat and naked barley, and conventional kernels of oat and hulled barley were richer in saturated palmitic acid.

Table 3

Content of crude lipids and percentage contribution of fatty acids in lipids of the analyzed cereals from organic (E) and conventional (C) cultivation system

Specification	Crude lipids (%)	14/0	16/0	18/0	18/1 (N9)	18/1 (N7)	18/2	18/3	20/0	20/1	20/2	22/0	22/2	24/0	SFA	UFA	MUFA	PUFA
		percentage contribution (%)													percentage contribution (%)			
Oat E	4.36	0.278	16.27	1.474	35.13	0.824	38.29	1.278	0.138	0.703	0.033	0.068	5.316	0.027	18.35	81.65	36.71	44.94
Oat C	4.49	0.269	16.53	1.502	34.24	0.766	37.29	1.275	0.152	0.667	0.046	0.070	6.954	0.071	18.68	81.32	35.73	45.59
Wheat E	1.43	0.105	17.02	0.963	14.89	0.896	60.13	4.248	0.150	0.689	0.068	0.107	0.069	0.111	18.67	81.33	16.67	64.66
Wheat C	1.53	0.083	13.62	0.117	33.67	0.521	46.77	3.459	0.301	0.566	0.071	0.097	0.304	0.097	14.49	85.51	34.89	50.62
Hulled barley E	1.35	0.343	21.52	1.141	11.55	0.510	56.06	7.024	0.209	0.641	0.111	0.228	0.077	0.142	23.78	76.22	12.91	63.32
Hulled barley C	1.45	0.362	22.56	1.310	12.57	0.666	56.50	1.972	0.000	0.000	0.087	0.241	0.136	0.158	24.88	75.12	16.39	58.73
Naked barley E	1.79	0.203	20.42	1.189	12.44	0.614	57.34	6.006	0.166	0.857	0.083	0.152	0.120	0.118	22.40	77.60	14.03	63.57
Naked barley C	1.66	0.201	20.46	1.211	13.00	0.644	56.83	5.815	0.158	0.899	0.075	0.162	0.130	0.083	22.46	77.54	14.68	62.87

SFA – saturated fatty acids, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids

CONCLUSIONS

1. Wheat grain from the organic cropping system was characterized by a higher content of Mn and a significantly higher content of Fe, Zn, Ca and Mg when compared to grain from conventional cultivation.

2. Lower content of iron and zinc was determined in organic grain of barley and oat but the cropping system was found not to exert the same effect on the content of Ca and Mg in any of the examined cereals.

3. Crude oil of organic cereals was richer in the most valuable, polyunsaturated fatty acids than that of conventionally grown crops

4. The study has demonstrated that the content of the macro- and micronutrients was more strongly dependent on a cereal species than a cultivation system.

REFERENCES

Bobko K., Biel W., Petryshak R., Jaskowska I. 2009. *Analysis of the chemical composition and the nutritional value of cereal from the ecological farm.* Fol. Pomer. Univ. Technol. Stetin. Agric., Aliment., Pisc. Zootech., 272(11): 5-12.

Barczak B., Nowak K., Kozera W., Majcherczak E. 2006. *Effect of fertilization with microelements on the content of cations in oat grain.* J. Elementol., 11(1): 13-20. (in Polish)

Mäder P., Hahn D., Dubois D., Gunst L., Alföldi T., Bergmann H., Oehme M., Amadó R., Schneider H., Graf U., Velimirov A., Fließbach A., Niggli U. 2007. *Wheat quality in organic and conventional farming: results of 21 year field experiment.* J. Sci. Food Agric., 87: 1826--1835.

Makarska E., Rachoń L., Michalak M., Szumiło G. 2006. *Macroelements and α-glucan content in hulled and naked cultivars of barley and oats in relation to chemical protection.* J. Elementol., 11(2): 175-182. (in Polish)

Hussain A., Larsson H., Kuktaite R., Johansson E. 2010. *Mineral composition of organically grown wheat genotypes: contribution to daily minerals intake.* Int. J. Environ. Res. Public Health., 7: 3442-3456.

PN-64/A-74039. *Cereal preparations. Determination of fat.* (in Polish)

Röhling R.M., Engel K.H. 2010. *Influence of input system (conventional versus organic farming) on metabolite profiles of maize (Zea mays) kernels.* J. Agric. Food Chem., 58: 3022-3030.

Ryan M.H., Derrick J.W. Dann P.R. 2004. *Grain mineral concentrations and yield of wheat grown under organic and conventional management.* J. Sci. Food Agric., 84: 207-216.

Szumiło G., Rachoń L. 2006. *Influence of electromagnetic field on yielding and quality of naked and hulled spring barley and oat.* Acta Agrophysica., 8(2): 501-508. (in Polish)

Wiśniowska-Kielian B., Klima K. 2007. *Comparison of microelement contents in the winter wheat grain from organic and conventional farms.* J. Res. Appl. Agric. Engin., 52(4): 100-103. (in Polish)

Zörb Ch., Niehaus K., Barsch A., Betsche T., Langenkamper G. 2009. *Levels of compounds and metabolites in wheat ears grains in organic and conventional agriculture.* J. Agric. Food Chem., 57: 9555-9562.

CONTENT OF BIOACTIVE COMPOUNDS IN SEMI-DRY RED WINE

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Abstract

The aim of the present work was to compare the content of bioactive compounds (polyphenols and minerals) in semi-dry wines. The material for the study consisted of 6 types of semi-dry red wine originating from Bulgaria (Sofino Melnik), Hungary (Loci), France (Bongeronde) and Spain (Don Kichot). The wines were subjected to organoleptic scoring assessment, followed by analyses of crude ash, anthocyanins, the total content of phenol compounds, zinc, manganese, copper, iron and magnesium. Additionally, the browning index was determined. All the samples attained positive organoleptic assessment. The highest score for the color was given to the wines from France and Spain. The total acidity in the analyzed red wines ranged between 4.97 g dm^{-3} (Spanish wine) and 5.56 g dm^{-3} (Bulgarian wine), which fulfills the quality requirements for grape wine. Grape wine contains from 1.0 to 4.0 g dm^{-3} of polyphenols. The analyzed wine samples contained a slightly lower concentration of phenolic compounds: 1.28 g dm^{-3} on average. The average content of p-coumaric acid in the examined samples was 1.85 mmol dm^{-3}. The concentration of anthocyanins, compounds responsible for the color of wine, was significantly lower in Bulgarian and Hungarian wine samples (26.05±0.25 mg dm^{-3} and 23.60±0.80 mg dm^{-3} respectively), in comparison with French (59.90±0.20 mg dm^{-3}) and Spanish wine particularly (75.90±2.40 mg dm^{-3}). All the analyzed wine samples, independently of their place of manufacture, achieved high organoleptic assessment, especially for clarity and color. The highest rated wines from France were characterized a high level of bioactive compounds: total polyphenols and anthocyanins. They also had a high content of crude ash and minerals such as Mg, Mn, Zn. Relatively high concentrations of polyphenols, anthocyanins and crude ash were also determined in Spanish wines.

Key words: semi-dry red wine, mineral elements, phenolic compounds, anthocyanins.

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ZAWARTOŚĆ ZWIĄZKÓW BIOAKTYWNYCH
W WINACH CZERWONYCH PÓŁWYTRAWNYCH

Abstrakt

Celem pracy było porównanie zawartości związków bioaktywnych (polifenole, antocyjany, taniny, składniki mineralne) w winach półwytrawnych. Materiał do badań stanowiły wina czerwone półwytrawne (po 6 prób w każdej grupie), pochodzące z Bułgarii (Sofino Melnik), Węgier (Loci), Francji (Bongeronde) i Hiszpanii (Don Kichot). Wina poddano punktowej ocenie organoleptycznej. Analizowano również zawartość popiołu surowego, garbników, kwasu szczawiowego, kwasowość ogólną, stężenie antocyjanów, zawartość związków fenolowych ogółem oraz zawartość cynku, manganu, miedzi, żelaza i magnezu. Oznaczono indeks zbrązowienia. Wszystkie poddane badaniom próby win uzyskały pozytywną ocenę organoleptyczną, a najkorzystniejszą ocenę pod względem barwy – wina pochodzące z Francji i Hiszpanii. Kwasowość ogólna analizowanych win czerwonych półwytrawnych wynosiła od 4,97 g dm^{-3} (wina hiszpańskie) do 5,56 g dm^{-3} (wina bułgarskie) i spełniała wymagania jakościowe dla win gronowych. Wina gronowe zawierają od 1,0 do 4,0 g dm^{-3} polifenoli. Badane próby win zawierały nieco mniej związków fenolowych, średnio 1,28 g dm^{-3}. Zawartość kwasu p-kumarowego wynosiła średnio 1,85 mmol dm^{-3}. Antocyjany to związki, które odpowiedzialne są za barwę win. Ich stężenie było istotnie niższe w winach bułgarskich oraz węgierskich (odpowiednio 26,05±0.25 mg dm^{-3} i 23,60±0.80 mg dm^{-3}), w porównaniu z winami francuskimi (59,90±0.20 mg dm^{-3}), a przede wszystkim hiszpańskimi (75,90±2,40 mg dm^{-3}). Wszystkie poddane badaniom wina, niezależnie od miejsca ich wytworzenia, uzyskały wysoką ocenę organoleptyczną, szczególnie w zakresie oceny klarowności i barwy. Najwyżej oceniono wina pochodzące z Francji, w których stwierdzono także wysoki poziom związków bioaktywnych – polifenoli ogółem i antocyjanów oraz wysoką zawartość popiołu surowego i składników mineralnych, takich jak Mg, Mn, Zn. Podobnie dość wysokie stężenie polifenoli, antocyjanów, a także popiołu surowego oznaczono w winach hiszpańskich.

Słowa kluczowe: wina czerwone półwytrawne, składniki mineralne, związki fenolowe, antocyjany.

INTRODUCTION

Red wine is a rich source of healthy components, such as phenolic acids, anthocyanins and (+)catechins (tannins). Polyphenols are believed to have an antioxidant effect, which contributes to protecting low-density lipoproteins (LDL) from oxidation (NIGDIKAR et al. 1998, ZIMA et al. 2005, AMAROWICZ et al. 2008). Wine contains hydroxybenzoic (mostly gallic and protocatechic) and hydroxycinnamic (mostly caffeic and p-coumaric) acids and high concentrations of some of their derivatives (HERNANDEZ-ORTE et al. 2006). Grapes and wine contain many mineral elements. The most important minerals include potassium, magnesium, calcium and sodium, whereas iron, boron, silicon and zinc occur only in insignificant amounts (ppm range), and there are merely trace quantities of aluminum, lead, cadmium, fluorine, copper or selenium (<1 mg dm^{-3}). The content of mineral elements is affected by numerous factors, such as the type of grapevine, climate and soil as well as the method of wine production (COOK et al. 1995, RUPASINGHE, CLEGG 2007).

With the growing awareness of the positive impact on health (the French paradox) and the increasing consumption of red wines, it is interesting to compare their chemical composition and organoleptic characteristics. As mentioned earlier, wine quality is significantly affected by the region in which grapevine grows, which is why wines from different parts of Europe were analyzed. The objective of the present work was to compare the content of bio-active substances (polyphenols, minerals) in semi-dry red wine originating from different vineyards in Bulgaria, Hungary, France and Spain.

MATERIAL AND METHODS

The material consisted of semi-dry red wine originating from Bulgaria (Sofino Melnik, 11% vol.), Hungary (Loci, 10.5% vol.), France (Bongeronde, 10% vol.) and Spain (Don Kichot, 11% vol.) produced in 2009.

Bulgarian wine Sofino Melnik is a multistrain wine from the south-western region of this country (the Mediterranean climate), more specifically from the Struma Valle. Hungary Loci is a multistrain red wine from Ěszak-Alföld. French semi-dry red wine Bongeronde is a multistrain wine made from grapes growing in Languedoc. Spanish wine Don Kichot is a multistrain wine from La Mancha. Each of the examined groups consisted of 6 wine types (6 wines x 4 countries) in two replications. In total, 48 samples were examined. The wine was subjected to organoleptic assessment. A panel of tasters consisted of 20 people (10 males and 10 females), aged from 35 to 45 years. The assessment was made on a 5-point scale in accordance with the standards PN-90 A-79120/02. Laboratory analyses were performed in order to determine the content of crude ash according to PN-90A-79120/09, the share of tannins, based on formation of insoluble tannins with heavy metal salts (AOAC, 1995). Total acidity was determined according to PN-90A-79120/07 and expressed in tartaric acid. Total anthocyanins were determined using the pH differential method described by LEE et al. (2005). The methods described by SOMERS and EVANS (1974) were used to determine the browning index of the wines. The measurements were carried out with a Unicam 5625 UV/VIS spectrophotometer. All samples were analyzed by HPLC-DAD (Waters) for phenolic acids. The analytical HPLC system (Gilson) employed consisted of two pumps 306, a detector UV-VIS DAD 170 and an injector 20 µL. Chromatographic separations were performed on a reversed phase column Symmetry C_{18} (5.0 µm, 250_4.6 mm i.d., Waters). Phenolic acids were separated using eluents, A: 1% acetic, B: 50% acetonitryle, both in water (v:v). Elution gradient was following: 0-10 min 92% A, 11-40 min 70% A, 41-55 min 60% A, 56-72 min 92% A. The monitoring wavelength was 320 nm. The results were calculated from the calibration curve.

The total content of phenolic compounds was determined by the spectrophotometric method of Folin-Ciocalteau (GORISTEIN et al. 2000) and ex-

pressed as gallic acid equivalent (GAE). For assays of minerals, the samples were pre-dried in a dryer at a temperature of 60ºC for 24 hours, and then dried at 105ºC for another 24 hours. Afterwards, the samples were weighed, incinerated in a muffle furnace at a temperature of 450ºC and solubilized in 6N of spectrally-pure hydrochloric acid. The content of zinc, manganese, copper, iron, and magnesium was determined in the mineralizates by the ASA method (PN-EN 14082:2004) using a UNICAM 939 spectrometer. Analysis of the content of oxalic acid was performed following the method presented by Brzozowska et al. (1999). The determination of the browning index was based on the procedure recommended by Wrolstad et al. (1982).

The results presented in the work are the arithmetic means obtained from 12 measurements (6 types of wine, two replications) analyzed statistically with Statistica ver. 5 software, and p=0.05 and p=0.01 regarded as a statistically significant value.

RESULTS AND DISCUSSION

All types of the analyzed wine obtained a positive organoleptic scoring (Table 1). The highest score for the color was given to the wines from France and Spain, while the Bulgarian and Spanish wine types had the best clarity. The aroma and flavor of the French wine obtained the highest score and this type of wine had the highest total score among all the analyzed types.

Table 1

Organoleptic assessment of semi-dry red wines

Wine origin	Number of points in a 5-point scale				General opinion
	C	K	A	T	
Bulgaria	3.8 ± 0.91	4.9 ± 0.38	3.4 ± 0.46	2.9 ± 0.35	satisfactory
Hungary	3.4 ± 0.54	4.6 ± 0.22	3.1 ± 0.26	3.2 ± 0.17	satisfactory
France	4.1 ± 0.55	4.6 ± 0.19	3.6 ± 0.30	3.6 ± 0.21	good
Spain	4.5 ± 0.47	4.8 ± 0.31	3.1 ± 0.14	2.6 ± 0.22	satisfactory

C – colour, K – clarity, A – aroma, T – taste

The key criterion while purchasing groceries is their sensory attractiveness and one of its elements is color. The color of wine red depends mainly of the anthocynins. These compounds are found predominantly in the grape skin and are released in the process of maceration. The longer the maceration period before decanting young wine, the richer the wine in dyes and tannins (Balík, Kumšta 2008).

The acidity of wine depends on its content of organic acids, such as tartaric, malic, citric, succinic or lactic acid (MAĆKIW 2003). This is a significant trait which gives wine its valuable properties enhancing digestion and ideally wine should contain 2.0-5.0 g dm^{-3} of organic acids (GAWLIK, BIALIK 1998). According to PN-A-79122:1996, the total acidity of grape wine should range from 3.5 to 9.0 g dm^{-3}, as converted into tartaric acid. The total acidity of the analyzed semi-dry red wine was from 4.97 g dm^{-3} in Spanish wine to 5.56 g dm^{-3} in Bulgarian wine, which met the requirements set for the quality of grape wine (Table 2). The total value of acidity in the analyzed wine was not affected by the amount of oxalic acid, which was significantly higher in Bulgarian and Hungarian wine than in the wine from France and Spain (Table 2).

Table 2

Content of oxalic acid and total acidity and browning in semi-dry red wines

Item	Wine origin				Mean
	Bulgaria	Hungary	France	Spain	
Oxalic acid (mg %)	1.80^a ± 0.61	1.71^a ± 0.21	5.67^b ± 0.95	5.49^b ± 1.56	3.66 ± 2.07
Total acidity (g dm^{-3})	5.56 ± 0.42	5.39 ± 0.54	5.06 ± 0.38	4.97 ± 0.27	5.24 ± 0.88
Browning index	2.97^a ± 0.16	3.37^{ab} ± 0.06	4.29^b ± 0.19	3.75^{ab} ± 0.11	3.59 ± 1.11

a, b – values in the same rows marked with different letters differ significantly at $p \leq 0.05$

Oxalic acid is fairly high in the concord grapes and ooccasionally, crystals of calcium oxalate form in wine. The development occurs late, commonly after bottling. The redox potential of most young wines stabilizes the complex formed between oxalic acid and metal ions, such as iron. However, as the redox potential of wine rises during aging, ferrous oxalate changes into the unstable ferric form. After dissociation, oxalic acid may bond with calcium, forming calcium oxalate crystals (JACKSON 2000).

The health-promoting value and the quality of wine also depend on its content of phenolic compounds (IWANOW et al. 2001). Grapes are a rich source of phenolic compounds and red grape wine contains from 1.0 to 4.0 g dm^{-3} of polyphenols (LOPEZ-VELEZ et al. 2003). The analyzed semi-dry red wine originating from France and Spain was relatively low in these compounds (1.45 g dm^{-3} in both replications). However, this value was significantly higher (p=0.05) compared with the wine from Bulgaria (1.25±0.03 g dm^{-3}) or Hungary (0.97±0.02 g dm^{-3}) – Table 3. Both the content of polyphenols and the share of anthocyanins and tannins, and hence the wine's color and its intensity, depend on the type of grapes, the degree of their maturity and the climate in which the fruit is cultivated (the warmer the climate, the darker the wine) (BALÍK et al. 2008), which can justify a higher concentration of the above compounds in French and Spanish wine varieties.

Content of biological activities compounds in semi-dry red wines

Item	Wine origin				Mean
	Bulgaria	Hungary	France	Spain	
Total phenolic (g dm^{-3})	$1.25^a \pm 0.03$	$0.97^a \pm 0.02$	$1.45^b \pm 0.03$	$1.45^b \pm 0.01$	1.28 ± 0.33
Anthocyanins (mg dm^{-3})	$26.05^a \pm 0.25$	$23.60^a \pm 0.80$	$59.90^b \pm 0.20$	$75.90^c \pm 2.40$	46.36 ± 20.11
p-coumaric acid (mmol dm^{-3})	$1.95^a \pm 0.44$	$2.84^b \pm 0.08$	$1.45^a \pm 0.01$	$1.17^a \pm 0.33$	1.85 ± 0.25
Tannins (%)	$2.57^a \pm 0.79$	$5.35^b \pm 0.98$	$4.68^b \pm 0.81$	$3.24^a \pm 0.59$	3.96 ± 1.23

a, b, c, d – values in the same rows marked with different letters differ significantly at $p \leq 0.05$

Numerous studies have proven very beneficial influence of polyphenols on human health (the French paradox) and have enabled us to determine which are most effective. The group of compounds with documented bioactive effects includes, among others, hydroxybenzoate and hydroxysuccinic acids.

A wide discrepancy in the number and concentrations of phenolic acids was found among the examined wines, which contained from five (French wines) to eighteen phenolic acids (Spanish wines). The concentration of p-coumaric acid was used for a comparative study of all the semi dry wines because it occurred in a high content in all the samples. According to Tuszyński and Sroka (1999), the content of p-coumaric acid in red wine is around 1.56 mmol dm^{-3}. The same content is quoted by Maćkiw (2003). Rastija et al. (2009) reported significant differences in the concentration of p-coumaric acid in Croatian red wines, where it ranged from 1.7 to 7.4 mg dm^{-3}. As regards p-coumaric acid in the analyzed wines (Table 3), its average content was 1.85 mmol dm^{-3}, being the highest in the Hungarian wine (2.84±0.08 mmol dm^{-3}).

The results concerning the total content of anthocyanins were confirmed by the total content of polyphenol compounds in the analyzed wine. Their concentration was significantly lower in the Bulgarian and Hungarian wine, (26.05±0.25 mg dm^{-3} and 23.60±0.80 mg dm^{-3}, respectively) compared to the wine from France (59.90±0.20 mg dm^{-3}) and Spain (75.90±2.40 mg dm^{-3}) – Table 3. Similar correlations were noted for the semi-sweet red wine analyzed by Czech (2007), where the highest anthocyanin content was recorded in Spanish wine and the lowest in the Bulgarian varieties. According to Tsanova-Savova et al. (2002), the content of anthocyanin in Bulgarian red wines varied from 22 to 274 mg dm^{-3}.

Anthocyanins are responsible for the color of wine. However, they are exposed to different factors during the manufacturing process and storage,

such as the presence of oxygen, metal ions or light, which lead to condensation processes and consequently brown polymer formation (PENG et al. 1998). An increased share of brown polymers deteriorates the color of wine, making it less vivid (CZAPSKI, WALKOWIAK-TOMCZAK 2008). Bulgarian wine was characterized by the lowest degree of browning (2.97±0.16), while this value was the highest in French wine (p=0.05) – Table 2.

The concentration of anthocyanins in red wine gives it a purple shade, whereas tannins are responsible for its lighter shade of orange or yellow. In time, the color of wine changes towards lighter shades as anthocyanins are less durable than tannins (MANIAK, KUNA-BRONIOWSKA 2007). The content of tannins in the analyzed wine samples ranged from 2.57±0.79% (Bulgarian wine) to 5.35±0.98% (Hungarian wine) – Table 3. The results revealed statistically significant differences at the level of p=0.05.

Tannins form colorful complexes with ion metals. They also affect the bitterness of wine and, under the effect of oxygen and high temperature, are oxidized into dark-colored compounds. The content of mineral elements in the analyzed semi-dry red wine samples is presented in Table 4. Analyzing the content of mineral elements, it should be noted that the number

Table 4

Content of dry ash (g dm^{-3}) and selected mineral elements (mg dm^{-3}) in semi-dry red wines

Item	Wine origin				Mean
	Bulgaria	Hungary	France	Spain	
Dry ash	0.89^a ± 0.03	1.19^a ± 0.08	1.63^b ± 0.03	2.56^c ± 0.11	1.57 ± 0.80
Copper	0.40^b ± 0.04	0.24^{ab} ± 0.06	0.13^a ± 0.04	0.09^a ± 0.03	0.21 ± 0.10
Magnesium	50.3^a ± 6.10	117.2^b ± 6.50	90.2^b ± 10.40	102.4^b ± 7.60	90.0 ± 29.45
Iron	6.41^b ± 0.96	9.47^c ± 0.86	5.98^b ± 0.73	4.67^a ± 0.95	6.63 ± 2.14
Zinc	1.26^a ± 0.09	2.08^b ± 0.16	3.06^c ± 0.17	1.61^a ± 0.12	2.00 ± 0.75
Manganese	0.67^a ± 0.11	1.13^b ± 0.06	0.94^b ± 0.05	0.32^a ± 0.04	0.76 ± 0.33

a, b, c – values in the same rows marked with different letters differ significantly at $p \leq 0.05$

of some microelements must be limited to maintain good health-promoting and organoleptic traits (COOK et al. 1995). An increase in the content of minerals may lead to turbidity and precipitation of lees in the wine. According to RĘCZAJSKA and JĘDRZEJCZAK (1998), the maximum limits in red wines are 5.0 mg dm^{-3} of zinc, 3.5 mg dm^{-3} of copper and 18.0 mg dm^{-3} of iron. Following the regulation by the Ministry of Health and Social Welfare, the acceptable content of zinc in wine is 5.0 mg dm^{-3}, and the content of copper in alcohol must not be higher than 1.0-4.0 mg dm^{-3}. Neither of these values was exceeded in any of the analyzed wine samples. The French wine was characterized by a significantly higher content of most analyzed miner-

al elements (magnesium, iron, zinc and manganese). The wine originating from Hungary also proved to be a rich source of minerals, particularly magnesium, iron and manganese. The Bulgarian wine had a significantly higher content of copper and iron, while the Spanish wine was richer in magnesium. The content of copper, manganese and iron in the analyzed wine samples was close to the results noted by BULIŃSKI et al. (1995) for red grape wine and MALIK and CZECH (2005). The concentration of zinc in the examined wines was significantly higher than in the wine studied by BULIŃSKI et al. (1995). Our results suggest some regularity related to the fact that certain metals may indirectly affect the degradation of anthocyanins by catalyzing the oxidation of ascorbic acid with the formation of H_2O_2 (PENG et al. 1998). Two elements participate in the processes of forming hydrogen peroxide, namely copper and iron. A higher content of these minerals in the Bulgarian wine may have led to the lowering of the content of anthocyanins in those wine samples.

The content of ash in wine, that is the components left after evaporation and roasting, depends mainly on the presence of minerals. The concentration of minerals in wine is slightly lower than in the fruit, which are used to proliferate yeast in the fermentation process (POGORZELSKI et al. 2005). The content of crude ash in the analyzed wine samples was significantly lower in the Bulgarian and Hungarian wines (0.89 ± 0.03 g dm^{-3}; 1.19 ± 0.08 g dm^{-3}, respectively). The highest share of crude ash was recorded in the Spanish wine (2.56 g dm^{-3}) – Table 4. According to WZOREK et al. (2005), the content of ash in red wine should not exceed 1.7 g dm^{-3}, whereas POGORZELSKI et al. (2005) suggest that the mean ash content in wine ranges from 1.8 to 2.5 g dm^{-3}. The analyzed semi-dry wine samples revealed a slightly lower crude ash concentration: 1.57 g dm^{-3} on average.

CONCLUSIONS

All the analyzed wine samples, regardless of their origin, achieved a high organoleptic assessment score, especially for clarity and color. The highest rated wine was made in France. It was characterized by a high level of bioactive compounds: total polyphenols and anthocyanins. It also had a high content of crude ash and minerals such as Mg^{+2}, Mn^{+2}, Zn^{+2}. Similarly, relatively high concentrations of polyphenols, anthocyanins and crude ash were determined in the Spanish wines. The results of our analyses showed a relationship between the content of bioactive compounds and the place of manufacture of wine. Consumption of wines, especially French ones, may contribute to increasing the supply of compounds producing beneficial effects in the human body.

REFERENCES

Amarowicz R., Narolewska O., Karamać M., Kosińska A., Weidner S. 2008. *Grapevine leaves as a source of natural antioxidants*. Pol. J. Food Nutr. Sci., 58(1): 73-78.

AOAC, 1995. *Official Methods of Association of Official Analytical Chemists*. 16th ed. Association of Official Analytical Chemists, Arlington, VA.

Balík J., Kumšta M. 2008. *Evaluation of color content in grapes originating from South Moravia*. Czech. J. Food Sci., 26: 18-24.

Balík J., Kyseláková M., Tříska J., Vrchotová N., Veverka J., Híc P., Totek J., Lefnerová D. 2008. *The changes of selected phenolic substances in wine technology*. Czech. J. Food Sci., 26: 3-12.

Brzozowska A., Czerwińska D., Kozłowska K., Morawiec M., Pietruszka B., Sulowska J., Wierzbicka E. 1999. *The toxicology of food – a practical guidebook*. Ed. SGGW, Warsaw. (in Polish)

Buliński R., Wyszogrodzka-Koma L., Marzec Z. 1995. *The examination of the content of selected metals in wine and alcoholic drinks*. Bromat. Chem. Toksykol., 28(3): 253-257. (in Polish; English abstract)

Cook J.D., Reddy M.B., Hurrell R.F. 1995. *The effect of red and white wines on nonheme-iron absorption in humans*. Am. J. Clin. Nutr., 61: 800-804.

Czapski J., Walkowiak-Tomczak D. 2008. *The kinetics of color changes in anthocyanins during heating dye solutions from Aronia berries, red grapes and elderberries*. Acta Agroph., 12(3): 625-636.

Czech A. 2007. *Bioactive compounds content in semi-sweet red wine*. Pol. J. Environ. Stud., 16(3A): 58-61.

Fuleki F., Francis F.J. 1968. *Quantitative methods for anthocyanins. Determination of total anthocyanins and degradation index for cranberry juice*. J. Food Sci., 33: 78-83.

Gawlik M., Bialik J. 1998. *Health-promoting properties of the substances present in wine*. Bromat. Chem. Toksykol., 21(4): 419-424. (in Polish; English abstract)

Goristein S., Caspi A., Zemser M., Trakhtenberg S. 2000. *Comparative contents of some phenolics in beer, red and white wines*. Nutr. Res., 20(1): 131-135.

Hernandez-Orte P., Bely M., Cacho J., Ferreira V. 2006. *Impact of ammonium additions on volatile acidity, ethanol, and aromatic compound production by different Saccharomyces cerevisiae strains during fermentation in controlled synthetic media*. Austr. J. Grape Wine Res., 12: 150-160.

Ivanov V., Carr A.C., Frei B. 2001. *Red wine antioxidants bind to human lipoproteins and protect them from metal ion-dependent and -independent oxidation*. J. Agric. Food Chem., 49(9): 4442-4449.

Jackson R.J. 2000. *Wine science: principles, practice, perception*. Published by Acad. Press (Elsev. Sci. & Technol. Books), pp. 364-365.

Lee J., Durst, R.W., Wrolstad, R.E. 2005. *Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study*. J. AOAC Int., 88: 1269-1278.

Lopez-Velez M., Martinez-Martinez F., Del Valle-Ribes C. 2003. *The study of phenolic compounds as natural antioxidants in wine*. Crit. Rev. Food Nutr., 43: 233-244.

Maćkiw E. 2003. *Selected components of wine and their health-promoting role*. Żyw. Czł. Metab., 30(3/4): 1088-1096. (in Polish; English abstract)

Malik A., Czech A. 2005. *Biological activities compounds in red wines*. Żyw. Czł. Metab. Supl. 1(2): 1076-1081. (in Polish; English abstrakt)

Maniak B., Kuna–Broniowska I. 2007. *Prediction of selected colour indices in red fruit wines*. Pol. J. Food Nutr. Sci., 57(3A): 83-87.

NIGDIKAR S.V., WILLIAMS N.R., GRIFFIN B.A., HOWARD A.N. 1998. *Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation in vivo*. Am. J. Clin. Nutr., 68: 258-265.

PENG Z., DUNCAN B., POCOCK K.F., SEFTON M.A. 1998. *The effect of ascorbic acid on oxidative browning of white wines and model wines*. Austr. J. Grape Wine Res., 4: 127-135.

POGORZELSKI E., KOBUS M., WILKOWSKA A. A. 2005. *Comparison of weighing and calculation methods in determining ash in wine*. Przem. Ferm. Ow.-Warz., 10: 28-30. (in Polish)

RASTIJA V., SRECNIK G., MEDIC-ŠARIC M. 2009. *Polyphenolic composition of Croatian wines with different geographical origins*. Food Chem., 115: 54-60.

RĘCZAJSKA W., JĘDRZEJCZAK R. 1998. *Determination of metallic elements in alcoholic drinks*. Przem. Ferm. Ow.-Warz., 6:18-20. (in Polish)

RUPASINGHE H.P.V., CLEGG S. 2007. *Total antioxidant capacity, total phenolic, mineral element, and histamine concentrations in wines of different fruit sources*. J. Food Comp. Anal., 20: 133-137.

SOMERS T.C., EVANS, M.E. 1974. *Wine quality: Correlations with colour density and anthocyanin equilibria in a group of young red wines*. J. Sci. Food Agric., 25: 1369–1379.

TSANOVA-SAVOVA S., DIMOVW S., RIBAROVA F. 2002. *Anthocyanins and color variables of bulgarian aged red wines*. J Food Comp. Analys., 15: 647-654.

TUSZYŃSKI T., SROKA P. 1999. *Resveratrol in wine – its occurrence, effects and determination methods*. Przem. Ferm. Ow.-Warz., 5: 13-17. (in Polish)

WROLSTAD R.E., CULBERTSON J.D., CORNWELL C.J., MATTICK L.R. 1982. *Detection of adulteration in blackberry juice concentrates and wines*. J. Offic. Anal. Chem., 65(6): 1417-1423.

WZOREK W., BONIN S., BASIAK A. 2005. *An attempt to use chitosan in its diluted form in wine stabilizing*. Żyw. Nauk. Technol. Jak., 1(42): 108-120. (in Polish)

ZIMA T., ALBANO E., INGELMAN-SUNDBERG M. 2005. *Modulation of oxidative stress by alcohol*. Alcohol. Clin. Exp. Res., 29: 1060-1065.

CONTENT OF NITRATES V AND III AND HEAVY METALS IN SELECTED *BRASSICA* VEGETABLES DEPENDING ON STORAGE

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Abstract

The study has been performed to analyze the content of nitrates V and III as well as lead and cadmium in *Brassica* vegetables, both fresh and after 5-month storage in a cool storeroom. The experimental material consisted of Chinese cabbage, red and white cabbage, savoy cabbage and Brussels sprouts. The content of nitrates in the plant material was determined with the spectrophotometric method based on Griess reaction, whereas concentrations of heavy metals were assayed with the AAS method after dry mineralization.

The 5-month storage period was found to decrease (by *ca* 65±5%) the content of nitrates V in savoy cabbage and Brussels sprouts. In turn, a *ca* 2-fold increase in the concentration of these compounds was determined in Chinese cabbage, and a similar tendency was observed in white cabbage. Chinese cabbage turned out to be the richest in the analyzed, undesirable elements and compounds, e.g. after storage it was characterized by an exceeded permissible level of nitrates V (750 mg kg^{-1} f.w.) and by the biggest, *ca* 10-fold, increase in concentrations of lead and cadmium. Besides, storage was observed to cause a significant increase in the content of nitrates III in the analyzed vegetables, except red cabbage, in which their content was shown to decrease by about 40%.

Concentrations of nitrates III and V, Pb^{2+} and Cd^{2+} in tissues of the cabbage plants should be monitored regularly in order to prevent their excessive accumulation in the food chain of man.

Key words: *Brassica* vegetables, storage, nitrates V, nitrates III, heavy metals.

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ZAWARTOŚĆ AZOTANÓW V I III ORAZ METALI CIĘŻKICH W WYBRANYCH WARZYWACH KAPUSTNYCH W ZALEŻNOŚCI OD CZASU PRZECHOWYWANIA

Abstrakt

Celem badań było przeanalizowanie zawartości azotanów V i III oraz ołowiu i kadmu w świeżych warzywach kapustnych i po ich 5-miesięcznym przechowywaniu w chłodnych magazynach. Materiał badawczy stanowiły: kapusta pekińska, kapusta głowiasta czerwona i biała, kapusta włoska oraz kapusta brukselska. W materiale roślinnym zawartość azotanów oznaczono metodą spektrometryczną, opartą na reakcji Griessa, natomiast metale ciężkie – metodą AAS po mineralizacji suchej.

Okres 5-miesięcznego przechowania wpłynął na obniżenie (o ok. 65±5%) zawartości azotanów V w główkach kapusty włoskiej i brukselskiej, natomiast ok. 2-krotny wzrost koncentracji tych związków odnotowano w kapuście pekińskiej, podobną tendencję zaobserwowano w przypadku kapusty białej. Kapusta pekińska okazała się warzywem najbardziej skażonym substancjami niepożądanymi, po przechowywaniu bowiem odnotowano przekroczenie (750 mg kg^{-1} ś.m.) zawartości azotanów V oraz największy, ok. 10-krotny wzrost zawartości ołowiu i kadmu. Przechowywanie spowodowało istotny wzrost zawartości azotanów III w analizowanych warzywach z wyjątkiem kapusty głowiastej czerwonej, w której nastąpił ok. 40% spadek koncentracji omawianych związków.

W celu zapobiegania nadmiernemu gromadzeniu się tych związków w łańcuchu pokarmowym człowieka, niezbędne jest regularne monitorowanie azotanów V i III oraz Pb^{2+} i Cd^{2+} w tkankach badanych roślin.

Słowa kluczowe: warzywa kapustne, przechowywanie, azotany V, azotany III, metale ciężkie.

INTRODUCTION

Vegetables are an essential component of human diet. They are a rich source of vitamins, minerals and dietary fibre. Unfortunately, they are also among the major sources of nitrates in foodstuffs (50-80%). Presence of some quantities of nitrates V and III in plants is a natural consequence of the nitrogen cycle in nature. Nevertheless, errors made during cultivation and storage of plant material coupled by limited oxygen access may trigger undesirable biochemical transformations, which in turn can modify the concentrations of nitrates. Although nitrates V do not pose any serious health problem, a product of their microbiological reduction, i.e. nitrates III, are highly toxic. In addition, genetic traits of vegetables that depend on a species or variety also influence accumulation of these compounds (RUTKOWSKA 1996, WOJCIECHOWSKA 2005, RUSINEK, CZECH 2007).

Another serious problem is food contamination with heavy metals. Lead and cadmium may enter the human body through the alimentary and respiratory systems and are capable of accumulating in human tissues. The major source of Pb and Cd are environmental pollutants, i.e. wastewater, solid

waste, dust emitted to the atmosphere, chemical fertilizers, and pesticides (Lo Coco et al. 2000, Yusuf et al. 2003, Khairiah et al. 2004, Rusinek, Czech 2007). The excess of these metals in food is implicated to raise the incidence of many diseases of the cardiovascular, respiratory and nervous systems. These metals are also involved in carcinogensis and mutagenesis (Steenland, Boffetta 2000, Radwan, Salama 2006).

Owing to the great popularity and high intake of *Brassica* vegetables in Poland, as well as to increasing environmental pollution, it seems highly advisable to monitor these crops in terms of concentrations of undesirable substances they may contain. The present study has been performed to analyze the content of nitrates V and III as well as lead and cadmium in *Brassica* vegetables, fresh and stored for 5 months in cool storerooms.

MATERIAL AND METHODS

The experimental material comprised fresh and stored *Brassica* vegetables, including: Chinese cabbage (*Brassica pekinensis* Rupr.), red cabbage (*Brassica oleracea var. capitata* L. *f. rubra*), white cabbage (*Brassica oleracea var. capitata* L. *f. alba*), savoy cabbage (*Brassica oleracea* L. *var. sabauda*), and Brussels sprouts (*Brassica oleracea L. var. gemmifera*). The vegetables originating from local producers were purchased in a hypermarket in Lublin (in October 2009). Some of the vegetables were stored for 5 months in cool storehouses, at the optimal temperature (0ºC) and air humidity (95-98%). Control measurements of the temperature and air humidity were carried out several times.

For each *Brassica* species, 6 samples were collected in each analytical period in order to determine levels of the undesirable components (nitrates V and III, lead and cadmium). The determinations were carried out in three replications. In total, 60 samples of *Brassica* vegetables were assayed.

Prior to chemical analyses, the vegetables underwent the necessary preparations (washing, fragmentation). The prepared plant material was divided into samples: 10 g of vegetables for analyses of nitrates V and III, and 5 g of vegetables for assays of heavy metals.

The content of nitrates V and III was determined with the spectrophotometric method (acc. to the Polish Standard PN-A-75112), based on Griess reaction. The determination consisted in diazotization of sodium nitrate III with sulfanilamide (Griess reagent I) and mixing with N-1-naphtylethylenediamine (Griess reagent II). The product of this reaction was two-phase, red-violet dye, whose color intensity was measured spectrophotometrically. Nitrates V were determined through direct reduction with cadmium to nitrates III.

For determination of lead and cadmium, the samples were pre-dried a drier at 60ºC for 24 h, and then dried at 105ºC for another 24 h. Afterwards, the samples were weighed, mineralized in a muffle furnace at 450ºC and solubilized in 6 N of spectrally pure hydrochloric acid. The mineralizate was determined for the content of heavy metals with the AAS method after dry mineralization (acc. to the Polish Standard PN-EN 14082), using a UNI-CAM 939 spectrometer. Lead content was assayed at $\lambda=217.0$ nm, whereas cadmium content – at $\lambda=228.8$ nm. The analytical range for Pb^{2+} and Cd^{2+} was: 0-50 µg dm^{-3} and 0-5 µg dm^{-3}, respectively.

The results were subjected to statistical analysis using Statistica ver. 5 software. The significance of differences between mean values was determined with the one-way analysis of variance test ANOVA.

RESULTS AND DISCUSSION

The 5-month storage period depressed (by *ca* 65±5%) the content of nitrates V in heads of savoy cabbage and Brussels sprouts (Table 1). In contrast, about a 2-fold increase in the concentration of these compounds was determined in Chinese cabbage and a similar tendency was observed in the case of white cabbage. Slightly different dependencies were noted while analyzing the impact of the 5-month storage on the content of nitrates III. The storage was observed to cause a significant increase in nitrates III in the analyzed vegetables, except for red cabbage, in which the concentration of these compounds decreased by about 40% (*p*=0.05) – Table 1. In turn, the biggest increase, by about 5-fold, in the concentration of nitrates III was determined in white cabbage, whereas the smallest one occurred in Brussels sprouts (*ca* 2-fold).

The binding EU regulations (*Regulation*…2006) limit the level of nitrates V only in vegetables, i.e. in lettuce and spinach. According to the previous *Regulation of the Minister of Health* (2003), the permissible level of these compounds in *Brassica* vegetables is 750 mg kg^{-1} f. w.

The content of nitrates V in both fresh and stored vegetables was far below the level of 750 mg kg^{-1} f.m., except in Chinese cabbage. The pretreatment of vegetable samples may have lowered the content of nitrate compounds, which are easily water soluble (MONDY, MUNSHI 1990). The stored Chinese cabbage was characterized by a high content of nitrates V, reaching 953.7 f.w. This might have resulted either from the excessive fertilization or from neglecting the pre-harvest interval.

LISIEWSKA and KMIECIK (1991) report that the accumulation of nitrate compounds is affected by light. Diminishing the light intensity or exposure time may increase the content of nitrates V in vegetables, because a deficient content of carbohydrates produced under insufficient light might be the fac-

Table 1

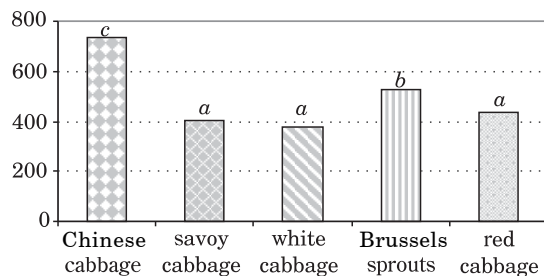Nitrate V and III content in the selected *Brassica* plants (mg kg^{-1} f.w.) – mean±SD

Brassica vegetable	Nitrates V		Nitrates III		$\overline{x}$	
	before storage	after storage	before storage	after storage	nitrates V	nitrates III
Chinese cabbage	518.9^A ± 52.28	953.7^B ± 37.81	0.034^A ± 0.002	0.115^B ± 0.024	736.3 ± 44.55	0.075 ± 0.013
Savoy cabbage	620.0^B ± 24.85	189.2^A ± 14.25	0.054^A ± 0.001	0.215^B ± 0.011	404.6 ± 19.55	0.135 ± 0.006
White cabbage	316.8^a ± 22.11	437.9^b ± 24.88	0.017^A ± 0.001	0.090^B ± 0.006	377.4 ± 23.50	0.054 ± 0.004
Brussels sprouts	754.8^B ± 64.45	303.2^A ± 38.11	0.035^A ± 0.001	0.080^B ± 0.009	529.0 ± 51.28	0.058 ± 0.005
Red cabbage	404.2 ± 24.48	471.7 ± 25.62	0.143^b ± 0.025	0.090^a ± 0.018	438.0 ± 25.05	0.117 ± 0.022
$\overline{x}$	522.9^A ± 37.43	471.1^B ± 28.13	0.05^A ± 0.006	0.118^B ± 0.014		

a, b – values marked with different letters differ significantly at $p \leq 0.05$
A, B – values marked with different letters differ significantly at $p \leq 0.01$

tor diminishing the reduction rate of these compounds. This factor might have caused an increased content of nitrates V and III in the vegetables analyzed in this study (Table 1).

Among the investigated *Brassica* vegetables, Chinese cabbage had the highest concentration of nitrates V (Figure 1). In contrast, the lowest concentration of NO_3^- ions was noted in white, savoy and red cabbage, with the value being about 45±5% lower than in Chinese cabbage ($p=0.05$). In

a, b, c – values marked with different letters differ significantly at $p \leq 0.05$

Fig. 1. The mean nitrates V content in the selected *Brassica* vegetables (mg kg^{-1} f.w.)

respect of nitrates III, their highest concentration was noted in savoy cabbage, higher than in white cabbage, Chinese cabbage or Brussels sprouts (Figure 2). The values were statistically significant and the differences reached about 50%. Red cabbage was characterized by a significantly lower (by 13%) content of nitrates III than savoys cabbage, and lower (by 50%) content of these compounds than in the other analyzed vegetables (Figure 2).

The content of nitrates V determined in this study was seven-fold lower than the value reported by MURAWA et al. (2008). Those authors determined the content of nitrates V in white cabbage at the level of 2 642.7 mg kg^{-1} f.m. In turn, the concentration of nitrates III reported by these authors equalled 0.13 mg kg^{-1} f.w., which exceeded by about 2.4-fold the values presented in Table 1. The research by LISIEWSKA and KMIECIK (1991) indicates that the content of nitrates V in savoy and red cabbage were higher by70% and 20%, respectively, than in the raw material analyzed in the present research. With respect to white cabbage, the results were comparable, whereas Brussels sprouts were characterized by a lower concentration of NO_2^- ions when compared to the values reported in our study. This might have been due to the stage of plant vegetation, for example, because the highest concentration of these compounds is observed in the early developmental stages, when the level of carbohydrates, the major source of energy to their reduction processes, is still low. According to AMR (2000) and MCKNIGHT et al. (1999), vegetables grown in heated greenhouses are characterized by a higher content of nitrates than cultivated on a field or in a tunnel, owing to lesser light intensity and higher nitrogen mineralization.
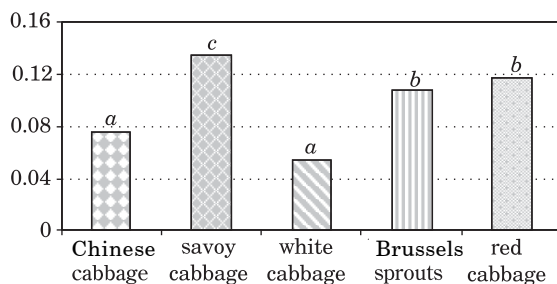
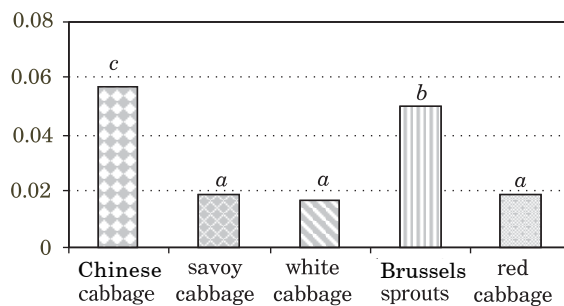*a, b, c* – values marked with different letters differ significantly at $p \leq 0.05$

Fig. 2. The mean nitrates III content in the selected *Brassica* vegetables (mg kg^{-1} f.w.)

Apart from nitrogen compounds, another group of xenobiotics likely to occur in plants are heavy metals, e.g. lead or cadmium. The uptake of heavy metals by plants is affected mostly by the grain size distribution of soil, which determines its absorption, and by the content of organic matter. For example, cadmium is very mobile in acid soils. The sources of cadmium are combustion of solid and liquid fuels, intensive fertilization with phosphate-based fertilizers, application of calcium and magnesium fertilizers, agricultural application of wastewater sludge, etc. The presence of lead is associated with the composition of soil, but unlike cadmium, lead is easily absorbed by plants from soils poor in organic matter, especially at low pH (SCOTT et al. 1996, VOUTSA et al. 1996, ZANIEWICZ-BAJKOWSKA 2002). When comparing the concentration of the two heavy metals (Pb^{2+} and Cd^{2+}) in the investigated *Brassica* vegetables, the highest levels were determined in Chinese cabbage (Figures 3, 4). In savoy, white and red cabbage, the concentrations of lead and cadmium were on a similar level and reached 0.031 ± 0.003 mg Pb kg^{-1}
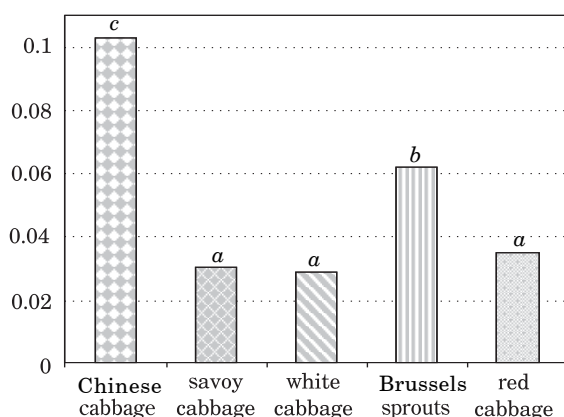
a, b, c – values marked with different letters differ significantly at $p \leq 0.05$

Fig. 3. The mean lead content in the selected *Brassica* vegetables (mg kg^{-1} f.w.)

a, b, c – values marked with different letters differ significantly at $p \leq 0.05$

Fig. 4. The mean cadmium content in the selected *Brassica* vegetables (mg kg^{-1} f.w.)

f.w. and 0.018 ± 0.002 mg Cd kg^{-1} f.w. Noteworthy is the fact that the permissible levels of lead and cadmium, i.e. 0.3 mg Pb kg^{-1} and 0.05 mg Cd kg^{-1} (*Regulation*…2006) were not exceeded in any of the *Brassica* species examined. Higher concentrations of heavy metals in Chinese cabbage than in collards were determined by BAHEMUKA and MUBOFU (1999). According to those authors, this was due to the differences in the morpho-physiological characteristics of vegetables, which was confirmed by SINGH and KUMAR (2006). In turn, as claimed by TAHVONEN and KUMULAINEN (1995), the accumulation of heavy metals depends on the part of a plant (roots>leaves>fruit>seeds), as well as the species or variety of plants cultivated under the same conditions. WONG et al. (1996) underlined the fact that Chinese cabbage belongs to vegetables characterized by higher accumulation of lead than other heavy metals, including cadmium, copper, zinc or nickel. This has been confirmed in the reported study, where the analyzed samples of Chinese cabbage had a higher concentration of lead than cadmium (Tables 2 and 3).

The availability of heavy metals is also greatly affected by environmental pollution. In a study by GONTARZ and DMOWSKI (2000), cabbage samples were characterized by the lead content of 0.45 mg kg^{-1} f.w. Such a high concentration of lead, exceeding the permissible level of contamination, might have resulted from the fact that these vegetables were cultivated in the vicinity of metal smelting plants, i.e. in an area with increased environmental pollution. According to the *Regulation of the Minister of Health* (2002), the content of lead in soil in industrial areas should not exceed 600 mg kg^{-1}. However, the content of lead in soils in the industrial areas, although below the stipulated permissible levels, is twice as high as in unpolluted regions, including the region of Lubelszczyzna.

As mentioned earlier, the uptake of elements is linked directly with their solubility, which in turn is affected by the following properties of soil: pH, content of organic matter, texture and redox potential. Hence, of outmost significance is the application of appropriate agricultural practices, especially rational organic and mineral fertilization. BEDNAREK et al. (2007) de-

Table 2

Lead content in the selected *Brassica* plants (mg kg^{-1}) – mean±SD

Brassica vegetable	(mg kg^{-1} f.w.)		(mg kg^{-1} f.w.)		$\overline{x}$	
	before storage	after storage	before storage	after storage	fresh weight	dry weight
Chinese cabbage	0.017^A ± 0.006	0.188^B ± 0.007	4.45^a ± 0.066	5.09^b ± 0.085	0.103^a ± 0.006	4.77 ± 0.076
Savoy cabbage	0.026^a ± 0.001	0.033^b ± 0.002	2.80^a ± 0.121	3.22^b ± 0.044	0.030^c ± 0.001	3.01 ± 0.032
White cabbage	0.021^A ± 0.002	0.037^B ± 0.001	2.50^a ± 0.032	2.88^b ± 0.062	0.029^c ± 0.001	2.69 ± 0.047
Brussels sprouts	0.025^A ± 0.005	0.099^B ± 0.007	1.31^A ± 0.045	1.99^B ± 0.052	0.062^b ± 0.006	1.65±0.049
Red cabbage	0.020^A ± 0.007	0.051^B ± 0.009	2.74^a ± 0.081	3.18^b ± 0.072	0.035^c ± 0.008	2.96 ± 0.077
$\overline{x}$	0.022^a ± 0.004	0.081^b ± 0.005	2.76^a ± 0.069	3.27b ± 0.063		

a, b – values marked with different letters differ significantly at *p*≤0.05
A, B – values marked with different letters differ significantly at *p*≤0.01

Table 3

Cadmium content in the selected *Brassica* plants (mg kg^{-1}) – mean±SD

Brassica vegetable	(mg kg^{-1} f.w.)		(mg kg^{-1} f.w.)		$\overline{x}$	
	before storage	after storage	before storage	after storage	fresh weight	dry weight
Chinese cabbage	0.010^A ± 0.001	0.104^B ± 0.006	2.62 ± 0.001	2.82 ± 0.008	0.057^a±0.004	2.72±0.005
Savoy cabbage	0.016^a ± 0.001	0.021^b ± 0.001	1.75^a ± 0.003	2.03^b ± 0.005	0.019^c±0.001	1.89±0.004
White cabbage	0.011^A ± 0.001	0.021^B ± 0.002	1.32^A ± 0.003	1.68^B ± 0.002	0.016^c±0.001	1.50±0.002
Brussels sprouts	0.023^A ± 0.001	0.077^b ± 0.002	1.22^A ± 0.003	1.56^B ± 0.005	0.050^b±0.001	1.39±0.004
Red cabbage	0.010^A ± 0.007	0.028^B ± 0.001	1.41^A ± 0.006	1.77^B ± 0.004	0.019^c±0.004	1.59±0.005
$\overline{x}$	0.014^A ± 0.002	0.050 ± 0.002	1.66^A ± 0.003	1.97B ± 0.005		

a, b – values marked with different letters differ significantly at $p \leq 0.05$
A, B – values marked with different letters differ significantly at $p \leq 0.01$

termined the content of cadmium in white cabbage to reach 0.006-
-0.028 mg kg^{-1}, similar to our results. The latter authors also observed
a significant correlation between the accumulation of this element in *Brassica* vegetables and its presence in soil (correlation coefficient 0.662). The low
content of cadmium in the analyzed vegetables may indicate a low pollution
level of the environment where they were cultivated, which is undoubtedly
typical of the region of Lubelszczyzna, and good agricultural practice (fertilization in particular). It is a very positive finding, showing that producers
adhere to the guidelines of the rational cultivation of vegetables sold on the
market.

The analyzed *Brassica* vegetables were observed to be characterized by
diversified tendencies for lead and cadmium accumulation in storage organs,
as affected by the process of storage. After the 5-month storage period, the
highest, about 10-fold, increase (per fresh weight) in the content of both
lead and cadmium was noted in Chinese cabbage, whereas the smallest increase (by about 25%) appeared in savoy cabbage (Tables 2, 3). The content
of these elements in the other analyzed *Brassica* vegetables was also significantly higher, reaching 80±15% in white cabbage. In Brussels sprouts, it
was 3.5±0.5-fold higher after the storage, and in red cabbage it rose by
2.5±0.5-fold. Such a high increase in Pb^{2+} and Cd^{2+} in the analyzed vegetables after the five-month storage was likely to be due to high water loss,
which reached about 14% in Chinese cabbage. In the other vegetables, the
water loss equalled to 10% in Brussels sprouts, 4.5% in red cabbage, and
1±0.5% in white and savoy cabbage.

CONCLUSIONS

1. The 5-month period of storage caused a decrease (by *ca* 65±5%) in the
content of nitrates V in savoy cabbage heads and in Brussels sprouts. In
contrast, a 2-fold increase in the concentration of these compounds was noted in Chinese cabbage, and a similar tendency was observed in white cabbage.

2. Chinese cabbage turned out to be the richest in the undesirable substances and characterized by an exceeded permissible level of nitrates V
(750 mg kg^{-1} f.w.) as well as the highest, *ca* 10-fold, increase in the content
of lead and cadmium after storage.

3. Storage caused a significant increase in the concentration of nitrates
III in all the analyzed vegetables except red cabbage, in which the level of
these compounds decreased by *ca* 40%.

4. In order to prevent excessive accumulation of nitrates V and III, Pb^{2+}
and Cd^{2+} in the food chain of man, it seems necessary to monitor regularly
their levels in tissues of edible plants.

REFERENCES

Amr. A. 2000. *Nitrate and nitrite content of some vegetables grown in central Jordan Valley.* Dirasat. Agricultural Sci., 27(3): 410-419.

Bahemuka T.E., Mubofu E.B. 1999. *Heavy metals in edible green vegetables grown along the sites of the Sinza and Msimbazi rivers in Dar es Salaam, Tanzania.* Food Chem., 66(1): 63-66.

Bednarek W., Tkaczyk P., Dresler S. 2007. *Content of heavy metals as a criterion for cabbage quality assessment.* Acta Agrophys., 10(1): 7-18. (in Polish)

Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. (in Polish)

Gontarz B., Dmowski Z. 2000. *Contens of heavy metals in soil and vegetables from gardens near the Hutmen metal smelting plant in Wroclaw.* Zesz. Probl. Post. Nauk Rol., 471: 913-919. (in Polish)

Khairiah J., Zalifah M.K., Yin Y.H., Aminha A. 2004. *The uptake of heavy metals by fruit type vegetable grown in selected agricultural areas.* Pak J. Biol. Sci., 7(8): 1438-1442.

Lisiewska Z., Kmiecik W. 1991. *Nitrates and nitrites in vegetables.* Part I. Post. Nauk Rol., 91(3): 11-24. (in Polish)

Lo Coco F., Monotti P., Noeelli V., Ceccon L., Adami G., Micali G. 2000. *Determination of cadmium and lead in vegetables by stripping chronopotentiometry.* Food Addit. Contam., 21(5): 441-446.

McKnight G.M., Duncan C.W., Leifert C., Golden M.H. 1999. *Dietary nitrate in man: friend or foe?.* Br. J. Nutr., 81(5): 349-358.

Mondy I., Munshi B. 1990. *Effect of nitrogen fertilization on glycoalkaloid and nitrate content of potato.* J. Agric. Food Chem., 38(2): 565-567.

Murawa D., Banaszkiewicz T., Majewska E., Błaszczuk B., Sulima J. 2008. *Nitrate and nitrite content in selected vegetables and potatoes commercially available in Olsztyn in 2003--2004.* Bromat. Chem. Toksykol., 41(1): 67-71. (in Polish)

PN-A-75112. 1992. *Fruits, vegetables and derived products. Determination of nitrites and nitrates content.* (in Polish)

PN-EN 14082. 2004. *Foodstuffs – Determination of trace elements – Determination of lead, cadmium, zinc, cooper, iron and chromium by atomic absorption spectrometry (AAS) after dry ashing.* (in Polish)

Radwan M.A., Salama A.K. 2006. *Market basket survey for some heavy metals in Egyptian fruits and vegetables.* Food Chem Toxicol., 44(8): 1273-1278.

Regulation of the Ministry for Environment of 9 September 2002 on soil and arable land quality standards. Journal of Laws no 165, item 1359. (in Polish)

Regulation of the Minister of Health of 13 January 2003 on the maximum levels of chemical and biological contaminants that may be present in food, food ingredients, allowed additional substances, substances that help in the processing or on food. (in Polish)

Rusinek E., Czech A. 2007. *Nitrate (V), nitrate (III) and heavy metal content in selected root vegetables depending on the shelf life (*Part I*).* Bromat. Chem. Toksykol., 40(1): 15-21. (in Polish)

Rutkowska G. 1996. *More about nitrates.* Chłodnictwo, 31(12): 38-40. (in Polish)

Scott D., Keoghan J.M., Allen B.E. 1996. *Native and low input grasses – a New Zealand high country perspective.* New Zealand Agric. J. Res., 39(4): 499-512.

Singh S., Kumar M. 2006. *Heavy metal load of soil, water and vegetables in peri-urban Delhi.* Environ. Monitor. Assess., 120(1-3): 79-91.

Steenland K., Boffetta P. 2000. *Lead and cancer in humans: where are we now?* Am. J. Ind. Med., 38(3): 295-299.

TAHVONEN R., KUMULAINEN J. 1995. *Lead and cadmium in some berries and vegetables on the Finnish market in 1991-1993.* Food Addit. Contam., 12(2): 263-279.

VOUTSA D., GRIMANIS A., SAMARA C. 1996. *Trace elements in vegetables grown in industrial areas in relation to soil and air particulate matter.* Environ. Pollut., 94(3): 325-335.

WOJCIECHOWSKA R. 2005. *Accumulation of nitrates and quality of horticultural products.* Wyd. Coperite, Kraków, 21-27. (in Polish)

WONG J.W., LI G.X., WONG M.H. 1996. *The growth of Brassica chinensis in heavy metal contaminated sludge compost from Hong Kong.* Biores. Technol., 58(3): 309-313.

YUSTUF A.A., AROWOLO T.A., BAMGBOSE O. 2003. *Cadmium, copper and nickel levels in vegetables from industrial and residential areas of Lagos City, Nigeria.* Food Chem. Toxicol., 41(3): 375-378.

ZANIEWICZ-BAJKOWSKA A. 2002. *Organic fertilization and liming of the soil and the content of cadmium in vegetables.* Part 1: *Direct influence.* Rocz. Nauk Rol. Ser. A, 116(1): 51-62. (in Polish)

ALUMINUM-BASED WINTER WHEAT BIOMASS AND GRAIN YIELD SPATIAL VARIABILITY IN ARABLE SOILS: CONCEPT AND FIELD TEST

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Abstract

The paper outlines a concept related to selecting a site for experimental purposes. Selection of an experimental plot most frequently relies on performing visual evaluation of a given site, followed by the establishment of a field trial. In general, the question of geochemical variability is 'intentionally' postponed! Verification of this approach has been undertaken, testing such parameters as soil pH and exchangeable aluminum (Al_{ex}) versus spatial (investigated area, $12\,672$ m^2) and downward (sampling depths, i.e., 0-20, 20-40 and 40-60 cm) distribution. Winter wheat biomass at tillering (BBCH29) and grain yield at harvest (BBCH99) were additionally considered. The results have revealed that pH values fluctuated between 3.6 and 4.4 with respective coefficients of variation (CV) ranging from 3.10 to 5.92%. The concentrations of Al_{ex} ranged from 38.0 to 144.9 mg kg^{-1}, corresponding to CV within 28.34 and 44.03%. The variograms and geostatistical maps have demonstrated the spatial as well as downward variability of these parameters. The spatial distribution of plant biomass followed quite closely the exchangeable aluminum (Al_{ex}) levels, which implies that natural soil parameters such as Al_{ex} are not easily compensated for by agricultural practices, for instance nitrogen application. The spatial *grain yield – Al_{ex}* dependence which emerged at harvest confirmed the variability observed at tillering (BBCH29). Thus, the spatial variability of pH, Al_{ex} and wheat biomass as well as grain yields (BBCH99) verified the approach to selecting an experimental site. It was demonstrated that selection of a research site on the basis of its appearance and shape alone may lead to misinterpretation of experimental results.

Key words: exchangeable aluminum, pH, wheat biomass, grain yield, spatial variability, geostatistics.

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WPŁYW GLINU NA PRZESTRZENNĄ ZMIENNOŚĆ BIOMASY I PLONU ZIARNA PSZENICY OZIMEJ NA GLEBACH UPRAWNYCH: KONCEPCJA I BADANIA TERENOWE

Abstrakt

Praca przedstawia koncepcję związaną z wyborem stanowiska na cele doświadczalne. To podejście opiera się na częstej ocenie wzrokowej danego stanowiska, a dalej – założeniu doświadczenia polowego. Zmienność geochemiczna bywa generalnie „celowo" odłożona! Przedstawioną koncepcję zweryfikowano za pomocą parametrów, takich jak pH oraz zawartość glinu wymiennego (Al_{ex}), pod względem przestrzennym (pole badawcze 12 672 m^2) i w głąb profilu glebowego (warstwy gleby: 0-20, 20-40 i 40-60 cm). Następnymi parametrami do opracowania przestrzennej zmienności były biomasa pszenicy ozimej w fazie krzewienia (BBCH29) oraz plon ziarna w fazie dojrzałości pełnej (BBCH99). W badaniach wykazano, że wartości pH wahały się między 3,6 a 4,4, a odpowiednie współczynniki zmienności (CV) wynosiły 3,10-5,92%. W przypadku Al_{ex}, jego zawartość zmieniła się w szerokich granicach (38,0-144,9 mg kg^{-1}), co odpowiadało wartościom CV od 28,34 do 44,03%. Opracowane wariogramy oraz mapy geostatystyczne wyraźnie podkreślały zmienność zarówno przestrzenną, jak i w głąb profilu glebowego badanych parametrów. Przestrzenne rozmieszczenie biomasy roślinnej postępowało zgodnie z zawartością Al_{ex}. Oznacza to, że naturalne czynniki glebowe, jak Al_{ex}, nie są łatwo zrównoważone zabiegami agrotechnicznymi, np. nawożeniem azotowym. Przestrzenna zależność: plon ziarna – Al_{ex}, która ujawniła się w fazie żniw, potwierdziła zmienność zaobserwowaną w fazie krzewienia (BBCH29). Zatem przestrzenna zmienność pH, Al_{ex} oraz zarówno biomasy pszenicy, jak i plonów ziarna (BBCH99) zweryfikowały koncepcję związaną z wyborem stanowisk na cele badawcze. Wykazano, że wybór stanowiska badawczego oparty tylko na wyglądzie terenu i jego kształcie może doprowadzić do błędnej interpretacji danych eksperymentalnych.

Słowa kluczowe: glin wymienny, pH, biomasa pszenicy, plon ziarna, zmienność przestrzenna, geostatystyka.

INTRODUCTION

The evaluation of agricultural practices requires good knowledge of mechanisms which control the geochemistry of minerals and interact with soil spatial variability (BUCHTER et al. 1991, DIIWU et al. 1998, HUANG et al. 2001, KIRCHMANN, THORVALDSSON 2000). The growth of crops is conditioned mainly by natural factors, of which climate and soil cover are dominant. For instance, poor soil fertility prevents crops from reaching the maximum growth and therefore impairs yields (KATYAL 2003). There have been reports that poor soil fertility leads to sparse plant cover, which promotes erosion, especially that 90% of plant available N and S, 50-60% of K, 25-30% of P and almost 70% of micronutrients are found in organic matter (STEVENSON, COLE 1999, PUGET, LAL 2005, DOLAN et al. 2006, MAIA et al. 2010). It is commonly held that samples taken close to one another have more similar properties than distant ones. However, classical statistics, where measured data are independent, is not in line to analyze the spatial dependency of a variable (BREJDA et al. 2000). At present, tools are needed to evaluate soil re-

sources in terms of spatial and temporal changes in soil quality in order to ascertain sustainability of farm practices (CORVIN, LESCH 2005, FLORIN et al. 2009).

The advent of precision agriculture and the environmental impact of excess nutrients encourage the application of geostatistical procedures for describing physical and chemical parameters and, on the other hand, nutrient spatial distribution in arable areas (YANG et al. 1995, NEWMAN et al. 1997, BORUVKA et al. 2005, VERMA et al. 2005, YANG, ZHANG 2008, DONG et al. 2009).

Traditionally, a field is regarded as a homogeneous unit. Thus, the agricultural practice and levels of inputs are basically uniform across a whole field, although it has long been known that yields can vary greatly within a field and that the intra-field yield variation is caused by variation in soils, years and soil x year interactions (MERCER, HALL 1911, JOERNSGAARD, HALMOE 2003, PANAYIOTOPOULOS et al. 2004). Selection of an arable area for experimental trials continues to be a challenge due to several constraints, i.e. (i) preceding crops, (ii) size of plots, (iii) date when a field trial begins, (iv) tillage characteristics, (v) soil physical and chemical 'homogeneity'. Whereas points (i) to (iv) are mostly modifiable and controllable, it appears that a more complex point (v) needs some operational approach, which will minimize (intentionally) soil heterogeneity. Therefore, the subjective assumption implying that an experimental area is morphologically homogeneous (except a slope), and therefore is expected to comply with experimental principles, has been broadly accepted. The same concerns mineral elements, including plant nutrients, generally considered geochemically homogeneous, both spatially and downward. Chemical soil tests performed before a field trial deal most specifically with liming evaluation and/or supplementation with macro- and micronutrients, but are less concerned with their in-field variability. The latter, however, is practically decisive for assessing plant biomass and yield stability structure on a small or large scale.

Acid soils are frequently low in calcium and magnesium but contain appreciable concentrations of aluminum in exchangeable and/or active forms. The problem of nutrient supply to growing crops surpasses the question of balanced amounts of applied fertilizers, as it also involves crop accessibility to soil natural nutrient pools (JANIK 2008, WŁODARCZYK et al. 2008). The latter is strictly connected with the growth of the root system in soil. There are some factors limiting roots' accessibility to nutrients even in soils of high natural fertility, the most important ones being soil acidity and related aluminum phytotoxicity (KIDD, PROCTOR 2001, ATKINSON et al. 2005). Spatial distribution of soil reaction (i.e. pH) and aluminum may probably reflect yield and plant biomass characteristics.

The purpose of the current paper has been to outline the problem of field variability that experimenters frequently face when selecting a site for trials. Most specifically, soil pH and exchangeable aluminum concentrations were the parameters considered in this study. The verification of this con-

cept was additionally undertaken by applying geostistical tools for evaluating winter wheat biomass at tillering (BBCH29) and grain yield at harvest (BBCH99).

MATERIAL AND METHODS

Field description

The experimental site was selected in the late summer 2006 and field trials were established at the same time in Gluszyna Lesna (52º14, N and 16º56, E), on a 300 hectare agricultural farm near Poznan (Poland). Soils under these trials belong to the agronomical categories comprising soils from IV to V classes, i.e. mostly sandy ones, vulnerable to chemical degradation, with low pH and relatively high concentrations of exchangeable and active forms of aluminum. The site for establishing the field trial was selected 'visually' by surveying the whole area (i.e. 300 ha) – Photo 1.

A site of 144 m x 88 m (i.e. 12 672 m^2) was delimitated and divided into 16 plots of 30 m x 22 m (i.e. 600 m^2). Technical paths occupied 3072 m^2.

Photo 1. Area selected 'visually' for a field trial

Soil samples (initial samples) were collected at the depths 0-20, 20-40 and 40-60 cm within the respective 16 plots under the preceding crop (late summer, Photo 1) before agricultural practices (i.e. tillage, and winter wheat sowing, Photo 2). Next, in the early spring 2007, the whole experimental site was divided into 200 subplots (4 m x 12 m), where nitrogen (ammonium nitrate) was applied in a dose of 120 kg ha^{-1} split as follows: 60 kg at regrowth and 60 kg at ear formation. Soil and plant samples were additionally collected at tillering (BBCH29) and at harvest (grain yields, BBCH99).

Photo 2. Experimental site preparation. 'Visually' homogeneous

Soils chemical analysis

Prior to chemical analyses, soil samples were air-dried, crumbled to pass through a 1.0 mm screen and stored in plastic bags. The pH was determined potentiometrically according to Polish Standard (1994) in 1.0 mol KCl dm^{-3}. Exchangeable aluminium (Al$_{ex}$) was determined according to LOGAN et al. (1985) and FILIPEK (1999), by applying 1 mol KCl for displacing Al ions. Recovered extracts were divided into two aliquots, of which one was directly titrated to determine the concentrations of H and Al, whereas the other one was titrated after Al precipitation with NaF. Exchangeable Al was obtained from the difference between these chemical tests. All analyses were performed in duplicates.

Geostatistical analysis

Data analyses were conducted in three steps: 1) normality tests (Kolmogorov-Smirnov test); 2) distribution by classical statistics (mean, minimum, maximum, standard deviation, coefficient of variation, skewness, kurtosis); 3) contour maps showing the spatial distribution of each tested variable. The best results were obtained by applying semivariogram analysis. In our study, the number of observations at the onset of the field trial was 16, for both pH and exchangeable aluminum (Al_{ex}) in each layer of soil. In such a case, the minimum curvature methods generally give good results. The principle of minimum curvature is two-dimensional interpolation, which allows drawing reasonable maps of geophysical data. As BRIGGS puts it, 'results are' not always as a draftsman would have them, but are an adequate substitute in most cases' (BRIGGS 1974). Spatial variability with interdependence is commonly described with a semivariogram (WARRICK et al. 1986, GOOVAERTS 1997, WEBSTER, OLIVER 2001). In geostatistics, the concept of variance from classical statistics is extended to semivariance. Considering a field experimental site with equally spaced samples and measurements of soil properties Z, a set of values $Z(x_1)$, $Z(x_2)...Z(x_n)$ at location x_1, x_2, x_n were obtained. The semivariance $\gamma(h)$ is estimated as:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=l}^{N(h)} [Z(x_i) - Z(x_i + h)]^2$$

where:

$N(h)$ is the number of pairs separated by lag distance h; $Z(x_i)$ is a measured sample value at point i; and $Z(x_i + h)$ is a measured sample value at point $i + h$.

Semivariograms, which graph the semivariance between spatially separate data points as a function of the distance, are well documented, particularly for the spatial relationship of soil properties (WARRICK et al. 1986, BUCHTER et al. 1991, BOCCI et al. 2000, FU et al. 2010). The creation of a semivariogram requires a large number of observations. The number of samples of plant biomass at tillering (BBCH29) and grain yield at harvest (BBCH99) was 200. The same applied to the exchangeable aluminum (Al_{ex}) content at both growth stages. A set of geostatistical maps is reported in the current paper.

RESULTS AND DISCUSSION

Soil pH – spatial and downward variability

The most striking finding about the soil of the experimental site was that it was very acid even far in the depth (Table 1) and the pH values

Statistical parameters for pH values within the experimental site (n=16)

Depth (cm)	Mean	Median	Min.	Max.	SD[a]	CV[b] (%)	Skewness	Kurtosis
0 – 20	3.9	3.9	3.6	4.1	0.12	3.10	-0.52	0.94
20 – 40	4.0	4.0	3.6	4.4	0.24	5.92	0.13	-0.66
40 – 60	4.0	4.0	3.7	4.4	0.19	4.76	0.05	-0.55

a – standard deviation; b – coefficient of variation

showed limited spatial variability. These observations were supported by the coefficients of variation (CV) ranging from 3.10 to 5.92% for the whole investigated area. This was practically unexpected for such a relatively large area (*ca* 12 672 m^2) subjected to intensive agricultural practices for a year. As it could be observed, the skewness is close to zero, which – along with the median quite similar to the mean – implies that the distribution of pH values within the soil layers is symmetric to the mean value. The same applies to kurtosis, which in fact assumed values different from zero. Therefore, it can be claimed that the distribution of pH values for a given depth does not differ from the normal distribution.

Such spatial distribution of pH values implies that pH homogeneity (*inter alia*) should not be strictly taken into consideration when establishing a field trial. According to YANG et al. (1995), conventional pH measurements treat soil pH as a random, independent variable for providing the mean pH of soil samples. This is assumed to represent the unsampled neighborhood. However, the measurement will be inadequate if spatially dependent heterogeneity of the soil property exists among the samples. Surface horizons are considered to exhibit more sensitivity to external influence (acid deposition, liming, N-fertilization) and their spatial variation is stronger (BORUVKA et al. 2005). But in the deepest mineral horizons, the effect of pedogenetic processes is more important, so it is difficult to clearly distinguish between effects of the particular factors. This has been illustrated by the spatial distribution of pH values as shown in Figure 1 for the layers 0-20, 20-40 and 40-60 cm. Moreover, correlation coefficients established for pH values between investigated soil layers exhibited the following values: 0-20 cm *versus* 20-40 cm: r=0.59; 20-40 cm *versus* 40-60 cm: r=0.62. It means, that pH values in the soil layer 0-20 cm are significantly positively correlated with those recorded for the 20-40 cm layer. The same applies to 20-40 cm and 40-60 cm.
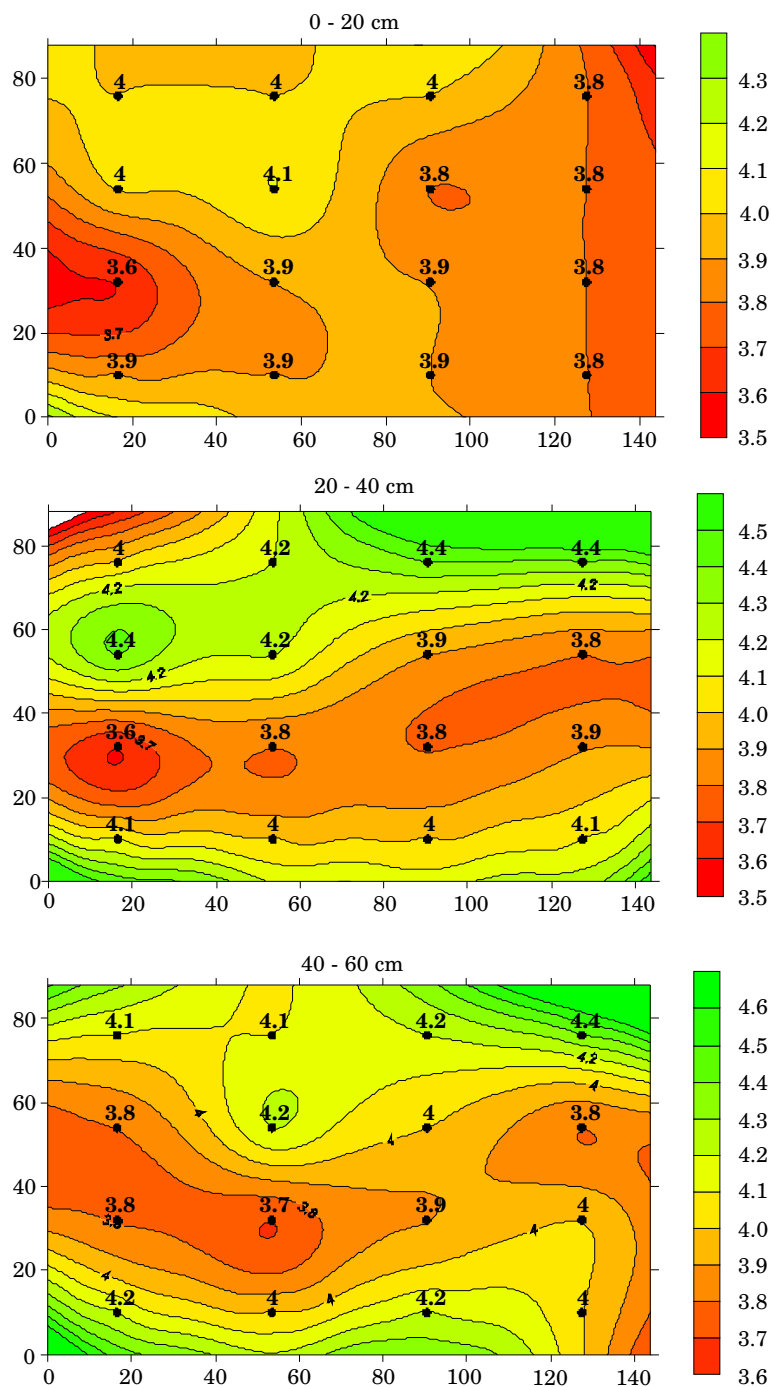
Fig. 1. Spatial distribution of pH in the investigated area (88 m x 144 m)

Variability of exchangeable aluminum (Al_{ex})

The verification of the concept was closely dependent on the concentrations of exchangeable aluminum (Al_{ex}) in soils within the investigated area. It was assumed that any chemical changes related to soil reaction (pH) may be directly reflected by correspondingly high or low Al concentrations. Moreover, the geochemistry of this metal in arable soils is strictly connected to its harmful impact on plants and this could be potentially expected under such extremely acid growth conditions as the ones in our study.

Table 2 contains detailed data on Al_{ex} spatial variability. Noteworthy is a wide range of the Al_{ex} content, especially in the deepest layer, i.e. 40--60 cm (27.20-144.90 mg kg^{-1}), unlike in the upper horizon 0-20 cm (38.00--96.00 mg kg^{-1}). Aluminum spatial variability (Figure 2) is well expressed by

Table 2

Statistical parameters for aluminum content within the experimental site (n=16)

Depth (cm)	Mean	Median	Min.	Max.	SD[a]	CV[b] (%)	Skewness	Kurtosis
	(mg kg^{-1})							
0 – 20	61.79	56.15	38.00	96.00	17.51	28.34	0.62	-0.64
20 – 40	58.86	55.20	29.00	117.70	24.59	41.79	1.00	0.80
40 – 60	71.56	67.00	27.20	144.90	31.50	44.03	0.92	0.56

a – standard deviation; b – coefficient of variation

high coefficients of variation, whose values for the layers 20-40 cm and 40--60 cm are double the ones in the 0-20 cm layer. The positive skewness and the fact that the medians were lower than the mean values are a proof of non-symmetrical Al_{ex} distribution within particular soil layers. The acidification as reported above is quite similar throughout the whole investigated soil profile. This implies that chemical reactions in upper soil layers directly influence geochemical processes located downward (Röver, Kaiser 1999). The same may be expected for aluminum, since its leaching or mobility in soil is strictly controlled by pH. Correlation coefficients obtained for the pairs 0-20 cm *versus* 20-40 cm and 20-40 cm *versus* 40-60 cm were respectively: r=0.72 and r=0.40. In practice, these results deserve attention when establishing a young plant stand within the layer 0-40 cm. While planning the horizontal (spatial) and vertical (downward) management of these soils, we must take into consideration the actual 'non-homogeneity' of the soil.

The spatial studies that are most often cited deal with large or very large areas considered to represent a given soil or plant parameter (Huang et al. 2001, Turgut et al. 2008, Kobierski et al. 2011). This approach has been exploited for several reasons, e.g. (i) the magnitude of an area to be investigated, (ii) reliability on previously elaborated soil agronomical classes, and (iii) *intentional* assumption that the area is homogenous! Such consider-
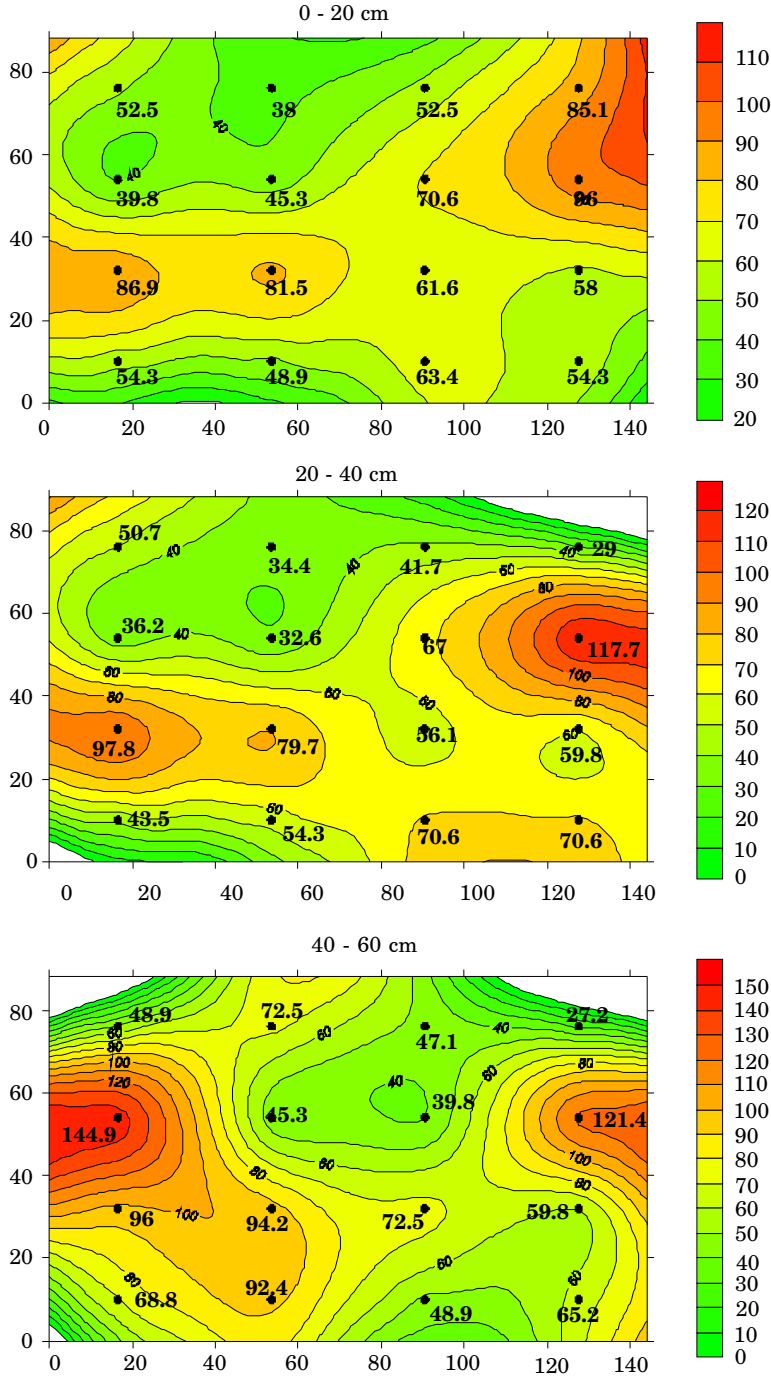
Fig. 2. Spatial distribution of exchangeable aluminum – Al_{ex} (mg kg^{-1}) in the investigated area (88 m x 144 m)

ations may be disastrous in terms of plant response to Al phytotoxicity and further disorders in the plant growth. On average, the coefficient of variation (CV) is *ca* 40%, which seems relatively high for an area of 12 672 m^2. The spatial variability of exchangeable aluminum (Al$_{ex}$) within such a relatively small area confirmed that the concept of selecting an experimental site most frequently based on visual appreciation/selection followed by agricultural practices may explain the up-to-date irregularities in harvested crops (BOUMA 1997, SAWYER et al. 2004).

Aluminum-induced biomass and grain yield spatial variability

Two phenological stages have been selected for this purpose, i.e., tillering (BBCH29) – Figure 3, when winter wheat growth is intensive, and har-
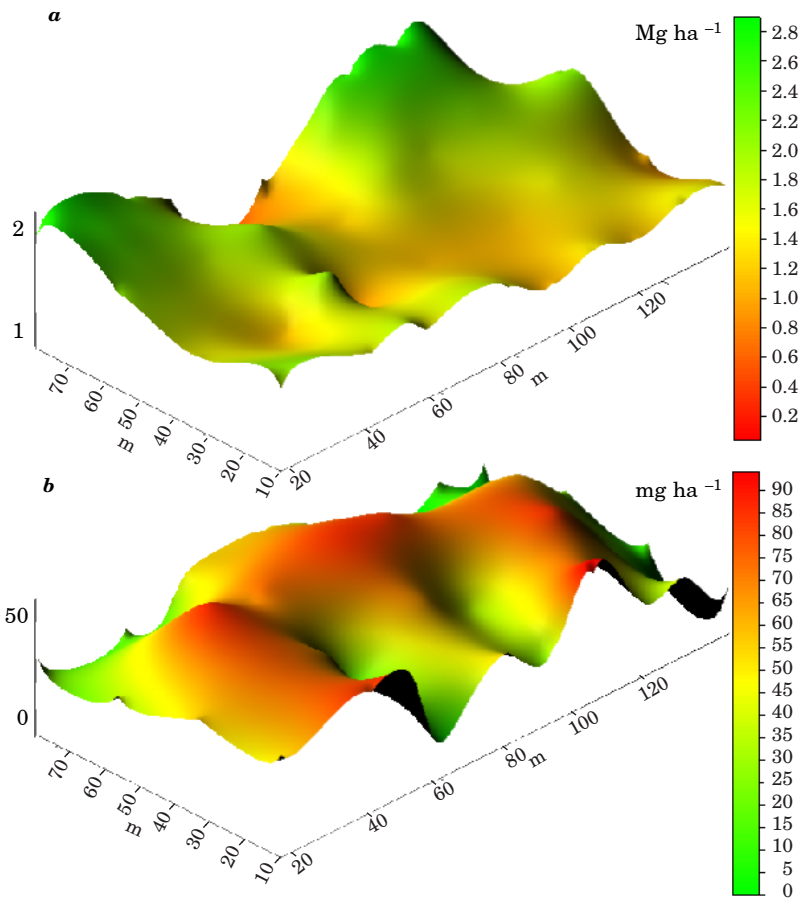
Fig. 3. Spatial variability of winter wheat biomass at tillering (*a*) and exchangeable aluminum (Al$_{ex}$) at the same growth stage (*b*)

vest (BBCH99) – Figure 4, so as to obtain a more detailed view on grain yield spatial distribution. As shown in Figure 3, the plant biomass spatial distribution resembled the distribution of exchangeable aluminum (Al_{ex}) concentrations, implying that natural soil parameters such as aluminum are not easily compensated for by agricultural practices, for instance nitrogen application. The distribution of both variables, i.e. winter wheat biomass and Al_{ex}, is spatially dependent. It seems that the visual homogeneity, previously considered as a site selection criterion, may not give a true picture of intra-field variability, which affects wheat biomass. For example, most of
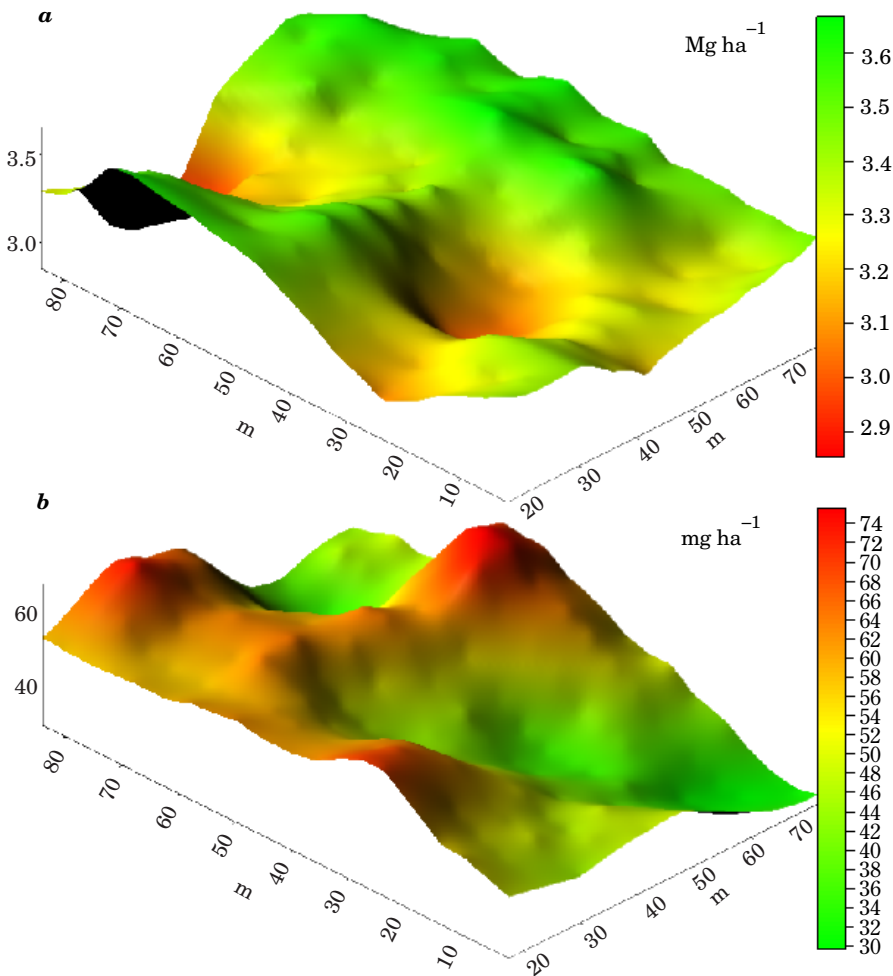
Fig. 4. Spatial variability of winter wheat grain yields at harvest (*a*) and exchangeable aluminum (Al_{ex}) at the same growth stage (*b*)

the high positions expressing the highest biomass corresponded with the lowest zones of Al$_{ex}$ concentrations. This implies that biomass spatial variability was closely dependent on exchangeable aluminum concentrations, i.e., naturally controlled.

Studies dealing with spatial variability of wheat yields are scarce (MO-HAMMADI 2002, WASHMON et al. 2002, CASA, CASTRIGNANŇ 2008) as compared to those concerning soil chemical properties, for instance plant nutritional status. The *grain yield – Al$_{ex}$* spatial dependence (Figure 4) which emerged at harvest is a topic rarely investigated by soil scientists or agronomists. The maps shown in Figure 4 were drawn by using the kriging method and parameters of semivariograms are summed up in Table 3. Until now, extension services have dealt with field heterogeneity by elaborating composite

Table 3

Parameters of theoretical semivariograms (n=200)

Parameter	Semivariogram	Nugget C$_0$	Scale C	Sill C+C$_0$	Range	Anisotropy	
						ratio	angle
		(mg kg^{-1})^2			(m)	(^{o})	
Exchangeable aluminum	spherical	912	240	1152	29.7	1	0
Wheat grain yields	exponential	(Mg ha^{-1})^2			34.9	2	106.2
		0.06	0.07473	0.13473			

soil samples consisting of a number of cores collected from 'visually homogeneous' agricultural lands. This practice 'intentionally' excludes spatial variability, first from soil chemical properties and next from plant biomass as well as yields (roots, grains). Yield increase and/or decrease is a direct consequence of inherent aluminum spatial variability, which most frequently is not included in a crop production forecast.

CONCLUSIONS

1. The experimental site for verifying the concept was selected *via* visual observation. This approach imitated *in situ* conditions frequently faced by field experimenters and agronomists.

2. The data have shown that pH values fluctuated between 3.6 and 4.4 with coefficients of variation (CV) ranging from 3.10 to 5.92%. In the case of Al$_{ex}$, its concentrations ranged from 38.0 to 144.9 mg kg^{-1}, corresponding to CV within 28.34 and 44.03%.

3. Variograms and geostatistical maps generated on the basis of pH and Al$_{ex}$ have emphasized the spatial as well as downward variability of these parameters. These characteristics outline the complex nature of biogeochemical reactions in arable soils.

4. Plant biomass spatial distribution resembled the distribution of exchangeable aluminum (Al$_{ex}$) levels. The spatial variability of Al$_{ex}$ and wheat biomass (BBCH29) as well as grain yields (BBCH99) rather than pH variability confirmed our concept related to the selection of sites for experimental purposes.

Acknowledgements

REFERENCES

Bocci S., CastrignanŇ A., Fornaro F., Maggiore T. 2000. *Application of factorial kriging for mapping soil variation at field scale*. Europ. J. Agronomy, 13:295-308.

Boruvka L., Mladkova L., Drabek O. 2005. *Factors controlling spatial distribution of soil acidification and Al forms in forest soils*. J. Inorg. Biochem., 99(9): 1796-1806.

Bouma J. 1997. *Precision agriculture: introduction to the spatial and temporal variability of environmental quality*. In: *Precision agriculture: spatial and temporal variability of environmental quality*. Lake J.V., Bock G.R., Goode J.A. (Eds.). Ciba Foundation Symposium 210, Wiley, Wageningen, The Netherlands, pp. 5-17.

Brejda J.J., Moorman T.B., Smith J.L., Karlen D.L., Allan D.L., Dao T.H. 2000. *Distribution and variability of surface soil properties at a regional scale*. Soil Sci. Soc. Am. J., 64:974-982.

Briggs I.C. 1974. *Machine contouring using minimum curvature*. Geophysics, 39(1): 39-48.

Buchter B., Aina P.O., Azari A.S., Nielsen D.R. 1991. *Soil spatial variability among transects*. Soil Technol., 4: 297-314.

Casa R., CastrignanŇ A. 2008. *Analysis of spatial relationships between soil and crop variables in a durum wheat field using a multivariate geostatistical approach*. Europ. J. Agronomy, 28: 331-342.

Clarke N., Danielsson L-G., Sparen A. 1996. *Analytical methodology for the determination of aluminum fractions in natural fresh waters*. Pure Appl. Chem., 68(8): 1597-1638.

Corvin D.L., Lesch S.M. 2005. *Characterizing soil spatial variability with apparent soil electrical conductivity*. Part II. *Case study*. Comp. Electron. Agric., 46: 135-152.

Diiwu J.Y., Ridry R.P., Dickenson W.T., Wall G.J. 1998. *Effect of tillage on the spatial variability of soil water properties*. Can. Agri. Engin., 40: 1-8.

Dolan M.S., Clapp C.E., Allmaras R.R., Baker J.M., Molina J.A.E. 2006. *Soil organic carbon and nitrogen in Minnesota soils as related to tillage, residue and nitrogen management*. Soil Till. Res., 89: 221-231.

Dong X.W., Zhang X.K., Bao X.L., Wang J.K. 2009. *Spatial distribution of soil nutrients after the establishment of sand-fixing shrubs on sand dune*. Plant Soil Environ., 55: 288-294.

Filipek T. 1999. *Principles and impacts of chemical inputs into agroecosystems*. Course Book. Ed. Filipek. Wyd. AR w Lublinie 242, pp. (in Polish)

Florin M.J., McBratney A.B., Whelan B.M. 2009. *Quantification and comparison of wheat yield variation across space and time.* Europ. J. Agronomy, 30: 212-219.

Fu W., Tunney H., Zhang C. 2010. *Spatial variation of soil nutrients in a dairy farm and its implications for site-specific fertilizer application.* Soil Till. Res., 106: 185-193.

Goovaerts P. 1997. *Geostatistics for natural resources evaluation.* Oxford University Press, New York, USA.

Huang X., Skidmore E.I., Tibke G. 2001. *Spatial variability of soil properties along a transect of CRP and continuously cropped land.* 10th Int. Soil Conservation Organization Meeting, pp. 641-647.

Janik G. 2008. *Spatial variability of soil moisture as information on variability of selected physical properties of soil.* Int. Agroph., 22: 35-43.

Joernsgaard B., Halmoe S. 2003. *Intra-field yield variation over crops and years.* Europ. J. Agronomy, 19: 23-33.

Katyal J.C. 2003. *Soil fertility management – a key to prevent desertification.* J. Ind. Soc. Soil Sci., 51: 378-387.

Kidd P.S., Proctor J. 2001. *Why do plants grow poorly on very acid soils: are ecologist missing the obvious?* J. Exp. Bot., 52(357): 791-799.

Kirchmann H., Thorvaldsson G. 2000. *Challenging targets for future agriculture.* Europ. J. Agronomy, 12: 145-161.

Kobierski M., Długosz J., Piotrowska A. 2011. *Spatial variability of different magnesium forms in Fluvisols formed from glacial till.* J. Elementol., 16(2): 205-214.

Logan K.A.B., Floate M.J.S., Ironside A.D. 1985. *Determination of exchangeable aluminum in hill soils.* Part 2. *Exchangeable aluminum.* Commun. Soil Sci. Plant Anal., 16(3): 309--314.

Maia S.M.F., Ogle M.S., Cerri C.C., Cerri, C.E.P. 2010. *Changes in soil organic carbon storage under different agricultural management systems in the Southwest Amazon Region of Brasil.* Soil Till. Res., 106: 177-184.

Mercer W.B., Hall A.D. 1911. *The experimental error in field trials.* J. Agric. Sci., 4: 107-132.

Mohammadi J. 2002. *Spatial variability of soil fertility, wheat yield and weed density in one--hectare field in Shahre Kord.* J. Agric. Sci. Technol., 4: 83-92.

Newman S., Reddy K.R., Debusk W.F., Wang Y., Shih G., Fisher M.M. 1997. *Spatial distribution of soil nutrients in a Northern Everglades Marsh: water conservation area 1.* Soil Sci. Soc. Am. J., 61: 1275-1283.

Panayiotopoulos K.P., Kostopoulou S., Hatjiyiannakis E. 2004. *Variation of physical and mechanical properties with depth in Alfisols.* Int. Agroph., 18: 55-63.

Polish Standardisation Committee, ref. PrPN-ISO 10390 (E). 1994. *Soil quality and pH determination.* First edition. (in Polish)

Puget P., Lal R. 2005. *Soil organic carbon and nitrogen in a Mollisol in Central Ohio as affected by tillage and land use.* Soil Till. Res., 80: 201-213.

Röver M., Kaiser E.A. 1999. *Spatial heterogeneity within the plough layer: low and moderate variability of soil properties.* Soil Biol. Biochem., 31: 175-187.

Sawyer J., Mallarino A., Killorn R. 2004. *Take a good soil sample to help make good decisions.* Iowa State University, University Extension. File Code: Agronomy 8-5

Stevenson F.J., Cole M.A. 1999. *Cycles of soil.* Carbon, *nitrogen, phosphorus, sulphur, micronutrients.* 2nd Ed. John Wiley & Sons, New York.

Turgut B., Aksakal E.L., Oztas T. 2008. *Assessment of spatial distribution patterns of soil properties in the EAARI-Experimental Station (Erzurum).* Int. Meeting on Soil Fertility Land Management and Agroclimatology, Turkey, pp. 165-173.

VERMA V.K., PATEL L.B., TOOR G.S., SHARMA P.K. 2005. *Spatial distribution of macronutrients in soils of Arid Tract of Punjab.* Ind. Int. J. Agri. Biol., 7(2): 295-297.

WARRICK A.W., MYERS D.E., NIELSEN D.R. 1986. *Geostatistical methods applied to soil science.* In: *Methods of soil analyses.* Part I. *Physical and mineralogical methods.* KLUTE A. (ed.). ASA and SSSA. Madison WI, pp. 53-73.

WASHMON C.N., SOLIE J.B., RAUN W.R., ITENFISU D.D. 2002. *Within field variability in wheat grain yields over nine years in Oklahoma.* J. Plant Nutrit., 25(12): 2655-2662.

WEBSTER R., OLIVER M.A. 2001. *Geostatistics for environmental scientists.* John Wiley & Sons, Chichester.

WŁODARCZYK T., STĘPNIEWSKI W., BRZEZIŃSKA M., PRZYWARA G. 2008. *Impact of different aeration conditions on the content of extractable nutrients in soil.* Int. Agroph., 22: 371-375.

YANG J., HAMMER R.D., BLANCHAR R.W. 1995. *Microscale pH spatial distribution in the Ap horizon of Mexico Silt Loam.* Soil Sci., 160(5): 371-375.

YANG Y., ZHANG S. 2008. *Approach of developing spatial maps of soil nutrients.* In: *International Federation for Information Processing (IFIP), vol. 258; Computer and Computing Technologies in Agriculture.* Vol. 1; Daoliang Li (Boston: Springer), pp. 565-571.

EFFECT OF FERTILIZATION WITH FARMYARD MANURE, MUNICIPAL SEWAGE SLUDGE AND COMPOST FROM BIODEGRADABLE WASTE ON YIELD AND MINERAL COMPOSITION OF SPRING WHEAT GRAIN

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Abstract

Both deficiency and excess of mineral components in cereal grain may impair its biological value, later interfering with the metabolic processes occurring in animal and human organisms. Potential accumulation of undesirable components in biomass should be taken into consideration since such compounds occur in large amounts in waste material. Therefore, it is necessary to assess the effect of waste-based fertilizers on both the amount of generated biomass and its chemical composition. The present investigations were conducted to determine the effect of fertilization with farmyard manure, municipal sewage sludge and compost from biodegradable materials on the yield and mineral composition of spring wheat grain. The field investigations ran for three years. Nitrogen in wheat grain was assessed using Kjeldahl's method, phosphorus by colorimetry, sulphur, copper, manganese and zinc by the ICP-AES method, and potassium, magnesium and sodium by flame photogrammetry. In the first year, organic fertilizers were observed to be less stimulating to yield formation than mineral fertilizers. Although the uptake of N, P and K from the applied organic fertilizers was impeded, their deficiency in wheat grain was preventable by balancing doses of these components. Despite different amounts of magnesium, sodium and sulphur applied to the soil with the fertilizers, the concentrations of these elements in grain were considerably stable in the years of the investigations. Copper and manganese concentrations in wheat grain were deficient, which significantly depressed grain fodder value and might indirectly determine the crop yield.

Key words: fertilization, sewage sludge, compost, mineral composition, spring wheat.

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WPŁYW NAWOŻENIA OBORNIKIEM, KOMUNALNYM OSADEM ŚCIEKOWYM I KOMPOSTEM Z BIODEGRADOWALNYCH MATERIAŁÓW NA PLONOWANIE I SKŁAD MINERALNY ZIARNA PSZENICY JAREJ

Abstrakt

Zarówno niedobór, jak i nadmiar składników mineralnych w ziarnie roślin zbożowych może obniżyć ich wartość biologiczną, co może niekorzystnie wpływać na procesy metaboliczne u zwierząt i ludzi. Należy brać pod uwagę możliwość akumulacji niepożądanych składników w biomasie, gdyż takie związki mogą występować w dużych ilościach w odpadach wykorzystywanych do nawożenia. Stąd też konieczna jest ocena wpływu nawozów wytwarzanych na bazie odpadów zarówno na ilość wytworzonej biomasy, jak i na jej skład chemiczny. Trzyletnie doświadczenie polowe wykonano w celu określenia wpływu nawożenia obornikiem, komunalnym osadem ściekowym oraz kompostem wyprodukowanych z materiałów biodegradowalnych na plonowanie i skład mineralny ziarna pszenicy jarej. Zawartość azotu w ziarnie pszenicy oszacowano metodą Kjedahla, fosfor kolorymetrycznie, siarkę, miedź, mangan i cynk za pomocą metody ICP-AES, natomiast potas, magnez i sód z użyciem fotogrametrii płomieniowej. W pierwszym roku stwierdzono, że nawozy organiczne miały słabszy wpływ plonotwórczy niż nawozy mineralne. Pomimo ograniczonego poboru N, P i K z zastosowanych nawozów organicznych, można było zapobiegać niedoborom tych pierwiastków w ziarnie przez bilansowanie dawek tych składników. Mimo zróżnicowanych ilości magnezu, sodu i siarki wprowadzonych do gleby wraz z nawozami, zawartość tych pierwiastków w ziarnie była znacząco stabilna w ciągu kolejnych lat badań. Zawartość miedzi i manganu w ziarnie pszenicy była niewystarczająca, co znacząco obniżyło wartość paszową ziarna pszenicy i mogło mieć pośredni wpływ na plonowanie.

Słowa kluczowe: nawożenie, osad ściekowy, kompost, skład mineralny, pszenica jara.

INTRODUCTION

Apart from the crop yield, another crucial aspect in the assessment of the value of cereal grain used as animal fodder is its content of macro- and microelements, which are an important source of mineral substances for animals. Concentrations of individual elements in cereal grain are highly varied depending on a cereal species and its form, soil abundance in nutrients, the weather conditions, plant protection and cultivation measures, including fertilization (Šramkova et al. 2009, Gondek, Gondek 2010). Fertilization may have direct or indirect influence on yielding and mineral content in grain by affecting speciation of elements in soil and the soil properties (Mijangos et al. 2006, Głąb, Gondek 2008, Rutkowska et al. 2009). Balanced application of natural and mineral fertilizers improves crop yields and poses no threat to the quality of biomass. The risk of adverse changes in yielding and chemical composition of crops may arise from soil fertilization with waste organic material whose chemical composition is not stable (Gasco, Lobo 2007).

Both deficiency and excess of mineral components in cereal grain may lead to its inferior biological value, thus disturbing the metabolic processes in an animal fed such grain. Potential accumulation of undesirable compo-

nents in biomass should be taken into consideration, since they may occur in large quantities in waste material. Therefore, it is necessary to assess the effect of these substances not only on the amount of biomass but also on its chemical composition (KALEMBASA, MALINOWSKA 2009).

With high quantities of plant nutrients in natural and organic fertilizers and waste organic material, fertilization with these substances on crop yields and their chemical composition can be expected to produce beneficial effects. This research was conducted to determine the effect of fertilization with farmyard manure, municipal sewage sludge and compost from biodegradable materials on the yield and mineral composition of spring wheat grain.

MATERIAL AND METHODS

The investigations were conducted as a field experiment set up 10 km west of Krakow (49º59' N; 19º41' E) under moderate climate. The data obtained from a meteorological station are presented in Tables 1 and 2. The soil under the experimental field was classified as Stagnic Gleysoils (FAO 1998). Table 3 shows selected properties of the soil prior to the experiment.

The experiment was set up according to the randomized block method. The plot area was 30 m^2. The experimental design comprised 5 treatments in four replications: without fertilization (0), NPK mineral fertilization (M) [110 kg N ha^{-1}, 58.6 kg P ha^{-1} and 120 kg K ha^{-1}]; swine FYM (SF) [14.30 t ha^{-1} of fresh mass], municipal sewage sludge from a mechanical-biological treatment plant (SS) [14.15 t ha^{-1} of fresh mass] and compost made from plant and other biodegradable waste (C) [6.46 t ha^{-1} of fresh mass]. Selected properties of the manure, municipal sewage sludge and compost are presented in Table 4.

Before the experiment (autumn 2004), the field was limed according to half the value of hydrolytic acidity (962.0 kg CaO ha^{-1}).

In the spring of the following year, after basic cultivation measures, the plots were evenly covered with farmyard manure, sewage sludge or compost and ploughed. Two weeks later, supplementary mineral fertilization was conducted and the fertilizer was mixed with the soil using a cultivator and harrow aggregate. The nitrogen dose supplied with the organic materials was 110.0 kg N ha^{-1}. Phosphorus and potassium were supplemented in mineral fertilizers to the equal level introduced with fertilizers in all the treatments (except the control), [phosphorus to 58.6 kg P ha^{-1} as single superphosphate and potassium to 120.0 kg K ha^{-1} as 60% potassium salt]. In the second and third year, doses of these elements were identical as in the first year but were introduced only in the form of mineral fertilizers, applied in order to supplement nutrients (nitrogen, phosphorus and potassium) removed with wheat yield.

Table 1

Monthly and periodic precipitation totals during the experiment (mm)

Year	Month												Σ Mar – Aug
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
2005	66.4	32.6	20.7	49.1	61.3	40.6	113.4	102.6	26.5	7.7	29.9	46.7	387.7
2006	57.5	48.5	60.1	56.5	51.9	89.1	14.1	104.1	17.2	31.9	20.9	16.1	375.8
2007	100.6	42.2	61.1	15.4	51.7	72.1	71.0	76.4	179.8	48.3	90.4	21.4	347.7
1961-1999	34.0	32.0	34.0	48.0	83.0	97.0	85.0	87.0	54.0	46.0	45.0	41.0	434.0

Table 2

Mean daily air temperature during the experiment (°C)

Year	Month												Mean Mar – Aug
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
2005	-1.2	-4.3	-0.2	6.8	11.4	14.4	17.6	15.4	12.5	7.1	3.9	-0.7	10.9
2006	-2.4	-3.0	0.2	5.6	10.9	15.0	18.6	15.6	13.4	9.1	6.3	0.9	11.0
2007	3.2	1.2	6.0	8.5	15.2	18.4	19.4	19.0	12.4	7.7	0.8	-1.1	14.4
1961-1999	-3.3	-1.6	2.4	7.9	13.1	16.2	17.5	16.9	13.1	8.3	3.2	-1.0	12.3

Table 3

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

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

The test plant was cv. Jagna spring wheat. In the first year, wheat was sown on 7 April 2005, then on 10 April 2006 and finally on 28 March 2007. The plant density was 485 plants per 1 m^2. During vegetation, chemical preparations were applied to protect wheat from weeds (spraying with 1 dm^3 ha^{-1} of Puma Universal herbicide, manufactured by Bayer CropScience, and 1 dm^3 ha^{-1} of Aminopielik Gold, manufactured by Makhteshim Agan Agro Poland S.A.) and from fungal diseases (spraying with 1 dm^3 ha^{-1} of Alert 375 S.C. fungicide, manufactured by Du Pont). The duration of the wheat growth period in each year depended on the weather conditions. Wheat was harvested at grain full maturity on 13 August 2005, on 3 August 2006 and on 31 July 2007. In order to determine grain yield, wheat was gathered from an area of 4 m^2, separately from each plot.

Sewage sludge used for the experiment originated from a municipal mechanical and biological sewage treatment plant located in Malopolska. Compost was produced from plant and other biodegradable waste in a composting plant located in Krakow, using the MUT-Kyberferm technology. The types and shares of waste in the composted biomass were: 25% grass, 20% wood pellets; 20% leaves; 10% market waste; 5% tobacco waste and 20% coffee production waste. Swine FYM was stored for 6 months on a manure plate.

The following were determined in the fresh samples of sewage sludge, compost and manure: dry mass by drying at 105ºC for 12 hrs, pH by potentiometery, conductivity with a conductometer and total nitrogen content af-

ter sample mineralization in concentrated sulphuric acid with Kjeldahl's method. Dried and ground material samples were used to determine the content of organic matter through annealing and the content of minerals after sample mineralization in a chamber furnace (at 450ºC for 5 hrs) and dissolution of the ash in diluted (1:2) v/v nitric acid. The phosphorus concentration was assessed by colorimetry on a Backamn DU 640 spectrophotometer and potassium was determined by flame photometry on a Philips PU 9100X apparatus. Heavy metal concentrations were determined using the ICP-AES method on a JY 238 Ultrace apparatus. The analyses were conducted according to the methodology described by Baran and Turski (1996) and Krzywy (1999) and the results are given in Table 4.

Wheat grain was crushed in a laboratory mill. Afterwards, crushed wheat grain samples were mineralized in concentrated sulphuric acid and nitrogen was determined with Kjeldahl's method. Sulphur was determined after mineralization of the material in concentrated nitric acid by with the ICP-AES method. The remaining elements were determined after sample mineraliza-

Table 4

Physical and chemical properties of the organic materials

Determination	Unit	Pig FYM (PF)	Sewage sludge (SS)	Compost (K)
Total N	g kg^{-1} d.m.^{b}	34.0	26.2	38.9
P	g kg^{-1} d.m.	12.8	8.2	5.8
K	g kg^{-1} d.m.	21.8	1.9	29.9
S	g kg^{-1} d.m.	4.76	9.66	3.61
Cu	mg kg^{-1} d.m.	156	103	33
Zn	mg kg^{-1} d.m.	284	1146	194
Mn	mg kg^{-1} d.m.	355	194	280
Cr	mg kg^{-1} d.m.	2.8	23.4	15.0
Pb	mg kg^{-1} d.m.	1.2	55.7	9.3
Cd	mg kg^{-1} d.m.	0.80	3.97	1.42
Ni	mg kg^{-1} d.m.	10.1	21.1	10.4
EC^a	mS cm^{-1}	2.89	1.69	2.62
pH (H$_2$O)	–	8.23	6.23	7.31
Organic matter	g kg^{-1} d.m.	831	414	531
Water content	g kg^{-1} f.m.^{c}	774	703	563
Ash	g kg^{-1} d.m.	169	586	469

a – EC = electrical conductivity
b – data are based on 105˚C dry matter weight
c – f.m. = fresh matter

tion in a chamber furnace (at 450ºC for 5 hrs) and dissolving the residue in diluted (1:2) nitric acid. Phosphorus was determined by colorimetry on a Backman DU 640 spectrophotometer, whereas the concentrations of potassium, magnesium and sodium were determined by flame photometry on a Philips PU 9100X apparatus. The concentrations of copper, manganese and zinc were determined using the ICP-AES method on a JY 238 Ultrace apparatus. The analyses were conducted according to the methodology described by Ostrowska et al. (1991).

Chemical analyses of the plant material were carried out in four replications, and the initial material (manure, sewage sludge, compost and soil) was analyzed in two replications. A plant reference sample NCS DC733448 (China National Analysis Center for Iron &Steel) or a soil reference sample AG-2 (*Agro*MAT) was assigned to each analyzed series and the result was regarded reliable if the estimated determination error did not exceed 5%.

The results were verified statistically using two-way ANOVA (factors: fertilization and years of the experiment) in a totally randomized design using f-Fisher test. Significance of differences between arithmetic means was verified on the basis of homogenous groups determined by Duncan's test at the significance level $\alpha<0.05$. All calculations were made using Statistica software (Stanisz 1998).

RESULTS AND DISCUSSION

The average spring wheat grain yield in the first year of the experiment from the treatments with organic fertilization was lower, between 0.30 t and 1.06 t of dry mass ha^{-1}, than the yield from the treatments where mineral NPK (M) fertilizers had been applied (Table 5). With respect to organic fertilization treatments, the lowest grain yield was harvested after the application of compost (C). In the third year, significantly the lowest yield of spring wheat grain dry mass was obtained from the treatment where only mineral fertilizers (M) had been used. An increase in wheat grain yield after the application of swine FYM (SF), sewage sludge (SS) and compost (C) in relation to the yield from the treatment where only mineral fertilizers (M) were used was 25%, 9% and 26%, respectively.

Fertilization with organic material usually has a weaker effect on the growth and development of plants than application of mineral fertilizers. The so-called residual effect is frequently missing because a considerable amount of nutrients from fertilizers is removed with harvest in the first year. The present study demonstrates a weaker effect of organic fertilizers than mineral ones in stimulating better yields. However, in the third year, organic fertilizers proved to be more effective. These results do not completely coincide with the research by Iżewska (2009), who demonstrated that

Table 5

Yields of grain spring wheat (t ha^{-1}± SD)

Objects	1st year	Relative	3rd year	Relative
No fertilization (0)	2.53 ± 0.15a	61	2.55 ± 0.12a	59
Mineral fertilization NPK (M)	4.13 ± 0.54d	100	4.30 ± 0.04de	100
Swine FYM (SF)	3.36 ± 0.19bc	81	5.39 ± 0.36f	125
Sewage sludge (SS)	3.83 ± 0.32cd	92	4.70 ± 0.41e	109
Compost (K)	3.07 ± 0.71ab	74	5.42 ± 0.13f	126

Means marked by the same letters did not differ significantly at $\alpha < 0.05$ according to Duncan's test.

higher doses of municipal sewage sludge significantly increased yields of spring rapeseed and winter triticale grain in comparison with the yield from plots receiving mineral fertilizers. Such a result did not occur when composted sewage sludge had been applied. However, it should be emphasized that under controlled conditions (pot experiment) plants have optimal moisture conditions, which may stimulate biochemical processes accelerating mineralization of organic matter supplied with the tested materials. Also JAMIL et al. (2004) and KHAN et al. (2007) reported a beneficial effect of fertilization with sewage sludge on wheat yielding. IBRAHIM et al. (2008), who investigated compost fertilization on wheat yield, point to the fact that amounts of nutrients supplied with fertilizers need to be balanced.

Concentrations of macroelements wheat grain dry mass from the treatments where fertilization had been applied were as follows: nitrogen 19.0 g - - 9.5 g kg^{-1}, phosphorus 4.61 g - 5.01 g kg^{-1}, potassium 3.97-5.32 g kg^{-1}, magnesium 0.83-1.13 g kg^{-1}, sodium 0.02-0.06 g kg^{-1}, sulphur 1.19-1.60 g kg^{-1} (Tables 6,7). Concentrations of nitrogen, potassium, magnesium and sulphur were significantly varied between the years of the experiment. Higher concentrations of these elements were detected in the grain from the third year of the research. The phosphorus and sodium concentrations in grain did not differ significantly between the years.

The nitrogen concentration in the first year was markedly the highest in wheat grain fertilized with mineral fertilizers, whereas in the third year much larger quantities of this element were assessed in wheat grain from the treatments where manure, sewage sludge or compost fertilization had been applied in the first year (Table 6). However, the statistical analysis of arithmetic means of the concentrations of this element in grain did not confirm the significance of differences.

Concentrations of phosphorus in wheat grain were relatively stable, irrespective of the year or applied fertilization (Table 6). Despite the lack of significant differences, there was a tendency towards increasing levels of this element in grain from the treatment where only mineral NPK ferti-

Table 6

Nitrogen, phosphorus and potassium content (g kg^{-1} d.m. ± SD) in grain of spring wheat

Treatments*	N		P		K	
	1st year	3rd year	1st year	3rd year	1st year	3rd year
(0)	20.1 ± 1.0ab	26.1 ± 0.3cd	5.00 ± 0.21a	4.99 ± 0.14a	4.54 ± 0.28b	5.27 ± 0.16c
(M)	24.6 ± 0.9c	27.7 ± 0.7de	4.68 ± 0.46a	5.01 ± 0.19a	3.97 ± 0.54a	5.32 ± 0.35d
(SF)	21.5 ± 2.1b	28.2 ± 1.8e	4.61 ± 0.24a	4.83 ± 0.25a	4.06 ± 0.13a	5.15 ± 0.23c
(SS)	20.5 ± 1.3ab	29.5 ± 0.5e	4.82 ± 0.18a	4.82 ± 0.15a	4.30 ± 0.17b	5.21 ± 0.12c
(K)	19.0 ± 1.2a	27.9 ± 1.0e	4.63 ± 0.17a	4.70 ± 0.23a	4.33 ± 0.18b	5.08 ± 0.15c

*see Table 5
Means marked by the same letters did not differ significantly at $\alpha < 0.05$ according
to Duncan's test.

lizers (M) were used. The increase in P in wheat grain from this treatment in the third year was over 7%. In the treatments where manure (SF) and compost (C) fertilization was applied, the increase in P concentrations in wheat grain in the third year versus the first one did not exceed 3.5%. According to the Danish feeding standards for cattle, the phosphorus concentrations in grain determined in our study were satisfactory (MŘLLER et al. 2000).

Potassium concentrations in spring wheat grain in the first year of the experiment were significantly the lowest following the application of mineral fertilizers (M) and manure (SF) – Table 6. Wheat grain fertilized with sewage sludge (SS) and compost (C) contained 8% and 9% more potassium, respectively, than the grain from the treatment where mineral fertilizers (M) were used. In the third year of the experiment, the highest concentrations of potassium were detected in wheat grain gathered from the treatment where mineral NPK fertilizers (M) were applied. Assessment of the fodder value of the analyzed material shows a deficient potassium concentration in grain from the first year of the research (MŘLLER et al. 2000).

Magnesium in grain from both the first and the third year of the investigations was on a similar level, independently of the applied fertilization (Table 7). On average, the Mg content from all fertilized treatments in the first year was 0.86 g kg^{-1} and in the third year – 1.09 g kg^{-1} of grain. Considering the fodder value, the magnesium concentration in grain from the first year was deficient, irrespectively of the applied fertilization (MŘLLER et al. 2000).

Our results demonstrate a relatively high increase in the sodium content in wheat grain harvested in the third year in comparison to the first year. Despite a considerable increase in this element, it continued to be deficient in respect the fodder value (MŘLLER et al. 2000). The applied fertilization did not differentiate the Na content in grain (Table 7).

The content of sulphur, like magnesium and sodium, was on a relatively low level (Table 7), independently of the fertilizers used. However, it was adequate considering the fodder value (MŘLLER et al. 2000). Nonetheless, it should be emphasized that an increased content of this element in wheat grain (from 7% to 27%) was observed in the third year, particularly after the application of sewage sludge (SS) and compost (C).

Beside the crop yield, plant chemical composition is conditioned by many factor s and their interactions. On the one hand, availability of nutrients is limited by the soil properties. On the other hand, their uptake is determined by the plant's genetic traits. Despite impeded absorption of nitrogen, phosphorus or potassium from organic fertilizers, by balancing their doses introduced to soil it was possible to diminish differences in concentrations of these components in wheat grain. Also, despite various amounts of magnesium, sodium or sulphur brought to soil with fertilizers, their levels in grain proved considerably stable in the years of the experiment, which was mainly because the wheat plants uptake of these elements was not excessive. A study by BOWSZYS et al. (2006) shows that concentrations of macronutrients are conditioned by the type of applied organic material and duration of its activity. As the above authors claimed, nitrogen concentrations in barley grain from treatments where composts were applied were higher only than in grain from an unfertilized treatment. Mineral fertilizers affected most strongly the concentration of this element. Fertilization with composts produced from municipal waste had no significant effect on concentrations of phosphorus or magnesium in barley grain. Analogously, in the present experiment (in the first year) the potassium content in spring wheat grain was higher following the application of sewage sludge and compost than in grain from the treatment receiving mineral fertilizers.

Zinc is important in plants, mainly because it co-activates many enzymes. In the first year of the experiment, the highest zinc concentrations were found in wheat grain fertilized with mineral fertilizers NPK (M) and compost (C) – Table 8. These concentrations differed significantly from the ones in wheat grain fertilized with manure (SF) and sewage sludge (SS). In the third year, the concentration of zinc content in grain increased markedly after the application of mineral fertilizers (M), manure (SF) and sewage sludge (SS) (the increases were 12.7%, 42.5% and 28.7%, respectively). With respect to the fodder value, zinc concentrations were optimal (GORLACH 1991).

Copper also belongs essential elements in plants, as it is a component of enzymes and proteins participating in many metabolic processes. The average copper concentration in wheat grain harvested in the first and third year of the experiment was 4.39 mg kg^{-1} of grain dry mass (Table 8), irrespectively of the applied fertilization. A significant increase in this element in wheat grain between the first and the third year was observed after manure (SF) application. The lowest copper concentration in the third year was found in grain from the treatment where only mineral fertilization has been applied (Table 8). Considering the fodder value, copper content was low (GORLACH 1991).

Table 7

Magnesium, sodium and sulphur content (g kg⁻¹ d.m. ± SD) in grain of spring wheat

Treatments*	Mg		Na		S	
	1st year	3rd year	1st year	3rd year	1st year	3rd year
(0)	0.88 ± 0.03ab	1.20 ± 0.04e	0.02 ± <0.01a	0.06 ± <0.01a	1.19 ± 0.07a	1.43 ± 0.04bc
(M)	0.89 ± 0.01ab	1.13 ± 0.04d	0.02 ± <0.01a	0.06 ± <0.01a	1.43 ± 0.05bc	1.54 ± 0.11cd
(SF)	0.83 ± 0.03a	1.07 ± 0.05c	0.02 ± <0.01a	0.06 ± <0.01a	1.36 ± 0.07b	1.54 ± 0.16cd
(SS)	0.87 ± 0.03ab	1.11 ± 0.01cd	0.02 ± <0.01a	0.06 ± <0.01a	1.37 ± 0.06b	1.60 ± 0.03d
(K)	0.87 ± 0.03ab	1.07 ± 0.02c	0.02 ± <0.01a	0.06 ± <0.01a	1.19 ± 0.11a	1.52 ± 0.04cd

*see Table 5
Means marked by the same letters did not differ significantly at $\alpha < 0.05$ according to Duncan's test.

Table 8

Zinc, copper and manganese content (mg kg⁻¹ d.m. ± SD) in grain of spring wheat

Treatments*	Zn		Cu		Mn	
	1st year	3rd year	1st year	3rd year	1st year	3rd year
(0)	41.1 ± 1.7b	51.3 ± 2.1e	4.78 ± 0.62d	2.58 ± 0.30a	31.5 ± 2.2f	17.8 ± 1.1ab
(M)	47.1 ± 3.7d	53.1 ± 2.0ef	4.54 ± 0.44cd	3.57 ± 0.19b	28.8 ± 2.0f	23.4 ± 1.5cd
(SF)	32.2 ± 0.9a	45.9 ± 2.4cd	4.11 ± 0.16c	4.76 ± 0.32d	25.1 ± 3.9de	16.1 ± 3.9a
(SS)	43.2 ± 1.9bc	55.6 ± 2.5f	4.62 ± 0.27cd	4.62 ± 0.16cd	31.8 ± 3.2f	28.5 ± 3.3bcd
(K)	47.5 ± 1.8d	47.5 ± 2.5d	4.29 ± 0.13cd	4.63 ± 0.31cd	32.1 ± 3.9f	32.1 ± 1.8f

*see Table 5
Means marked by the same letters did not differ significantly at $\alpha < 0.05$ according to Duncan's test.

Manganese is equally important for good fodder value. Mn content in the analyzed grain was low (GORLACH 1991). Fertilization with sewage sludge (SS) and compost (C) favourably affected concentrations of this element. In the third year, the Mn content decreased in comparison with the first year, except the treatment where compost (C) had been applied. An average decline in the grain manganese concentrations was 4.43 mg kg^{-1} of dry mass.

According to GORLACH (1991), both excess and deficiency of micronutrients in fodders is dangerous to animals. The content and bioavailability of micronutrients in soil are modified by many factors, such as soil pH, organic matter content, soil sorption capacity. But they also depend on the plant's ability for their absorption and on fertilization. A study by RUSZKOWSKA et al. (1996) demonstrated that producing long-term production of high yields requires control over the plant supply with micronutrient, a conclusion which supports the purposefulness of the present experiment.

The concentrations of all the analyzed micronutrients, except zinc, in wheat grain were deficient, which evidently depresses the grain's fodder value and may also indirectly affect the crop yield, especially that manganese or copper are part of enzymes and proteins which participate in specific metabolic processes (RÜEGSEGGER et al. 1990, PRASAD 1995, YERUELA 2005). Much of these elements is arrested in the root system, which strongly inhibits their transport to biomass, a source of animal feed (GONDEK, FILIPEK-MAZUR 2003). Limited bioavailability of these elements might have resulted from the soil properties, particularly its sorptive characteristics. Lower copper content in crop yields from highly productive fields was also pointed out by CZUBA (1986). Our results demonstrated stronger influence of the applied organic materials than that of manure on the content of the analyzed micronutrients in wheat grain.

Right proportions of the nutrients are crucial for assessment of biomass fodder value. In the analyzed wheat grain, the of K : Mg ratio was relatively stable and, independently of the year, ranged from 4.46 to 4.99 (Table 9).

The N:S ratio is important for the proper nitrogen economy in plant. In the present investigations, values of this parameter in wheat grain harvested in the first year from the treatments fertilized with manure (SF) and sewage sludge (SS) were 15.8 and 15.0 respectively (Table 9). A higher value of this parameter was demonstrated for wheat grain fertilized with mineral materials (M) and compost (C). In the third year, the value of this ratio increased significantly in wheat grain from all fertilized treatments, which resulted from the increase in nitrogen in grain. The highest increase in the N:S value between the first and third year (by 23%) appeared in wheat grain fertilized with sewage sludge (SS).

Significant diversification, both in the impact of the applied fertilization and between the years of the research, was observed for the P:Zn ratio (Table 9). In the first year, the highest value of this ratio was in wheat grain fertilized with manure (SF) and compost (C). The lowest value was

Table 9

Value of select ratio of quantitative elements in grain of spring wheat

Treatments*	K : Mg		N : S		P : Zn	
	1st year	3rd year	1st year	3rd year	1st year	3rd year
(0)	5.14 ± 0.35d	4.39 ± 0.06a	16.9 ± 0.49bc	18.0 ± 0.33c	121.5 ± 1.8e	97.2 ± 3.3bc
(M)	4.46 ± 0.44ab	4.72 ± 0.21abc	17.3 ± 0.90bc	18.0 ± 0.97c	100.1 ± 5.5bc	94.4 ± 1.3ab
(SF)	4.81 ± 0.18cd	4.79 ± 0.13bcd	15.8 ± 1.63ab	18.4 ± 0.79c	135.0 ± 8.2f	105.4 ± 2.8cd
(SS)	4.93 ± 0.26cd	4.71 ± 0.10abc	15.0 ± 0.93a	18.5 ± 0.15c	111.7 ± 7.9d	86.8 ± 3.9a
(K)	4.99 ± 0.07cd	4.76 ± 0.05bc	16.0 ± 2.09ab	18.3 ± 0.79c	126.0 ± 4.4ef	98.8 ± 4.2bc

*see Table 5

Means marked by the same letters did not differ significantly at $\alpha < 0.05$ according to Duncan's test.

observed in wheat grain receiving mineral fertilizers. In the third year, the value of P:Zn ratio declined in grain from all the treatments. Statistically significant differences were computed for all the treatments except the one enriched exclusively with mineral NPK fertilizers (M).

CONCLUSIONS

1. In the first year of the experiment, organic materials were less effective than mineral fertilizers in stimulating higher yields. A superior yield forming effect of organic materials compared with mineral fertilization was obtained in the third year.

2. Balancing N, P and K dose, despite worse uptake of these components from organic materials, minimizes the differences in their concentrations in wheat grain.

3. Despite various quantities of magnesium, sodium and sulphur in organic materials, the content of these elements in grain remained considerably stable in the years of the experiment.

4. The content of all micronutrients except zinc in wheat grain was deficient, which evidently depressed the grain fodder value. It may have also indirectly determined the crop yield.

REFERENCES

BARAN S., TURSKI R. 1996. *Specialist exercises in waste and sewage disposal*. Wyd. AR w Lublinie, 1-136. (in Polish)

BOWSZYS T., WIERZBOWSKA J., SĄDEJ W. 2006. *Chemical composition of plants grown on soil fertilized with municipal waste compost*. Zesz. Probl., Post. Nauk Rol., 512, 55-61. (in Polish)

CZUBA R. 1986. *Changes of component content in crops in the area of Poland depending on fertilization*. In: Proceedings of symposium *"Effect of fertilization of yield quality"* ART, Olsztyn, 24-25.06.1986, 34-42 pp. (in Polish)

FAO-ISRIC-SICS, 1998. *World reference base for soil resources*. World Soil Resources Reports 84, FAO, Rome.

GASCÓ G., LOBO M.C. 2007. *Composition of Spanish sewage sludge and effect on treated soil land olive trees*. Waste Manage., 27(11): 1494-1500.

GŁĄB T., GONDEK K. 2008. *Effect of organic amendments on morphometric properties of macropores in stagnic gleysol soil*. Pol. J. Environ. Stud., 17(2): 209-214.

GONDEK K., FILIPEK-MAZUR B. 2003. *Biomass yields of shoots and roots of plants cultivated in soil amended by vermicomposts based on tannery sludge and content of heavy metals in plant tissues*. Plant, Soil Environ., 49(9): 402-409.

GONDEK K., GONDEK A. 2010. *The influence of mineral fertilization on the field and content of selected macro- and microelements in spring wheat*. J. Res. Appl. Agricult. Engin., 55(1): 30-36.

GORLACH E. 1991. *The content of trace elements in fodder plants as a measure of their quality*. Zesz. Nauk. AR w Krakowie, 262(34): 13-22. (in Polish)

Ibrahim M., Hassan A., Iqbal M., Valeem E.E. 2008. *Response of wheat growth and yield to various levels of compost and organic manure.* Pak. J. Bot., 40(5): 2135-2141.

Iżewska A. 2009. *The impact of manure, municipal sewage sludge and compost prepared from municipal sewage sludge on crop yield and content of Mn, Zn, Cu, Ni, Pb, Cd in spring rape and spring triticale.* J. Elementol., 14(3): 449-456.

Jamil M., Qasim M., Umarand M., Rehman K. 2004. *Impact of organic wastes (sewage sludge) on the yield of wheat (Triticum aestivum L.) in calcareous soil.* Intern. J. Agric. Biol., 6(3): 465-467.

Kalembasa D., Malinowska E. 2009. *Influence of sewage sludge fertilization on heavy metal content in biomass of silver grass during field experiment.* Environ. Protect. Engin., 35(20): 149-155.

Khan M.A., Kazi T.G., Ansari R., Mujtaba S.R., Khanzada B., Khan M.A., Shirazi M.U., Mumtaz S. 2007. *Effects of un-treated sewage sludge on wheat yield, metal uptake by grain and accumulation in the soil.* Pak. J. Bot., 39(7): 2511-2517.

Krzywy E. 1999. *Environmental management of sewage and sludge.* Wyd. AR w Szczecinie, 1-145. (in Polish)

Mijangos J., Pérez R., Albizu I., Garbisu C. 2006. *Effect of fertilization on tillage on soil biological parameters.* Enzyme Microbiol. Technol., 40: 100-106.

Mřller J., Thřgersen R., Kjeldsen A.M., Weisbjerg M.R., Sřegaard K., Hvelplund T., Břrsting CH.F. 2000. *Composition and feeding value of feedstuffs for cattle.* Ed. The National Department of Cattle Husbandry and Danish Institute of Agricultural Sciences, 1-51 pp.

Ostrowska A., Gawliński A., Szczubiałka Z. 1991. *Methods of analysis and assessment of soil and plant properties.* Issued by the Institute of Environmental Protection, 1-324. (in Polish)

Prasad M. N. W. 1995. *Inhibition of maize leaf chlorophylls, carotenoids and gas exchange functions by cadmium.* Photosynthetica, 31: 635-640.

Rüegsegger A., Schmutz D., Brunold C. 1990. *Regulation of glutathione synthesis by cadmium in Pisum sativum L.* Plant Physiol., 93: 1579-1584.

Ruszkowska M., Sykut S., Kusio M. 1996. *Plant supply in microelements under conditions of diversified fertilization in long-term lysimetric experiment.* Zesz. Probl. Post. Nauk Rol., 434: 43-47.

Rutkowska B., Szulc W., Łabętowicz J. 2009. *Influence of soil fertilization on concentration ofmicroelements in soil solution of sandy soil.* J. Elementol., 14(2): 349-355.

Šramkova Z., Gregová E., Šturdik E. 2009. *Chemical composition and nutritional quality of wheat grain.* Acta Chim. Slov., 2(1): 115-138.

Stanisz A. 1998. *Straightforward course of statistics basing on programme.* Statistica PL examples from medicine. Ed. by Statsoft Poland, 1-362. (in Polish)

Yruela J. 2005. *Copper in plants.* Braz. J. Plant Physiol., 17(1): 145-156.

POTATO TUBER CONTENT OF MAGNESIUM AND CALCIUM DEPENDING ON WEED CONTROL METHODS

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Abstract

Studies were conducted on samples of potato tubers harvested in a field experiment carried out at Zawady Experimental Farm of the University of Natural Sciences and Humanities in Siedlce in 2005-2007. The experimental design was a randomised complete block in a split-plot arrangement with 2 cultivars (Irga and Balbina) as main plots, and 4 weed control technologies (with the following herbicides: Plateen 41.5 WG, Racer 250 EC and Sencor 70 WG) as subplots. There was also a control plot with no chemical control of weeds. The purpose of the study was to determine magnesium and calcium content in the dry matter of edible potato tubers depending on weed control methods based on some herbicides. Magnesium and calcium content in the dry matter of potato tubers was determined by the AAS method. The content of the elements depended significantly on cultivars, weed control methods and weather conditions during the growing season. The herbicides Plateen 41.5 WG and Sencor 70 WG significantly increased the magnesium content in the dry matter of the tubers of potato whose cultivation included weed control based on Racer 250 EC and control potato (only mechanical weed control). The highest magnesium content was determined in the tubers of potato sprayed with Sencor 70 WG (metribuzin) – 1.032 g kg^{-1}, on average. Moreover, the experimental herbicides increased the tuber contents of calcium compared with the control, by 0.016 g kg^{-1}, on average. There were determined different magnesium and calcium contents in the experimental cultivars. Balbina accumulated more and Irga less magnesium and calcium, on average 1.138 and 0.575 g kg^{-1} as well as 0.890 and 0.510 g kg^{-1}, respectively. Weather conditions in the study years significantly affected the potato tuber contents of magnesium and calcium.

Key words: magnesium, calcium, potato, herbicides.

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ZAWARTOŚĆ MAGNEZU I WAPNIA W BULWACH ZIEMNIAKA W ZALEŻNOŚCI OD ZABIEGÓW ODCHWASZCZAJĄCYCH

Abstrakt

Badania wykonano na próbach bulw ziemniaka pochodzących z doświadczenia polowego przeprowadzonego w latach 2005-2007 w Rolniczej Stacji Doświadczalnej Zawady, należącej do Uniwersytetu Przyrodniczo-Humanistycznego w Siedlcach. Eksperyment założono metodą losowanych podbloków. Czynnikami eksperymentu były: 2 odmiany ziemniaka jadalnego – Irga, Balbina i 4 sposoby pielęgnacji z uwzględnieniem herbicydów: Plateen 41,5 WG, Racer 250 EC, Sencor 70 WG oraz obiekt kontrolny bez ochrony chemicznej. Celem badań było określenie zawartości magnezu i wapnia w suchej masie bulw ziemniaka jadalnego w zależności od zabiegów odchwaszczających z uwzględnieniem niektórych herbicydów. Zawartość magnezu i wapnia w suchej masie bulw ziemniaka oznaczono metodą ASA. Zawartość pierwiastków zależała istotnie od odmian, sposobów zwalczania chwastów i warunków pogodowych w okresie wegetacji. Herbicydy Plateen 41,5 WG i Sencor 70 WG wpływały istotnie na zwiększenie koncentracji magnezu w suchej masie bulw ziemniaka w odniesieniu do pielęgnacji z użyciem preparatu Racer 250 EC i obiektu kontrolnego (pielęgnacji mechanicznej). Największą zawartość tego makroskładnika stwierdzono w bulwach ziemniaka opryskiwanych herbicydem Sencor 70 WG (metribuzin) – średnio 1,032 g kg^{-1}. Ponadto stosowane w doświadczeniu herbicydy wpływały na zwiększenie koncentracji wapnia w odniesieniu do obiektu kontrolnego, przeciętnie o 0,016 g kg^{-1}. Badane odmiany różniły się istotnie pod względem zawartości magnezu i wapnia, i więcej tych składników gromadziły bulwy odmiany Balbina – odpowiednio 1.138 g kg^{-1} i 0,575 g kg^{-1}, a mniej odmiany Irga – średnio 0,890 g kg^{-1} i 0,510 g kg^{-1}. Warunki pogodowe w latach badań istotnie różnicowały zawartość magnezu i wapnia w bulwach ziemniaka.

Słowa kluczowe: magnez, wapń, ziemniak, herbicydy.

INTRODUCTION

Plant-derived food is the major source of nutrients for man. One of such plants is potato so potato tuber content of nutrients is very important (Boligłowa, Dzienia 1999, Nemutlu, Özaltm 2005, Abulude et al. 2006).

Potato tubers contain 1-1.2% mineral compounds, the most basic being potassium, magnesium, calcium and phosphorus (Kolasa 1993). Macroelements perform important building functions, are an integral part of enzymes, and play an important role as regulators of metabolic processes (Stefańska et. al. 2003). A recommended daily intake of Mg and Ca by man is around 300-400 and 800-1000 mg, respectively (Hendrix et al. 1995). Both the shortage and excess of the elements result in disturbed metabolism in plants and animals (Szynal, Sykut 1992, Rivero et al. 2003). Magnesium and calcium contents in potato tubers are conditioned by varietal properties, soil, weather conditions during growth and agrotechnological practices, including weed control and mineral fertilization (Boligłowa, Dzienia 1999, Mazurczyk, Lis 2001, Tekalign, Hammer 2005, Gugała, Zarzecka 2009, Miles, Buchman 2009, Wichrowska et al. 2009). The literature on the subject reveals that the effect

of herbicides on minerals in potato tubers or cereal grains has not been fully explained and remains dubious (Brzozowska 2008, Gugała, Zarzecka 2009).

Hence, the purpose of this study was to determine magnesium and calcium contents in edible potato tubers depending on different weed control methods incorporating some herbicides.

MATERIALS AND METHODS

Potato tubers were harvested in a field experiment conducted at the Zawady Experimental Farm of the University of Natural Sciences and Humanities in Siedlce over 2005-2007. The experiment was set up on the soil of very good rye complex. Selected soil chemical properties prior to experiment set-up are presented in Table 1. The experimental design was a randomised complete block in a split-plot arrangement with 2 cultivars (Irga

Table 1

Chemical properties of the soil

Specification	2005	2006	2007
Organic matter (g kg^{-1})	11.5	11.3	14.0
Soil pH (1 M KCl)	6.74	5.50	4.99
Content of available nutrients (mg kg^{-1}):			
P	43.0	55.4	99.4
K	85.6	179.4	149.4
Mg	145.2	50.0	39.0

and Balbina, both mid-early cultivars) as main plots, and 4 weed control practices as subplots (Table 2). The herbicides were applied prior to potato plant emergence. The same farmyard manure amount of 25 t ha^{-1} was applied. Mineral fertilizers were applied at the following rates: 90 kg ha^{-1} N, 32.9 kg ha^{-1} P and 112.1 kg ha^{-1} K. The area of one plot was 25 m^2. Potatoes were harvested at the technological maturity stage in early September.

Potato tubers, later used for chemical analysis, were sampled from plots during harvest. Initially prepared samples (cleaned and mixed) were stored till analysed (for two days) in paper bags at the temperature of 18-20˚C. Then, they were analysed for magnesium and calcium using atomic absorption spectrometry – AAS (Azcue, Murdoch 1994). The chemical analyses were performed on dry material in three replications. The material was washed in tap and then distilled water and ground down. Next, it was dried until a constant weight was reached initially at 70˚C, and then 105˚C. Dried potatoes (1.0 g of a sample) were ground and a mixture of HNO$_3$ (7 ml) and H$_2$O$_2$ (1 cm^3) was added. Then, the plant material was mineralised in

Table 2

Factors of the experiment

Factor I – Cultivars
1. Irga 2. Balbina
Factor II – Weed control methods
1. Control object – mechanical weeding until and after potato rising 2. Plateen 41.5 WG (metribuzin + flufenacet) 2.0 kg ha^{-1} 3. Racer 250 EC (fluorochloridon) 3.0 dm^3 ha^{-1} 4. Sencor 70 WG (metribzin) 1.0 kg ha^{-1}

a laboratory oven Ethos plus during 1 cycle (0.5 h). Following filtration, magnesium and calcium contents were determined. Mg and Ca contents are expressed as g kg^{-1} dry matter.

The results of the study were statistically analysed by means of variance analysis. Mean separation between variables was obtained by Tukey's test at the significance level of p=0.05. Weather conditions the growing seasons under study varied (Table 3).

Table 3

The mean temperature and rainfalls from April to September at Zawady in 2005-2007

Year	Meteorological conditions from April to September			
	rainfalls sum (mm)	in comparison to long term	mean temerature (°C)	in comparison to long term
2005	268.8	-2.3%	15.0	+2.0%
2006	358.6	+30.3%	15.8	+7.5%
2007	308.2	-12.0%	15.4	+4.8%
1987-2000	275.2	-	14.7	-

RESULTS AND DISCUSION

There are very few data in the literature concerning the influence of plant protection agents, including herbicides, on mineral contents in potato tubers (ABULUDE et al. 2006, ZARZECKA et al. 2009).

Under the conditions of the experiment discussed, the dry matter of potato tubers contained between 0.875 and 1.149 g kg^{-1} magnesium (Tables 4, 5). There was found a significant effect of cultivars, weed control methods and weather and moisture conditions on magnesium concentration. Tuber content of magnesium was similar to the values reported by other authors (BRETZLOFF 1971, YILDRIM, TOKUSOÐLU 2005, TEKALIGN, HAMMES 2005, ZARZECKA et al. 2009).

Content of magnesium in dry mass of potato tubers depending of cultivars (g kg^{-1} d.m.)

Weed control methods (II)	Cultivars (I)		Mean value
	Irga	Balbina	
1. The control object – mechanical weeding	0.877	1.124	1.001
2. Plateen 41.5 WG	0.900	1.147	1.024
3. Racer 250 EC	0.867	1.133	1.000
4. Sencor 70 WG	0.914	1.149	1.032
Mean	0.890	1.138	1.014

LSD$_{0.05}$ for:
    cultivar – I
    weed control methods – II
    interaction I x II
			0.024
			0.029
			n.s.

n.s. – not significant

Content of magnesium in potato tubers depending on years of study (g kg^{-1} d.m.)

Weed control methods (II)	Years (III)			Mean value
	2005	2006	2007	
1. The control object – mechanical weeding	1.039	0.875	1.088	1.001
2. Plateen 41.5 WG	1.072	0.895	1.104	1.024
3. Racer 250 EC	1.022	0.885	1.094	1.000
4. Sencor 70 WG	1.079	0.899	1.119	1.032
Mean	1.053	0.889	1.10	1.014

LSD$_{0.05}$ for:
    weed control methods – II
    years – III
    interaction II x III
				0.029
				0.038
				n.s.

n.s. – not significant

The cultivars contained significantly different magnesium amounts; Balbina accumulated more and Irga less magnesium, on average 1.138 and 0.890 g kg^{-1}, respectively. Many workers (KARIM et al. 1997, GUGAŁA, ZARZECKA 2009, MILES, BUCHMAN 2009) have found that potato tuber chemical composition is primarily influenced by the genotype.

Plateen 41.5 WG and Sencor 70 WG significantly increased magnesium concentrations in potato tuber dry matter compared with Racer 250 EC and the control (mechanical weed control). The tubers of potato sprayed with Sencor 70 WG (metribuzin) contained most magnesium, on average 1.032 g kg^{-1}. Similar changes were reported by ZARZECKA and GĄSIOROWSKA (2000) who used herbicide mixtures to control weeds in potatoes, and BRZOZOWSKA (2008) who applied herbicides in winter wheat and examined its grain.

Calcium content in potato tuber dry matter was 0.543 g kg^{-1}, on average (Table 6). Similar tuber contents of this element were reported by KARIM et al. (1997), KOLBE and STEPHAN-BECKMANN (1997), ZARZECKA and GĄSIOROWSKA (2000). Statistical analysis confirmed that calcium content significantly depended on the weed control methods, cultivars and weather conditions in the study years.

Table 6

Content of calcium in potato tubers (g kg^{-1} d.m.)

Weed control methods (II)	Cultivars (I)		Mean value
	Irga	Balbina	
1. The control object – mechanical weeding	0.488	0.564	0.526
2. Plateen 41.5 WG	0.529	0.582	0.556
3. Racer 250 EC	0.507	0.574	0.541
4. Sencor 70 WG	0.514	0.581	0.548
Mean	0.510	0.575	0.543

LSD$_{0.05}$ for:
    cultivar – I
    weed control methods – II
    interaction I x II
			0.009
			0.020
			n.s

n.s. – not significant

Herbicides increased calcium concentration compared with the control where no chemicals were applied, by 0.016 g kg^{-1}, on average. These findings agree with earlier studies by ZARZECKA et al. (2002). What is more, in the study by GUGAŁA and ZARZECKA (2010), the herbicides they applied in field pea cultivation increased seed contents of calcium compared with the control. By contrast, BRZOZOWSKA (2008) demonstrated that crop protection chemicals – herbicides – had no significant effect on calcium concentration in winter wheat grain.

The genetic traits of the cultivars significantly determined calcium contents. More calcium was accumulated by Balbina than Irga, on average 0.575 g kg^{-1} and 0.510 g kg^{-1}, respectively. The cultivars responded with an increased tuber content of calcium to the herbicides applied, however, there was found no interaction between the experimental factors. The influence of cultivar on calcium concentration has been reported by TEKALIGN and HAMMES (2005), MILES and BUCHMAN (2009), ZARZECKA et al. (2009).

According to BAĆMAGA et al. (2007), pesticides applied under field conditions to protect crops do not usually influence macroelements contents in plants, but it is easier for a crop plant to take up more individual nutrients when there is no competition with weeds.

In general, weather conditions in the study years significantly influenced magnesium and calcium contents in potato tuber dry matter (Tables 5,7).

Content of calcium in potato tubers depending on years of study (g kg^{-1} d.m.)

Weed control methods (II)	Years (III)			Mean value
	2005	2006	2007	
1. The control object – mechanical weeding	0.558	0.447	0.574	0.526
2. Plateen 41.5 WG	0.594	0.469	0.605	0.556
3. Racer 250 EC	0.577	0.458	0.587	0.541
4. Sencor 70 WG	0.582	0.465	0.597	0.548
Mean	0.578	0.460	0.591	0.543

LSD$_{0.05}$ for:
    weed control methods – II
    years – III
    interaction II x III
				0.020
				0.015
				n.s.

n.s. – not significant

Less magnesium and calcium were accumulated in 2006, when the weather was wet and warm, and more in 2005 and 2007, when the temperature was moderate and the rainfall was quite evenly distributed. The works by ZARZECKA and GĄSIOROWSKA (2000) as well as WADAS et al. (2008) have confirmed that there is an effect of weather conditions on magnesium and calcium contents

CONCLUSIONS

1. The herbicides Plateen 41.5 WG and Sencor 70 WG significantly affected the potato tuber dry matter contents of magnesium and calcium. In turn, Racer 25 EC had less impact, in particular on magnesium concentration.

2. Varietal traits influenced the concentration of both the elements. More magnesium and calcium in tuber dry matter was determined in Balbina compared with Irga.

REFERENCES

ABULUDE F.O., OSEKE M.O., OGUNTOKUN M.O., ADESANYA W.O., AFOLABI O.O. 2006. *Calcium and magnesium contents of selected nigerian foods.* Adv. Food Sci., 28: 181-185.

AZCUE J., MURDOCH A. 1994. *Comparison of different washing, ashing, and digestion methods for the analysis of trace elements in vegetation.* Intern. J. Environ. Chem., 57:151-162.

BAĆMAGA M., KUCHARSKI M., WYSZKOWSKA J. 2007. *Impact of crop protection chemicals on plants and animals.* J. Elementol., 12(2): 135-148.

BOLIGŁOWA E., DZIENIA S. 1999. *Impact of foliar fertilisation of plant on the content of macroelements in potato.* Elec. J. Polish Agric. Univ. Agronomy, 2(2) http://www.ejpau.media.pl/volume2/issue2/agronomy/art-05.html.

BRETZLOFF C.W. 1971. *Calcium and magnesium distribution in potato tubers.* Am. J. Potato Res., 48(3): 97-104.

BRZOZOWSKA I. 2008. *Macroelements content in winter wheat grain as affected by cultivation and nitrogen application methods.* Acta Agroph., 11: 23-32. (in Polish)

GUGAŁA M., ZARZECKA K. 2009. *Effect of cultivation factors on magnesium content in and removal by the potato tuber crop.* J Elementol., 14(2):249-256.

GUGAŁA M., ZARZECKA K. 2010. *The effect of weed control methods on magnesium and calcium content in edible pea seeds (Pisum sativum L.).* J Elementol., 15(2): 269-280.

HENDRIX P., CAUWENBERGH R.V., ROBBERECHT H.J., DEELSTRA H.A. 1995. *Measurement of the daily dietary calcium and magnesium intake in Belgium, using duplicate portion sampling.* Z Lebensm. Unters Forsch., 201: 213-217.

KARIM M.S., PERCIVAL G.C., DIXON G.R. 1997. *Comparative composition of aerial and subterranean potato tubers (Solanum tuberosum L).* J. Sci. Food Agric., 75: 251-257.

KOLASA K.M. 1993. *The potato and human nutrition.* Am. Potato J., 70: 375-384.

KOLBE H., STEPHAN-BECKMANN S. 1997. *Development, growth and chemical composition of the potato crop (Solanum tuberosum L.). Tuber and whole plant.* Potato Res., 40: 135-153.

MAZURCZYK W., LIS B. 2001. *Variation of chemical composition of tubers of potato table cultivars grown under deficit and excess of water.* Pol. J. Food Nutr. Sci., 10(51): 27-30.

MILES G.P., BUCHMAN J.L. 2009. *Impact of zebra chip disease on the mineral content of potato tubers.* Am. J. Potato Res., 86: 481-489.

NEMUTLU E., ŐZALTM N. 2005. *Determination of magnesium, calcium, sodium, and potassium in blood plasma samples by capillary zone electrophoresis.* Anal. Bioanal. Chem., 383: 833-838.

RIVERO R.C., SUAREZ P.S., RODRIGUEZ E.M., MARTIN J.D., ROMARA C.D. 2003. *Mineral concentrations in cultivars of potatoes.* Food Chem., 83: 247-253.

STEFAŃSKA E., KARCZEWSKI J., OSTROWSKA L., CZAPSKA D. 2003. *The content of magnesium and the zinc in day – long alimentary right of students of Medical Academy in Białystok.* J. Elementol., 8(1): 31-39. (in Polish)

SZYNAL J., SYKUT A. 1992. *Effect of selected herbicides on protein content and amino acids composition in winter wheat grain.* Bromat. Chem. Toksykol., 25: 243-249. (in Polish)

TEKALIGN T., HAMMES P.S. 2005. G*rowth and productivity of potato as influenced by cultivar and reproductive growth. Growth analysis, tuber yield and quality.* Sci. Hort., 105: 29-44.

WADAS W., JABŁOŃSKA-CEGLAREK R., KUROWSKA A. 2008. *Effect of using covers in early crop potato culture on the content of phosphorus and magnesium in tubers.* J. Elementol., 13(2): 275-280.

WICHROWSKA D., WOJDYŁA T., ROGOZIŃSKA I. 2009. *Concentrations of some macroelements in potato tubers stored at 4ºC and 8ºC.* J. Elementol., 14 (2): 373-382.

YILDRIM Z., TOKUSOĐLU Ő. 2005. *Some analytical quality characteristic of potato (Solanum tuberosum L.) minitubers (cv. NIF) developed via in vitro cultivation.* Electron. J. Environ. Agric. Food Chem., 4(3): 916-925.

ZARZECKA K., GĄSIOROWSKA B. 2000. *Impact of some herbicides on the chemical composition of potato tubers.* Elec. J. Polish Agric. Univ. Agronomy, 3(3), http://www.ejpau.media.pl/volume3/issue1/agronomy/art-04.html.

ZARZECKA K., GUGAŁA M., GĄSIOROWSKA B., MAKAREWICZ A. 2002. *Fluctuations in magnesium and calcium concentrations in potato tubers under the influence of herbicides and their mixtures.* J. Elementol., 7(4): 309-315. (in Polish)

ZARZECKA K., GUGAŁA M., ZADROŻNIAK B. 2009. *Impact of insecticides on magnesium and calcium contents in potato tubers.* J. Elementol., 14(1): 189-195.

EFFECTS OF WATERBORNE COPPER ON GILLS CATALASE AND BLOOD BIOCHEMISTRY IN GILTHEAD SEABREAM (*SPARUS AURATA* L.)

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Abstract

Cu is a transition metal essential for every organism, but an excess can lead to toxicity caused by oxidative stress, which can disturb the natural antioxidant defence systems. Since catalase (CAT) is a major enzyme involved in the decomposition of oxygen peroxide, the main goal of this study was to evaluate changes in the concentration and activity of CAT by means of enzymatic and immunohistochemical methods. Modifications in the blood biochemistry profile of Cu-exposed gilthead sea bream (*Sparus aurata* L.) were also studied. In gills of the exposed fish, Cu significantly increased throughout the study to a maximum of 6.9 ± 4.7 µg g^{-1} wet weight at the end of the treatment. Immunohistochemistry (IHC) evidenced a brown cytoplasmic stain in the epithelial cells of the primary and secondary lamellae and in pillar cells, while enzyme activity was localized in the epithelium and pillar cells of both primary and secondary lamellae and appeared as a strong cytoplasmic stain particularly at the base of the primary lamellae. IHC and enzymohistochemistry (EHC) quantitative analysis suggested that the main variations in amount and activity of the enzyme were recorded 15 days after exposure (both IHC and EHC expressed a decrease in CAT in exposed fish compared to controls, $P<0.0001$) and 28 days after exposure (IHC recorded increased CAT in exposed specimens compared to controls, $P<0.0001$; EHC evidenced a decrease in CAT in exposed subjects compared to controls, $P<0.0001$). The biochemical blood profile was monitored with a standard blood biochemistry panel. An increase in plasma urea was evident only in exposed fish, while – as a function of time – a decrease of glucose in both exposed and control fish was apparent. The three investigation methods evidenced that CAT was effective against Cu toxicity, and the increase in plasma urea could be considered a suitable metabolic marker of Cu exposure in fish.

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WPŁYW MIEDZI W WODZIE NA KATALAZĘ W SKRZELACH I BIOCHEMIĘ KRWI U DORADY (*SPARUS AURATA* L.)

Abstrakt

Miedź jest metalem przejściowym niezbędnym w każdym organizmie, lecz jej nadmiar może prowadzić do skutków toksycznych powodowanych przez stres oksydacyjny, który zakłóca naturalne systemy obrony antyoksydacyjnej. Ponieważ katalaza (CAT) jest jednym z głównych enzymów uczestniczących w rozkładzie nadtlenku tlenu, głównym celem badań była ocena zmian zawartości i aktywności CAT za pomocą metod enzymatycznych i immunohistochemicznych. Badano również zmiany w biochemicznym profilu krwi u dorady (*Sparus aurata* L.) poddanej działaniu Cu. Stwierdzono znacząco wyższe stężenie tego metalu w skrzelach ryb, na które oddziaływała miedź, aż do maksymalnej zawartości 6,9±4,7 μg g^{-1} mokrej masy pod koniec doświadczenia. Badania immunohistochemiczne (IHC) ujawniły brązową plamkę cytoplazmatyczną w komórkach nabłonka blaszek pierwszego i drugiego rzędu oraz w komórkach filarowych, podczas gdy aktywność enzymatyczna została zlokalizowana w nabłonku oraz w blaszkach pierwszego i drugiego rzędu, i ujawniła się jako silna plamka cytoplazmatyczna, szczególnie u podstawy blaszek pierwszego rzędu. Analiza ilościowa wyników badań IHC oraz enzymohistochemicznych (EHC) sugeruje, iż główna zmienność w ilości oraz aktywności enzymu nastąpiła w 15. dniu po ekspozycji (zarówno IHC, jak i EHC wykazały spadek zawartości CAT u ryb wystawionych na działanie Cu w porównaniu z kontrolą, *P*<0,0001) oraz w 28. dniu po ekspozycji (IHC wykazało wzrost CAT u ryb wystawionych na Cu w porównaniu z kontrolą, *P*<0,0001; EHC ujawniło spadek CAT u ryb doświadczalnych w porównaniu z kontrolą, *P*<0,0001). Biochemiczny profil krwi monitorowano za pomocą standardowego zestawu biochemicznego krwi. Wzrost zawartości mocznika we krwi ujawnił się jedynie u ryb poddanych działaniu Cu, natomiast spadek zawartości glukozy – z upływem czasu – wystąpił u ryb doświadczalnych i kontrolnych. Trzy zastosowane metody badawcze udowodniły, iż CAT skutecznie przeciwstawia się toksyczności Cu, a wzrost zawartości mocznika we krwi można uznać za odpowiedni marker metaboliczny oznaczający wystawienie ryb na działanie miedzi.

Słowa kluczowe: miedź, *Sparus aurata*, katalaza, skrzela, biochemia krwi, immunohistochemia, enzymohistochemia.

INTRODUCTION

Cu is an essential trace element for all organisms, but an excess can result in reactive oxygen species (ROS) production (GAETKE, CHOW 2003) and lipid peroxidation (HALLIWELL, GUTTERIDGE 1992). Recently, changes have been reported in *Sparus aurata* in the expression of proteins involved in the inflammation/immunity network (ISANI et al. 2011).

Defense systems against oxyradicals include catalase (CAT) among other enzymes. CAT is a Fe^{3+} dependent metalloenzyme fundamental to all living organisms exposed to oxygen and is usually located in the matrix of cellular peroxisomes (NOVIKOFF, NOVIKOFF 1973)**,** where CAT has been immunohisto-

chemically evidenced in primary cell cultures from fish (*Danio rerio) (*KRYSKO et al. 2010). CAT is present in nearly all cells exposed to oxygen, but its distribution in gilthead sea bream *Sparus aurata* (L.) gills is still unknown. Gilthead sea bream was chosen as a model for our study due to the available knowledge of its basic biochemical response to Cu (ISANI et al. 2003, 2011) and economic importance.

Since studies concerning blood biochemistry, immunohistochemistry (IHC) and enzymohistochemistry (EHC) for CAT on fish tissues exposed to a toxic amount of Cu are lacking, the aims of this work are: 1) to evaluate the effects of Cu exposure on blood biochemistry; 2) to test the methods for CAT location; 3) to show any variation in quantity and activity of the enzyme in *S. aurata* gills after Cu exposure.

MATERIALS AND METHODS

All experimental procedures were approved by the Ethics and Scientific Committee of the University of Bologna and were carried out in accordance with European legislation regarding the protection of animals used for experimental and other scientific purposes (Council Directive 86/609/EEC).

Experimental design

Eighty juvenile gilthead sea bream (*S. aurata*), average wt 65±5 g, were obtained from farmed stock. The fish were maintained in ten 500L tanks (at a density of eight fish/tank) filled with natural sea water taken from 300 m offshore Cesenatico (FC, Italy). Before the experimental exposure, fish were acclimatised for 15 days at 13˚C and at a salinity of 32.0±2.6‰.

The work was performed during January-February 2009 when water temperature drops below 13˚C and *S. aurata* experience the natural period of starvation (IBARZ et al. 2007). Therefore, fish were not fed throughout the Cu exposure time avoiding water contamination and additional Cu intake with the diet.

$CuSO_4$ was added to 5 tanks (40 specimens) to give a nominal Cu concentration of 0.5 mg dm^{-3}, while the fish in the other 5 tanks (40 specimens) served as controls. The waterborne concentration of Cu was chosen based on the evidence of previous studies (ISANI et al. 2003). Cu concentrations in water were verified by atomic absorption spectrometry (AAS). On day 0 (T0) prior to Cu exposure and after 15 (T1), 28 (T2) and 50 (T3) days fish were randomly chosen from each tank, sacrificed and tissues immediately sampled for metal, IHC and EHC analysis.

Sample collection

Gills were sampled from 32 control and 24 exposed fish and divided into two aliquots: 1) one for metal analysis was stored at -20ºC; 2) the other one was cut into halves, one for routine histology and IHC and the other for EHC: the samples for histology and IHC were formalin-fixed and paraffin-embedded, then processed according to routine procedures and cut at 4 µm; one section was stained with Haematoxylin and Eosin (H&E) and a further serial section was used for CAT IHC; for EHC, fish gill specimens were frozen in liquid nitrogen and stored at -80ºC for 7 days before processing and 4 µm-thick sections were cut with a cryostat.

Cu determination

Cu determination in gill samples and sea water were determined as reported by ISANI et al. 2011. The accuracy of the method was evaluated by calibration to an international standard (CRM 278).

Blood collection and biochemistry analyses

Blood samples from 21 control and 16 exposed specimens were collected and treated as previously reported (ISANI et al. 2011). Plasma samples were immediately analysed with an automated biochemical analyzer (Olympus AU400). A standard biochemical profile was chosen, including: glucose, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), gamma glutamyl transferase (GGT), Ca, P, Mg, Na, Cl and K.

Immunohistochemistry

Four-m-thick paraffin-embedded tissue sections were dewaxed and rehydrated in decreasing ethanol solutions. Endogenous peroxidase was blocked by immersion in 0.3% hydrogen peroxide in methanol. Sections were then rinsed in Tris buffered saline (TBS) and antigen was retrieved with citrate buffer (2.1 g citric acid monohydrate/litre distilled water), pH 6.0, and heating for two 5 min periods in a microwave oven at 750 W, followed by cooling at room temperature for 20 min. A reduction of background staining was achieved with Protein Block Serum Free (cod. X0909 DAKO, Milan, Italy) for 10 min. The primary antibody (cod. C0979 Sigma, St. Louis, Missouri, USA), diluted (1:300) in PBS containing bovine serum albumin, was incubated with the tissue sections overnight at 4ºC. Sites of antibody binding were identified with Dako LSAB+ System-HRP code K0690. 3,3'-Diaminobenzidine (DAB) (cod. D5637 Sigma) was used as chromogen (0.05% in TBS 0.05 M pH 7.2 and H_2O_2: concentration 0.05% for 10 min at room temperature). The sections were then counterstained with Papanicolaou hematoxylin. In negative control sections, the primary antibody was replaced with phosphate buffered saline (PBS) containing bovine serum albumin.

Enzymohistochemistry

EHC CAT was performed according to C<small>AJARAVILLE</small> et al. (1993) on gill frozen specimens.

Control reactions were carried out with incubation media lacking H_2O_2, DAB or containing only imidazole (0.01 M) (C<small>AJARAVILLE</small> et al. 1993).

Quantitative analysis of CAT

CAT IHC: ten fields per each gill section, randomly chosen, were analysed to obtain a total number of 260 fields. Digital images of the selected fields have been achieved with a digital camera (Leica DFC320, Solms, Germany) mounted on a Leica DMLB (Solms, Germany) microscope and connected with a PC. All images were obtained using a 63x objective and a 10x eyepiece, yielding a final magnification of 630x. Images were acquired with an image analysis software Leica QWIN at 24-bit, saved as JPEG format. Each selected field had an area of $2.1x10^{-2}$ mm^2. For image analysis a standard grid with sixteen horizontal-lines was set up and superimposed to each image. Only the positive cells with nucleus adjacent to or cutting one of the lines were counted and the data expressed as the mean number of positive cells assessed irrespectively of the location (stroma or epithelium of the lamellae).

CAT EHC: images (400x magnification, $5.4x10^{-2}$ mm^2 per field) were acquired using the same tools as for IHC. Intensity of cytoplasmic staining was estimated in each of the 260 images, automatically measuring the integrated optical density with the image analysis software Leica QWIN.

Statistical analysis

The recorded values of IHC and EHC quantification were expressed as means ±SE (Standard Error) of the ten measured fields. For each case, CAT EHC and IHC quantitative data were tested for normality using the Shapiro-Wilk W-test. As the data distribution was not normal, the non-parametric Spearman rank order correlation test was utilized. Values of $P<0.05$ were considered significant. Blood biochemistry parameters were analysed by a two ways full factorial design time exposure with the R-statistics program.

RESULTS

Cu concentration

Cu analysis in the sea water samples after 50 days of exposure resulted in 0.35±0.05 mg dm^{-3}, indicating that 70-80% of the nominal dose of Cu was present in the exposure tanks at the end of the experiment. Cu concentrations in gills of controls at T0, T1, T2 and T3 did not change significantly;

while in exposed fish, metal concentrations increased significantly reaching a maximum of 6.9±4.7 µg g^{-1} wet weight at the end of the treatment ($P<0.05$) – Figure 1.
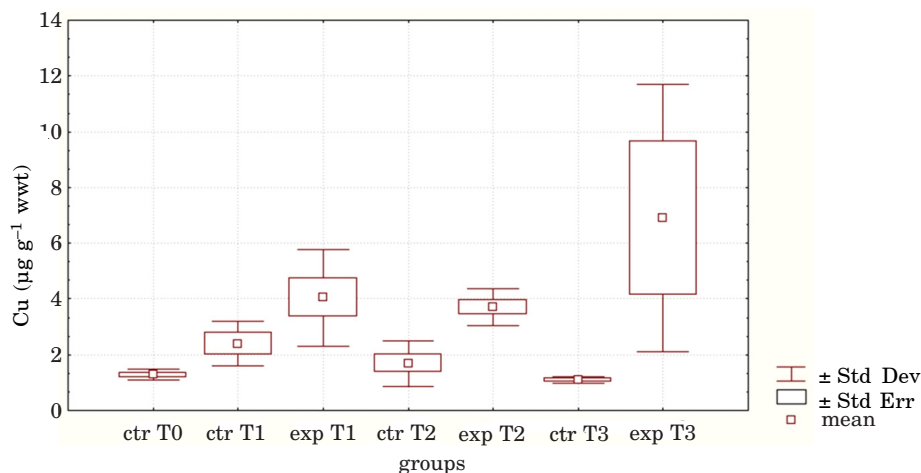

Fig. 1. Cu concentrations in gills of control (N=32) and exposed (N=24) fish. Significant increases were noted in treated fish ($P<0.05$)

Blood biochemistry

Only plasma urea significantly increased in Cu exposed fish ($P<0.01$). A significant decrease in glucose ($P<0.01$) and a significant increase for CK and ALP ($P<0.05$) were observed in control and exposed fish, as a function of time. The free ions did not show significant variations with the exception of a significant increase ($P<0.05$) of P both in control and exposed fish, as a function of time (Table 1).

Macro- and microscopic aspects

No mortality and no gross lesions were detected in the gills of exposed and control subjects. On histological ground no lesions were recorded.

CAT IHC and quantitative analysis

Brown intracytoplasmic positivity was evident in both exposed and control fish only in the epithelial cells of the primary and secondary lamellae and in pillar cells of the latter (Figure 2a). Scant positivity was noted in stromal cells. Quantitative analysis for CAT presence (Figure 3a), expressed by IHC, demonstrated a significant decrease in exposed subjects compared to controls at T1 ($P<0.0001$), an increase in positivity in exposed fish compared to controls ($P<0.0001$) at T2; similar values in positivity were obtained in exposed and control fish ($P=0.09$) at T3.

Table 1

Plasma biochemical parameters in control and treated specimens of gilthead seabream. Values are expressed as mean±standard error (SE)

Item	Glu* (mg dl⁻¹)	Urea** (mg dl⁻¹)	Ca (mg dl⁻¹)	P* (mg dl⁻¹)	Na (mg dl⁻¹)	K (mg dl⁻¹)	Cl (mg dl⁻¹)	Mg (mg dl⁻¹)	AST (U l⁻¹)	ALT (U l⁻¹)	ALP* (U l⁻¹)	CK* (U l⁻¹)	GGT (U l⁻¹)
1	2	3	4	5	6	7	8	9	10	11	12	13	14
T0 (*N*=6)													
Mean	104	4.9	8.7	6.2	218	5.2	140	3.5	120	5.1	108	Nd	Nd
SE	14	0.8	0.6	0.7	4	0.5	3	0.2	29	0.1	18		
Min-Max	46-142	2.7-8.5	7.1-11.4	3.9-9.4	204-229	3.8-6.8	134-152	3-4.4	36-267	5-5.2	54-187		
T1 C (*N*=6)													
Mean	80	5.2	6.9	6.4	250	4.6	125	2.9	48	4.6	108	1274	9.7
SE	12	0.7	0.7	0.4	8	0.3	7	0.2	10	0.9	12	561	0.7
Min-Max	50-121	3.2-7.8	4.8-8.5	5.2-7.5	226-274	3.7-5.9	103-146	2.5-3.7	29-62	3-8	58-137	137-3475	7.7-11.4
T1 E (*N*=6)													
Mean	72	11.9	6.6	5.9	236	4.3	135	3.3	50	5.2	108	2685	8.9
SE	2	3.7	0.8	0.8	8	0.4	11	0.1	18	2	9	1035	0.8
Min-Max	67-76	5.5-26.4	4.8-8.5	3.1-7.4	224-260	3.5-5.3	105-155	2.9-3.6	14-114	1-12	92-136	710-5887	6.1-11.2

cont. Table 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14
T2 C (N=5)													
Mean	81	6.0	7.8	7.0	248	4.6	131	2.9	78	5.8	119	5461	10.7
SE	6	0.2	0.1	0.6	5	0.4	2	0.2	19	0.8	3	1430	0.1
Min-Max	67-98	5.3-6.5	7.5-8.4	5-8.2	233-260	3.9-5.3	127-135	2.6-3.2	21-104	4-7	112-126	1448-6217	10.4-11.1
T2 E (N=6)													
Mean	53	7.7	7.2	6.2	241	3.9	125	3.5	60	5	76	4184	10.2
SE	4	0.8	0.9	0.5	4	0.1	6	0.5	9	0.5	9	942	0.8
Min-Max	35-67	5.0-10.6	4.3-10.6	4.4-7.4	233-261	3.4-4.1	103-145	2.6-6.1	30-80	3-6	48-112	1080-7377	7.3-13.4
T3 C (N=4)													
Mean	61	5.6	9.5	7.7	221	4.4	148	3.4	36	6.5	169	2755	12.3
SE	5	0.2	0.3	0.4	3	0.2	3	0.2	9	0.7	18	772	0.3
Min-Max	50-71	5.1-5.9	9.0-10.5	6.7-8.7	215-230	4-4.7	143-154	3.0-3.6	19-58	5-8	136-206	1282-4873	11.8-13.1
T3 E (N=4)													
Mean	57	7.5	7.9	9.1	225	4.6	142	4.1	75	9.5	141	4927	10.7
SE	5	0.2	0.6	1.6	2	0.2	6	0.5	23	2.3	35	1227	0.6
Min-Max	46-68	6.9-8.0	6.9-9.4	5.6-12.9	218-229	4.2-4.7	128-156	3.1-5.4	33-142	6-16	69-234	2529-6321	9.7-12.4

* Values presented significant variations as a function of time: glucose $P<0.01$; phosphorus $P<0.05$; alkaline phosphatase $P<0.01$ and creatine kinase $P<0.05$.
** Values presented significant variations as a function of Cu exposure: urea $P<0.01$.

CAT EHC and quantitative analysis

Enzyme activity was localized in the epithelium and pillar cells of both primary and secondary lamellae and appeared as a strong cytoplasmic stain particularly at the base of the primary lamellae (Figure 2b). Low positivity was observed in the connective tissue of the blood vessel walls of primary lamellae. Some positive spots were recorded within the branchial arch stroma. Quantitative analysis of enzyme activity in positive areas, evaluated as integrated optical activity, showed a significant decrease in exposed subjects
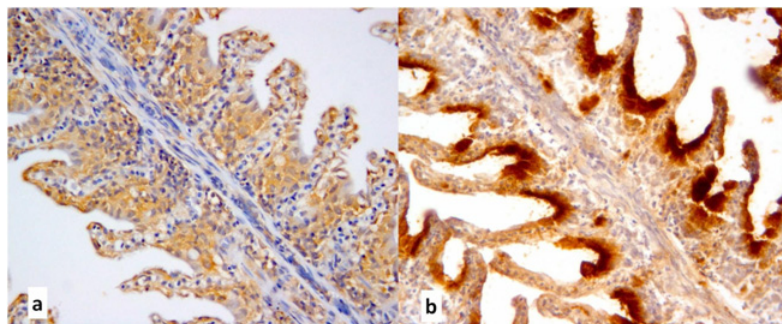
Fig. 2. Gills of the same control specimen: *a* – CAT IHC: pulverulent brown positivity in the cytoplasm at the base of secondary lamellae 40x; *b* – CAT EHC: enzyme activity is evidenced by brown colour in the epithelial cells of secondary lamellae 40x

compared to controls at T1 and T2 (*P*<0.0001) – Figure 3b. At T3 the activity of the enzyme in the exposed and control fish reached equal values (*P*=0.68).

Correlation analysis of CAT data

No evident correlation (*R*=0.3; *P*=0.12) was found between CAT IHC and EHC by linear regression analysis.

a

b
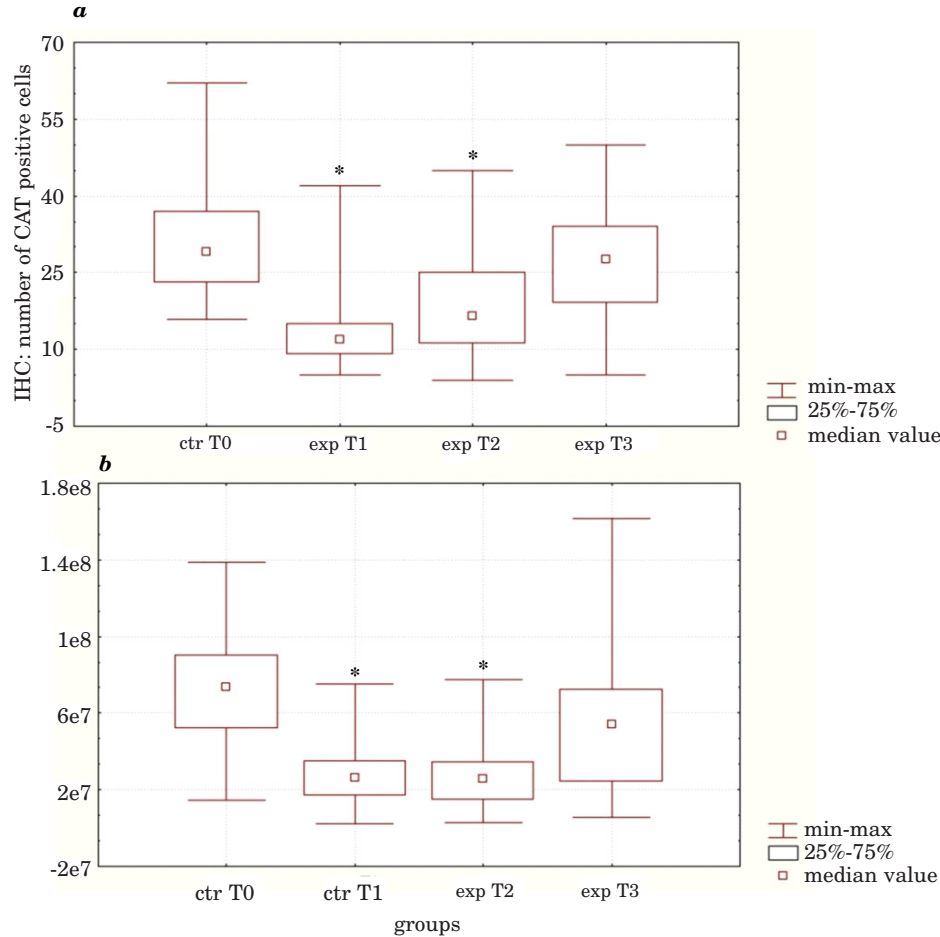
Fig. 3. CAT IHC (*a*) and CAT EHC (*b*) quantitative analysis.
*Indicates significant differences with respect to T0 (*P*<0.0001).

DISCUSSION

Cu accumulation in gills

Cu exposure has resulted in a significant increase in metal (5.3 folds) concentration in gills of exposed fish. Similar accumulation rates were found in *S. aurata* exposed for 20 days to 0.1 mg dm^{-3} and 0.5 mg dm^{-3} (ISANI et al. 2003) and in black sea breams exposed to 0.05 mg dm^{-3} for 20 days (DANG et al. 2009). Cu concentrations in gills originate from mechanisms that regulate uptake, sequestration and export; as concerns membrane transport, also in gilthead sea bream the presence of the Ctr1 was demonstrated

(MINGHETTI et al. 2008). Moreover, Cu can be bound to mucus glycoproteins, which are present on gills surface and effectively bind diffusible cations such as Cu (SPEARE, FERGUSON 2006), hence representing the first defensive line against waterborne Cu.

Blood biochemistry

Measurements of blood parameters are commonly used as a diagnostic tool in mammals, while they are not extensively applied to fish. The basal values of plasma parameters measured in T0 fish were in the range of those reported for other control or unexposed marine teleosts (FOLMAR 1993) and were indicative of a healthy status of fish used in the present work. Changes in blood biochemistry could be related to unsuitable environmental conditions (BARCELLOS et al. 2004), including chronic exposure to chemical pollutants (FOLMAR 1993). In the present experiment, Cu exposure determined a significant increase in plasma urea. In fish, urea is mostly used as an osmolyte or is excreted as a waste of ammonia detoxification after its biosynthesis in the liver (GROSELL et al. 2004). The gills are the major site of urea excretion, through an urea transporter protein, which was indentified in several marine species (WALSH et al. 2001). The higher plasma urea levels in Cu-treated fish could be due to the impairment of gills nitrogen excretory function. The plasma urea increase could derive directly from the inhibition of the urea transporter, or indirectly from the inhibition of ammonia excretion; this metabolite in turn should be transformed in urea by the liver in order to avoid systemic toxicity. In both cases, the higher urea plasma concentration could derive from a molecular damage of gill transport systems. Accordingly, the measure of blood urea nitrogen, routinely determined in mammals as an index of renal function, could represent in fish a simple and useful biomarker of gill function. We also found a significant decrease of glucose as a function of time in both control and exposed fish. These changes could be due to starvation and are in agreement with data reported in river lamprey (EMELYANOVA et al. 2004). The observed increase in ALP may be linked to alteration of P metabolism (WHYTE 2010) and together with changes of CK activity could be related to muscle proteolysis that naturally occurs during food deprivation (SEILIEZ et al. 2008).

CAT IHC and EHC

The IHC findings assessed CAT presence located mostly in the cytoplasm of epithelial cells at the base of the primary lamellae; to a lesser extent CAT was found in the epithelium of the secondary lamellae, where Cu is absorbed through the Ctr1 mediated transport (MINGHETTI et al. 2008). The role of CAT at these sites was to prevent the damaging of the epithelium in the form of membrane lipid peroxidation operated by Cu redox activity (CRAIG et al. 2007). The granular pattern found in the present paper confirmed CAT presence in peroxisomes.

In accordance with Guderley et al. (2003), starvation induced an increase in CAT presence. The significant decrease in CAT presence noted in our cases at T1 compared to T0 was consequent to Cu toxicity. The following increase of CAT concentration from T1 to T3 was partially due to starvation and in part to the protective role of CAT against metal-induced oxidative stress (FIRAT, KARGIN 2010).

CAT presence disclosed by IHC was integrated by EHC results indicating enzyme activity particularly at the base of the primary lamellae. The significant decrease in CAT activity at T1 and T2 in the gills of treated fish could be related to Cu exposure either by direct binding of the metal to the enzyme or by ROS-induced inhibition as reported by CRAIG et al. (2007) in *Danio rerio*. At T3, the increase in enzyme activity up to control levels may indicate an acclimation to waterborne Cu exposure or, more likely, the activation of protection mechanisms, such as an increase in metallothionein (MT) (ISANI et al. 2003) and/or CAT expression (CRAIG et al. 2007) in heavy metal exposed fish. CRAIG et al. (2007) examined the genes encoding for major proteins (including CAT) involved in combating oxidative stress in *D. rerio* and found no significant changes in the expression of CAT in the gills. However, similarly to our T1 data, they noted a decrease in CAT activity within 48 hours post exposure, which could suggest a rapid rise in oxidative stress due to sub-lethal doses of Cu and a comparably rapid response of defense mechanisms. The biphasic responses of CAT expression and its enzymatic activities were consistent with an increase of CAT gene transcription and a detoxifying activity of MT. At T3, Cu toxicity may be neutralized by the increase in CAT expression, which in turn appeared as a rise in enzymatic activity.

IHC positivity did not necessarily coincided with EHC positivity. As two CAT isoforms were recognized (BAILLY et al. 2004), one active (55 kDa) and one inactive (59 kDa), we assumed that when the inactive isoform is present, only IHC results positive, whereas both methods reveal the active isoform.

All the three main investigation methods, i.e. analytical chemistry, IHC-EHC and blood biochemistry, have proven effective in highlighting responses to Cu exposure: increased Cu levels were associated with an initial decrease in CAT expression and activity and with increased plasma urea that could be considered a metabolic marker of gills function in exposed fish.

REFERENCES

BAILLY C., LEYMARIE J., LEHNER A., ROUSSEAU S., COME D., CORBINEAU F. 2004. *Catalase activity and expression in developing sunflower seeds as related to drying*. J. Exp. Bot., 55(396): 475-483.

BARCELLOS L.J.G., KREUTZ, L.C., DE SOUZA, C., RODRIGUES, L.B., FIOREZE, I., QUEVEDO, R.M., CERICATO, L., SOSO, A.B., FAGUNDES, M., CONRAD, J., DE ALMEIDA LACERDA L., TERRA S. 2004. *Hematological changes in jundià (Rhamdia quelen Quoy and Gaimard Pimelodidae) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects*. Aquaculture, 237: 229-236.

CAJARAVILLE M.P., URANGA J.A., ANGULO E. 1993. *Light microscopic catalase histochemistry in mussel digestive gland tissue*. Histol. Histopathol., 8: 537-546.

CRAIG P.M., WOOD C.M., McCLELLAND G.B. 2007. *Oxidative stress response and gene expression with acute Cu exposure in zebrafish (Danio rerio)*. Am. J. Physiol. Regul. Integr. Comp. Physiol., 293: 1882-1892.

DANG F., ZHONG H., WANG W.X. 2009. *Copper uptake kinetics and regulation in a marine fish after waterborne copper acclimation*. Aquat. Toxicol., 94: 238-244.

EMELYANOVA L.V., KOROLEVA E.M., SAVINA M.V. 2004. *Glucose and free amino acids in the blood of lampreys (Lampetra fluviatilis L.) and frogs (Rana temporaria L.) under prolonged starvation*. Comp. Biochem. Physiol. Mol. Integr. Physiol., 138: 527-532.

FIRAT O., KARGIN F. 2010. *Response of Cyprinus carpio to copper exposure: alterations in reduced glutathione, catalase and proteins electrophoretic patterns*. Fish Physiol. Biochem., 36(4): 1021-1028.

FOLMAR L.C. 1993. *Effects of chemical contaminants on blood chemistry of teleost fish: a bibliography and synopsis of selected effects*. Environ. Toxicol. Chem., 12: 337-375.

GAETKE L.M., CHOW C.K. 2003. *Copper toxicity, oxidative stress, and antioxidant nutrients*. Toxicology, 189: 147-163.

GROSELL M., McDONALD M.D., WALSH P. J., WOOD C.M. 2004. *Effects of prolonged copper exposure in the marine gulf toadfish (Opsanus beta). II. Copper accumulation, drinking rate and Na$^+$/K$^+$-ATPase activity in osmoregulatory tissues*. Aquat. Toxicol.. 68: 263-275.

GUDERLEY H., LAPOINTE D., BEDARD M., DUTIL J.D. 2003. *Metabolic priorities during starvation: enzyme sparing in liver and white muscle of Atlantic cod, Gadus morhua L.* Comp. Biochem. Physiol., 135: 347-356.

HALLIWELL B., GUTTERIDGE M.C. 1992. *Biologically relevant metal ion-dependent hydroxyl radical generation*. FEBS J., 307: 108-112.

IBARZ A., BELTRAN M., FERNANDEZ-BORRAS J., GALLARDO M.A., SANCHEZ J., BLASCO J. 2007. *Alterations in lipid metabolism and use of energy depots of gilthead sea bream (Sparus aurata) at low temperatures*. Aquaculture, 262: 470-480.

ISANI G., ANDREANI G., MONARI M., CARPENČ E. 2003. *Metal concentrations (Cu, Zn and Cd) and metallothionein expression in Sparus aurata exposed to waterborne copper*. J. Trace Elem. Med. Biol., 17: 17-23.

ISANI G., ANDREANI G., CARPENČ E., DI MOLFETTA S., ELETTO D., SPISNI E. 2011. *Effects of waterborne Cu exposure in gilthead sea bream (Sparus aurata): A proteomic approach*. Fish Shellfish Immunol., 31: 1051-1058.

KRYSKO O., STEVENS M., LANGENBERG T., FRANSEN M., ESPEEL M., BAES M. 2010. *Peroxisomes in zebrafish: distribution pattern and knockdown studies*. Histochem. Cell Biol., 134: 39-51.

MINGHETTI M., LEAVER M.J., CARPENČ E., GEORGE S.G. 2008. *Copper transporter 1, metallothionein and glutathione reductase genes are differentially expressed in tissues of sea bream (Sparus aurata) after exposure to dietary or water-borne copper*. Comp. Biochem. Physiol. C Toxicol. Pharmacol., 147: 450-459.

NOVIKOFF A.B., NOVIKOFF P.M. 1973. *Microperoxisomes*. J. Histochem. Cytochem., 21: 963-966.

SEILIEZ I., PANSERAT S., SKIBA-CASSY S., ., VACHOT C., KAUSHIK S., TESSERAUD S. 2008. *Feeding status regulates the polyubiquitination step of the ubiquitin-proteasome-dependent proteolysis in rainbow trout (Oncorhynchus mykiss) muscle*. J. Nutr., 138: 487-491.

SPEARE D.J., FERGUSON H.W. 2006. *Systemic pathology of fish*. 2th edn. Scotian Press, London; 25-62.

WALSH P.J., WANG Y., CAMPBELL C.E., DE BOECK G., WOOD C.M. 2001. *Patterns of nitrogenous waste excretion and gill urea transporter mRNA expression in several species of marine fish*. Mar. Biol., 139: 839-844.

WHYTE M.P. 2010. *Physiological role of alkaline phosphatase explored in hypophosphatasia*. Ann. New York Acad. Sc., 1192: 190-200.

NON-INVASIVE QUALITY DETERMINATION OF SPINACH UNDER SIMULATED SALE CONDITIONS AND PREDICTION OF POSSIBLE CHANGES

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Abstract

Chlorophyll fluorescence, especially the optimum quantum yield Fv/Fm, is used to quantify the physiological state of plants, because stress, infections or wilting may lower the optimum quantum yield. The characteristics of chlorophyll fluorescence have attracted our attention for its use to determine the quality of spinach, because the measurement of Fv/Fm during storage can be performed quickly and non-destructively, delivering information about spinach quality and its sale suitability.

Applicability of modern techniques for quick and non-invasive *in vivo* determination of such parameters as quality and freshness of spinach under simulated sale conditions was tested in our study. In this experiment, application of chlorophyll fluorescence for quantification of quality as well as physical and physiological changes which occur in fresh products was verified. The experiment was conducted on spinach, a product characterized by rapid appearance of postharvest losses. Spinach was harvested as leafy vegetable for direct consumption and stored for 5 days (16 h in a cooling room at 2-4˚C, 96-98% relative humidity, followed by 8 h at room temperature: *ca* 22ºC, 65% relative humidity). Variants were represented by different cultivation conditions (fertilizer, leaf application of Previcur, $CaCl_2$ and Cerone). The investigations revealed that fresh mass losses in the postharvest phase could be described by changes of Fv/Fm spinach leaves adapted to darkness by a linear relationship of parameters (r=0.77). The disadvantage of chlorophyll fluorescence measurement was the less precise determination of the critical loss of fresh mass of 3%. Instead, losses of 4 to 5% resulted in a the decrease of optimum quantum yield below the critical value of 0.80, which indicates the occurrence of postharvest stress. Leaf application

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of pesticides negatively influenced Fv/Fm. This relationship may be used to detect the application of plant protection products shortly before harvest, before the grace period is over. Hence, the determination of freshness using chlorophyll fluorescence offers, to a limited extent, protection of consumers.

Further investigations showed that optimum quantum yield was linearly and positively correlated with the calcium content in spinach leaves. This relationship may indicate worse cell membrane integrity and reduced cell wall stability postharvest. Additionally, the decrease of Fv/Fm during storage may indicate the occurrence of cold stress as well as altering processes (senescence) and loss of quality of spinach, independent of its cultivation method. In these investigations, the relationship between chlorophyll fluorescence and color changes of leaves, content of chlorophyll, total nitrogen and water soluble carbohydrates, as well as respiration intensity and ethylene emission could not be found.

NIEINWAZYJNE OZNACZANIE JAKOŚCI SZPINAKU PODCZAS SYMULOWANYCH WARUNKÓW SPRZEDAŻY ORAZ PRZEWIDYWANIE MOŻLIWYCH ZMIAN

Abstrakt

Fluorescencja chlorofilu, w szczególności optymalne wykorzystanie kwantów Fv/Fm, stosowana jest do oznaczania stanu fizjologicznego roślin, gdzie występowanie stresu, chorób czy procesów więdniecia może spowodować zmniejszenie optymalnego wykorzystania kwantów. W praktyce fluorescencja chlorofilu ma specjalne zastosowanie, ponieważ pomiary podczas przechowywania mogą zostać przeprowadzone szybko i bezdestrukcyjnie, natychmiast informując o stanie jakości szpinaku oraz jego przydatności do sprzedaży.

Możliwość zastosowania nowoczesnych technik w celu szybkiego i nieinwazyjnego oznaczenia *in vivo* takich parametrów, jak jakość i świeżość szpinaku podczas symulowanych warunków sprzedaży, stanowiła tematykę badań. W badaniach zweryfikowano zastosowanie fluorescencji chlorofilu do określenia zmian jakościowych, fizycznych i fizjologicznych, zachodzących w świeżym produkcie odznaczającym się szybkim występowaniem strat pozbiorczych – szpinaku będącym warzywem liściowym przeznaczonym do bezpośredniego spożycia, składowanym 5 dni (16 h w chłodni: 4ºC, 96% wilg. wzgl.; 8 h w temperaturze pokojowej: 22-23ºC, 70% wilg. wzgl.), z uwzględnieniem różnych warunków uprawy (nawożenie, stosowanie dolistne Previcuru, $CaCl_2$ oraz Cerone). Badania wykazały, że straty świeżej masy w fazie pozbiorczej mogły zostać opisane za pomocą zmian Fv/Fm zmierzonych w przystosowanych do ciemności liściach szpinaku ze względu na liniową zależność parametrów (r^2=0,77). Negatywny aspekt pomiaru fluorescencji chlorofilu stanowiła mała dokładność określenia krytycznej dla jakości handlowej szpinaku granicy strat świeżej masy wielkości 3%. Dopiero straty od 4 do 5% spowodowały zmniejszenie optymalnego wykorzystania kwantów poniżej krytycznej granicy 0,80, wskazującej na wystąpienie stresu pozbiorczego. Ponadto dolistne zastosowanie pestycydów negatywnie wpłynęło na pomiar Fv/Fm, co jednak może znaleźć zastosowanie w wykrywaniu stosowania środków ochrony roślin na krótko przed zbiorem, jeszcze przed upływem okresu karencji. Dlatego też oznaczenie stopnia świeżości za pomocą fluorescencji chlorofilu umożliwia również, w pewnym stopniu, ochronę konsumenta. Dalsze badania wykazały, że optymalne wykorzystanie kwantów Fv/Fm skorelowane było liniowo pozytywnie z zawartością wapnia w liściach szpinaku. Zależność ta wskazywałaby na zmniejszenie zarówno integracji błon komórkowych, jak i zmniejszenie stabilizacji ścian komórkowych podczas okresu pozbiorczego. Ponadto zmniejszenie wartości Fv/Fm podczas przechowywania może wskazywać na wystąpienie stresu

chłodu i procesów starzenia (senescencji) oraz utraty jakości badanego szpinaku, niezależnie od sposobu jego uprawy. W badaniach nie stwierdzono zależności między fluorescencją chlorofilu a zmianami barwy liści szpinaku, zawartością chlorofilu, azotu ogółem i węglowodanami rozpuszczalnymi w wodzie oraz intensywnością oddychania i emisją etylenu.

INTRODUCTION

Vegetables, especially their leaves, are highly perishable commodities. Harvested plants or plant organs must still be regarded as living tissue that is subjected to continuous changes with respect to its metabolism. They require energy for maintenance of cell organisation, membrane permeability, metabolism and metabolite transport within the tissue. Instead of photosynthetic assimilates, the required energy in harvested plants is supplied by oxidative breakdown of certain organic substances stored in the tissue (KEUTGEN 2000). From the consumer's point of view, most of the postharvest changes are undesirable because they result in plant and tissue senescence as well as tissue breakdown. They cause freshly harvested vegetables to deteriorate, shrivel, loose the green colour and to turn yellow due to chlorophyll degradation and lead to distinct shifting of sensory quality, especially visual deterioration and loss of palatability. In consequence, quality and shelf-life sustainability of leafy vegetables depends strongly on their vitality (FERRANTE, MAGGIOREA 2007). Recently, new kinds of *in vivo* analyses have provided detailed information on intrinsic value and storage as well as processing effects on fruits and vegetable (RUIZ-ALTISENTA et al. 2010, PAPAGEORGIOUA, GOVINDJEE 2011). These include the option of on-line control of quality characteristics of vegetable products during cultivation, storage and manufacture. Quality control as well as quality and shelf-life prediction are of prime importance to guarantee an optimal product. In practice, quality analyses should be fast, simple, robust, reproducible and reliable. Ideally, they should simultaneously describe chemical and physical parameters and, of course, should be non-destructive.

Recently, consumers focus not only on a good-looking product, but also ask for an excellent quality with regard to the nutritive value. Quality evaluation is a tool to ensure that new and existing products meet the consumers' requirements. New kinds of analyses should provide detailed information about ingredients and the effects of storage and processing. This includes the option of using on-line instruments to control the quality characteristics of vegetable products during cultivation, storage and manufacture. Quality control as well as quality and shelf-life prediction are of prime importance to guarantee an optimal product quality. In practice, quality analyses must be fast, simple, robust, reproducible and reliable. Ideally, they should simul-

taneously describe chemical and physical parameters and, of course, should be non-destructive. Chlorophyll fluorescence is a technique with a great potential to fulfill these requirements. It enables non-destructive measurements to determine a plants' physiological state, its vitality and its 'stress history'. It describes not only visible damages but also an eventual early stage of deterioration a few days before visible damage symptoms occur. In addition, chlorophyll fluorescence is able to identify effects of potentially toxic substances such as pesticides that have been applied to a plant a few days before harvest. To summarize, the chlorophyll fluorescence technique can be used to record the effect of biotic and abiotic stress factors (KEUTGEN 2000, RUIZ-ALTISENTA et al. 2010, PAPAGEORGIOUA, GOVINDJEE 2011), to predict changes of nutritive characters, the maturity state of the harvested or stored plant organ and thus shelf-life (DEELL et al. 1999, KEUTGEN 2000). Chlorophyll fluorescence potentially allows studies that range from maturity assessment to the prediction of quality attributes during the postharvest period and effects of postharvest stress on product quality.

Contemporary research into postharvest physiology is focusing on the development of instrumental methods for non-destructive quality assessment. This kind of research is empirical, correlating analytical parameters with quality properties for single products under variable conditions. This approach is quite helpful in developing new methods to monitor and control production and storage processes. However, in many cases a better understanding of the plant's physiological and metabolic basis for quality degradation is needed to identify relationships that are determined by vegetable quality but not by pre- or postharvest environmental conditions. It is the aim of the proposed research project to develop objective quality criteria for spinach and broccoli using non-destructive methods for vegetable quality assessment.

MATERIAL AND METHODS

Spinach plants (*Spinacia oleracea* L. cv. San Felix F1) were grown on experimental fields at Marhof Research Station near Bonn at two sowing dates: 2nd and 16th April of two consecutive years. Two different forms of nitrogen were applied as fertiliser (220 kg N ha^{-1}) with 27% of nitrogen, where ammonium nitrate (KAS) consisted of 13.5% nitrate and 13.5% ammonium, whereas basammon-nitrogen (BAS) consisted of 6.8% nitrate, 18.2% ammonium and 2% nitrification blocker Dicyandiamid (DCD), respectively. Plants were cultivated on brown soil (70-78 soil points), with a thickness of 0.75 to 1.20 m, situated on sand and gravel, of middle water permeability and of middle to high water storage capacity, with the ground water level at 13 m depth. The soil (0-30 cm) was characterized by an pH$_{KCl}$ – value of 6.6, P$_2$O$_5$ 0.43 g kg^{-1} soil, K$_2$O 0.24 g kg^{-1} soil, and MgO 18 g kg^{-1} soil. On

average the content of nitrate (V) and ammonia before fertilization were 39.36 ± 12.87 and 17.4 9.0 kg N ha^{-1}, respectively. No additional fertilization with phosphorus and potassium were applied. During the growing period from April to June the mean temperatures of the air was 15.7ºC and of the soil in the depth of 10 cm of 13.8ºC (depth of mean root mass of spinach). The mean precipitation in the vegetation time was on average 221 mm. Depending on spinach requirements, automatic watering from a computer controlled pouring car at the tensiometer value of -120 mb was applied (150 ml of water per plant). Spinach was harvested twice from each experimental field (22nd and 27th May, 4th and 9th June, respectively) and kept under simulated sale conditions for 5 days. Prior to the experiments, harvested spinach was washed in cool tap water at about 12ºC and drained using a hand centrifuge. Clean spinach was placed in Euro-Pool-System-Boxes D. Over night (16 h) plants were stored in darkness in closed 400 l containers at 2-4ºC and 96-98% relative humidity. To establish high humidity in the containers, air was forced with a pump through distilled water. During the day (8 h), spinach was kept in diffuse daylight at room temperature of about 22ºC and 65% relative humidity similar to conditions on the fresh market. Beside the kind of fertilisers, other experimental factors were different leaf applications during the growth period: Previcur 0.2% (active substance: propamocarb-HCl 66.5%) against *Peronospora* sp., $CaCl_2$ water solution (15, 30, 45, 60 mmol dm^{-3}) for quality improvement (PERUCKA et al. 2007), and Cerone as a precursor of ethylene (50, 100, 150, 200 mmol dm^{-3}) in the amount of 2 dm^{-3} m^{-2} each. Samples of 80 spinach plants were used for each combination (4 replicates with 20 plants each). Analyses were carried out during storage to investigate quality changes continuously. Samples for chlorophyll fluorescence measurement and for chemical analyses were taken from the same plant (rosette). Water loss was determined for all plants within a single Euro-Pool-System-Box.

Chlorophyll fluorescence measurements were conducted in a photosynthesis laboratory at the temperature of 25ºC and atmospheric CO_2 between 400-600 ppm, using a portable pulse-amplitude-modulation Fluorometer PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany). The plant tissues were dark-adapted for 30 min prior to the analyses. Fo (minimal fluorescence), Fm (maximal fluorescence), Fv (= Fm-Fo, variable fluorescence) and optimum quantum yield (= Fv/Fm) were determined three times on each representative of fully expanded and old leaves, with at least four replicates per treatment. For setting of Fo, the leaf was illuminated with low modulated light (650 nm, <0.1 µmol m^{-2} s^{-1}) and then for Fm after a light pulse (665 nm, 3200 µmol m^{-2} s^{-1}) (KEUTGEN 2000).

To detect colour changes and discoloration, the colour attributes were determined daily, recorded in L*, a*, b* CIE co-ordinates of the spinach leaves using a Chroma-Meter II Reflectance, Minolta, Japan. On each leaf blade, three measurements were taken between the main veins of a leaf.

The leaf samples for intrinsic compounds were freeze-dried and ground for chlorophyll, total N and carbohydrate analyses. Chlorophyll content (Chl a, Chl b and total Chl) was determined according to WELLBURN (1994) from methanol extracts and measured at 649 nm and 665 nm using a spectrophotometer Lambda 5/15 (Perkin-Elmer). Residues of the methanol extractions were used for starch analyses. Starch was enzymatically hydrolysed to glucose, which was detected by HPLC (Knauer, Berlin, Germany). Starch content was calculated according to LOPEZ-HERNANDEZ et al. (1994). Soluble carbohydrate content was determined from water extracts using HPLC as described by KEUTGEN (2000). Total N was determined by a modified Kjeldahl method through reduction of the organic and inorganic N into ammonium by acid hydrolysis (CHEN et al. 1997). Content of total N was measured colorimetrically in a SFAS - 5100 Skalar Auto-Analyser (Skalar GmbH, Erkelenz, Germany).

The experimental design was a completely randomised one. The data were analysed using correlation and multiple regression procedures of the statistical program SPSS for Windows, release 9.01. standard version, Copyright SPSS Inc., 1989-1999. Significances of differences were defined at P=0.05.

RESULTS AND DISCUSSION

The application of chlorophyll fluorescence for the estimation of concentration of intrinsic parameters in vegetables is a less investigated issue. Investigations on different plants indicate a correlation between chlorophyll fluorescence and content of nitrogen, ascorbic acid, carotenoids or soluble sugars and starch (PAPAGEORGIOUA, GOVINDJEE 2011). In spinach, quality changes during storage could at least partially be detected using chlorophyll fluorescence of dark-adapted leaves. During the storage under simulated sale conditions, a decrease of the optimum quantum yield Fv/Fm (Figure 1) was detected. On the third day of the storage, a drop of Fv/Fm below the value of 0.80 was observed. For comparison, values between 0.82 and 0.80 are typical of healthy, fresh plants. A decrease of Fv/Fm may also indicate the occurrence of cold stress during the storage time and/or the loss of membrane integrity due to senescence processes. The optimum quantum yield Fv/Fm was identified as a meaningful parameter, because the relationship between Fv/Fm and fresh mass losses was significantly linear (Figure 2; r=0.77). The fresh mass loss represents an important, if not the main parameter for the quantification of spinach freshness in practice. By definition, a fresh mass loss of 3% during the storage represents a critical value. With higher losses, spinach is regarded as non-marketable. In the present experiment, wilting symptoms and deterioration of spinach quality did not
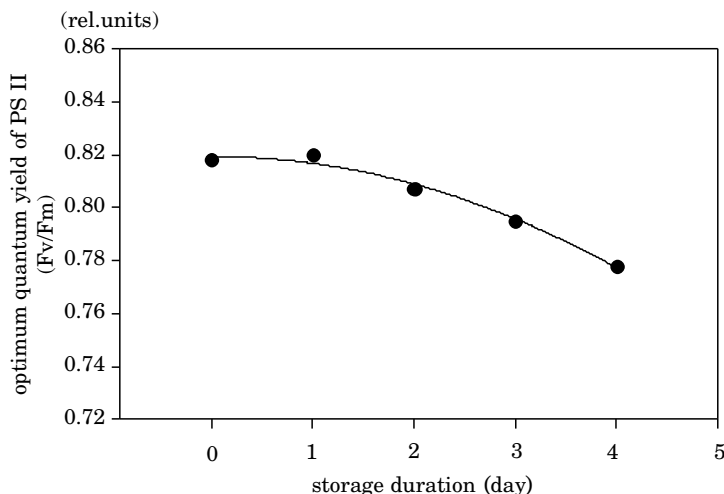
Fig. 1. Changes of optimum quantum yield of PS II (Fv/Fm) in spinach under simulated sale conditions (mean values of 4 experiments)
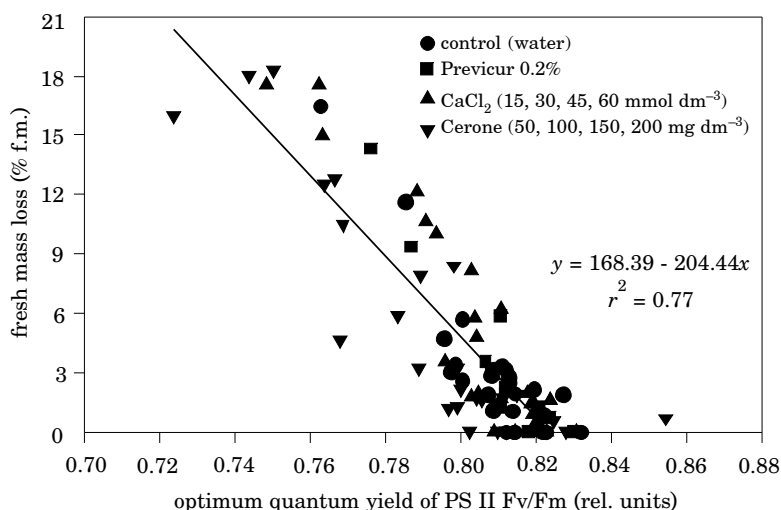

Fig. 2. Linear relationship between the optimum quantum yield of PS II (Fv/Fm) and fresh mass losses in spinach leaves during storage under simulated sale conditions (values are means of 4 experimental replications)

become evident before the third day, when the fresh weight loss reached 5-6%. During the storage of spinach under simulated sale conditions (16 h cool storage/8 h sale keeping), a loss of 4-5%, which is close to the critical value of 3%, corresponded with values for the optimum quantum yield at or below 0.80.

The correlation between optimum quantum yield of PS II and calcium content was positive and high as well as independent on used cultivation technique (Figure 3). A low content of calcium coincided with a low value of Fv/Fm. Spinach plants with the calcium content below 9 g kg^{-1} of dry matter were already non-marketable during the storage under simulated sale conditions. This relation may be explained by the significance of calcium in cell walls and cell membranes, where it supports the anti-senescence reactions. Ca^{2+} is important for the selective permeability and membrane integrity (PALTA 1996). Furthermore, Ca^{2+} stabilizes the cell membranes by linking the phosphate- and carboxylic groups of phospholipids present on the membrane surface. The fact that the lower optimum quantum yield coincides with a low content of calcium may indicate an impairment of membrane integrity.
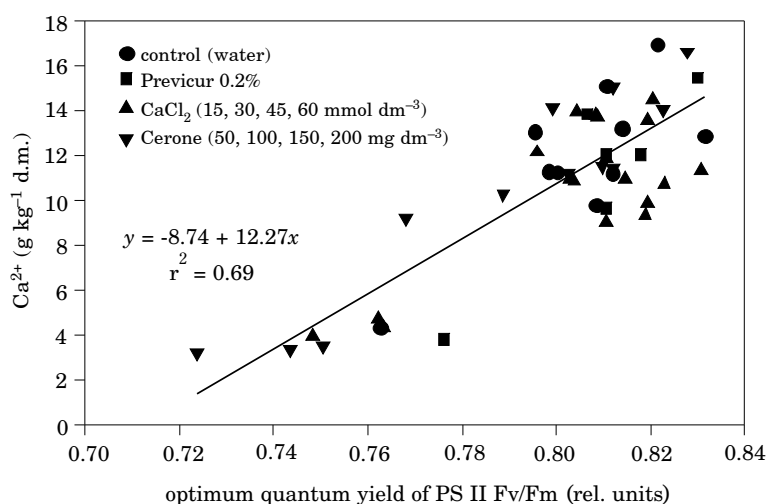
Fig. 3. Correlation between optimum quantum yield of PS II (Fv/Fm) and content of calcium in spinach leaves stored under simulated sale conditions (mean values of 4 experiments)

Considering all measurements of the investigated variants, the relationship between Fv/Fm and starch content in leaves was best explained by a non-linear function (Figure 4). In the case of a starch content up to 10 g kg^{-1} of dry matter, the relationship showed linear character. Based on these results it can be concluded that spinach leaves with a starch content higher than 5 g kg^{-1} of dry matter will exhibit good shelf-life behavior. However, the content of starch in spinach is quite low compared to the other carbohydrates. Nevertheless, this parameter seems to be suitable for the prediction of shelf-life.
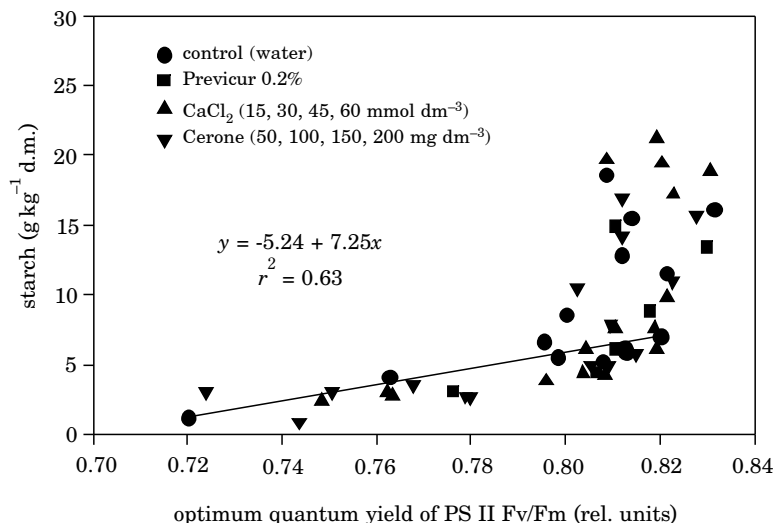
Fig. 4. Linear relationship between optimum quantum yield of PS II (Fv/Fm) and starch content in spinach leaves stored under simulated sale conditions (values are means of 4 experimental replications)

The investigations did not reveal any kind of relationship between chlorophyll fluorescence parameters and respiration intensity, ethylene emission, changes of leaf colour and intrinsic parameters such as total nitrogen, soluble carbohydrate and chlorophyll content. Indeed, chlorophyll fluorescence depends on the chlorophyll content. However, the optimum quantum yield is not directly correlated with the latter. Primarily, a high chlorophyll loss causes a decrease of Fv/Fm (KEUTGEN 2000). This can at least partly explain why no correlation between the chlorophyll content and Fv/Fm was observed in the presented investigations. In the present work, chlorophyll losses during storage were quite low.

CONCLUSIONS

The investigations showed that the quality of spinach during the storage under simulated sale conditions can be evaluated quickly and easily by chlorophyll fluorescence. In commercial practice, freshness is quantified by the water loss during storage. At a fresh mass losses of 3%, spinach is classified as non-marketable. The chlorophyll fluorescence, especially the optimum quantum yield Fv/Fm, is a good tool for the determination of spinach quality state independently from cultivation techniques. Fv/Fm < 0.80 corresponded to a loss of fresh mass of 4 to 5% and more under simulated

sale conditions. The Fv/Fm value can be lowered by cold stress, pesticides or infections, which can be used for the protection of interests of consumers and retailers. However, it is important to mention that successful application of chlorophyll fluorescence as an indicator of quality requires very good and broad knowledge of postharvest physiology, especially of the quality parameter changes combined with the maturity and senescence of the investigated plant.

REFERENCES

CHEN K., HU G., LENZ F. 1997. *Effects of CO_2 concentration on strawberry*. V. *Macronutrient uptake and utilization*. Angew. Bot., 71: 189-194.

DEELL J., VAN KOOTEN O., PRANGE R., MURR D. 1999. *Application of chlorophyll fluorescence. Techniques in postharvest physiology*. Hortic. Rev., 23: 69-107.

FERRANTE A., MAGGIOREA T. 2007. *Chlorophyll a fluorescence measurements to evaluate storage time and temperature of Valeriana leafy vegetables*. Postharvest Biol. Tech., 45(1): 73-80.

KEUTGEN A. 2000. *Modification on spinach quality by selected pre- and postharvest treatments*. Beiträge zu Agrarwissenschaften 24, Witterschlick/Bonn: Verlag P. Wehle.

LOPEZ-HERNANDEZ L., GONZALEZ-CASTRO M. J., VAZQUEZ-BLANCO M. E., VAZQUEZ-ODERIZ M. L., SIMAL-LOZANO J. 1994. *HPLC determination of sugar and starch in green beans*. J. Food Sci., 59(5): 1048-1049.

PALTA J.P. 1996. *Role of calcium in plant responses to stress: linking basic research to the solution of practical problems*. Hort Sci., 31: 51-57.

PAPAGEORGIOUA G.C., GOVINDJEE 2011. *Photosystem II fluorescence: Slow changes – Scaling from the past*. J. Photoch. Photobio. B, Special Issue on Recent Progress in the Studies of Structure and Function of Photosystem II, 104 (1-2): 258-270.

PERUCKA I., NURZYŃSKI J., OLSZÓWKA K. 2007. *Influence of $CaCl_2$ on mineral nutrients content in lettuce leaves*. J. Elementol., 12(3): 181-190.

RUIZ-ALTISENTA M., RUIZ-GARCIAA L., MOREDAA G.P., LUB R., HERNANDEZ-SANCHEZA N., CORREAA E.C., DIEZMAA B., NICOLAĎC B., GARCÍA-RAMOSD J. 2010. *Sensors for product characterization and quality of specialty crops-A review*. Comput. Electron. Agric., 74(2): 176-194.

WELLBURN A. R. 1994. *The spectral determination of chlorophylls a and b, as well as carotinoids, using various solvents with spectrophotometers of different resolution*. J. Plant Physiol., 144: 307-313.

THE EFFECT OF DIFFERENT DIETARY SODIUM LEVELS ON BLOOD ELECTROLYTES, GROWTH PERFORMANCE AND FOOT PAD DERMATITIS INCIDENCE IN TURKEYS*

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Abstract

The objective of this study was to determine the effect of different dietary sodium levels on serum macroelement concentrations, growth performance and incidence of foot pad dermatitis (FPD) in female turkeys raised to six weeks of age. The influence of a low-sodium diet (without additional sodium) and diets supplemented with NaCl to increase sodium content by 0.5, 1.0, 1.5, 2.0 and 2.5 g kg^{-1} was compared. The dietary electrolyte balance (DEB) was high (242-248 mEq kg^{-1}) due to a high potassium content (11.0 g kg^{-1}).

Turkeys fed the low-sodium diet were characterized by significantly lower concentrations of chloride ($p=0.002$) and phosphorus ($p<0.001$), and significantly higher magnesium levels ($p<0.001$), compared with the other groups.

The lowest dietary inclusion of sodium (0.5 g kg^{-1}) contributed to a significant increase in the body weights of turkeys at six weeks of age ($p<0.001$), and it improved feed conversion efficiency ($p<0.001$) in comparison with the low-sodium diet. No significant differences in body weight and feed conversion ratio followed experimental treatments with higher dietary NaCl addition. Diets supplemented with different amounts of sodium had no effect on litter moisture content, whereas the incidence of FPD increased significantly following the addition of dietary sodium at 1.0 g kg^{-1} to 2.5 g kg^{-1}, compared with the low-sodium diet. The results of our study indicate that moderate sodium supplementation (0.5 g kg^{-1}) of a sodium-deficient diet significantly increased the body weights of young

turkeys, while it did not increase the incidence of FPD. A further increase in the sodium content of experimental diets did not increase the body weights of birds, and it enhanced the occurrence of FPD which was not related to increased litter wetness.

Key words: sodium, blood minerals, growth performance, turkeys.

WPŁYW ZRÓŻNICOWANEGO POZIOMU SODU W DIECIE NA POZIOM ELEKTROLITÓW KRWI, WZROST INDYKÓW I WYSTĘPOWANIE ZAPALENIA SKÓRY PODUSZKI STOPY

Abstrakt

Celem badań było określenie wpływu różnych poziomów sodu w diecie na zawartość makroelementów w surowicy, wzrost ptaków i występowanie zapalenia skóry stóp (FPD) u indyczek w wieku sześciu tygodni życia. Porównano efekty podawania diety niskosodowej (bez dodatkowego źródła sodu) i diet z dodatkiem NaCl w ilości 0,5; 1,0; 1,5; 2.0 i 2,5 g kg^{-1}. Ze względu na wysoką zawartość potasu w dietach (11,0 g kg^{-1}), bilans elektrolitów (DEB) był również wysoki (242-248 mEq kg^{-1}).

U indyków żywionych dietą o niskiej zawartości sodu wykazano znacznie niższy poziom chlorku ($p=0,002$) i fosforu ($p<0,001$), a znacznie wyższy poziom magnezu ($p<0,001$) w surowicy krwi, w porównaniu z innymi grupami. Niewielkie zwiększenie zawartości Na w diecie (0,5 g kg^{-1}) spowodowało istotny wzrost masy ciała indyków w wieku sześciu tygodni ($p<0,001$) i poprawę wykorzystania paszy ($p<0,001$), w porównaniu z dietą bez dodatku Na. Nie stwierdzono istotnych różnic w masie ciała i wykorzystaniu paszy u indyków otrzymujących diety z dodatkiem NaCl. Dodatek NaCl do diety nie wpłynął na wilgotność ściółki, natomiast występowanie FPD istotnie nasiliło się w grupach otrzymujących dodatek 1,0 i 2,5 g kg^{-1} Na, w porównaniu z dietą bez dodatku sodu.

Stwierdzono, że umiarkowany dodatek sodu (0,5 g kg^{-1}) do diety ubogiej w ten pierwiastek istotnie zwiększa masę ciała młodych indyków, a nie zwiększa częstości występowania FPD. Dalszy wzrost zawartości sodu w diecie nie zwiększa masy ciała ptaków i wilgotności ściółki, a nasila występowanie FPD.

Słowa kluczowe: sód, elektrolity we krwi, wyniki odchowu, indyki.

INTRODUCTION

The optimization of dietary mineral balance so as to meet the requirements of growing birds is an important consideration in modern intensive poultry farming. The most common reason for insufficient bone mineralization leading to skeletal deformities (including leg bone abnormalities) are mineral metabolism disorders, in particular a deficiency of calcium and phosphorus (RAO et al. 2003, VENÄLÄINEN et al. 2006, MAJEWSKA et al. 2009). Leg deformities and abnormalities adversely affect the welfare and growth rate of birds, reduce their activity and decrease feed consumption (NÄÄS et al. 2009). In turkeys, leg problems result for instance from selection for a rapid increase in body weight and breast muscle yield (CRESPO et al. 2000, MIKULSKI et al. 2009). The fast growth rate of turkeys is often accompanied by nutrient and vitamin D deficiencies (FARUGA et al. 2009, TATARA et al. 2011, ZHOU et al. 2011).

A high litter moisture content due to nutritional factors may lead to leg diseases, including foot pad dermatitis, in growing birds (FRANCESCH, BRUFAU 2004, MAYNE et al. 2007). The dietary sodium intake is often increased in broilers to stimulate their growth (VIERA et al. 2003, BORGES et al. 2003, JANKOWSKI et al. 2011a, b), which results in excess litter wetness. Numerous experiments on chickens have shown that diets with an increased sodium content lead to higher water consumption followed by a higher excreta moisture content (MURAKAMI et al. 2001, BORGES et al. 2003, MUSHTAQ et al. 2007). When compared to chickens, there is a scarcity of data regarding the effects of increased dietary sodium intake in turkeys, with respect to the growth rate of birds, mineral metabolism disorders and incidence of FPD. The above problem is of particular importance in young turkeys fed soybean-based diets rich in potassium. An increased potassium content of feed components increases DEB, also in low-sodium diets (JANKOWSKI et al. 2011a,b), which may have a negative impact on production results in growing turkeys.

The objective of this study was to determine the effect of different inclusion levels of sodium in potassium-rich diets with high DEB on serum macroelement concentrations, growth performance, and incidence of foot pad dermatitis (FPD) in turkeys raised to six weeks of age.

MATERIAL AND METHODS

The experiment was carried out at the Research Laboratory of the Department of Poultry Science, University of Warmia and Mazury in Olsztyn, on 336 female BUT-10 turkeys kept for six weeks in three-tier battery cages, each with a floor area of 0.5 m^2. The turkeys were randomly divided into six groups of eight replicates, each of seven birds. The temperature and lighting program was consistent with the recommendations of the British United Turkeys Ltd. The birds were fed *ad libitum* and they had free access to water. The composition and nutritional value of low-sodium (without sodium addition) basal diets (1-3 weeks and 4-6 weeks) are presented in Table 1. Basal diets were mixed thoroughly with a premix prepared under laboratory conditions (at a ratio of 99:1), which contained precisely determined amounts of NaCl, so as to increase the sodium content of diets by 0.5, 1, 1.5, 2 and 2.5 g kg^{-1}, relative to the control low-sodium diet. Samples of experimental diets were assayed for the content of sodium and potassium by atomic absorption spectrometry, and chloride – by the biamperometric technique. The analyses were performed at the National Feed Laboratory in Lublin (Poland), and their results were used to calculate the dietary electrolyte balance (DEB), with the following formula: DEB = Na mEq kg^{-1} + K mEq kg^{-1} – Cl mEq kg^{-1} (MONGIN 1981).

Table 1

Composition and calculated nutrient content of basal starter (1-3 weeks) and grower
(4-6 weeks) diets (%)

Specification	1 - 3 weeks	4-6 weeks
Composition (%):		
wheat	26.22	34.47
maize	20.00	18.00
soybean	41.70	36.50
potato protein	5.00	4.45
soybean oil	2.17	2.20
limestone	1.62	1.45
monocalcium phosphate	2.27	2.02
L-Lysine 99 MonohydroCL	0.28	0.25
DL-Methionine 99	0.24	0.21
Premix[1]	0.50	0.45
Nutritional value (calculated):		
ME (kcal kg^{-1})	2801	2851
crude protein (%)	27.99	25.98
lysine (%)	1.75	1.58
methionine (%)	0.66	0.60
Met + Cys (%)	1.10	1.02
threonine (%)	1.08	0.98
tryptophan (%)	0.35	0.32
Ca (%)	1.26	1.12
available P (%)	0.65	0.60
Na (%)	0.03	0.02

[1]Content per kg of premix: vitamin A – 2 400 000 IU, vitamin D_3 – 1 000 000 IU, vitamin E
– 20 g, vitamin K_3 – 1.2 g, vitamin B_1 – 1 g, vitamin B_2 – 1.6 g, vitamin B_6 – 1.2 g, vitamin
B_{12} – 6 mg, biotin (H) – 60 mg, Fe – 2 g, Mn – 24 g, Zn – 22 g, Cu – 4 g, J – 600 mg, Se – 60 mg,
panthotenic acid – 5 g, nicotinic acid – 16 g, folic acid – 600 mg, choline chloride – 80 g

On day 40, excreta samples were collected underneath the cages in each
group, for three hours, to determine the moisture content of droppings. On
day 42, the incidence and severity of foot pad dermatitis (FPD) were as-
sessed based on the scoring system developed by HOCKING et al. (2008).

At 42 days, all turkeys were weighed individually and seven birds repre-
senting an average body weight of each group were selected for blood sam-
pling from the jugular vein to determine the concentrations of selected ele-

ments. The analyses were performed by the Analytical Laboratory at the Municipal Hospital in Olsztyn (Poland), using the COBAS INTEGRA 400 PLUS system (Roche, France). The concentrations of Ca, P and Mg were determined by the colorimetric method, and the levels of Na, K and Cl – by indirect potentiometry.

The results were processed statistically by one-way analysis of variance (ANOVA) in an orthogonal design. The significance of differences was estimated by Duncan's test. The calculations were performed using the Statistica software package ver. 8.1.

RESULTS AND DISCUSSION

The concentrations of sodium, chloride and potassium in experimental diets are shown in Table 2. Our analysis of the sodium content of diets revealed that it corresponded to the assumed dietary sodium levels but differed (lower or higher) from the value of 1.7 g kg^{-1} recommended for the youngest turkeys (NRC 1994). The concentrations of chloride (1.7-5.9 g kg^{-1}) and potassium (11.0 g kg^{-1}) in the experimental diets were higher than the respective nutrient requirements of young turkeys, determined at 1.5 g kg^{-1} for Cl and 7 g kg^{-1} for K (NRC 1994). DEB was high (235-249 mEq kg^{-1}), similar to the value recommended for broiler chickens (MONGIN 1981) and higher than the value of 211 mEq kg^{-1} recommended for turkeys (NRC 1994). High DEB in poultry diets results from a high potassium content

Table 2

Sodium supplementation, sodium, potassium, chloride content (g kg^{-1}) and dietary electrolyte balance (DEB, mEq kg^{-1}) in experimental diets

Content in diet (g kg^{-1})	Na addition (g kg^{-1})					
	0	0.5	1.0	1.5	2.0	2.5
1-3 weeks of feeding						
Na	0.34	0.85	1.34	1.94	2.31	2.82
K	11.0					
Cl	1.7	2.7	3.2	4.3	5.2	5.9
DEB (mEq kg^{-1})	248	242	249	245	235	246
4-6 weeks of feeding						
Na	0.31	0.78	1.21	1.75	2.08	2.72
K	10.03					
Cl	1.5	2.4	2.9	3.9	4.7	5.3
DEB (mEq kg^{-1})	233	228	234	229	222	238

of cereals and soybean meal, the major feed components (JANKOWSKI et al. 2011a). The potassium content of diets may be reduced by partial replacement of soybean meal with feed components of animal origin, including fish meals (MURAKAMI et al. 2000).

The serum concentrations of macroelements are presented in Table 3. Turkeys fed the low-sodium diet were characterized by lower sodium concentrations, significantly lower concentrations of chloride (p=0.002) and phosphorus (p<0.001), and significantly higher magnesium levels (p<0.001), compared with the other groups. The only statistically significant difference observed among groups of turkeys fed diets supplemented with NaCl was lower serum phosphorus concentration in birds receiving 1.0 and 2.5 g kg^{-1}

Table 3

Concentrations of selected minerals in blood serum

Mineral element	Na addition (g kg^{-1})						SEM	p
	0	0.5	1.0	1.5	2.0	2.5		
Na (mmol l^{-1})	141.4	149.6	148.3	149.7	145.7	147.6	1.11	0.256
Cl (mmol l^{-1})	104.7^b	110.3^a	110.1^a	109.3^a	109.7a	109.7a	0.483	0.002
K (mmol l^{-1})	3.56	3.85	3.55	3.28	2.71	3.27	0.112	0.069
Ca (mg dl^{-1})	13.73^a	13.27^{ab}	12.77^b	13.31^{ab}	13.29^{ab}	12.94^b	0.100	0.090
P (mg dl^{-1})	6.3^c	9.21^a	7.63^b	8.56^a	8.89^a	7.77	0.202	<0.001
Mg (mg dl^{-1})	2.83^a	2.34^b	2.37^b	2.37	2.41	2.46	0.035	<0.001

a,b, c – values with different superscript letters within a column are different at $P \leq 0.01$

additional sodium per kg feed. The results of studies investigating the effects of dietary sodium intake on blood electrolyte levels in chickens are inconclusive: an increased sodium content of diets had no influence on the serum concentrations of sodium and potassium (MUSHTAQ et al. 2005), it had no effect on blood calcium and chloride levels (OLANREWAJU et al. 2007), and it decreased the blood concentrations of sodium and potassium (MUSHTAQ et al. 2007). In an experiment similar to ours (TYKAŁOWSKI et al. 2011), a low-sodium diet decreased serum sodium and chloride levels, and increased calcium, magnesium and phosphorus concentrations, compared with chickens fed a sodium-supplemented diet. In the present study, the low-sodium diet surprisingly did not affect the serum Na concentration but decreased the Cl level in the serum.

The lowest dietary inclusion of sodium (0.5 g kg^{-1}) contributed to a significant increase in the body weights of turkeys at three and six weeks of age (p<0.001), in comparison with the low-sodium diet (Table 4). As a result, feed intake per kg body weight gain during 1-3 and 4-6 weeks was significantly (P<0.001) lower in the group fed a diet with the lowest sodium

Table 4

Body weight and feed efficiency ratio in turkeys fed diets with different
inclusion levels of sodium

Specification	Na addition (g kg^{-1})						SEM	p
	0	0.5	1.0	1.5	2.0	2.5		
Body weight (g)								
3 weeks	323^a	415^b	416^b	413^b	427^b	424^b	64	<0.001
6 weeks	1055^a	1474^b	1463^b	1478^b	1497^b	1443^b	259	<0.001
FCR (kg kg^{-1})								
1-3 weeks	2.09^a	1.75^b	1.76^b	1.81^b	1.80b	1.74^b	0.027	<0.001
4-6 weeks	2.45^a	2.02^b	2.02^b	1.99^b	2.03^b	2.15^b	0.031	<0.001
1-6 weeks	2.35^a	1.95^b	1. 97^b	1.94^b	1.97^b	2.04^b	0.027	<0.001

a,b, c – values with different superscript letters within a column are different at $P \leq 0.01$

addition, as compared to the group whose diet was not supplemented with
NaCl. No significant differences in body weight and feed conversion ratio
were found between groups of turkeys fed diets with different NaCl addi-
tion. In many experiments, the growth performance of birds and feed con-
version efficiency were improved when the sodium content of feed was in-
creased from 0.0 to 3.0 g kg^{-1} (OVIEDO-RONDON et al. 2001, VIEIRA et al. 2003,
BORGES et al. 2003, JANKOWSKI et al. 2011a, b). Such a trend was not observed
in our study, in which an increase in the body weights of young turkeys in
response to sodium supplementation at 0.5 g kg^{-1} was similar to that noted
when sodium addition was two- to five-fold higher.

There were no significant differences in excreta moisture content be-
tween turkeys fed diets with different sodium levels (Table 5). Different re-
sults were reported for broiler chickens at the final stage of rearing (MU-
RAKAMI et al. 2001, BORGES et al. 2003, MUSHTAQ et al. 2007). It seems that
relatively low feed consumption, as compared with the body weights of young
turkeys, does not produce the undesirable effect observed in broilers.

Table 5

Dry matter content of excreta (%) and foot pad dermatitis (FPD) scores in turkeys fed diets
with different inclusion levels of sodium

Specification	Na addition (g kg^{-1})						SEM	p
	0	0.5	1.0	1.5	2.0	2.5		
DM content	18.6	18.4	18.5	18.4	19.0	17.4	0.243	0.365
FPD	3.0c	3.2bc	3.4b	3.5b	3.8ab	4.0a	0.050	<0.001

a,b, c – values with different superscript letters within a column are different at $P \leq 0.01$

The incidence of FPD was the lowest in turkeys fed a NaCl-unsupplemented diet, significantly higher in the groups receiving diets supplemented with sodium at 1.0, 1.5 and 2.0 g kg^{-1}, and the highest in turkeys fed a diet with 2.5 g additional sodium per kg feed (in the latter case, insignificant only in comparison to the dietary level 2.0). This may suggest that a considerable increase in dietary sodium supply rather excess litter wetness raises the incidence of FPD in growing turkeys. The symptoms of FPD may intensify due to metabolic disorders caused by some diet ingredients, e.g. biogenic amines, and the specific composition of fat (MAYNE 2005).

CONCLUSIONS

Components of diets fed to young growing turkeys are sodium-deficient, while their potassium content and DEB values exceed the recommended levels. Such diets significantly decrease the concentrations of chloride and phosphorus and increase magnesium levels in the blood serum of turkeys. Moderate sodium supplementation (0.5 g kg^{-1}) of a low-sodium diet seems to be enough to increase the body weights of young turkeys, without raising the incidence of FPD. A further increase in the sodium content of the experimental diets did not increase the body weights of birds, but exacerbated the severity of FPD.

REFERENCES

BORGES S.A., FISCHER DA SILVA A. V., ARIKI J., HOOGE D.M., CUMMINGS K.R. 2003. *Dietary electrolyte balance for broiler chickens under moderately high ambient temperatures and relative humidities.* Poul. Sci., 82: 301-308.

CRESPO R., STOVER S.M., TAYLOR K.T., CHIN R.P., SHIVAPRASAD H.L. 2000. *Morphometric and mechanical properties of femora in young adult male turkeys with and without femoral fractures*. Poult. Sci., 79: 602-608.

FARUGA A., PYDYNKOWSKA K., JANKOWSKI J., KOZŁOWSKI K., PŁUDOWSKI P. 2009. *Comparison of the effect of cholecalciferol and 25-hydroxycholecalciferol on the characteristic features of femoral and tibia bones in male fattening turkeys.* Arch. Geflügelk., 74(4): 237-241.

FRANCESCH M., BRUFAU J. 2004. *Nutritional factors affecting excreta/litter moisture and quality*. World's Poult. Sci., J., 60: 64- 75.

HOCKING P.M, MAYNE R.K., ELSE R.W., FRENCH N.A., GATCLIFFE J. 2008. *Standard European footpad dermatitis scoring system for use in turkey processing plants*. World's Poult. Sci. J., 64(3): 323 – 328.

JANKOWSKI J., ZDUŃCZYK Z., JUŚKIEWICZ J., KWIECIŃSKI P. 2011a. *Effect of different dietary sodium levels on the growth performance of broiler chickens, gastrointestinal function, excreta moisture and tibia mineralization.* J. Anim. Feed Sci., 20: 93-106.

JANKOWSKI J., JUŚKIEWICZ J., ZDUŃCZYK Z., ŚMIECIŃSKA K., KWIECIŃSKI P. 2011b. *Effects of the inclusion level and source of dietary sodium from different sodium salts on performance and meat characteristics of broiler chickens*. Arch. Anim Nutr., 65(3):186-202.

MAJEWSKA T., MIKULSKI D., SIWIK T. 2009. *Silica grit, charcoal and hardwood ash in turkey nutrition*. J. Elementol., 14(3): 489-500.

MAYNE R.K. 2005. *A review of the aetiology and possible causative factors of foot pad dermatitis in growing turkeys and broilers*. World's Poult. Sci. J., 61: 256-267.

MAYNE R.K., ELSE R.W., HOCKING P.M. 2007. *High litter moisture alone is sufficient to cause footpad dermatitis in growing turkeys*. Brit. Poult. Sci., 48(5): 538-545.

MIKULSKI D., JANKOWSKI J., ZDUŃCZYK Z., WRÓBLEWSKA M., SARTOWSKA K., MAJEWSKA T. 2009. *The effect of selenium source on performance, carcass traits, oxidative status of the organism, and meat quality of turkeys*. J. Anim. Feed Sci., 18: 318-330.

MONGIN P. 1981. *Recent advantages in dietary anion-cations balance: Applications in poultry.* Proc. Nutr. Soc., 40: 285-294.

MURAKAMI A.E., SALEH E.A., WATKINS E., WALDROUP P.W. 2000. *Sodium sources and level in broiler diets with and without high levels of animal protein.* J. Appl. Poult. Res., 9: 53-61.

MURAKAMI A. E., OVIEDO-RONDON E. O., MARTINS E. N., PEREIRA M. S., SCAPINELLO C. 2001. *Sodium and chloride requirements of growing broiler chickens (twenty-one to forty-two days of age) fed corn-soybean diets.* Poult. Sci., 80: 289-294.

MUSHTAQ T., SARWAR M., NAWAZ H., ASLAM M. M., AHMAD T. 2005. *Effect and interactions of dietary sodium and chloride on broiler starter performance (hatching to twenty-eight days of age) under subtropical summer conditions.* Poult. Sci., 84: 1716-1722.

MUSTHAQ T., ASLAM MIRZA M., ATHAR M., HOOGE D.M., AHMAD T., AHMAD G., MUSHTAQ M.M.H., NOREEN U. 2007. *Dietary sodium and chloride for twenty-nine to forty-two-day-old broiler chickens at constant electrolyte balance under subtropical summer conditions.* Appl. Poult. Res., 16: 161-170.

NÄÄS I.A., PAZ I.C.L.A., BARACHO M.S., MENEZES A.G., BUENO L.G.F., AMLEIDA I.C.I., MOURA D.J. 2009. *Impact of lameness on broiler well-being.* J. Appl. Poult. Res., 8: 432-439.

NRC. 1994. *Nutrient requirements of poultry.* 9th rev. Ed. Ed. National Academy Press, Washington, DC.

OLANREWAJU H.A., THAXTON J.P., DOZIER W.A., BRANTON S.L. 2007. *Electrolyte diets, stress, and acid-base balance in broiler chickens*. Poult. Sci., 86, 1363-1371.

OVIEDO-RONDON E. O., MURAKAMI A.E., FURLAN A.C., MOREIRA I., MACARI M. 2001. *Sodium and chloride requirements of young broiler chickens fed corn-soybean diets (one to twenty-one days of age).* Poult. Sci., 80: 592-598.

RAO R.S.V., RAJU M.V.L.N., SHARMA R.P., NAGALAKSHMI D., REDDY M.R. 2003. *Lameness in chickens: alleviation by dietary manipulation.* Poult. Intern., 42: 56-61.

TATARA M.R, KRUPSKI W., JANKOWSKI M., ZDUŃCZYK Z., JANKOWSKI J., STUDZIŃSKI T. 2011. *Effects of dietary calcium content and vitamin D source on skeletal properties in growing turkeys.* Brit. Poult. Sci., 52(6): 718-729.

TYKAŁOWSKI B., STENZEL T., MIKULSKI D., JANKOWSKI J., ZDUŃCZYK Z., JUSKIEWICZ J., KONCICKI A. 2011. *Level of electrolytes and percentage of T-lymphocyte subpopulations in blond of broiler chickens fed mixtures with different contents of sodium chloride.* Bull. Vet. Inst. Pulawy, 55: 333-337.

VENÄLÄINEN E., VALAJA J., JALAVA T. 2006. *Effect of dietary metabolisable energy, calcium and phosphorus on bone mineralization, leg weakness and performance of broiler chickens.* Brit. Poult. Sci., 47(3): 301-310.

VIEIRA S.L., PENZ A.M., POPHAL S., GODOY DE ALMEIDA J. 2003. *Sodium requirements for the first seven days in broiler chicks.* J. Appl. Poult. Res., 12: 362-370.

ZHOU Z.L., RATH N.C., HUFF G.R., HUFF W.E., RASAPUTRA K.S., SALAS C., COON C.N. 2011. *Bone characteristics of 16 wk-old-turkeys subjected to different dietary supplements and stimulated stress.* Intern. J. Poult. Sci., 10(5): 332-337.

PHYTOPLANKTON METRICS RESPONSE TO THE INCREASING PHOSPHORUS AND NITROGEN GRADIENT IN SHALLOW LAKES*

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Abstract

A new phytoplankton-based method (the Phytoplankton Metric for Polish Lakes – PMPL) for the ecological status assessment of lakes has been developed in Poland. This method has not been validated and tested on independent data, hence the aim of the study was to check the applicability of this method and the sensitivity of different phytoplankton metrics along pressure gradient. Phytoplankton data were collected from 33 Polish lowland lakes (20 stratified, 13 non-stratified), during the growth season in the period from 2005 to 2009. The biomass of phytoplankton was calculated from cell volume measurements and chlorophyll *a* concentration was determined spectrophotometrically by the alcohol method. Total nitrogen and total phosphorus concentrations were determined by standard methods (PN-EN ISO 6878:2006p.7 and PN-EN 25663:2001, respectively) and water transparency was measured using a Secchi disk.

For comparability, an ecological status was determined according to three phytoplankton-based methods: Polish, German and Hungarian. Polish metrics showed a strong correlation with the other metrics (German, Hungarian), which are broadly and successfully used for assessment in Europe. However, a statistically significant correlation was found between all the metric values and the main parameters: total phosphorus, total nitrogen and Secchi disk visibility. The metrics based on quantitative phytoplankton features were more sensitive to the growth of eutrophication than others, based on indicator taxa. Total phosphorus demonstrated a stronger relationship with the tested metrics than total nitro-

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gen. Phytoplankton metrics reacted quite well to pressure gradients because of the decreasing tendency of metric values along the TP and TN gradient and an increasing tendency along the Secchi disk visibility gradient. The multimetric PMPL may be recommended as useful for the assessment of the ecological status of Polish lakes.

Key words: WFD, ecological status assessment, phytoplankton-based method, PMPL, PSI, Q Index.

ODPOWIEDŹ METRIKSÓW FITOPLANKTONOWYCH NA WZROST GRADIENTU FOSFORU I AZOTU W JEZIORACH PŁYTKICH

Abstrakt

W Polsce opracowano nową metodę do oceny stanu ekologicznego jezior na podstawie fitoplanktonu (polski multimetriks fitoplanktonowy – PMPL). Metoda ta nie została zwalidowana i przetestowana na niezależnych danych. Celem badań było sprawdzenie możliwości zastosowania tej metody oraz wrażliwości różnych metriksów fitoplanktonowych na gradient presji. Dane fitoplanktonowe zebrano z 33 polskich jezior nizinnych (w tym 20 stratyfikowanych i 13 niestratyfikowanych), w sezonie wegetacyjnym w latach 2005-2009. Biomasę fitoplanktonu oszacowano metodą pomiaru objętości komórek, stężenie chlorofilu a w wodzie określono spektrofotometryczną metodą alkoholową. Zawartość fosforu i azotu ogólnego oznaczono standardowymi metodami (odpowiednio PN-EN ISO 6878:2006p.7 i PN-EN 25663:2001), natomiast widzialność wyznaczono z użyciem krążka Secchi'ego. Ocenę stanu ekologicznego określono według trzech metod fitoplanktonowych: polskiej, niemieckiej i węgierskiej.

Polskie metriksy wykazywały silną korelację z innymi metriksami (niemieckimi i węgierskim), które są szeroko stosowane do oceny stanu ekologicznego w Europie. Statystycznie istotne korelacje wykazano również między wartościami metriksów a głównymi parametrami presji, fosforem i azotem ogólnym oraz widzialnością krążka Secchi'ego. Metriksy oparte na cechach ilościowych fitoplanktonu były bardziej wrażliwe na wzrost stopnia eutrofizacji niż inne, oparte na taksonach wskaźnikowych. Fosfor ogólny wykazywał silniejszy związek z testowanymi metriksami niż azot ogólny. Zaobserwowano tendencję do zmniejszania się wartości metriksów wzdłuż gradientu fosforu i azotu ogólnego oraz tendencję do wzrostu wzdłuż gradientu widzialności. Świadczą one o tym, że metriksy fitoplanktonowe dobrze reagują na presję, a multimetriks PMPL może być rekomendowany jako przydatny do oceny stanu ekologicznego polskich jezior.

Słowa kluczowe: RDW, ocena stanu ekologicznego, metoda fitoplanktonowa, PMPL, PSI, Q indeks.

INTRODUCTION

Phytoplankton, like other biological quality elements, responds to multiple environmental factors and, being a good indicator of water quality with respect to eutrophication, is required for the implementation of the Water Framework Directive (WFD) – EC2000/60/WE. Several methods based on phytoplankton parameters (chlorophyll a, biomass, indicator taxa, phytoplankton assemblage structure) for the ecological status assessment of lakes are

still being developed or have already been applied in the EU countries (PADIS-ÁK et al. 2006, MISCHKE et al. 2008, PTACNIK et al. 2009). At present, the assessment criteria in Poland are based only on chlorophyll a concentration in water as a measure of phytoplankton abundance (*The Regulation...* 2008, No. 162, Item 1008). In 2009, on a commission from the Chief Inspectorate for Environmental Protection, a phytoplankton-based method for the assessment of the ecological status of lakes was developed (the Phytoplankton Metric for Polish Lakes – PMPL; HUTOROWICZ et al. 2011), which, apart from chlorophyll a, also accounts for the total phytoplankton biomass and the biomass of Cyanoprokaryota. The latter index covers the biomass of blue-green algae and their share in the total phytoplankton biomass. Therefore, it may be treated as an element in the assessment based on the taxonomic composition of phytoplankton assemblage. Each newly developed method for assessment ought to be a suitable tool to measure the composed anthropogenic damage and should be checked before being included in monitoring programs (NõGES et al. 2009). The Polish method has not yet been validated and tested on independent data. Hence, the aim of this study was to check the usefulness of the Polish method for the ecological status assessment and compare the sensitivity of different phytoplankton metrics to the increase of phosphorus and nitrogen gradient in Polish shallow lakes. Additionally, Secchi disk visibility was taken into account as an important factor of light conditions in water.

MATERIALS AND METHODS

Phytoplankton and chlorophyll a data were collected from 33 different lowland lakes (20 stratified, 13 non-stratified) during the growth season in the period from 2005 to 2009 (totally 39 – 'lake years'). The lakes were situated in north-eastern Poland, on postglacial deposits, belonging to the Western Europe Unit and Eastern Europe Unit. These data were obtained within the framework of the National Environmental Monitoring and within the framework of the Polish-Norwegian project tilted 'deWELopment' – 'Development and validation of methods for integrated assessment of ecological status of rivers and lakes to support river basin management plans'.

According to the Water Framework Directive 2000/60/WE requirement, lakes are classified as:
– lowland lakes;
– small- (50–100 ha) and medium-sized (100-1000 ha) natural reservoirs;
– very shallow (mean depth <3 m) and shallow (mean depth 3-15 m) lakes;
– calcareous lakes;
– lakes with Schindler's ratio (the ratio of total catchment's area to lake volume) below 2 and above 2;

– lakes with different mixing types: stratified and non-stratified, according to Polish abiotic typology (KOLADA et al. 2005) belong to 2a, 3a, 5a, 6a and 2b, 3b, 6b, respectively.

In stratified lakes, samples were collected from the euphotic zone – in spring and autumn and from the epilimnion in summer; in non-stratified lakes they were taken from the whole depth. Samples were taken at one-meter intervals and then integrated. The analyses of phytoplankton were conducted according to UTERMÖHL's method (1958) as described in the guidance standard CEN 2004. The total biomass was calculated from cell volume measurements (PLIŃSKI et al. 1984). Chlorophyll a concentrations were determined spectrophotometrically by the alcohol method (NUSCH 1980). Additional samples were collected for chemical analysis of water, mainly total nitrogen and total phosphorus concentrations, which were determined by standard methods. Water transparency was measured using Secchi disks and expressed as the Secchi disk visibility (SV).

The ecological status was determined according to three phytoplankton-based methods: Polish, German and Hungarian. The new Polish method, called the PMPL, comprises a multimetric based primarily on the quantitative and qualitative parameters which characterise phytoplankton (HUTOROWICZ et al. 2011). The PMPL includes three constituent metrics: 'Metric Chlorophyll a' (MC), 'Metric Total Biomass' (MTB) and 'Metric Biomass of Cyanoprokaryota' (MBC), and its final value is an averaged value of these three components.

The German multimetric Phytoplankton Seen Index (PSI) is based on three metrics: 'Metric Biomass' (MB), 'Metric Algal Classes' (MAC) and 'Metric Phytoplankton Taxa Seen Index' – PTSI (MISCHKE et al. 2008). The 'Metric Biomass' takes into account the total biomass, the mean concentration of chlorophyll a and the maximum concentration of chlorophyll a. The 'Metric Algal Classes' is based on the percentage shares in the total biomass of the taxonomic groups typical of the particular water quality classes. The PTSI (Phytoplankton-Taxa-Seen-Index) includes the trophic value of indicator taxa, taking into account their differentiation in the particular abiotic types of lakes.

In the Hungarian method, the Q Index (the phytoplankton assemblage index) was calculated on the basis of Colin Reynolds' concept of functional groups (REYNOLDS et al. 2002, PADISÁK et al. 2006). The Q Index is based on the ascription of the factor F to each of the 33 functional groups, depending on the reservoir type and experts' knowledge. It takes into account the relative share of the functional group biomass in the total biomass.

For comparability and further analyses, the values of all metrics were transferred to standardised Ecological Quality Ratio (EQR), which takes into account the measured value and reference value. EQR is set into the range from 0 (the worst) to 1 (the best quality) and class boundaries given in Table 1.

Table 1

Class boundaries of the ecological status of lakes according
to metrics values transferred to standardised
Ecological Quality Ratios

EQR normalized	Ecological status
0.8-1.0	high
0.6-0.8	good
0.4-0.6	moderate
0.2-0.4	poor
0-0.2	bad

The concentrations of total phosphorus and total nitrogen and water transparency (Secchi disk visibility) were chosen as proxies to express the degree of eutrophication. They were used as stressor parameters for phytoplankton development. The analysis of the relationship among the calculated metrics and between them and the environmental parameters was based on the Spearman rank correlation, at the significance level of $p<0.05$. The variability of phytoplankton metric values was analysed with reference to the average seasonal concentrations of nutrients and water transparency. The content of TP ranged from 0.023 to 0.125 mg dm^{-3} and TN from 0.742 to 3.258 mg dm^{-3} and SV varied from 0.65 to 4.88 m.

RESULTS

The tested phytoplankton-based methods classified the surveyed lakes from a high to bad ecological status (Figure 1). The diversified assessments of the status were obtained using different methods because they characterise the assemblage of planctic algae in different ways (in the case of reservoirs with an extremely different quantitative and qualitative phytoplankton structure). Nevertheless, about 40% of lakes were assessed as being at least in good status (high plus good status) according to both Polish and German methods, whereas only about 25% were assessed as such by the Q Index. The ecological status of the other natural reservoirs was assessed, depending on the used method, mainly as moderate or poor. The most divergent assessments were obtained with respect to the bad ecological status. The PMPL, PSI and Q Index classified about 6%, 0% and 29% of lakes as being in this state, respectively.

The Polish phytoplankton-based method was closely connected with the German and Hungarian methods. There was a statistically significant correlation observed between the analysed metric values (Table 2). The strongest
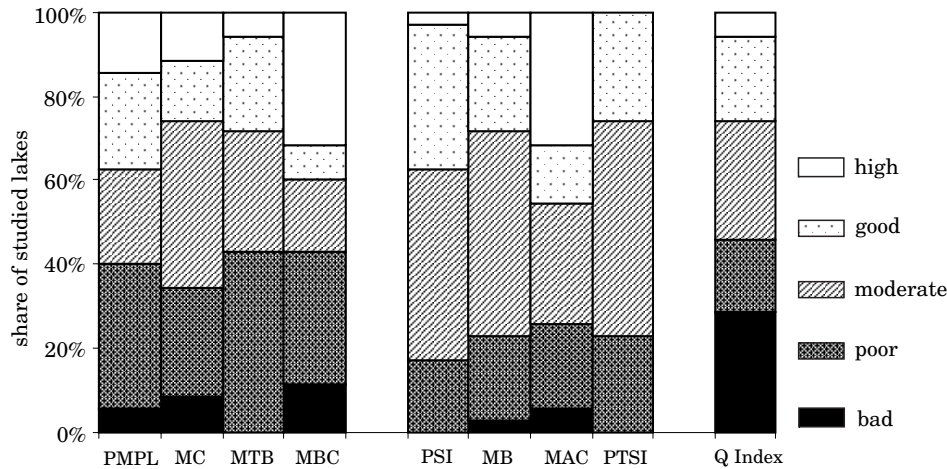
Fig. 1. The ecological status assessment of lakes (*n*=39) based on three phytoplankton methods: Polish (PMPL), German (PSI) and Hungarian (Q Index)

Explanation: PMPL – Phytoplankton Metric for Polish Lakes, MC – Metric Chlorophyll a, MTB - Metric Total Biomass, MBC – Metric Biomass of Cyanoprokaryota, PSI – Phytoplankton Seen Index, MB – Metric Biomass, MAC – Metric Algal Classes, PTSI – Phytoplankton Taxa Seen Index

Table 2

The Spearman rank correlation between the metrics of the Polish method and metrics of the other methods, *n* = 39, *p*<0.05*

Tested metrics		Polish method			
		PMPL	MC	MTB	MBC
German method	PSI	*0.822*	*0.732*	*0.756*	*0.731*
	MB	*0.834*	*0.830*	*0.786*	*0.633*
	MAC	*0.774*	*0.632*	*0.769*	*0.717*
	PTSI	0.281	0.277	0.095	0.301
Hungarian method	Q Index	*0.650*	*0.432*	*0.567*	*0.698*

*Statistically significant correlations *(p<0.05)* are given in italics (the key to symbols is given in Fig. 1)

dependence was noted between the PMPL and PSI (*r*=0.822) and their partial metrics, especially based on the chlorophyll *a* concentration, the total biomass and the biomass of particular taxonomic groups. The exception was the PTSI metric which was dependent on the occurrence of the indicator species, and that one was not significantly correlated between the two method. A distinctly lower, but statistically significant relationship was observed between the Polish multimetric and Q Index describing taxonomic composition.

In respect of all the tested metrics, a statistically significant correlation was found with the main parameters expressing the eutrophication degree, e.g. total phosphorus, total nitrogen and Secchi disk visibility (Tabble 3). The strongest dependence occurred between the PSI and nutrient concentrations: PSI:TP (r=-0.793) and PSI:TN (r=-0.653). A slightly weaker relationship was observed with partial metrics. The lowest statistically significant correlation was noted between the PTSI and all relevant parameters. The Polish multimetric correlated significantly with the total phosphorus and total nitrogen, but the weakest dependence was observed with the MBC metric. The Q Index also showed quite a good relationship with environmental stressors. In nearly all the cases, total phosphorus was found to be more strongly correlated with phytoplankton metrics than total nitrogen. Regarding the light conditions in water, a significant relationship was found with Secchi disk visibility. The highest correlation coefficient was identified for the Q Index (r=0.812).

Table 3

The Spearman rank correlation between the main physicochemical parameters and the metrics values, expressed as EQR normalized for all studied lakes, $n = 39$, $p<0.05*$

Tested metrics		Physicochemical parameters		
		Secchi disk visibility	total phosphorus	total nitrogen
Polish method	PMPL	0.695	-0.629	-0.435
	MC	0.527	-0.633	-0.407
	MTB	0.599	-0.544	-0.445
	MBC	0.682	-0.481	-0.391
German method	PSI	0.799	-0.793	-0.653
	MB	0.720	-0.725	-0.521
	MAC	0.744	-0.700	-0.522
	PTSI	0.355	-0.375	-0.386
Hungarian method	Q Index	0.812	-0.502	-0.533

*Statistically significant correlations ($p<0.05$) are given in italics (the key to symbols is given in Fig. 1)

Considering the lakes of different water mixing types, the most common statistically significant correlation was found between phytoplankton data and environmental parameters in stratified lakes (Table 4). However, the total phosphorus concentration was slightly more strongly correlated with the Polish and German metrics in non-stratified lakes. The Hungarian index responded reversely. The total nitrogen concentration was significantly related to the PSI in stratified lakes and to the Q Index in non-stratified

Table 4

The Spearman rank correlation between the main physicochemical parameters
and the metrics values, expressed as EQR normalized for stratified lakes,
n=23 and non-stratified, n=16, p<0.05*

Tested metrics		Physicochemical parameters					
		Secchi disk visibility		total phosphorus		total nitrogen	
		S	NS	S	NS	S	NS
Polish method	PMPL	*0.677*	*0.597*	*-0.462*	*-0.679*	-0.316	-0.311
	MC	*0.626*	*0.565*	*-0.509*	*-0.776*	-0.378	-0.389
	MTB	*0.686*	*0.509*	*-0.462*	*-0.568*	*-0.457*	-0.261
	MBC	*0.548*	*0.671*	-0.352	-0.421	-0.161	-0.432
German method	PSI	*0.663*	0.464	*-0.666*	*-0.890*	*-0.642*	-0.402
	MB	*0.877*	0.297	*-0.660*	*-0.779*	*-0.572*	-0.200
	MAC	*0.557*	0.179	*-0.507*	*-0.765*	*-0.512*	-0.094
	PTSI	0.020	*0.727*	-0.378	-0.341	-0.060	*-0.626*
Hungarian method	Q Index	*0.671*	*0.662*	*-0.729*	-0.024	-0.322	*-0.741*

S – stratified lakes, NS – non-stratified lakes, *statistically significant correlations (p<0.05)
are given in italics (the key in Fig. 1)

lakes. The composition metrics in the MBC and PTSI usually demonstrated a negligible response to nutrients regarding the two different types of lakes. The relationship with the Secchi disk visibility was stronger in stratified than in non-stratified lakes.

The examined lakes (very shallow and shallow) display considerable gradients in terms of nutrient enrichment and water transparency. Our analysis of the phytoplankton metrics towards the chosen parameters showed significant trends along the eutrophication gradient corresponding to an appropriate ecological status from high to bad, according to the direction of trophy growth. The values of all metrics decreased with the increasing TP and TN gradients, but increased consistently with the Secchi disk visibility gradient (Figures 2, 3). The metrics based on quantitative phytoplankton features (biomass and chlorophyll a concentration) of the PMPL (MC, MTB) and PSI (MB, MAC) reacted to the growth of eutrophication degree much stronger than others, based on composition structure (MBC, PTSI) – Figure 2.
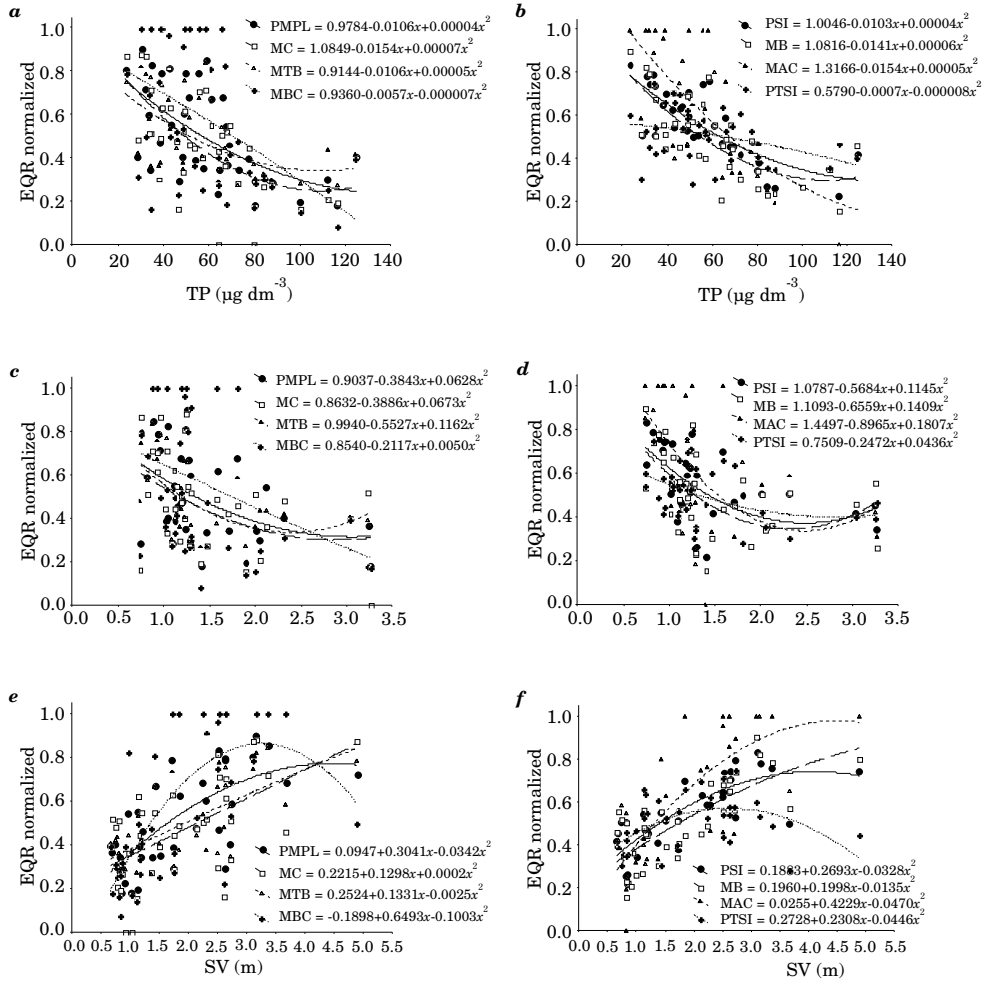
Fig. 2. Relationships for the Polish (PMPL, MC, MTB, MBC) and German (PSI, MB, MAC, PTSI) phytoplankton metrics along the total phosphorus (TP), total nitrogen (TN) and Secchi disk visibility (SV) gradients
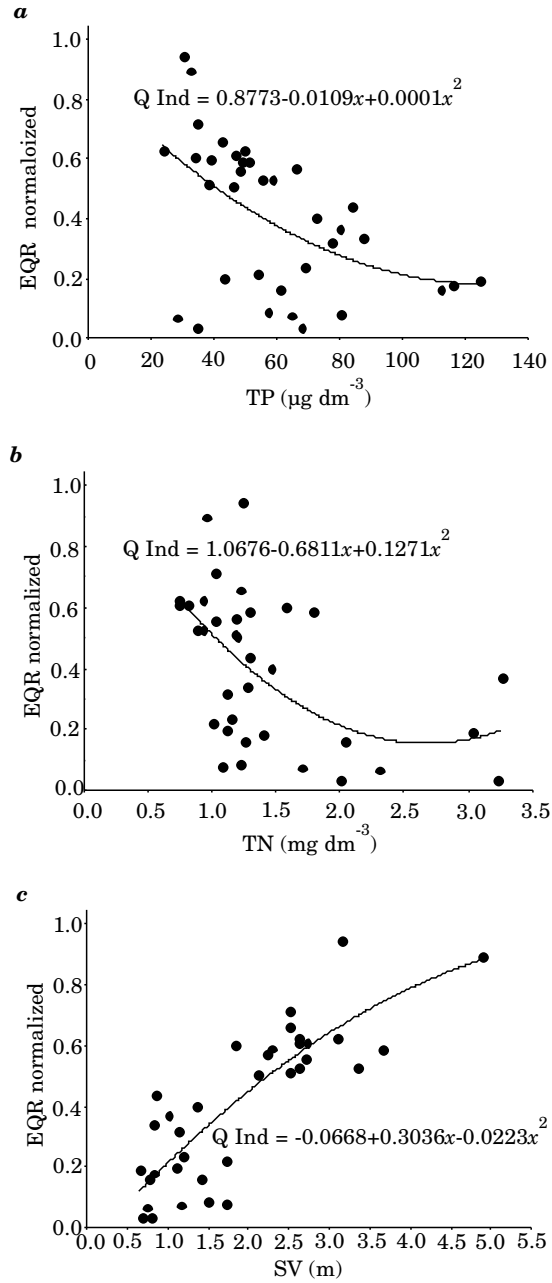
a

$$Q\ Ind = 0.8773 - 0.0109x + 0.0001x^2$$

EQR normaloized

TP (µg dm^{-3})

b

$$Q\ Ind = 1.0676 - 0.6811x + 0.1271x^2$$

EQR normalized

TN (mg dm^{-3})

c

$$Q\ Ind = -0.0668 + 0.3036x - 0.0223x^2$$

EQR normalized

SV (m)

Fig. 3. Relationships for the Hungarian Q Index along the total phosphorus (TP), total nitrogen (TN) and Secchi disk visibility (SV) gradients

DISCUSSION

Phytoplankton, as the earliest component in the water food web, reacts rapidly to nutrient enrichment. Species-specific strategic adaptations to the different physicochemical parameters and nutrient availability allow some estimates of population variability (DEVLIN et al. 2009). Their response to specified environmental conditions are essential to being a very useful indicator. Therefore, according to the WFD planctic algae together with bentic flora, macroinvertebrates and fish are included as the biological quality elements (BQEs) for the ecological status assessment of lakes. Both phytoplankton biomass and taxonomic composition with bloom-forming occurrence should be applied for this purpose (WFD, Annex V). Each BQE (expressed as proper index or metric) relates to different pressures in degree to be operational for water management. Several phytoplankton-based methods for ecological status assessment of lakes, coastal and estuarine water bodies exist in European countries (LEPISTÖ et al. 2006, PADISÁK et al. 2006, MISCHKE et al. 2008, PTACNIK et al. 2009, DEVLIN et al. 2009). The newly elaborated Polish method corresponds to the conditions and types of lowland lakes in Poland. The applicability of this method is currently being tested using an intercalibration process (EC 2010) to ensure that the 'good' ecological status represents the same level of water quality in different areas in Europe (EC 2005).

Among all the 39 'lake years' only about 40% of lakes were assessed as having at least a 'good' ecological status. This status is defined as slight changes in the composition and abundance of planktonic taxa compared to the type-specific communities (EC2000/60/WE). The good ecological status is determined on the basis of both biological and non-biological (physicochemical and hydromorphological) elements and is required for all natural surface waters in European countries by 2015. Most of the surveyed lakes demonstrated signs of higher distortion resulting from human activity and the phytoplankton biomass was significantly more disturbed than under conditions of the good status. Persistent blooms occurred in those lakes (NAPIÓRKOWSKA-KRZEBIETKE et al. 2009), up to possible production of toxins producing.

The Polish phytoplankton multimetric and its component metrics showed a strong correlation with the other analysed metrics (German, Hungarian), which are used broadly and successfully for assessment in Europe (CROSSETTI, DE M. BICUDO 2008, HAJNAL, PADISÁK 2008, KAIBLINGER et al. 2009). The consistency between the lake assessment by the German method and the Polish classification based on chlorophyll a concentration was also found for selected Polish lakes in the previous research (PASZTALENIEC, PONIEWOZIK 2010), despite the fact that the German PSI index covers a much larger number of phytoplankton parameters than the PMPL and primarily takes into account a much greater degree the species structure of the algal assemblage.

The impact of the metric 'Biomass of Cyanoprokaryota' in the final assessment is very significant, as it brings to the assessment elementary information on the structure of the phytoplankton biomass (the share of blue-green algae in the total biomass). This group of phytoplankton is very important and is commonly used as a partial metric for assessment in Poland as well as in other countries, mainly Belgium, Lithuania and the United Kingdom (EC 2010). The importance of blue-green algae is recognized as a good indicator for phytoplankton response to eutrophication (LYCHE SOLHEIM et al. 2008).

The surveyed lakes are under various anthropogenic pressures, e.g. the predominance of arable land in most of the lakes' catchment areas, existing village buildings, tourist facilities, various forms of tourism and point-sources of pollution entering directly the lakes or the Wel River. Additionally, hydromorphologic differences were also observed between the lakes. A whole range of environmental variables affected the final ecological status assessments of the lakes. This corresponded quite well with the eutrophication degree; as a rule of the thumb, a worse ecological status (worse water quality) equals a higher trophy status. Phosphorus and nitrogen are widely recognized as key nutrients and – for pressure determination – total rather than available forms are used (mostly soluble reactive phosphorus, nitrate and ammonium), which are characterized by much higher lability (MOSS et al. 2003). The total phosphorus demonstrated a stronger relationship with the used and tested metrics than total nitrogen, confirming that phosphorus is usually the limiting nutrient for the growth of phytoplankton in lakes (SCHINDLER 1977, GUILDFORD, HECKY 2000). In most cases, phytoplankton is controlled by P concentrations (e.g. DOKULIL, TEUBNER 2005) and TP can be a very good predictor of its development (LYCHE SOLHEIM et al. 2008, NÕGES et al. 2009). Despite this fact, total nitrogen can correlate very strongly with phytoplankton biomass and chlorophyll a concentration (CARSTENSEN, HENRIKSEN 2009). The N:P ratio in water columns of the surveyed lakes usually reached values above the critical value of about 17 (ZDANOWSKI 1982). Only in a few cases lower ratios of N:P were recorded which, indeed, corresponded quite well with Cyanoprokaryota dominance in the summer phytoplankton of the examined lakes (NAPIÓRKOWSKA-KRZEBIETKE et al. 2009). Despite the facts that the Secchi disk visibility reflects effects of both phytoplankton and inorganic and detrital turbidity, as well as dissolved colour substances in the water (MOSS et al. 2003) and that phytoplankton is adaptable to low light availability by producing more chlorophyll per unit biomass (GREISBERGER, TEUBNER 2007), the water transparency was also taken into account as a proxy for eutrophication. The visibility of Secchi disk is usually treated as a very convenient summary of many features in lakes.

The current results show a quantitative and qualitative impact-response change across a stressor gradient. In view of the strong correlation between the values of the Polish phytoplankton metrics and the pressure factors, as

well as the strong correlation between the values of the PMPL and PSI metrics, the PMPL may be recommended as useful for the assessment of the ecological status of lakes.

CONCLUSIONS

The newly developed Phytoplankton Metric for Polish Lakes (PMPL) is significantly correlated with other widespread metrics (German and Hungarian). The strongest correlation was observed with the Phytoplankton Seen Index (PSI). All phytoplankton metrics were related to stress gradients and a statistically significant correlation was found with the selected physico-chemical parameters expressing the degree of eutrophication (total phosphorus, total nitrogen and Secchi disk visibility). The strongest dependence occurred in the case of the PSI multimetric and its components. The decreasing tendency of metrics values along the TP and TN gradient and the increasing tendency along the Secchi disk visibility gradient confirmed the ecologically important relationship to environmental stressors. The partial metrics of the PMPL (MC, MTB) and PSI (MB, MAC) based on quantitative phytoplankton features (chlorophyll a and biomass) were more sensitive to the growth of eutrophication degree than other metrics based on indicator taxa (MBC, PTSI). The Q Index classified the studied lakes more rigorously than the PMPL and PSI, but responded very similarly to a trophy increase.

These conclusions, in turn, enable us to recommend the Phytoplankton Metric for Polish Lakes as a useful method for the assessment of the ecological status of lakes. The intercalibration process of this method will confirm its applicability to assessment of lakes comparable to water quality assessments in different areas of Europe.

REFERENCES

CARSTENSEN J., HENRIKSEN P. 2009. *Phytoplankton biomass response to nitrogen inputs: a method for WFD boundary setting applied to Danish coastal waters*. Hydrobiologia, 633: 137-149.

CEN 2004. *Water quality – Guidance standard for the routine analysis of phytoplankton abundance and composition using inverted microscopy (Utermöhl technique)* CEN TC 230/WG2/TG3/N83.

CROSSETTI L.O., DE M. BICUDO C. E. 2008. *Phytoplankton as a monitoring tool in a tropical urban shallow reservoir (Garças Pond): the assemblage index application*. Hydrobiologia, 610: 161-173.

DEVLIN M., BARRY J., PAINTING S., BEST M. 2009. *Extending the phytoplankton tool kit for the UK Water Framework Directive: indicators of phytoplankton community structure*. Hydrobiologia, 633:151-168.

DOKULIL M.T., TEUBNER K. 2005. *Do phytoplankton communities correctly track trophic changes? An assessment using directly measured and palaeolimnological data*. Freshwater Biol., 50: 1589-1593.

EUROPEAN COMMISION 2000. *Directive of the European Parliament and of the Council 2000/60/EC establishing a framework for community action in the field of water policy*. Official Journal 2000 L 327/1, European Commission, Brussels.

EUROPEAN COMMISION 2005. *Common implementation strategy for the water framework directive (2000/60/ec). Guidance on the Intercalibration process 2004-2006*. Luxemburg, Office for Official publications of the European Communities. http://circa.europa.eu/Public/irc/env/wfd/library.

EUROPEAN COMMISION 2010. *WFD Intercalibration Phase 2: Milestone 2 report (for ECOSTAT meeting 8-9 April 2010)*, European Commission, Directorate General Jrc and Joint Research Centre, Institute of Environment and Sustainability.

GREISBERGER S., TEUBNER K. 2007. *Does pigment composition reflect phytoplankton community structure in differing temperature and light conditions in a deep alpine lake? An approach using HPLC and delayed fluorescence (DF) techniques*. J. Phycol., 43: 1108--1119.

GUILDFORD S.J., HECKY R. E. 2000. *Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship?* Limnol. Oceanogr., 45: 1213-1223.

HAJNAL É., PADISÁK J. 2008. *Analysis of long-term ecological status of Lake Balaton based on the ALMOBAL phytoplankton database*. Hydrobiologia, 599: 227-237.

HUTOROWICZ A., NAPIÓRKOWSKA-KRZEBIETKE A., PASZTALENIEC A., HUTOROWICZ J., LYCHE SOLHEIM A., SKJELBRED B. 2011. *Phytoplankton*. In: *Ecological status assessment of the waters in the Wel River catchment. Guidelines for integrated assessment of ecological status of rivers and lakes to support river basin management plans*. SOSZKA H. (ed). IFI Olsztyn, 143-168. (in Polish)

KAIBLINGER C., ANNEVILLE O., TANDONLEKE R., RIMET F., DRUART J.C., GUILLAR J., DOKULIL M.T. 2009. *Central European water quality indices applied to long-term data from pre-alpine lakes: test and possible improvements*. Hydrobiologia, 633: 67-74.

KOLADA A., SOSZKA H., CYDZIK D., GOŁUB M. 2005. *Abiotic typology of Polish lakes*. Limnologica, 35: 145-150.

LEPISTÖ L. HOPAINEN A-L., VUORISTO H., REKOLAINEN S. 2006. *Phytoplankton assemblages as a criterion in the ecological classification of lakes in Finland*. Boreal Environ. Res., 11: 35-44.

LYCHE SOLHEIM A., REKOLAINEN S., MOE S.J., CARVALHO L., PHILLIPS G., PTACNIK R., PENNING W.E., TOTH L.G., O'TOOLE C., SCHARTAU A-K.L., HESTHAGEN T. 2008. *Ecological threshold responses in European lakes and their applicability for the Water Framework Directive (WFD) implementation: synthesis of lakes results from the REBECCA project*. Aquat. Ecol., 42: 317-334.

MISCHKE U., RIEDMÜLLER U., HOEHN E., SCHÖNFELDER I., NIXDORF B. 2008. *Description of the German system for phytoplankton-based assessment of lakes for implementation of the EU Water Framework Directive (WFD)*. In: *Bewertung von Seen mittels Phytoplankton zur Umsetzung der EU-Wasserrahmenrichtlinie*. MISCHKE U., NIXDORF B. (eds.). Aktuelle Reihe 2/2008, Bad Saarow, Freiburg, Berlin. University of Cottbus, 117-146.

Moss B.D. et al. 2003. *The determination of ecological status in shallow lakes – a tested system (ECOFRAME) for implementation of the European Water Framework Directive*. Aquat. Conserv., 13(6): 507-549.

Napiórkowska-Krzebietke A., Pasztaleniec A., Hutorowicz A. 2009. *Phytoplankton – element in ecological status assessment for lakes of the Wel River catchment area*. Teka Kom. Ochr. Kszt. Środ. Przyr. – OL PAN, 6: 200-2005.

Nõges P., van de Bund W., Cardoso A.C., Solimini A.G., Heiskanen A-S. 2009. *Assessment of the ecological status of European surface waters: a work in progress*. Hydrobiologia, 633: 197-211.

Nusch E.A. 1980. *Comparison of different methods for chlorophyll and phaeopigment determination*. Ergebn. Limnol., 14: 14-36.

Padisák J., Borics G., Grigorszky I., Soróczki-Pintér E. 2006. *Use of phytoplankton assemblages for monitoring ecological status of lakes within the Water Framework Directive: the assemblage index*. Hydrobiologia, 553: 1-14.

Pasztaleniec A., Poniewozik M. 2010. *Phytoplankton based assessment of the ecological status of four shallow lakes (Eastern Poland) according to Water Framework Directive – a comparison of approaches*. Limnologica, 40: 251-259.

Pliński M., Picińska J., Targoński L. 1984. *Method defining the biomass of marine phytoplankton by means of computers*. Zesz. Nauk. WBiNoZ Gdansk University, 10: 129-155. (in Polish)

Ptacnik R., Solimini A.G., Brettum P. 2009. *Performance of a new phytoplankton composition metric along a eutrophication gradient in Nordic lakes*. Hydrobiologia, 633: 75-82.

Reynolds C.S., Huszar V., Kruk C., Naselli-Flores L., Melo S. 2002. *Towards a functional classification of the freshwater phytoplankton*. J. Plankton Res., 24: 417-428.

Schindler D.W. 1977. *Evolution of phosphorus limitation in lakes*. Science, 195: 260-262.

The Regulation of the Minister of the Environment of 20 August 2008 for the status classification of surface water bodies. The Official Journal of the Laws of 2008, No. 162, Item 1008.

Utermöhl H. 1958. *Zur Vervollkommung der quantitativen Phytoplankton-Methodik*. Mitt. internat. Verein. Limnol., 9: 1-38. (in German)

Zdanowski B. 1982. *Variability of nitrogen and phosphorus contents and lake eutrophication*. Pol. Arch. Hydrobiol., 29: 541-597.

EFFECT OF YEARS, FERTILIZATION AND GROWING REGIONS ON THE CONTENT AND FORMS OF POTASSIUM IN SOIL*

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Abstract

The dynamics of potassium (K) in soil was investigated in long-term small-plot field trials at seven stations, where five combinations of fertilization were tested. The objective of the experiment was to estimate the effect of the weather conditions, type of growing region and increasing doses of fertilizers on the content of water soluble K (Sol-K), exchangeable K (Ex-K) and acid soluble K (Ac-K) in soils. The weather conditions had the strongest effect on the total variability of the Sol-K (34.9%), to a lesser extent on the variability of Ex-K (14.1%) and only negligibly on the Ac-K (0.3%). In the individual years of the experimental period, the content of water soluble and Ex-K was particularly affected, chiefly by the weather and due to different demands of crops for this nutrient (leguminous plants consume high amounts and cereals relatively low amounts of K). In fairly dry years (e.g. 1982), the level of potassium (Sol-K and Ex-K) in soil was higher than in years with precipitation above the average. The content of soluble and exchangeable K was positively influenced by the application of potassium fertilization (0.989, 0.994, respectively). Compared to the control, the content of Sol-K and Ex-K in the variant where the fertilizer dose was the highest increased by 69.3 and 55.3%, respectively. Potassium fertilization (compared to the control) raised the level of the Ac-K, but not significantly, also implying that the effect of fertilization on the total variability of the Ac-K (0.6%) was virtually none. The effect of the growing region on the total variability of the potassium content was higher wherever the potassium bonding to the the sorption soil complex was stronger. The diffe-

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rences between the average levels of Sol-K characterizing the growing regions were not statistically significant. The levels of Ex-K and Ac-K differed significantly in the sugar-beet growing region, where the reserve was more than 40% higher than in the potato growing region.

Key words: long-term field trials; water soluble K; exchangeable K; acid soluble K.

WPŁYW LAT, NAZWOŻENIA I REGIONU UPRAWY NA ZAWARTOŚĆ I FORMY POTASU W GLEBIE

Abstrakt

Dynamikę zmian zawartości potasu (K) w glebie badano w trakcie wieloletnich doświadczeń polowych małopoletkowych prowadzonych w siedmiu stacjach doświadczalnych, z zastosowaniem pięciu kombinacji nawożenia. Celem doświadczenia było ocenienie wpływu warunków pogodowych, rodzaju regionu, gdzie prowadzono uprawę, oraz wzrastających dawek nawożenia na zawartość w glebie K rozpuszczalnego w wodzie (Sol-K), K wymiennego (Ex-K) oraz K rozpuszczalnego w kwasach (Ac-K). Warunki pogodowe wywarły najsilniejszy wpływ na całkowitą zmienność Sol-K (34,9%), w mniejszym stopniu – na zmienność Ex-K (14,1%), a ich wpływ na zmienność Ac-K był niemal niezauważalny (0,3%). W poszczególnych latach doświadczenia zawartość K rozpuszczalnego w wodzie i wymiennego podlegała szczególnym wpływom badanych czynników, zwłaszcza warunkom pogodowym i zróżnicowanemu zapotrzebowaniu roślin uprawnych na ten składnik odżywczy (rośliny strączkowe zużywają duże ilości potasu, rośliny zbożowe zaś potrzebują niewielkich ilości tego pierwiastka). W stosunkowo suchych latach (np. 1982) zawartość potasu (Sol-K i Ex-K) w glebie była wyższa niż w latach z opadami powyżej średniej. Na zawartość K rozpuszczanego i wymiennego dodatnio wpływało zastosowanie nawożenia potasowego (odpowiednio 0,989 i 0,994). W porównaniu z kontrolą, zawartość Sol-K i Ex-K w wariancie z najwyższą dawką nawozu wzrosła odpowiednio o 69,3 i 55,3%. Nawożenie potasem (w porównaniu z kontrolą) wpłynęło na zwiększenie zawartości Ac-K, aczkolwiek nie był to wzrost statystycznie znaczący, co wskazuje tym samym, że wpływ nawożenia na całkowitą zmienność Ac-K (0,6%) był niemal zerowy. Wpływ regionu uprawy na całkowitą zmienność zawartości potasu był silniejszy w przypadku silniejszego wiązania potasu z kompleksem sorpcyjnym gleby. Różnice między średnimi zawartościami Sol-K w obu regionach nie były istotne statystycznie. Zawartości Ex-K i Ac-K różniły się statystycznie w regionie uprawy buraka cukrowego, gdzie ich rezerwa była o 40% wyższa niż w regionie uprawy ziemniaka.

Słowa kluczowe: wieloletnie doświadczenie polowe, K rozpuszczalny w wodzie, K wymienny, K rozpuszczalny w kwasie.

INTRODUCTION

Potassium is an essential element for plants and animals, and is considered a major nutrient element together with nitrogen and phosphorus (OBORN et al. 2005). Generally, it is not a limiting element in agricultural systems, but it is a major nutrient for crop quality and yield in intensive grass or lucerne dominated systems and rotations dominated by potatoes

and green vegetables (WHITEHEAD 2000). In agricultural production, including many organic farming systems, K has become a limiting element, especially in areas dominated by coarse-textured or organic soils (GOULDING, LOVELAND 1986). Its deficiency may reduce plant production quantitatively as well as qualitatively and typical visible deficiency symptoms are chlorosis and necrosis on the tips and margins of mature leaves.

The total content of K depends on the parent material, soil type and mineralogy. Traditionally, four forms of soil K are recognized: structural or mineral K, non-exchangeable K, also referred to as fixed K, exchangeable K and K in water solution (SPARKS 1987). Exchangeable and water solution K are often considered readily available to plants (SPARKS 1987), while interlayer and fixed K is frequently described as slowly or potentially available and structural K as almost unavailable (PAL et al. 1999, řGAARD et al. 2001). The acid soluble K stands for that part of soil K which forms a transitional form between the exchangeable and non- exchangeable forms, which only minimally contribute to plant nutrition. It is the soil K which is released from inter-lamellar spaces of some potassium alumo-silicates after the disintegration of their crystal lattice. According to HUDCOVA and VOPLAKAL (1981), this fraction more adequately characterizes the natural reserve of potassium in the soil than exchangeable potassium.

There is a dynamic equilibrium between the phases of soil K (SPARKS 1987), hence all factors that affect this equilibrium reactions will indirectly affect the volume of different K forms.

The objective of this study was to determine the effect of year, mineral fertilizers, organic manure and types of growing regions on the content of potassium forms in the soil.

MATERIAL AND METHODS

Experimental design

The experiment was established at 7 sites in potato (altitude 400-650 m a.s.l., annual average temperature 5-8ºC, annual average precipitation 550- -900 mm, predominant cambisols) and sugar-beet growing regions (altitude 250-350 m a.s.l., annual average temperature 8-9ºC, annual average precipitation 500-650 mm, predominant chernozems and haplic luvisols) as a long-term, small-plot stationary trial. It was conducted by the Central Institute for Supervising and Testing in Agriculture between 1982 and 1998. Table 1 gives the characteristics of the sites.

The experiment comprised 5 combinations of fertilization, in 3 replications. Table 2 shows the average content of N, P and K, which were applied in organic fertilizers (farm manure) and mineral fertilizers at three levels (low – $N_1P_1K_1$, medium – $N_2P_2K_2$, high – $N_3P_3K_3$). In both production re-

Table 1

Characteristics of the sites

Site	Growing regions	Altitude (m a.s.l.)	Annual average		Soil type	Soil textural class
			temperature (°C)	precipitation (mm)		
Horazd'ovice	potato	470	7.4	573	Cambisols	sandy s.
Svitavy	potato	460	6.5	624	Cambisols	sandy s.
Chrastava	potato	345	7.1	798	ha Luvisols	loamy s.
Staňkov	potato	370	8.3	443	ha Luvisols	clay s.
Pusté Jakartice	sugar.-beet	295	8.0	640	ha Luvisols	loamy s.
Uherský Ostroh	sugar.-beet	196	9.2	551	ha Luvisols	loamy s.
Zatec	sugar.-beet	247	8.3	451	Chernozems	clay s.

soil type (FAO soil taxonomy), soil textural class SHEPARD 1954)

Table 2

Treatments of the experiment

Var. No.	Treatments of fertilization	Doses of nutrients in applied fertilizers (kg ha^{-1} year^{-1})					
		nitrogen		phosphorus		potassium	
		PGR	SGR	PGR	SGR	PGR	SGR
1	no fertilization	0	0	0	0	0	0
2	manure	25*	25*	8*	8*	35*	35*
3	manure + $N_1P_1K_1$	25*+58**	25*+58**	8*+23**	8*+21**	35*+57**	35*+51**
4	manure + $N_2P_2K_2$	25*+88**	25*+87**	8*+35**	8*+33**	35*+90**	35*+81**
5	manure + $N_3P_3K_3$	25*+117**	25*+115**	8*+51**	8*+49**	35*+131**	35*+119**

PGR – potato growing regions, SGR – sugar-beet growing regions;
* – doses of nutrients applied in organic fertilizers;
** – doses of nutrients applied in mineral fertilizers

gions, mineral fertilizers containing phosphorus and potassium were supplied as a reserve in autumn of the preceding year. The source of phosphorus was granulated super-phosphate; potassium was applied as potassium chloride. Nitrogen was applied in ammonium sulphate during pre-sowing soil preparation and ammonium nitrate with lime was applied as additional foliar nutrition. Organic fertilizers were applied twice in the crop rotation; 40 t ha^{-1} of good-quality farm manure was incorporated in the sugar-beet growing region under maize for silage and under sugar-beet, and in the potato growing region – under potatoes (the average doses of nutrients applied in manure are shown in the Table 2).

The crops in the experiment were grown in regular crop rotations. Before 1989 (1981-1989) nine plots were used for crop rotations in both growing regions; from 1990 (1990-1997 and 1998-2005) the crop rotations were arranged in eight plots; 50% of the crops were cereals. The crops grown in the experimental years are mentioned in the Table 3. Table 4 shows the weather conditions during the experiment.

Table 3

The crops grown in the sampling years

Year	Potato growing regions	Sugar-beet growing regions
1982	potatoes	sugar beet
1987	spring barley	spring barley
1991	clover	alfalfa
1995	spring barley	spring barley
1998	oat with underseeding clover	oat with underseeding alfalfa

Table 4

Weather conditions in potato and sugar-beet growing regions during the experiment

Growing regions	Average	Years				
		1982	1987	1991	1995	1998
Potato	temperature (ºC)	7.8	6.6	7.2	7.9	8.2
	sum of precipitation (mm)	533	735	540	738	645
Sugar-beet	temperature (ºC)	8.8	7.6	8.2	9.0	9.1
	sum of precipitation (mm)	456	645	497	629	601

long term average sum of precipitation and temperature in potato growing regions (664 mm and 7.6ºC) and in sugar-beet growing regions (544 mm and 8.6ºC)

Soil sampling and analysis

Soil was sampled at all the sites in autumn 1982, 1987, 1991, 1995 and 1998. Soil samples were taken from the 0-30 cm depth. Samples were dried naturally and passed through a 2-mm sieve. The solution potassium of the soil in its dry state (in the text abbreviated as Sol-K) was determined using distilled water solution [ratio of soil to solution of 1:5 (w/v)] (JAVORSKÝ et al. 1987) and measured by atomic absorption spectrometry (AAS) on a Carl Zeiss Jena AAS-30 apparatus. Exchangeable K of the soil in its dry state (in the text abbreviated as Ex-K) was estimated by the method of JONES (1990), with the soil extracted with solution Mehlich III (NH_4F, NH_4NO_3, CH_3COOH, HNO_3 and EDTA) [ratio of soil to solution of 1:10 (w/v)]. The concentration of K in the extract was measured by atomic absorption spectrometry (AAS). K-HNO_3 was extracted by boiling a soil sample in its dry state in 1 M HNO_3 for 10 min [ratio of soil to solution of 1:10 (w/v)] (NEUBERG 1985). After filtration, the K concentration in the extract was measured by atomic absorption spectrometry (AAS). The exchangeable K value was subtracted from the K-HNO_3 value and the difference is called acid soluble K (in the text abbreviated as Ac-K).

Statistical evaluation

The results of chemical soil analyses were computer-processed and set up using the Microsoft Excel editor. The Statistica 7.1 programme was used for determination of the overall characteristics. Arithmetic means were calculated when evaluating the results. In order to elaborate the significance of differences among the arithmetic means of each characteristic, we used one- and two-way analysis of variance, followed by testing at a 95% ($p < 0.05$) level of significance using Tukey's test.

RESULTS AND DISCUSSION

The results of the long-term trial showed that the amount of potassium representing the above forms and its spatial and temporal variability primarily depended on the bonding of potassium to the sorption complex of the soil and exchangeability of the potassium pool. This conclusion is based on a big difference in the effects produced by individual factors (weather during the year, fertilization and growing region) expressing the overall variability of the respective K forms, which was particularly evident between the intensively (water soluble and exchangeable potassium) and extensively (acid soluble potassium) available potassium.

The effect of the weather conditions of the year

The variability of amounts of water soluble and exchangeable forms of potassium monitored in the individual experimental years (Table 5) was mainly attributed to the changeable weather and to the different demands of the crops for this nutrient. The conclusions of JOUANY et al. (1996), BERRY et al. (2003) and HOLMQVIST et al. (2003) confirmed that the climate in a given year was one of the basic factors affecting the K content in soil and had the strongest effect on the total variability of Sol-K (Table 6). The highest K content was detected in the first experimental year and was statistically significantly higher [$F_{(4; 125)} = 6.50$, $p=0.001$] than in 1991 and 1995, i.e. by 42.3 and 24.4%, respectively (Table 5). The high content of Sol-K detected in 1982 was probably due to the low sum of precipitation of the year, which was below the long-term average. This is confirmed by HUDCOVA and FURST (1982), ZENG and BROWN (2000) and MENGEL et al. (2001), who stated that the content of water in the soil considerably affected the amount of Sol-K. POSS et al. (1991) further discovered that in dry years the uptake

Table 5

Average content of soil soluble K, exchangeable K and acid soluble K (mg kg^{-1} DM soil)

Factors	Soil soluble K		Exchangeable K		Acid soluble K	
	mg kg^{-1} ± SE	v	mg kg^{-1} ± SE	v	mg kg^{-1} ± SE	v
Years						
1982	27.9 [b] ± 1.80	38.1	174.0 [c] ± 10.4	35.3	579 [a] ± 40.3	41.1
1987	21.8 [ab] ± 1.51	41.1	120.4 [ab] ± 8.5	41.9	607 [a] ± 39.8	38.8
1991	16.1 [a] ± 1.13	41.7	108.8 [a] ± 9.6	52.2	589 [a] ± 40.1	40.2
1995	21.1 [a] ± 1.58	44.4	129.4 [ab] ± 10.2	46.8	586 [a] ± 37.7	38.0
1998	22.1 [ab] ± 1.89	50.7	147.0 [bc] ± 10.3	41.6	621 [a] ± 44.1	42.0
Fertilization						
No fertilization	16.6 [a] ± 1.21	43.0	105.3 [a] ± 7.6	42.7	573 [a] ± 38.8	40.1
Manure	17.8 [a] ± 1.27	42.2	120.6 [ab] ± 9.7	47.4	580 [a] ± 39.6	40.4
Manure + N$_1$P$_1$K$_1$	22.6 [ab] ± 1.42	37.2	142.2 [abc] ± 10.0	41.6	613 [a] ± 40.6	39.2
Manure + N$_2$P$_2$K$_2$	23. 9 [b] ± 1.78	44.1	147.9 [bc] ± 10.4	41.5	607 [a] ± 41.4	40.3
Manure + N$_3$P$_3$K$_3$	28.1 [b] ± 2.03	42.7	163.5 [c] ± 11.8	42.7	610 [a] ± 41.6	40.3
Growing regions						
PGR	21.2 [a] ± 0.86	40.7	114.8 [a] ± 4.5	38.9	507 [a] ± 18.1	35.7
SGR	22.5 [a] ± 1.36	52.5	164.1 [b] ± 8.1	42.8	715 [b] ± 29.0	35.0

SE – standard error, v – variation coefficient (%), PGR – potato growing regions;
SGR – sugar-beet growing regions; Variants with identical letters express statistically insignificant differences ($P<0.05$ – statistical significance at a 95% level of significance).

Relative effect of factors on total variability of examined forms of K (%)

Factors	Water soluble K		Exchangeable K		Acid soluble K	
A: years	34.9	***	14.1	***	0.3	ns
B: fertilization	47.8	***	12.6	***	0.6	ns
C: growing regions	4.4	ns	68.9	***	94.7	***
A × B	1.2	ns	0.4	ns	0.0	ns
A × C	3.2	ns	1.0	ns	1.0	ns
B × C	1.7	ns	0.8	ns	0.2	ns
A × B × C	1.3	ns	0.2	ns	0.1	ns

* $\alpha = 0.05$, ** $\alpha = 0.01$, *** $\alpha = 0.001$, ns = not significant

of potassium from the soil and from fertilizers decreased, while the content of K in the soil increased. Table 5 shows that also the content of Ex-K was statistically the highest in 1982 [F150 (4; 125) = 7.15, $p<0.001$]. Most of this form of K was absorbed and hence the reserve in the soil was the lowest in 1991, when red clover was incorporated in the crop rotation, confirming the fact that of all leguminous plants absorb most of potassium (PAL et al. 1999, WANG et al. 2000). In 1987, 1995 and 1998, the average amount of Ex-K ranged between 120 and 147 mg kg^{-1}. The crop rotations then included cereals, which – according to KUHLMANN and WEHRMANN (1984) and ASKEGAARD et al. (2003) – have a relatively low demand for potassium. This development corresponds with the conclusions of MENGEL et al. (2001), who stated that changes in the content of Ex-K are caused by the succession of crops in crop rotations. The trend in time-related variability of this potassium fraction was closely correlated with the trend in Sol-K. The conclusions of BRAR et al. (1986) confirmed this fact. However, the weather conditions of the year affected the exchangeable form of K to a lesser extent (Table 6) and their effect on the overall variability of the Ac-K was virtually zero. This corresponds to the statistically insignificant differences between the content discovered in the individual years of the experiment [F (4;125) = 0.11, $p=0.979$] – Table 5. The greatest difference was observed between the years 1982 and 1998; the Ac-K increased by 7.3%.

The effect of fertilization

Fertilization was the decisive factor affecting the variability of the content of the Sol-K (Table 6) [F (4;125) = 8.91, $p<0.001$]. The correlation coefficient $r=0.989$ expresses the positive effect of this factor on the water soluble form of K and is consistent with the opinion of HUDCOVA and SIROVY (1981), HUDCOVA (1989), HEMING (2004) and FORTUNE et al. (2005), who discovered that the concentration of the Sol-K is dependent primarily on potassium fertilization. In our trial, fertilization with 160 kg K ha^{-1} increased the content of

this form of K by 69.7% compared to the unfertilized variant (Table 5). BANSAL et al. (2002) also confirmed the positive effect of 177 fertilization; they discovered that in the course of 10 years the average content of Sol-K in unfertilized soil decreased from 14.5 to 11.3 mg kg^{-1}. Analogously, the correlation coefficient (0.994) of Ex-K showed its strong dependence on increasing doses of K fertilizers. HUDCOVA (1985) and ASKEGAARD and ERIKSEN (2002) reached the same conclusions; the latter found a positive correlation between Ex-K and increasing doses of fertilizers, also in combination with liming and particularly with organic fertilization. In the present experiment, the statistically significantly lowest content of Ex-K was monitored in the control variant (Table 5) and the highest one appeared in variant 5 [F (4;125) = 6.42, $p < 0.001$]. HRTANEK (1987) likewise reported an increased content of the Ex-K from 117 mg kg^{-1} (unfertilized variant), to 174 mg kg^{-1} (variant fertilized with 113 kg K ha^{-1}) to 244 mg kg^{-1} in the variant fertilized with the highest dose (226 kg K ha^{-1}). RICHTER et al. (2002) likewise discovered that this form of K was much dependent on fertilization ($r=0.762 – 0.964$). These authors further reported that after the application of 1 kg of K fertilizers the level of exchangeable potassium increased by 0.15-0.42 mg kg^{-1} depending on the soil type In our experiment, the increase was very much the same, ranging between 0.30 and 0.50 mg kg^{-1}. The data given in the table 5 indicate that potassium fertilization (compared to the control) also increased the level of the Ac-K. However, among the individual variants, including the control, no significant differences were found (Table 5) [F (4;125) = 0.19, $p=0.944$]. The effect of fertilization on the total variability was negligible (Table 6). Also HUDCOVA and SIROVY (1981) proved that fertilization did not considerably affect the acid soluble potassium. But other results presented by HUDCOVA and VOPLAKAL (1981) indicated that intensive NPK fertilization increased the content not only of Ex-K, but also of forms with stronger potassium bonds (acid soluble K and total K). Also TAKAC and PESLOVA (1994) proved that especially potassium supplied in fertilizers produced this form. On the contrary, HRTANEK (1987) discovered that increasing doses of fertilizers had no effect on the content of Ac-K.

The effect of the growing region

The results confirmed that the growing region is an important factor affecting mainly the content of the Ac-K. Table 6 shows that the effect of the factor on its total variability increased with the increasing intensity of the bonding of potassium to the sorption soil complex. The differences between the average content of Sol-K characterizing the growing regions were not statistically significant (Table 5) [F (1; 125) = 0.82, $p=0.367$]. A significant difference was detected in the Ex-K [F (1; 125) = 34.99, $p<0.001$] and Ac-K [F (1; 125) = 30.32, $p<0.001$]. The amounts of both of these fractions of potassium were 40 % higher in the sugar beet growing region than in the potato growing region (Table 5).

REFERENCES

ASKEGAARD M., ERIKSEN J. 2002. *Exchangeable potassium in soil as indicator of potassium status in an organic crop rotation on loamy sand.* Soil Use Manage., 18: 84-90.

ASKEGAARD M., ERIKSEN J., OLESEN J.E. 2003. *Exchangeable potassium and potassium balances in organic crop rotations on coarse sand.* Soil Use Manage., 19: 96-103.

BANSAL S.K., PASRICHA N.S., IMAS P. 2002. *Monitoring changes in potassium fertility in major benchmark soil series in India under long-term cropping.* In: *Feed the soil to feed the people – The role of potash in sustainable agriculture.* JOHNSTON A.E. (Ed). IPI Golden Jubilee Congress, Basel, Switzerland.

BERRY P.M., STOCKDALE E.A., SYLVESTER-BRADLEY R., PHILIPPS L., SMITH K.A., LORD E.I., WATSON C.A., FORTUNE S. 2003. *N, P and K budgets for crop rotations on nine organic farms in the UK.* Soil Use Manage., 19: 112-118.

BRAR M.S., SUBBARAO A., SEKHON G.S. 1986. *Solution, exchangeable and nonexchangeable potassium in five soil series from the alluvial soils region of northern India.* Soil Sci., 142: 229-234.

FORTUNE S., ROBINSON J.S., WATSON C.A., PHILIPPS L., CONWAY J.S., STOCKDALE E.A. 2005. *Response of organically managed grassland to available phosphorus and potassium in the soil and supplementary fertilization: field trials using grass-clover leys cut for silane.* Soil Use Manage., 21:370-376.

GOULDING K.W.T., LOVELAND P.J. 1986. *The classification and mapping of potassium reserves in soils of England and Wales.* Eur. J. Soil Sci., 37: 555-565

HEMING S.D. 2004. *Potassium balances for arable soils in southern England 1986-1999.* Soil Use Manage., 20:410-417.

HOLMQVIST J., OGAARD A.F., OBORN I., EDWARDS A.C., MATTSSON L., SVERDRUP H. 2003. *Application of the PROFILE model to estimate potassium release from mineral weathering in Northem European agricultural soils.* Eur. J. Agron., 20:149-163.

HRTANEK B. 1987. *Potassium fractions in the long-fertilized soils of Slovakia.* Rost. Vyr., 33:673-680.

HUDCOVA O. 1985. *The effect of fertilizing and liming on the content of available potassium in the soil.* Rost. Vyr., 31: 361-368

HUDCOVA O. 1989. *The influence of long-term fertilizing on the factors of soil potassium regime.* Rost. Vyr., 35: 9-16

HUDCOVA O., FURST Z. 1982. *The effect of gradated fertilization rates and different fertilization methods on the changes in the soil fixation of potassium.* Rost. Vyr., 28: 1233-1240

HUDCOVA O., SIROVY V. 1981. *The effect of gradated fertilizer application rates on the strength of potassium bonds in soils.* Rost. Vyr., 27: 517-523

HUDCOVA O., VOPLAKAL K. 1981. *The effect of high fertilizer application rates on the potassium regime in soil.* Rost. Vyr., 27: 289-298

JAVORSKÝ P., FOJTÍKOVÁ D., KALAŠ V., KRATOCHVÍL J., SCHWARZ M. 1987. *Chemical analysis in agricultural laboratories.* Ministry of Agriculture and Nutrition of the Czech Republic, Prague, Czech Republic. (in Czech)

JONES J.B. 1990. *Universal soil extractants – their composition and use.* Commun. Soil Sci. Plant Anal., 21: 1091-1101.

JOUANY C., COLOMB B., BOSC M. 1996. *Long-term effects of potassium fertilization on yields and fertility status of calcareous soils of south-west France.* Eur. J. Agron., 5: 287-294.

KUHLMANN H., WEHRMANN J. 1984. *K-Fertilization Recommendation on the Foundation of 81 Experiments with Small Grains and Sugar-Beets on Loes Soils in the Southern Part of Lower Saxony (West-Germany).* Zeitschrift fur Pflanzenernahrung und Bodenkunde, 147: 349-360.

MENGEL K., KIRKBY E.A., KOSEGARTEN H., APPEL T. 2001. *Principles of plant nutrition.* Kluwer Academic Publishers, Netherlands.

NEUBERG J. 1985. *Complex methodic of plant nutrition. 2nd part.* UVTIZ, Prague. (in Czech)

OBORN I., ANDRIST-RANGEL Y., ASKEKAARD M., GRANT C.A., WATSON C.A., EDWARDS A.C. 2005. *Critical aspects of potassium management in agricultural systems.* Soil Use Manage., 21:102–112.

ŘGAARD A.F., KROGSTAD T., LOES A.K. 2001. *Potassium uptake by grass from a clay and a silt soil in relation to soil tests.* Acta Agric. Scand. Sect. B-Soil Plant Sci., 51: 97-105.

PAL Y., WONG M.T.F., GILKES R.J. 1999. *The forms of potassium and potassium adsorption in some virgin soils from south-western Australia.* Aust. J. Soil Res., 37: 695-709.

POSS R., FARDEAU J.C., SARAGONIT H., QUANTIN P. 1991. *Potassium release and fixation in Ferralsols (Oxisols) from Southern Togo.* Eur. J. Soil Sci., 42: 649-660.

RICHTER R., HLUSEK J., TRAVNIK K. 2002. *Changes in soil available potassium with varying intensity of fertilization.* In: *Feed the soil to feed the people – the role of potash in sustainable agriculture.* JOHNSTON A.E. (Ed), IPI Golden Jubilee Congress, Basel, Switzerland

SHEPARD F.P. 1954. *Nomenclature based on sand-silt-clay ratios.* J. Sediment. Res., 24: 151-158.

SPARKS D.L. 1987. *Potassium dynamics in soils.* Adv. Soil Sci., 6: 1-63.

TAKAC J, PESLOVA H. 1994. *Potassium fertilization influence on fruit quality.* Agrochemia, 34: 175-178.

WANG J.G., ZHANG F.S., CAO Y.P., ZHANG X.L. 2000. *Effect of plant types on release of mineral potassium from gneiss.* Nutr. Cycl. Agroecosyst., 56: 37-44.

WHITEHEAD D.C. 2000. *Nutrient elements in grassland: Soil-plant-animal relationships.* CABI Publishing, Wallingford, UK.

ZENG Q.P., BROWN P.H. 2000. *Soil potassium mobility and uptake by corn under differential soil moisture regimes.* Plant Soil, 221:121-134.

ATMOSPHERIC RADON CONCENTRATION AROUND A PHOSPHOGYPSUM STACK AT WISLINKA (NORTHERN POLAND)*

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Abstract

The measurements of atmospheric radon concentration were carried out for one year at the turn of 2008/2009, using Kodak LR 115 passive track detectors. The average atmospheric radon activity near the phosphogypsum stack was 104 Bq m^{-3}. This is below the level accepted for indoor air. The results indicate that there are strong positive correlations between radon concentration and temperature (r=0.9) or atmospheric pressure (r=0.9) and a negative correlation between radon concentration and humidity (r=-0.7) or wind velocity (r=-0.7). Moreover, for all monitoring points the correlation between radon atmospheric concentrations measured in four seasons of the year were analyzed. The correlation coefficients are as follows: winter-summer 0.7, winter-autumn 0.2, winter-spring 0.2. Influence of radon exhalation from the stack was especially distinct in winter when the background radon activity was low. Spring and autumn farmland cultivation works increase radon exhalation from the soil, so that the contribution of radon emitted from the stack was less obvious.

K e y   w o r d s: radon activity concentration, phosphogypsum stack, correlation coefficients of radon concentration, meteorological parameters.

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STĘŻENIE RADONU W ATMOSFERZE W OKOLICY HAŁDY FOSOFGIPSÓW W WIŚLINCE (PÓLNOCNA POLSKA)

Abstrakt

Pomiary stężenia radonu w atmosferze przeprowadzono w ciągu jednego roku, na przełomie lat 2008/2009, z użyciem detektorów Kodak LR-115. Średnie stężenie radonu w atmosferze wynosiło 104 Bq m^{-3}, co jest wartością poniżej dopuszczalnego stężenia tego gazu w budynkach mieszkalnych. Wykazano silne dodatnie korelacje między stężeniem radonu i temperaturą (r=0.9), i ciśnieniem atmosferycznym (r=0.9) oraz ujemne korelacje między stężeniem radonu a wilgotnością powietrza (r=-0.7) oraz prędkością wiatru (r=-0.7). Ponadto obliczono korelację między stężeniami radonu zarejestrowanymi w wyznaczonych punktach pomiarowych w poszczególnych sezonach. Współczynniki korelacji w sezonach: zima – lato, zima – jesień, zima – wiosna wynoszą odpowiednio: 0,7; 0,2; 0,2. Wpływ ekshalacji radonu z hałdy na stężenie radonu w atmosferze jest szczególnie widoczny w sezonie zimowym, w którym poza obszarami sąsiadującymi z hałdą rejestruje się najniższe w roku stężenie tego gazu. Wiosną i jesienią prace rolnicze wpływają na wzrost ekshalacji radonu z gruntu, przez co udział radonu, który wydostał się z hałdy, staje się mniej wyrazisty.

Słowa kluczowe: stężenie radonu, hałdy fosfogipsowe, współczynniki korelacji między stężeniem radonu a parametrami meteorlologicznymi.

INTRODUCTION

The monitoring of atmospheric radon activity around a phosphogypsum stack located on the Baltic coast in northern Poland was carried for four seasons from autumn 2008 to autumn 2009. Phosphogypsum, a byproduct of phosphate processing, is a well known source of radon (Rabi, Mohamad 2006, Dueńas et al. 2007, Lysandrou et al. 2007). Phosphates, especially sedimentary ones, have a high uranium content. In deposits found in Marocco and Florida, it reaches 1500-1700 Bq kg^{-1} (Skorovarov et al. 1998). Concentrations of the activity of natural radionuclides in phosphogypsum in different countries are listed in Table 1 (Beretka, Mathew 1985, Fourati, Faludi 1988, Kobal et al. 1990, Laiche, Scott 1991, Luther et al. 1993, Rutherford et al. 1994, Burnett et al. 1996, Hull, Burnett 1996, Papastefanou et al. 2006, Borrego et al. 2007, Abril et al. 2009, Dueńas et al. 2010). High radium and low uranium concentrations in phospogypsum are connected with radionuclide separation during the wet acid process. Because of the high radium content, using phosphogypsum in civil engineering (Rabi, Silva 2006, Reijnders 2007, Tayibi et al. 2009, Máduar et al. 2011) and agriculture (Papastefanou et al. 2006, Abrill et al. 2008, 2009) is controlled. In agriculture, the EPA radium activity limit is set at of 370 Bq kg^{-1} (EPA, *Federal Register* 1999).

The main aim of this study was to evaluate the environmental impact of a phosphogypsum stack on radon activity concentration. Another objective was to describe the seasonal variation of this phenomenon.

Table 1

Concentrations of natural radionuclides in phosphogypsum in different countries

Country/region	^{226}Ra	^{210}Pb	^{238}U	^{232}Th	^{228}Ra	^{40}K	References
USA, Central Florida	907 (505-1353)	860 (578-11833)	132 (45-368)				Hull and Burnett, 1996
	820 (340-2000)		76				May and Sweeney, 1984 a,b fide in Papastefanou et al., 2006
	1100 (836-1230)	1370 (1270-1430)	130 (93-190)				Horton et al., 1988 fide in Papastefanou et al., 2006
	1140 (844-1670)						Guidry, 1990 fide in Papastefanou et al., 2006
	1140	1370	130				Rutherford et al., 1994
USA, North Florida	433 (270-598)	435 (347-553)	130 (23-452)				Hull and Burnett, 1996
	500 (477-548)						Rossler et al., 1979 fide in Papastefanou et al., 2006
USA, Louisiana	1100 (700-1700)						Laiche and Scott, 1991
USA, Mississippi	780						Mullins and Mitchell, 1990 fide in Papastefanou et al., 2006
Canada, Alberta	890			5.8			Luther et al., 1993
Australia	451			10			Beretka and Mathew, 1985
	500		10				Rutherford et al., 1994
Sweden	15		390				Rutherford et al., 1994
Hungary	1093				68		Fourati and Faludi, 1988
Yugoslavia	390						Kobal et al., 1988
Greece	633	476	28	3	3	8	Papastefanou et al., 2006
Spain	620		140				Borrego et al., 2007
Spain	730 (670-790)						Abrill et al., 2009
Spain	647			8		33	Dueñas et al.., 2010
China	85	82	15				Burnett et al., 1996
Indonesia	473	480	43				Burnett et al., 1996
India	510	490	60				Burnett et al., 1996
Egypt	100						Burnett et al., 1996

MATHERIAL AND METHODS

The examined phosphogypsum stack is located in the northern part of Poland, close to the Baltic Sea (Figure 1A), southeast of Gdansk, in the vicinity of a small village called Wiślinka.

The climate of the area transitional between maritime and continental. It is also strongly influenced by the lowland topography of this part of Eu-
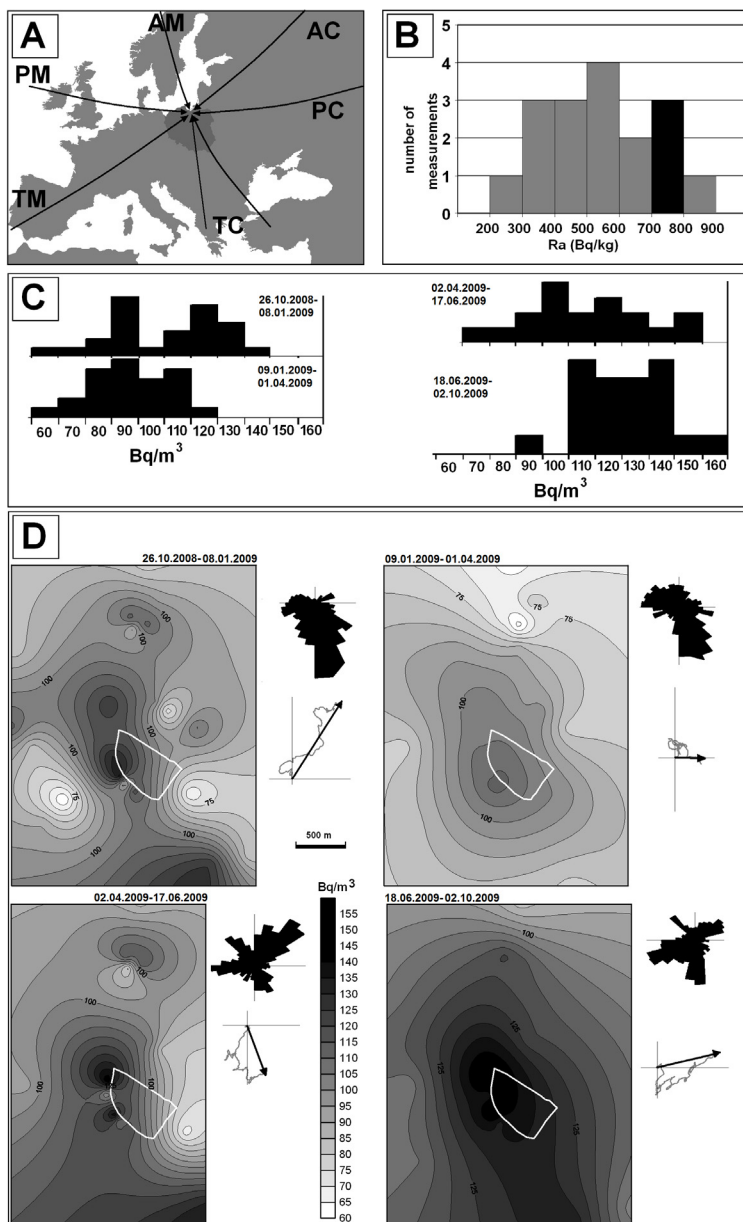
Fig. 1. Results of atmospheric radon monitoring: A) Area of the investigation and main directions of atmospheric air migration, *B)* Distribution of radium equivalent activity of the gamma dose for the Wiślinka phospogypsum stack. Black class column corresponds to gamma spectrometric laboratory measurement of Wiślinka phosphogypsum, C) Seasonal variation of average radon activity concentration, D) Seasonal changes in spatial distribution of atmospheric radon activity around the phosphogypsum stack connected with the cumulative air movement vector and wind directions

rope, resulting in air masses moving quickly from the Atlantic Ocean or the North Sea. Arctic marine, arctic continental, polar marine, polar continental, tropical marine and tropical continental air masses enter this area depending on regional meteorological conditions.

The main pressure systems that affect the weather are the Icelandic low (stronger in winter) and the Azores anticyclone (more active in summer), and the changing atmospheric fronts from Asia: the East Asian high in winter and the South Asian low in summer. However, the Baltic Sea is the major contributor to the local climate.

This part of Poland has predominantly westbound winds, caused by the eastward movement of barometric lows from the Atlantic (Figure 1A). In summer months, westerlies (WSW) prevail, whereas in other seasons easterlies (NE) are more significant. The winds are typically weak to moderate, with velocity between 2 to 10 m s^{-1}. Strong and very strong winds occur at the seaside, causing storms; there their velocity may exceed 30 m s^{-1}. Southern and northern winds of local significance are sea and land breezes. In that area, the southern direction of winds is promoted by the local relief, dominated by the broad N-S Vistula River valley.

Spring starts in March, with temperatures varying from -1 to +20ºC, and lasts until June. Summer is quite warm, with the temperatures recorded during our study up to 31.3ºC. In autumn, days become colder until December, when winter begins and the temperature oscillates between a few degrees above 0ºC, dropping to -16.7ºC during our study. Winters last from December to March and include shorter or longer periods of snow. The ground is frozen from December to March. The average number of cloudy days a year, with the sky more than 80% overcast, is 120-160; there are 30-50 sunny days, with cloudiness below 20%. The annual precipitation ranges between 400 mm and 750 mm. The maximum precipitation occurs in autumn.

This area is a lowland and partly a depression, situated in the delta of the Vistula River. The fertile soils developed on alluvial muds encourage intensive agricultural activity. Locally, the subsoil bedrock consists of glacial sediments of the Pleistocene era as well as lacustrine and shallow marine sediments connected with various stages of Baltic Sea history.

The phosphogypsum stack covers an area of 36 ha and is 46 m high. Phosphogypsum dumping started in 1972. Since 1998, phosphogypsum stack has been reclaimed in the parts where the disposal was finished. Mixtures of water, sewage and grass seeds were used for rehabilitation of its surface.

Because of the ongoing reclamation works, the direct access to the tailing area was impossible, so the radiological characteristics of its material are based on the gamma dose data obtained by courtesy of the Fosfory Ciech company (BIERNACKA et al. 2005). Gamma dose variation in the tailing area varies in the range 144-400 nSv h^{-1}, which is an equivalent of radium activity in the range 337-937 Bq kg^{-1} (Figure 1B). Radium activity of the

Wiślinka phosphogypsum, according to the gamma spectrometric laboratory measurements obtained by courtesy of Greenpeace (personal communication), is of the order 870 Bq kg^{-1}. Published data on radium activity from other phosphogypsum stacks show that in Moroccan phosphorites it varies in the range 580±30 to 670±35 Bq kg^{-1} (BOLÍVAR et al. 2009). This corresponds to the modal class distribution of the diagram presented in Figure 1B.

The monitoring of atmospheric radon activity was carried out from autumn 2008 to summer 2009 in 30 sites (some of the detectors during exposition were lost) localized around the phosphogypsum stack at Wiślinka. The measurements were carried out using Kodak LR-115passive track detectors. The detectors were placed in chambers designed for protection against insulation and humidity. The chambers were fixed on trees at a height of 1.5 m above the ground. The detectors were exposed in the periods: 26.10.2008--8.01.2009, 9.01.2009-1.04.2009, 2.04.2009-17.06.2009 and 18.06.2009-2.10.2009, roughly corresponding to the seasons: autumn, winter, spring and summer. After exposure, the detectors were transported to a laboratory, where they were etched for 1.5 h in 2.5 N NaOH at 60ºC. Tracks generated by the alpha-particle impact were counted using an optical microscope at the magnification of 100x. Density of the tracks was converted into radon concentration using calibration coefficients as described by SRIVASTAVA et al. (1995).

Data on the temperature, humidity, atmospheric pressure, wind velocity and direction were obtained by courtesy of the Foundation Armaag, which provides information on meteorological conditions in the Gdansk agglomeration. Their measurements were carried out in an automatic measuring station in the vicinity of the phosphogypsum stack. Average values for one-hour periods were obtained. Temporal variation in temperature, pressure, humidity and wind velocity was analyzed for series covering the radon monitoring period. In respect of winds, not only distribution of wind directions and velocity but also the combined parameter called cumulative air movement was analyzed.

RESULTS AND DISCUSSION

The average radon concentration for the whole measurement period was 104 Bq m^{-3}, which is significantly higher than 4.4 Bq m^{-3} and 6.5 Bq m^{-3} obtained for whole Poland by BIERNACKA et al. (1991) and Jagielak et al. (1998), respectively. Comparable results have been reported from Bad Gastein in Austria (30-100 Bq m^{-3}; POHL-RÜLING, HOFMANN 2002) and from the coal mining town Nowa Ruda in Poland (98 Bq m^{-3}; TCHORZ-TRZECIAKIEWICZ, SOLECKI 2011).

The seasonal variation in radon concentration in the atmosphere is presented in Figures 1C and 2. The highest values were measured in summer (the average 100 Bq m^{-3}), and the lowest – in winter (the average 88 Bq m^{-3}), whereas in spring and autumn the radon concentrations were intermediate.

The seasonal variation in meteorological parameters such as temperature, atmospheric pressure, relative humidity, wind velocity, wind direction and cumulative air movement is shown in Figure 2 and 1D. The average temperature varied from 1ºC in winter to 17ºC in summer; the average atmospheric pressure was from 1009 hPa in winter to 1015 hPa in spring; the average relative humidity was from 71% in spring to 84% in autumn; the average wind velocity was 2.1 m s^{-1}.

Correlation of the seasonal variation in the atmospheric radon concentration with the meteorological parameters is presented in Figure 3A. Pearson's correlation coefficients are shown above the trend lines. The correlation coefficients indicate that radon concentration was strongly positively correlated with temperature (r=0.9) and pressure (r=0.9). Correlation with humidity and wind speed was negative (inverse) and the same in both cases (r=-0.7).

The positive correlation between temperature and radon concentration is consistent with the results obtained by ISRAELSSON et al. (1972), MORISS and FRALE (1989), STRANDEN et al. (1991), SUNDAL et al. (2008), PRASAD et al. (2008). Those researchers have noticed that higher temperature increases radon exhalation from the ground and thus increases radon concentration in the atmosphere. The negative correlation between relative humidity and radon concentration is the result of negative correlation between temperature and relative humidity. It is commonly known that temperature increase results in the decrease of relative humidity.

Frozen soil and snow cover reduce radon exhalation from the ground (YAMAZAWA et al. 2005). In the investigated area, the snow cover and frost kept the winter average value of radon concentration the lowest of all seasons. The present results (Figure 1D) indicate that radon anomaly around the stack was more distinct. The most probable explanation is that the snow cover was thinner on the stack and thicker on the ground around the stack. Moreover the wind velocity and cumulative air movement vector were the lowest in winter (Figure 1D). The highest concentrations of radon were measured near the stack; further away the values were lower. Apart from the strong influence of the stack, the effect of the wind direction was noticeable. The concentric contours indicating radon concentrations around the stack are stretched in the NW-SE direction. It was only in winter that the relation between radon activity in the atmosphere and the distance from the stack and wind direction was so clear.

The positive correlation between atmospheric pressure and radon concentration indicates that increase in atmospheric pressure results in higher radon concentration. This is consistent with the results obtained by COSMA

et al. (1999) and Cɪɢᴏʟɪɴɪ et al. (2009), but contradicts the findings of Cʟᴇ-
ᴍᴇɴᴛs and Wɪʟᴋᴇɴɪɴɢ (1974) and Dᴏ̋ʀʀ and Mᴜ́ɴɴɪᴄʜ (1990). The latter con-
cluded that a 1% drop in atmospheric pressure results in an increase of
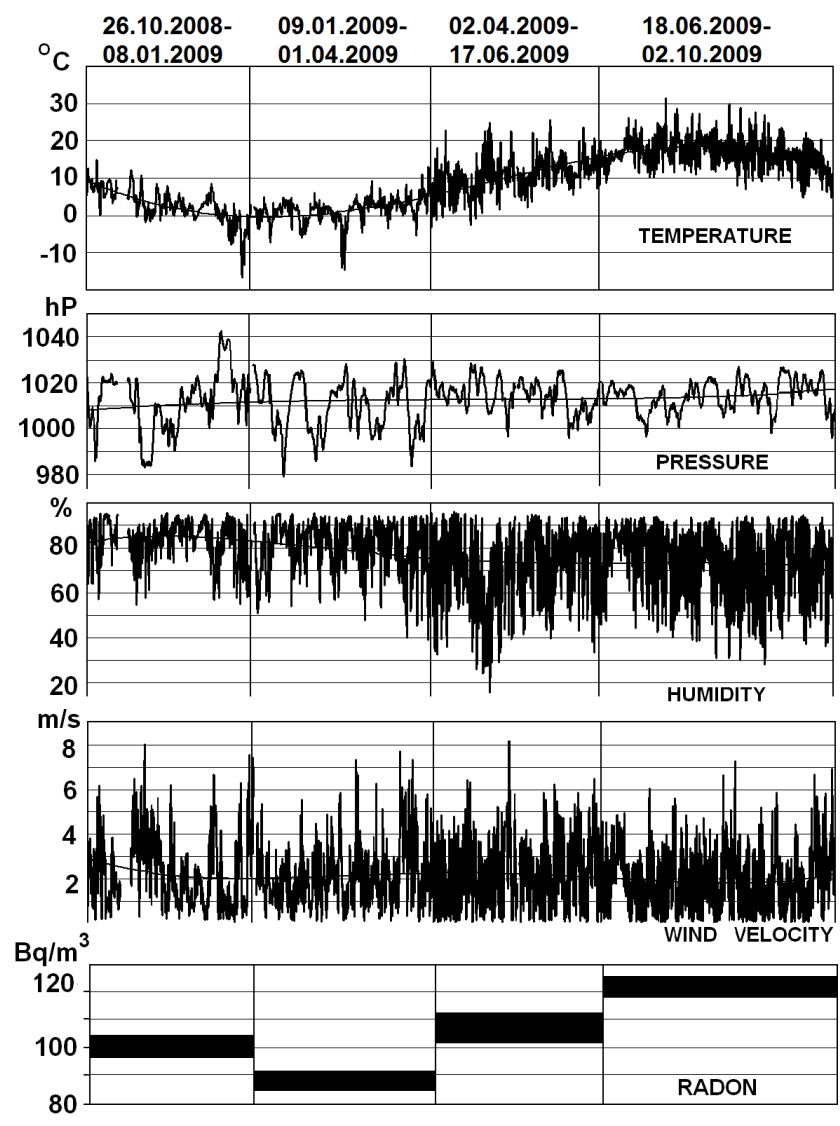radon exhalation by 6%.

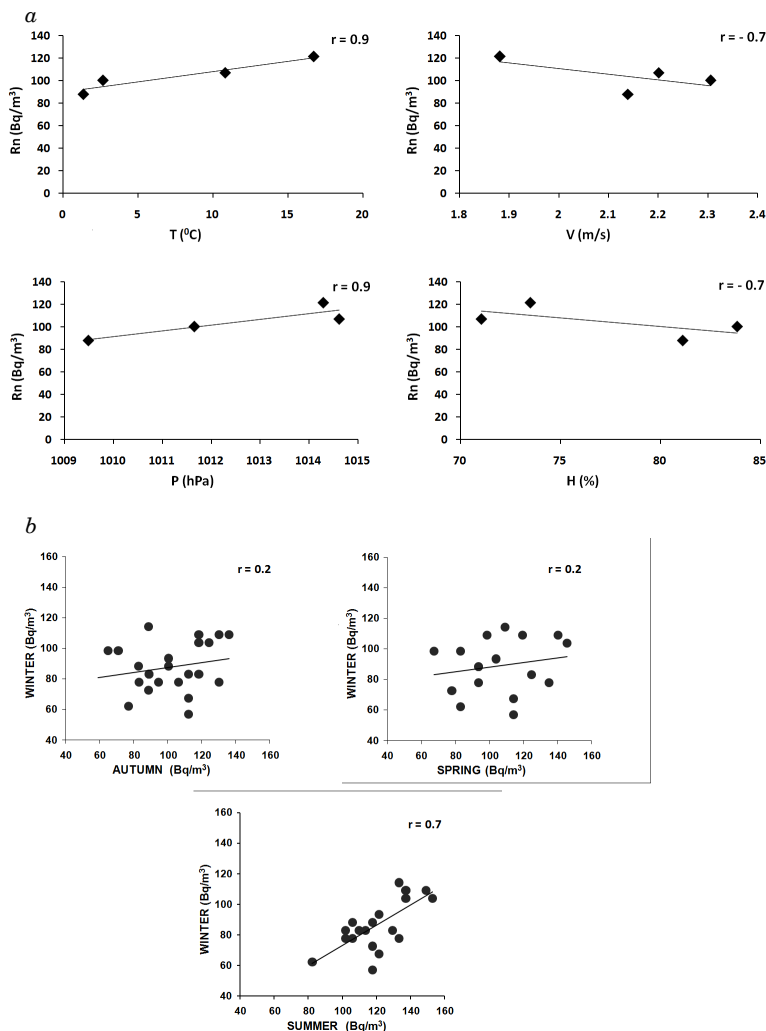Fig. 2. Correlation of atmospheric radon activity with meteorological parameters

Fig. 3. Correlations of atmospheric radon activity concentration: a – with meteorological parameters, b – for results obtained at measurements points in various seasons

For all the monitoring points, the correlation between radon atmospheric concentration measured in various seasons was analyzed. The correlation coefficients were as follows: 0.7 for winter-summer, 0.2 for winter-autumn and 0.2 for winter-spring (Figure 3b). The positive correlation coefficient between winter and summer (r=0.7) means that the spatial distribution pattern of the measured values was similar in both seasons. The correlation coefficients between winter-autumn and winter-spring (r=0.2) indicate that there is no correlation between these seasons.

The lack of correlation of winter measurements with autumn and spring ones cannot be explained only by the changes of wind direction, because in the summer the wind direction was different than in the winter, but the correlation between these two seasons is still significant. There must be an additional factor other than wind direction which modifies the spatial distribution of the atmospheric radon concentration. The whole area around the stack is rural in character and most of the farming practice takes place in autumn and spring. In these seasons, the plant cover is less dense than in summer. Soil cultivation causes loosening of the soil structure, which can stimulate radon exhalation from the ground. The surveys done by CHAUHAN and CHAKARVARTI (2002), SHWEIKANI et al. (1995) support the hypothesis that loose soil structure is a factors that facilitates radon transport. Radon exhalation from cultivated fields strongly influenced its distribution pattern around the stack.

CONCLUSIONS

The measured radon concentrations do not exceed the values accepted for indoor air. The influence of radon exhalation from the stack was especially distinct in winter, against the background of generally low values of winter radon activity. Spring and autumn soil cultivation increased radon exhalation from the soil, so that the contribution of radon emitted from the stack was less obvious.

REFERENCES

ABRILL J.M., GARCÍA-TENORIO R., ENAMORADO S.M., HURTADO M.D., ANDREU L., DELGADO A. 2008. *The cumulative effect of three decades of phosphogypsum amendments in reclaimed marsh soils from SW Spain: ^{226}Ra, ^{238}U, and Cd contents in soils and tomato fruit.* Sci. Total. Environ., 403: 80-88.

ABRIL J.M., GARCÍA-TENORIO R., MANJÓN G. 2009. *Extensive radioactive characterization of phosphogypsum stack in SW Spain: ^{226}Ra, ^{238}U, ^{210}Po concentrations and ^{222}Rn exhalation rate.* J. Hard. Matter., 164: 790-197.

ABRILL J.M., GARCÍA-TENORIO R., PERIÁŃEZ R., ENAMORADO S.M., ANDREU L., DELGADO A. 2009. *Occupational dosimetric assessment (inhalation pathway) from the application of phosphogypsum in agriculture in South West Spain.* J. Environ. Radioactiv., 100: 29-34.

BERETKA J., MATHEW P.J. 1985. *Natural radioactivity of Australian building materials, industrial wastes and by-products.* Health Phys., 48: 87-95.

BIERNACKA M., HENSCHKE J., JAGIELAK J. 1991. *Radiation atlas of Poland. Central Laboratory for Radiological Protection*. Warsaw. (In Polish)

BIERNACKA M., ISAJENKO K., MAMONT-CIEŚLA K., ŻAK A. 2005. *Radiological research in the vicinity of a phosphogypsum stack in Wiślinka*. Central Laboratory for Radiological Protection. (In Polish)

BOLÍVAR J.P., PÉREZ-MORENO J.P., MAS J.L., MARTÍN J.E., SAN MIGUEL E.G., GARCÍA-TENORIO R. 2009. *External radiation assessment in a wet phosphoric acid production plant*. Appl. Radiat. Isotopes, 67: 1930-1938.

BORREGO E., MAS J.L., MARÍTN J.E., BOLÍVAR J.P., VACA F., AGUADO J.L. 2007. *Radioactivity levels in aerosol particles surrounding a large TENORM waste repository after application of preliminary restoration work*. Sci. Total Environ., 377: 27-35.

BURNETT W.C., SCHULTZ M.K., CARTER D.H. 1996. *Radionuclide flow during the conversion of phosphogypsum to ammonium sulfate*. J. Environ. Radioactiv., 32: 33-51.

CHAUHAN R.P., CHAKARVARTI S.K. 2002. *Radon diffusion through soil and fly ash: effect of compaction*. Radiat. Meas., 35: 143-146.

CIGOLINI C., POGGI P., RIPEPE M., LAIOLO M., CIAMBERLINI C., DELLE DONE D., ULIVIERI G., COPPOLA D., LACCANA G., MARCHETTI E., PISCOPO D., GENCO R. 2009. *Radon surveys and real time monitoring at Stromboli volcano: Influence of temperature, atmospheric pressure and tidal forces on ^{222}Rn degassing*. J. Volcanol. Geoth. Res., 184: 381-388.

CLEMENTS W.E., WILKENING M.H. 1974. *Atmospheric pressure effect on ^{222}Rn transport across the earth-air interface*. J. Geophys. Res., 79 (33): 5025-5029.

COSMA C., BACIU C., RISTOIU D. 1999. *Some aspects of radon potential in soil land underground waters in the Somesul Mic Hudrographic basin (North-Western Romania)*. Proc. 5th Int. Conf. on Rare Gas Geochemistry, 305-314.

DÖRR H., MÜNNICH K.O. 1990. *^{222}Rn flux and soil air concentration profiles in West-Germany. Soil ^{222}Rn as tracers for gas transport in the unsaturated soil zone*. Tellus, 42: 20-28.

DUEŃAS C., FERNÁNDEZ M.C., CAŃETE S., PÉREZ M. 2010. *Radiological impacts of natural radioactivity from phosphogypsum piles in Huelva (Spain)*. Radiat. Meas., 45: 242-246.

DUEŃAS C., LIGER E., CAŃETE S., PÉREZ M., BOLÍVAR J.P. 2007. *Exhalation of 222Rn from phosphogypsu piles located at the Southwest of Spain*. J. Environ. Radioactiv., 95: 63-74,

EPA, 1999. *Federal Register*. Part VII. 40 CFR. Part 61. *Natural emission standards for hazardous air pollutants*. Subpart R Rule: *National emission standards for radon emission from phosphogypsum stacks*. Section 61204. *Distribution and use of phosphogypsum for agricultural purposes* (64FR 5574, February 3, 1999), U.S. Environmental Protection Agency, Washington, DC 20460, 64(22): 5574-5580.

FOURATI A., FALUDI G. 1988. *Changes in radioactivity of phosphate rocks during the process of production*. J. Radioanal. Nucl. Ch., 125: 287-293.

GOH T.B., OSCARSON D.W., CHESLOCK M., SHAYKEWICH C. 1991. *Fluence rate of radon from soil: effect of sorption barriers, moisture content, and temperature*. Health Phys., 61 (3): 359--365.

HULL C.D., BURNETT W.C. 1996. *Radiochemistry of Florida phosphogypsum*. J. Environ. Radioactiv., 32: 213-237.

ISRAELSON S., KNUDSEN E., UNGETHŮM E., DAHLGREN L. 1972. *On the natural alpha- activity near the ground*. Tellus, 24: 368-379.

JAGIELAK J., BIERNACKA M., HENSCHKE J., SOSIŃSKA A. 1998. *Radiation atlas of Poland*. Central Laboratory for Radiological Protection. Warsaw. (In Polish)

KOBAL I., BRAJNIK D., KALUZA F., VENGUST M. 1990. *Radionuclides in effluents from coal mines, a coal-fired power plant and a processing plant in Zasanje, Slovenia (Yugoslawia)*. Health Phys., 58: 81-85.

LAICHE T.P., SCOTT M.L. 1991. *A radiological evaluation of phosphogypsum*. Health Phys. 60: 691-693.

LUTHER S.M., DUDAS M.J., RUTHERFORD P.M. 1993. *Radioactivity and chemical characteristic of Alberta phosphogypsum.* Water Air Soil Poll., 69: 277-290.

LYSANDROU M., CHARALAMBIDES A., PASHALIDIS I. 2007. *Radon emanation from phosphogypsum and related minerals in Cyprus*. Radiat. Meas., 42: 1583-1585.

MÁDUAR M.F., CAMPOS M.P., MAZZILLI B.P., VILLAVERDE F.L. 2011 *Assessment of external gamma exposure and radon levels in a dwelling constructed with phosphogypsum plates*. J. Hazard. Mater., 190: 1063-1067

MORRIS R.C., FRALEY L. 1989. *Effects of vegetation, a clay cap and environmental variables on ^{222}Rn fluency rate from reclaimed U mill tailings*. Health Phys., 56: 431-440.

PAPASTEFANOU C., STOULOS S., IOANNIDOU A. MANOLOPOULOU M. 2006. *The application of phosphogypsum in agriculture and radiological impact*. J. Environ. Radioactiv., 89: 188-198.

POHL-RÜLING J., HOFMANN W. 2002. *Investigation of cancer mortality in the Gastein Valley, an area of high-level natural radiation*. Int. Congr. Ser., 1236: 27-29.

PRASAD Y., PRASAD G., GUSAIN G.S., CHOUBEY V.M., RAMOLA R.C. 2008. *Radon exhalation rate from soil samples of South Kumaun Lesser Himalays, India.* Radiat. Meas., 43: 369-374.

RABI J.A., ds SILVA N.C. 2006. *Radon exhalation from phosphogypsum building boards: symmetry constraints impermeable boundary conditions and numerical simulation of a test case.* J. Environ. Radioactiv., 86: 164-175.

RABI J.A., MOHAMAD A.A. 2006. *Parametric modeling and numerical simulation of natural-convective transport of radon -222 from a phosphogypsum stack into open air.* Appl. Math. Model., 30: 1546-1560.

REIJNDERS L. 2007. *Cleaner phosphogypsum, coal combustion ashes and waste incineration ashes for application in building materials*: *A review*. Build. Environ., 42: 1036-1042.

RUTHERFORD P.M., DUDAS M.J., SAMEK R.A. 1994. *Environmental impacts of phosphogypsum*. Sci. Total Environ., 149: 1-38.

TAYIBI H., CHOURA M., LÓPEZ A.F., ALGUACIL F.J., LÓPEZ-DELGADO A. 2009. *Environmental impact and management of phosphogypsum.* J. Environ. Manage., 40: 2377-2386.

SHWEIKANI R., GIADDUI T.G., DURRANI S.A. 1995. *The effect of soil parameters on the radon concentration values in the environment*. Radiat. Meas., 25: 581-584.

SKOROVAROV J.I., RUZIN L.I., LOMONOSOV A.V., TSELITSCHEV G.K. 1998. *Solvent extraction cleaning phosphoric acid in fertilizer production*. J. Radioanal. Nucl. Ch., 229: 111-116.

SRIVISTAVA D.S., SINGH P., RANA N.P.S., NAQVI A.H., AZAM A., RAMACHANDRAN T.V., SUBBA RAMU M.C. 1995. *Calibration factor for LR-115 Type II track detectors for environmental radon measurements.* Nucl. Geophys., 9: 487-495.

SUNDAL A.V., VALEN V., SOLDAL O., STRAND T. 2008. *The influence of meteorological parameters on soil radon levels in permeable glacial sediments*. Sci. Total Environ., 389: 418-428.

TCHORZ-TRZECIAKIEWICZ D.E., SOLECKI A.T. 2011. *Seasonal variation of radon concentrations in atmospheric air in the Nowa Ruda area (the Sudety Mountains) of southwest Poland*. Geochem. J., 45: 491-496.

YAMAZAWA H., MIYAZAKI T., MORIIZUMI J., IIDA T., TAKEDA S., NAGARA S., SATO K., TOKIYAWA, T. 2005. *Radon exhalation from a ground surface during cold snow season*. Int. Cong. Ser., 1276: 221-222.

RESPONSE OF PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.) TO SOIL CONTAMINATION WITH ZINC

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Abstract

Phytoremediation is one of the ways of removing toxic metals from soil. Phytoremediation relies on using plants which are highly capable of absorbing heavy metals to remove them from soil. In order to determine the effect of the degree of soil contamination with zinc on the production of biomass and concentration of zinc in ryegrass, a pot experiment was carried out, consisting of 6 treatments in which gradually increasing doses of zinc were used: control treatment (without zinc), 25, 50, 100, 200 and 400 mg Zn kg^{-1} off soil. The experiment was conducted on two types of soil, different in the cation exchange capacity: sand and sandy loam. The tested plant was perennial ryegrass cv. Nira.

The toxic effect of zinc on the growth of ryegrass was much more pronounced in the case of plants grown on sand than on sandy loam. When ryegrass was grown on sand, the toxic effect of zinc was observable even when the lowest rate of zinc had been introduced to soil: the biomass of the first cut was considerably depressed. On sandy loam, the toxic effect of zinc was not manifested until the highest rate of the contaminant had been introduced to soil (400 mg Zn kg^{-1} of soil).

The experiment has demonstrated the presence of a strong, statistically verified correlation between the content of zinc in soil extracted in 1 M HCl and the concentration and uptake of this metal in the following cuts of ryegrass. The concentration of zinc in grasses from the first cut was very high: 1660 mg Zn kg^{-1} d.m. on sand and 1200 mg Zn kg^{-1} d.m. on sandy loam. A one-year cultivation of perennial ryegrass only slightly lowered the content of zinc in soil. Although the concentration of zinc in harvested grass was very high, the total uptake was just a small percentage (1-2%) of the quantity of this metal introduced to soil. Perennial ryegrass cv. Nira is capable of accumulating very high amounts of zinc and is well tolerant to a high content of Zn in soil, which is why it can be used for sowing on land polluted with this heavy metal.

Key words: zinc, phytotoxicity, phytoremediation, perennial ryegrass.

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**REAKCJA ŻYCICY TRWAŁEJ (*LOLIUM PERENNE* L.)
NA ZANIECZYSZCZENIE GLEBY CYNKIEM**

Abstrakt

Jednym ze sposobów oczyszczania gleb z toksycznych metali jest fitoremediacja, polegająca na wykorzystaniu roślin o dużej zdolności do pobierania znacznych ilości metali ciężkich do usuwania ich z gleby. W celu określenia wpływu stopnia zanieczyszczenia gleby cynkiem na produkcję biomasy i koncentrację cynku w życicy trwałej wykazano doświadczenie wazonowe, składające się z 6 obiektów, na których zastosowano wzrastające ilości cynku: obiekt kontrolny (bez Zn), 25, 50, 100, 200 i 400 mg Zn kg^{-1} gleby. Doświadczenie prowadzono na dwóch glebach, różniących się pod względem pojemności sorpcyjnej: piasku i glinie piaszczystej. Rośliną testowaną była życica trwała, odmiany Nira.

Toksyczne działanie cynku na wzrost życicy ujawniło się w znacznie większym stopniu w przypadku uprawy na piasku niż na glinie piaszczystej. W doświadczeniu na piasku już po zastosowaniu najmniejszej dawki cynku (25 mg Zn kg^{-1} gleby) nastąpiło istotne zmniejszenie masy pierwszego odrostu traw, natomiast na glinie piaszczystej toksyczne działanie cynku ujawniło się dopiero po największym zanieczyszczeniu gleby tym metalem (400 mg Zn kg^{-1} gleby).

Badania wykazały istnienie silnej, statystycznie udowodnionej korelacji między zawartością cynku w glebie, ekstrahowanego 1 M HCl, a koncentracją oraz pobraniem Zn z plonem kolejnych odrostów życicy. Wraz ze wzrostem zanieczyszczenia gleby cynkiem istotnie zwiększała się koncentracja oraz pobranie tego metalu przez trawy. Stężenie cynku w życicy pierwszego odrostu, rosnącej na glebie o największym zanieczyszczeniu tym metalem, było bardzo wysokie i wynosiło 1660 mg Zn kg^{-1} s.m. na piasku oraz 1200 mg Zn kg^{-1} s.m. na glinie piaszczystej. Jednoroczna uprawa życicy trwałej tylko nieznacznie wpłynęła na zmniejszenie zawartości cynku w glebie. Pomimo bardzo wysokiej koncentracji cynku w trawach całkowite pobranie stanowiło tylko niewielką część (1-2,5%) ilości tego metalu wprowadzonego do gleby. Życica trwała odmiany Nira charakteryzuje się zdolnością do akumulacji bardzo dużych ilości cynku oraz dobrze znosi wysoką koncentrację Zn w glebie, dlatego może być wykorzystywana do zasiedlania terenów zanieczyszczonych tym metalem ciężkim.

Słowa kluczowe: cynk, fitotoksyczność, fitoremediacja, życica trwała.

INTRODUCTION

The emission of zinc from industrial sources, compared to that of other trace elements, is relatively high. Moreover, zinc in soil is a highly mobile element, which makes it a serious threat to the natural environment. In Poland, the percentage of soils to a greater or lesser degree contaminated with zinc is estimated to equal *ca* 11.3% (TERELAK et al. 1995).

Soil contamination with zinc causes retardation of plant growth, and its accumulation in crops may reach values dangerous to animals and people (BARAN, JASIEWICZ 2009). The phytotoxic effect of zinc depends mainly on soil properties, such as soil reaction, organic matter content orsoil absorbing complex, as well as the species of a crop and its current developmental phase (ROSZYK et al. 1988, RUSZKOWSKA 1991, ŁYSZCZ, SPIAK et al. 2000, KABATA-PENDIAS 2002, BARAN et al. 2008, GAMBUŚ et al. 2004).

Reclamation of soils burdened with high levels of heavy metals is a difficult and expensive process, which is why it is not conducted on a large scale. Phytoremediation is one of the ways in which excess heavy metals are removed from soil. Phytoremediation means growing plants which are highly capable of absorbing heavy metals from soil, thus removing them from the soil environment. Hyperaccumulators are the plant species which can contain up to 1-2% of a toxic element. In theory, such plants could remove as much as 200-1000 kg of metals per 1 ha. However, as there are no detailed agronomic recommendations concerning the cultivation of such plants, these species usually yield too little biomass. Phytoremediation can also involve typical crops. Although they do not accumulate as much of trace metals as hyperaccumulators, these plants produce much more biomass and can therefore improve the effects of soil remediation. The advantages of phytoremediation techniques are their low cost and easy application. The disadvantages include low efficacy and the problem of handling the biomass which contains large amounts of toxic elements (SAS-NOWOSIELSKA et al. 2004, KARCZEWSKA 2005, MARECIK et al. 2006).

The aim of this study was to evaluate the usefulness of perennial ryegrass, cv. Nira for phytoremediation of soils contaminated with zinc.

MATERIAL AND METHODS

This study has been performed as a strict pot experiment set up in 2007 in a greenhouse at the University of Warmia and Mazury in Olsztyn. The experiment consisted of 6 treatments in which increasing quantities of zinc in the form of $ZnSO_4 \cdot 7H_2O$ were added to soil prior to sowing ryegrass: control treatment (no Zn added), 25, 50, 100, 200 and 400 mg Zn kg^{-1} of air dry soil.

The experiment was carried out on two soils, sand and sandy loam, different in the sorption capacity. The content of organic carbon was 5.67 g kg^{-1} in sand and 10.05 g kg^{-1} in sandy loam. Some of the physicochemical properties of the soils are presented in Table 1.

The pots were filled with 8.0 kg of air dry soil mass. The tested plant was perennial ryegrass (*Lolium perenne* L.) cv. Nira. The grass was cut three times in the early phase of heading. Each treatment comprised four replications.

The nitrogen, phosphorus, potassium and magnesium fertilization was identical in all the fertilized treatments. When setting up the experiment, 0.4 g N, 0.2 g P, 0.4 g K and 0.08 g Mg per pot were applied. After the first cut, additional 0.5 g N, 0.4 g K and 0.1 g Mg were introduced to soil, and after the second cut the soil was amended with 0.25 g N, 0.2 g K and 0.05 g Mg per pot. Nitrogen was applied as NH_4NO_3; phosphorus was used in the form of KH_2PO_4; potassium was introduced as KH_2PO_4 and KCl, whereas magnesium was taken as $MgSO_4 \cdot 7H_2O$.

Some physicochemical properties and texture class of soils used in experiment

Texturec-lasses USDA classifi-cation	Soilfraction			C org. g kg^{-1} of soil	pH$_{KCl}$	Hh mmol H$^+$ kg^{-1} of soil	Base saturation mmol (+) kg^{-1} of soil	CEC mmol (+) kg^{-1} of soil	mg Zn kg^{-1} of soil
	diameter (mm)								
	2.0-0.05	0.05-0.002	< 0.002						
Sandy loam	72.3	25.3	2.4	10.05	6.16	14.1	57.0	71.1	14.03
Sand	86.7	12.1	1.2	5.67	5.72	8.3	18.3	26.6	7.19

Grass seeds were sown after three weeks of the incubation of soil with the applied salts. After emergence, 20 plants of ryegrass were left in each pot. The soil moisture was maintained as the level of 70% of the maximum water capacity.

In order to determine the initial concentration of Zn in soil (extraction in 1 M HCl) in all the treatments, an additional trial was set up in which soil was incubated for three weeks with the same quantities of zinc per pot as used in the plant growing experiment.

Dried and ground plant samples were mineralized (separately for each pot) in a mixture of concentrated nitric (V), chloric (VII) and sulphuric (VI) acids, in a volumetric ratio between the acids equal 40:10:1. Zinc was determined in the mineralized samples with the atomic absorption spectrometric method.

Prior to the experiment, the soils were analyzed to determine the following parameters: pH in 1 M KCl by potentiometry, content of organic carbon by Kurmies method (WALINGA et al. 1992), base saturation and hydrolytic acidity (Hh) by Kappen method, Zn concentrations by flame AAS after soil extraction in 1 M HCl and soil particle size distribution using the laser diffraction method (BUURMAN et al. 1997).

After the third cut, soil samples were taken from each pot. They were dried and passed through a 1 mm mesh sieve. Afterwards, zinc in soil samples was determined with the AAS method, following soil extraction in 1 M HCl.

Statistical processing of the data involved analysis of variance for a one-factor pot experiment in a completely random, orthogonal arrangement. Significance of differences between the treatment means was verified using t-Student test. Correlations between selected traits were determined with the analysis of correlation and regression.

RESULTS AND DISCUSSION

In the treatments set up on sand, as the soil contamination with zinc increased (treatments with 25, 50, 100, 200 and 400 mg Zn kg^{-1} soil), grass yields from all harvested cuts tended to decrease (Table 2). Astrong negative correlation appeared for pots filled with zinc contaminated soil between the content of zinc in soil and the harvested mass of the three cuts (Figure 1). In the first cut, a significant decrease in the yield was already noticeable in response to the lowest zinc rate (25 mg Zn kg^{-1} of soil); in the second cut, it was the second smallest rate, i.e. 50 mg Zn kg^{-1} of soil, that led to

Table 2

Effect of soil contamination with zinc on the yielding of perennial ryegrass

Zn rate (mg kg^{-1} of soil)	Yields of perennial ryegrass (g of d.m. pot^{-1})							
	sand				sandy loam			
	1st cut	2nd cut	3rd cut	total yield	1st cut	2nd cut	3rd cut	total yield
0	18.40	22.73	16.03	57.16	27.97	19.90	21.37	69.24
25	15.97	21.13	15.93	53.03	27.60	21.73	20.80	70.13
50	14.97	20.57	15.70	51.24	29.67	21.03	20.73	71.43
100	14.33	19.50	14.23	48.06	28.63	21.03	20.17	69.83
200	12.73	19.47	14.27	46.47	29.70	22.03	20.00	71.73
400	8.83	17.13	13.40	39.36	24.37	20.73	20.50	65.60
$LSD_{0.01}$	1.81	2.00	1.41		2.50	3.11	2.94	
$LSD_{0.001}$	2.55	2.83	1.99		3.53	4.40	4.15	

$y = 0.00007x^2 - 0.0661x + 55.879$

$R^2 = 0.950***$

mg Zn kg^{-1} of d.m.

mg Zn kg^{-1} of soil
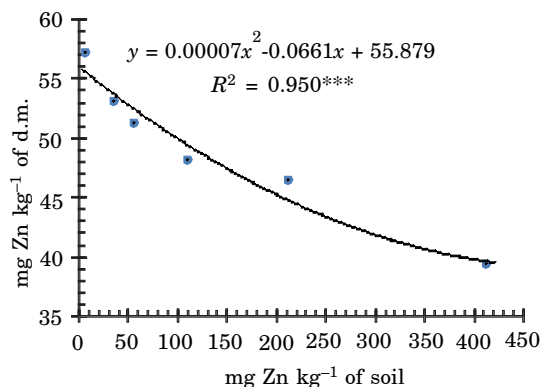
Fig. 1. Total yield of perennial ryegrass as affected by zinc content in sand (extracted in 1 M HCl)

a significantly lower grass yield; with respect to the third cut, it was not until the rate of 100 mg Zn kg^{-1} had been introduced to soil that the grass yield declined significantly. The largest and significantly high decrease in the grass biomass occurred on the soil with the highest rate of the contaminant, i.e. 400 mg Zn kg^{-1} of soil. In that treatment, the biomass of ryegrass was lower by 52% in the first, 25% in the second and 16% in the third cut than in the control treatment. In a study conducted by BARAN (2011) on sand, the rates of 250 and 750 mg Zn kg^{-1} of soil depressed the mass of aerial parts of maize by 10% and 25%, respectively.

Excess zinc is accumulated mainly in the below-ground parts of plants, impairing their function. WERYSZKO-CHEMLEWSKA et al. (2000) demonstrated that a high content of zinc in the substrate caused damage to root cells of horse bean and led to a decreased leaf area and mass. SAGARODY et al. (2009) report that toxic quantities of zinc reduce the uptake of such essential nutrients as nitrogen, magnesium, potassium and iron by plants. They also cause depressed concentrations of chlorophyll in leaves, with an adverse effect on the process of photosynthesis, leading to a retarded growth of plants.

In the present experiment, the emergence of plants growing on sand contaminated with 200 mg or 400 mg Zn kg^{-1} of soil was evidently worse and the plants developed more slowly. As the supply of mobile forms of Zn in the substrate was being depleted, the toxic effect produced by the metal was considerably weakened. Thus, the negative effect of this element was the strongest in the first cut of ryegrass, but proved much weaker in the last cut.

Contrary results were obtained in the other experiment, set up on sandy loam (Table 2). Statistically verified, the toxic effect of zinc in the soil with a larger CEC was not observable until the highest degree of soil contamination with zinc was tested (400 mg Zn kg^{-1}), but even then it appeared only in the first ryegrass cut. No significant differences were detected between the mass of plants harvested from the treatments which had received zinc in rates less than 400 mg kg^{-1} (25, 50, 100 and 200 mg Zn kg^{-1}). The results suggest that the adverse effect of zinc is dependent to a great degree on the type of soil, and mainly its CEC. On very light soil, even a small rate of added zinc (25 mg Zn kg^{-1} of soil) significantly depressed the biomass of ryegrass, whereas on sandy loam a significant decline in the grass yield was observed only under the influence of 400 mg Zn kg^{-1} of soil. It is obvious that a higher percentage of organic and mineral colloids in the soil with the texture of sandy loam had a protective action and reduced the concentration of active zinc forms in soil. This assumption is supported by the research carried out by SPIAK (1996), in which the value of the zinc migration coefficient, which reflects the ratio of the concentration of this metal in plants to its content in soil, largely depended on the quantity of the soil fraction less than 0.02 mm in diameter. On light soil, following the application of 500 mg Zn kg^{-1}, the value of this index was 36.1, whereas on medium and heavy soil, it fell to 12.3 and 5.9, respectively. GAMBUŚ et al.

(2004), KABATA-PENDIAS (2002) and MERCIK et al. (2004) also report that solubility and mobility of zinc in soil are depressed when the content of clay fractions, humus and aluminium, iron and manganese oxides increases. This finding explains a much weaker toxic effect of zinc in sandy loam than in sand.

Perennial ryegrass can be regarded as a species well tolerant to high content of zinc in soil. The decrease in the mass of cut grass caused by the highest rate of zinc contamination was relatively small, especially on sandy loam. Relatively good tolerance of grasses to high zinc concentrations in the substrate has also been reported by PASCHKE et al. (2000).

The gradually increasing levels of zinc contamination of soil caused a very high rise in the concentration of this metal in ryegrass (Figures 2-7). Zinc permeated into plant cells very readily. In the experiment run on sand, following the application of 400 mg Zn kg^{-1} of soil, the concentration of this metal rose to 1629 mg Zn kg^{-1}d.m. in the first grass cut (43-fold more
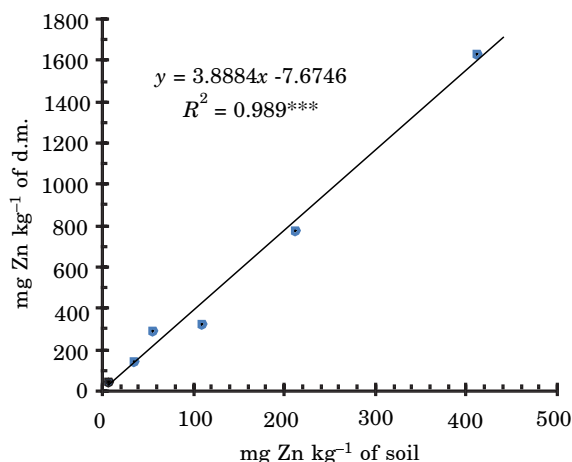
Fig. 2. Zinc concentration in the first cut of perennial ryegrass
as affected by zinc content in sand (extracted in 1 M HCl)

than in the treatment with the natural soil content of zinc, Table 3). This element was luxuriously taken up by plants, which confirms very high mobility of zinc in soil. In the trials conducted on sand, a radical increase in the concentration of zinc in the first grass cut was also observed in the pots with a very small amount of this metal introduced to soil (25 and 50 mg Zn kg^{-1} of soil). The concentration of zinc in plants harvested from those treatments increased to 139.8 mg and 287.7 mg Zn kg^{-1} d.m. of plants, respectively. For comparison, the ryegrass harvested from the control treatment contained 37.8 mg Zn kg^{-1} d.m. Similar concentrations of this metal in grasses growing on soils unpolluted with zinc have been observed by

Effect of soil contamination with zinc on the concentration
of this element in perennial ryegrass

Zn rate (mg kg⁻¹ of soil)	Zn concentration (mg Zn kg⁻¹ d.m.)					
	sand			sandy loam		
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
0	37.76	31.69	44.40	54.40	40.28	38.98
25	139.76	53.97	66.73	106.36	53.22	45.85
50	287.67	77.40	93.27	157.22	77.45	59.92
100	317.22	122.58	115.65	307.62	112.96	74.92
200	773.04	300.92	274.83	639.33	208.58	131.78
400	1629.13	1042.55	695.56	1223.41	311.11	187.23

KUCHARCZYK and MORYL (2010). In plants grown for fodder, the threshold level of this microelement is 100 mg Zn kg⁻¹ d.m. The fact that zinc can permeate so easily into plant cells may pose a high risk on farmlands used for growing crops and just very slightly polluted with this metal.

The concentration of zinc in the second and third cut of ryegrass grown on sand also increased significantly following the application of increasing rates of zinc (Figures 3 and 4). When 400 mg Zn kg⁻¹ had been added to soil, the concentration of zinc in the second and third cuts rose 33- and 15-fold, respectively, compared to the control. It should be noticed, however, that the concentrations of zinc in grasses from the second and third cuts were around 2.5-fold smaller than in the ryegrass from the first cut on corresponding treatments. The determination coefficients, which indicate the

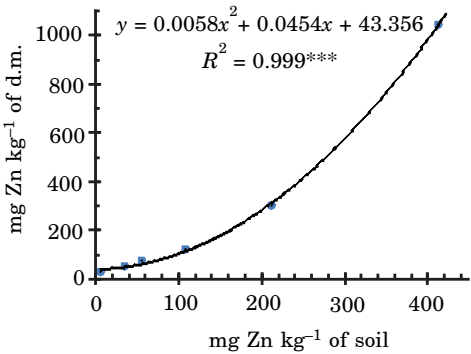$y = 0.0058x^2 + 0.0454x + 43.356$

$R^2 = 0.999***$

Fig. 3. Zinc concentration in the second cutting of perennial ryegrass
as affected by zinc content in sand (extracted in 1 M HCl)
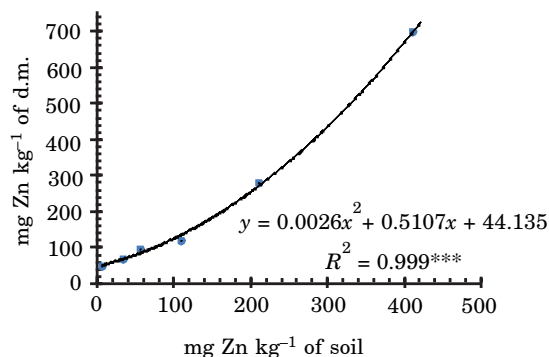
Fig. 4. Zinc concentration in the third cut of perennial ryegrass
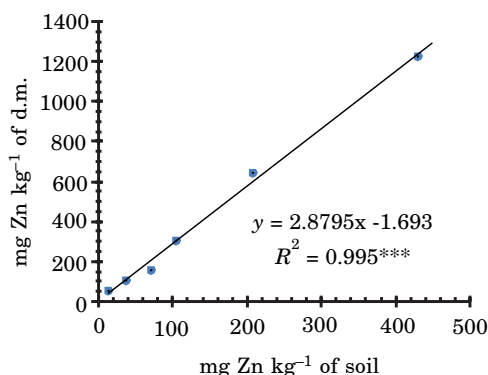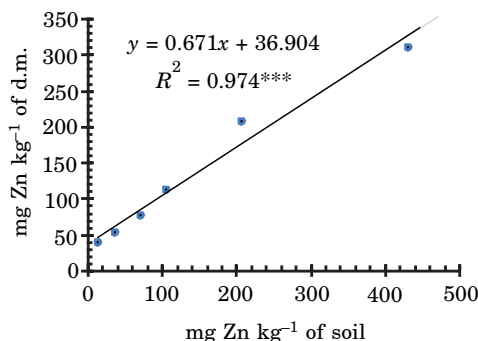as affected by zinc content in sand (extracted in 1 M HCl)

Fig. 5. Zinc concentration in the first cut of perennial ryegrass
as affected by zinc content in sandy loam (extracted in 1 M HCl)

relationship between the concentration of Zn in plants and its content in soil (extracted in 1 M HCl) were very high and reached 0.998, 0.999 and 0.999 respectively for the first, second and third cut.

The concentrations of zinc in grasses growing on sand were higher than in plants harvested from the trials on sandy loam (at the corresponding levels of zinc contamination). Particularly big differences in Zn concentration in plants grown on the two different types of soil appeared in the third cut after the application of the two highest rates of zinc, i.e. 200 and 400 mg Zn kg^{-1} (Table 3).

Fig. 6. Zinc concentration in the second cutting of perennial ryegrass
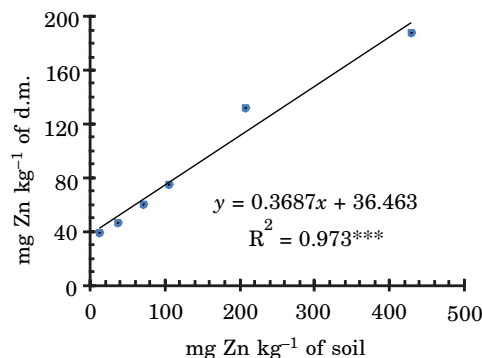as affected by zinc content in sandy loam (extracted in 1 M HCl)

Fig. 7. Zinc concentration in the third cut of perennial ryegrass
as affected by zinc content in sandy loam (extracted in 1 M HCl)

Ryegrass does not require large amounts of zinc to satisfy its nutritional demand. Nonetheless, it absorbed the metal in the amounts proportional to its content in soil. According to MISZTAL and LIGĘZA (1996), zinc is one of the most mobile elements and it is its mobility that ensures such an easy uptake of this heavy metal by plants. KICIŃSKA and HELIOS-RYBICKA (1995) reported that the concentration of zinc in plants was largely dependent on the content of this element in soil.

The concentration of zinc ranging between 15 and 50 mg Zn kg^{-1} of plant dry matter is sufficient to cover the plant's demand for this element. CHANEY and MARSCHNER (after BROADLEY et al. 2007) report that symptoms of a toxic response to zinc appear on leaves most often when the con-

centration of the metal exceeds 300 mg Zn kg^{-1} d.m., although more sensitive plants can reveal such symptoms at a content of zinc as low as 100 mg Zn kg^{-1} d.m. of leaves. Other studies also suggest that the response of plants to soil contamination with zinc depends strongly on a plant species (BARAN et al. 2008, BARAN, JASIEWICZ 2009, SPIAK et al. 2000, STANISŁAWSKA-GLUBIAK, KORZENIOWSKA 2005). In the experiment set up on sandy loam, even when the concentration of zinc reached 639 mg Zn kg^{-1} of dry matter of plants, the yield of ryegrass was high and showed no signs of the toxic influence of zinc. In contrast, when ryegrass grew on sand, the mass of grass decreased significantly at the content of zinc equal 140 mg Zn kg^{-1} d.m. These results demonstrate expressly that the content of organic and mineral colloids in soil is a factor that conditions the toxic influence of zinc on plants, and its seems to be a more important factor than the species of plants or the concentration of zinc in soil.

In the subsequent cuts of ryegrass, the concentration of zinc tended to decline in the treatments established on either of the two types of soil: sand or loam. However, a decrease in the concentration of zinc in the following grass cuts was much bigger in the treatments set up on loam than on sand. In the treatment with the highest zinc contamination dose (400 mg Zn kg^{-1} of soil), the third cut of ryegrass grown on loam contained about 6.5-fold less zinc than the first one (1233 mg Zn kg^{-1} d.m. in the first cut and 187 mg Zn kg^{-1} d.m. in the third one); in ryegrass grown on sand, the analogous difference was about 2.3-fold (1629 mg Zn kg^{-1} d.m. in the first cut and 696 mg Zn kg^{-1} d.m. in the third one). The results suggest that the solubility, and consequently the bioavailability of zinc and its diffusion to plant roots were diminishing considerably as the plants continued to grow. This process was much more intensive in the soil with a larger sorption complex. The activity of zinc in the experiment carried out on sandwas on a constant, high level until the vegetative season terminated. Therefore, perennial ryegrass growing on soil with a low content of soil colloids accumulated high amounts of this heavy metals, regardless of the cut. In turn, zinc in the soil of the texture of sandy loam most probably quickly transformed into a hardly mobile form, which contributed to a big decrease in the concentration of Zn in the tissues of plants harvested in the second and third cut.

The uptake of zinc by ryegrass growing on sand and on sandy loam increased proportionally to the degree of soil contamination with this metal (Table 4). Despite a very high concentration of zinc in grasses growing on heavily polluted soil, the total Zn uptake in the yield from the three cuts was very small compared to the amount of this metal introduced to soil. Consequently, growing ryegrass for one season did not have any considerable effect on the content of zinc in soil after harvest (Table 5). The total uptake of Zn by ryegrass growing on sand was just 1.1-2.2% of the zinc introduced to soil. When ryegrass was grown on loam, the percentage of removed zinc was similar, i.e. 1.3-2.5%. In a study by GAWOREK et al. (2003)

Table 4

Effect of soil contamination with zinc on Zn uptake by perennial ryegrass

Zn rate (mg pot^{-1})	Zn uptake (mg Zn pot^{-1})							
	sand				sandy loam			
	1st cut	2nd cut	3rd cut	total yield	1st cut	2nd cut	3rd cut	total yield
0	0.69	0.72	0.71	2.12	1.52	0.80	0.83	3.15
200	2.23	1.14	1.06	4.43	2.94	1.16	0.95	5.05
400	4.31	1.59	1.46	7.36	4.66	1.63	1.24	7.53
800	4.55	2.39	1.65	8.59	8.81	2.38	1.51	12.70
1600	9.84	5.86	3.92	19.62	18.99	4.60	2.64	26.23
3200	14.39	17.86	9.32	41.57	29.81	6.45	3.84	40.10

Table 5

Effect of soil contamination with zinc on the content of this metal in the soil
(extraction with 1 M HCl) after harvest of perennial ryegrass

Zn rate (mg kg^{-1} of soil)	Sand		Sandy loam	
	Zn content in soil after incubation with ZnSO$_4$·7H$_2$O	Zn content in soil after harvest	Zn content in soil after incubation with ZnSO$_4$·7H$_2$O	Zn content in soil after harvest
	mg Zn kg^{-1} of soil			
0	7.13	12.82	13.88	15.11
25	35.05	31.23	37.7	37.69
50	56.19	54.76	71.94	67.82
100	109.47	101.44	106.03	105.95
200	211.35	191.90	207.82	207.18
400	411.65	382.52	430.3	429.23

on the efficacy of phytoremediation of soils contaminated with heavy metals, the amount of Zn removed with plant yields did not exceed 2.5% of the content of this metal in the mobile form. A similar level of the uptake of zinc was determined by ANTONKIEWICZ and JASIEWICZ (2003), who investigated maize. Another reason why phytoremediation techniques do not prove to be very effective is the fact that soil samples analyzed after plant harvest contain some quantities of small roots, in which the concentration of heavy metals is much higher than in the aerial parts (BARAN, JASIEWICZ 2009, ZALEWSKA 2010). This is certainly reflected in the results of post-harvest soil chemical analyses.

The uptake of zinc by the yield of the second and third cut of ryegrass, having applied from 200 mg to 1600 mg Zn per pot, as well as the total Zn uptake by the yield of all the three cuts were similar in the trials established on sand and loam in the corresponding treatments. In contrast, a two-fold smaller uptake of Zn in the yield from the first cut of ryegrass growing on the sand most heavily polluted with zinc, compared to the uptake of this metal by plants growing on loam, was due to a strongly depressed mass of plants growing on very light soil with the highest dose of the contaminant.

The determination coefficients for the relationship between the Zn uptake and the content of this metal in soil were very high, for both sand and for sandy loam (Figures 8 and 9).
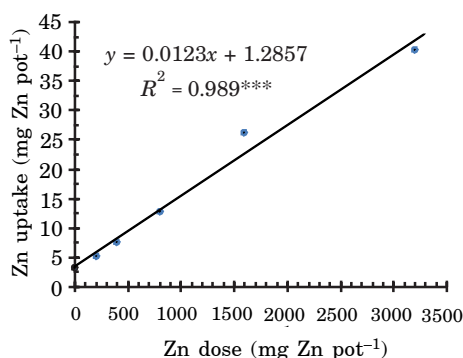
$$y = 0.0123x + 1.2857$$
$$R^2 = 0.989***$$

Fig. 8. Zinc uptake by total yield of perennial ryegrass
as affected by zinc dose per pot on sand
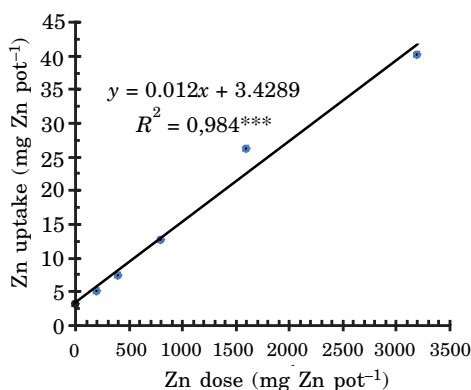
$$y = 0.012x + 3.4289$$
$$R^2 = 0{,}984***$$

Fig. 9. Zinc uptake by total yield of perennial ryegrass
as affected by zinc dose per pot on sandy loam

CONCLUSIONS

1. The toxic effect of zinc on the growth of ryegrass was much more pronounced on sandthan on sandy loam. In the trial carried out on sand, even the smallest dose of zinc (25 mg Zn kg^{-1} of soil) significantly depressed the yield of the first grass cut, whereas on sandy loam, the toxic effect of zinc did not occur until the highest rate of this metal had been introduced to soil (400 Zn kg^{-1} of soil).

2. As the soil contamination with zinc increased, the concentration and uptake of this metal by ryegrass increased significantly. A very high concentration of Zn in ryegrass growing on soil contaminated with this metal suggests that zinc can easily permeate into plant roots and is very mobile in soil.

3. A one-year cultivation of perennial ryegrass did not have any larger effect on decreasing the content of zinc in soil. Although zinc appeared in very high concentrations in the cut grasses, its total uptake from a pot equalled just 1.1 to 1.5% of the amount of this metal introduced to soil.

4. Perennial ryegrass cv. Nira is characterized by good capability of accumulating very high amounts of zinc and is tolerant to a high content of Zn in soil, which is why it can be grown on areas contaminated with this heavy metal.

5. There is a strong, statistically verified correlation between the content of zinc in soil, extracted in 1 M HCl, and the Zn concentration and uptake by plants. Zinc extraction in 1 M HCl can be used for evaluation of the degree of soil contamination with this metal.

REFERENCES

Antonkiewicz J., Jasiewicz Cz. 2003. *Assessment of the suitability of maize (Zeamays L.) for phytoextraction of cadmium, lead, nickel, copper and zinc from soilcontaminated with theseelements.* Acta Sci. Pol. Formatio Circumiectus, 2(1): 61-69. (in Polish)

Baran A. 2011. *Response of maize to zinctoxiccontent in soil.* Proceedings of ECOpole, 5(1): 155-160. (in Polish)

Baran A., Jasiewicz Cz. 2009. *Toxiccontent of zinc and cadmium in soil to different plant species.* Ochr. Środ. Zasob. Nat., 40: 157-164. (in Polish)

Baran A., Jasiewicz Cz., Klimek A. 2008. *Response of plants to the toxiccontent of zinc and cadmium in soil.* Proc. of ECOpole, 2 (2): 417-422. (in Polish)

Broadley M.R., White P.J., Hammond J.P., Zelko I., Lux A. 2007. *Zinc in plants.* New Phytol, 173: 677-702.

Buurman P., Pape Th., Muggler C.C. 1997. *Laser grain-size determination on soil genetic studies. 1. Practical problems.* Soil Sci., 162(3): 211-218.

Gambuś F., Rak M., Wieczorek J. 2004. *Effect of somesoilproperties on phytoavailability and solubility of zinc, copper and nickel in soil.* Zesz. Probl. Post. Nauk Rol., 502: 71-79. (in Polish)

GWOREK B., JESKE K., KWAPISZ J. 2003. *Assessment of the effectiveness of phytoremediation of soilscontaminated with heavy metals, using the sequentialextractionmethod.* Arch. Ochr. Środ., 29(4): 71-79. (in Polish)

KABATA-PENDIAS A. 2002. *Biogeochemistry of zinc.* Zesz. Nauk. Kom. PAN, 33: 11-18. (in Polish)

KICIŃSKA A., HELIOS-RYBICKA E. 1995. *Heavy metals in the soil-plant system overanareaaffected by a zincsmelter.* Zesz. Probl. Post. Nauk Rol., 418(2): 751-759. (in Polish)

KARCZEWSKA A. 2005. *New aspects of the problem of reclamation of soilspolluted with heavy metals.* Rocz. Gleb., 56 (1/2): 18-189. (in Polish)

KUCHARCZAK E., MORYL A. 2010. *Content of metals in crops originating from the region of Zgorzelec and Bogatynia.* Part 2. *Arsenic, chromium, zinc, copper.* Ochr. Środ. Zasob. Nat., 43: 7-16. (in Polish)

ŁYSZCZ S., RUSZKOWSKA M. 1991. *Differentiatedresponse of some plant species to excesszinc.* Rocz. Glebozn., 42 (3/4): 215-221. (in Polish)

MARECIK R., KRÓLICZAK P., CYPLIK P. 2006. *Phytoremediation – an alternative to traditional methods of decontamination.* Biotechnologia, 3(74): 88-97. (in Polish)

MERCIK S., STĘPIEŃ W., MATYSIAK B. 2004. *Mobility and uptake of copper and zinc by plantsdepending on soilproperties.* Zesz. Probl. Post. Nauk Rol., 502: 235-245. (in Polish)

MISZTAL M., LIGĘZA S. 1996. *Effect of the reaction and moisture of soilcontaminated by a zincsmlter on the content of heavy metals in the soilsolution.* Zesz. Probl. Post. Nauk Rol., 434: 879-883. (in Polish)

PASCHKE M.W., REDENTE E.F., LEVY D.B. 2000. *Zinc toxicity thresholds for important reclamation grass species of the Western United States.* Environ. Toxicol. Chem., 19(11): 2751-2756.

ROSZYK E., ROSZYK S., SPIAK Z. 1988. *Soilconcentration of zinctoxic to plants.* Rocz. Glebozn., 37: 57-69. (in Polish)

SAGARDOY R., MORALES F., LÓPEZ-MILLAN A., ABADIA A., ABADIA J. 2009. *Effects of zinc toxicity on sugar beet (Beta vulgaris L.) plants grown in hydroponics.* Plant Biol., 11(3): 339-350.

SAS-NOWOSIELSKA A., KUCHARSKI R., MAŁKOWSKI E., POGRZEBA M., KRYŃSKI K., KUPERBERG, J.M., KRZYŻAK J.2004. *Handling the yieldafterphytoextraction – a problem to be solved.* Ochr. Powietrza Probl. Odpadów, 38 (5): 180-184. (in Polish)

SPIAK Z. 1996. *The currentstate of research on excess heavy metals in soils and plants.* Zesz. Probl. Post. Nauk Rol., 434: 769-775. (in Polish)

SPIAK Z. ROMANOWSKA M., RADOŁA J. 2000. *The content of zinc in soilstoxic to differentplants.* Zesz. Probl. Post. Nauk Rol., 471: 1125-1134. (in Polish)

STANISŁAWSKA-GLUBIAK E., KORZENIOWSKA J. 2005. *Criteria for evaluation of the toxicity of zinc to plants.* Wyd. IUNG-PIB, Puławy, 1-12. (in Polish)

TERELAK H., PIOTROWSKA M., MOTOWICKA-TERELAK T., STUCZYŃSKI T., BUDZYŃSKA K. 1995. *Content of heavy metals and copper in soilsundercroppedfields in Poland, and theircontamination with thesecomponents.* Zesz. Probl. Post. Nauk Rol., 418: 45-58. (in Polish)

WALINGA I., KITHOME M., NOVOZAMSKY N., HOUBA V.J.G., VANDER LEE J.J. 1992. *Spectrophotometric determination of organic carbon in soil.* Comm. Soil. Sci. Plant. Anal., 23: 1935-1944.

WERYSZKO-CHMIELEWSKA E., MICHOŃSKA M., KOSTRZEWSKA-KUCZUMOW J. 2000. *Changes in the anatomy of horse bean (Vicia faba L. var. minor Harz.) stems under stress caused by excess zinc.* Bibl. Frag. Agr., 8: 297-302. (in Polish)

ZALEWSKA M. 2010. *Effect of soil contamination by Pb, Ni and Cd and VA mycorrhizal fungi on yield and heavy metal concentration in roots and aboveground biomass of oat.* Ecol. Chem. Eng. A, 17(2): 11-19.

THE ROLE OF MAGNESIUM IN MIGRAINE PATHOGENESIS. POTENTIAL USE OF MAGNESIUM COMPOUNDS IN PREVENTION AND TREATMENT OF MIGRAINE HEADACHES

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Abstract

One of the major functions of magnesium is the maintenance of proper electric potential of neurons. Pathological conditions associated with systemic magnesium deficiencies may be associated with disturbance of numerous neurophysiological processes. These may include neuron function, transmission of nerve impulses, neuromuscular transmission, muscle contraction and vasomotor reflex. Therefore, magnesium deficiencies, particularly those associated with hypomagnesemia, are a source of problems for neurologists.

Migraine is one of the most common neurological disorders. Despite many years of research, pathophysiology of migraine has not been elucidated. The predominant opinion is that the onset of migraine headaches is associated with cerebral vascular spasms. Based on the available knowledge of biological functions of magnesium, at least several feasible mechanisms for prevention or reduction of the intensity of migraine attacks by magnesium ions have been proposed. The goal of this review is to summarize the literature re-

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ports on magnesium in the pathogenesis of migraine and to identify the potential uses for magnesium compounds in prevention and treatment of migraine headaches.

Key words: magnesium, migraine, migraine headache prevention, treatment of migraine attacks.

ROLA MAGNEZU W PATOGENEZIE MIGRENY. MOŻLIWOŚCI ZASTOSOWANIA ZWIĄZKÓW MAGNEZU W PROFILAKTYCE I LECZENIU MIGRENOWYCH BÓLÓW GŁOWY

Abstrakt

Jedną z najważniejszych funkcji magnezu jest utrzymywanie odpowiedniego potencjału elektrycznego komórek nerwowych. W stanach patologii związanych z ogólnoustrojowymi niedoborami magnezu może dochodzić do zakłócenia rozmaitych procesów neurofizjologicznych. Należą do nich m.in.: funkcjonowanie neuronów, przewodzenie impulsu nerwowego, przekaźnictwo nerwowo-mięśniowe i skurcz mięśni oraz odruch wazomotoryczny. Z powyższych względów stany niedoborów magnezu, zwłaszcza te przebiegające z hipomagnezemią, stały się problemem w praktyce klinicznej lekarza neurologa.

Migrena jest jednym z najczęściej występujących schorzeń neurologicznych. Pomimo wielu lat badań, jej patofizjologia nie została jednoznacznie wyjaśniona. Aktualnie dominuje pogląd, iż pojawianie się migrenowych bólów głowy ma związek ze skurczem naczyń mózgowych. Na podstawie dostępnej wiedzy dotyczącej biologicznych funkcji magnezu zasugerowano istnienie przynajmniej kilku możliwych mechanizmów, za których pośrednictwem jony magnezu mogłyby zapobiegać pojawianiu się ataków migreny lub ograniczać ich nasilenie. Celem pracy było podsumowanie dostępnych w piśmiennictwie doniesień dotyczących roli magnezu w patogenezie migreny, a także wskazanie możliwości wykorzystania preparatów magnezu w profilaktyce i leczeniu migrenowych bólów głowy.

Słowa kluczowe: magnez, migrena, profilaktyka migrenowych bólów głowy, leczenie napadów migreny.

INTRODUCTION

Magnesium is present in all tissues, being the second most common intracellular cation (after potassium). Magnesium plays many roles in the human system: it contributes to intracellular energy storage and expenditure, acts as a cofactor for more than 300 enzymes, is required for the proper course of nucleic acid synthesis, and is involved in cell division and growth, as well as in operation of ion channels, receptors and transport systems.

One of the major functions of magnesium is the maintenance of proper electric potential of neurons (Hu et al. 2006). In order for this function to be carried out properly, the system must be armed with structures protecting the central nervous system from sudden drops of magnesium levels. These include the blood-brain barrier and choroid plexus, which effectively prevent fluctuations in cerebrospinal fluid magnesium levels in physiological condi-

tions (MCKEE et al. 2005). Pathological conditions associated with systemic magnesium deficiencies may be associated with disturbance of numerous neurophysiological processes. These may include neuron function, transmission of nerve impulses, neuromuscular transmission, muscle contraction and vasomotor reflex (SONTIA, TOUYZ 2007, DRIBBEN et al. 2010). Therefore, magnesium deficiencies, particularly those associated with hypomagnesemia, are a source of problems for neurologists. These problems are very serious since, as demonstrated in studies conducted by independent teams of researchers, the number of patients with hypomagnesemia is usually underestimated (DE FEO 2009). In addition, low plasma magnesium levels are more common in patients presenting at hospital admission rooms or emergency units and may affect as much as 65% of population with proper creatinine levels, contributing to increased mortality among these patients (WHANG, RYDER 1990, SAFAVI , HONARMAND 2007).

MIGRAINE

Migraine is one of the most common neurological disorders. According to different sources, migraine affects 4 to 20% of population and is a common cause of patients' referral to the neurologist's office. The disorder is more common in females than in males, albeit the age of onset is younger in males (KURTH et al. 2009). Although most reports mention the disorder as affecting adults, it has been recently pointed out that children might also be affected, with the incidence in this age group being underestimated (ABU-AREFEH et al. 2010). Migraine headaches come 19th in the WHO rank of disorders excluding patients from normal functioning in their environment. It was estimated that employees in the US miss a total of 110 million workdays per year, which significantly reduces the productivity index (HU et al. 1999, STANG et al. 2001, BUSE et al. 2009).

Although migraine headaches have been the subject of research for many decades, and the term "migraine" dates back to the ancient times, migraine classification criteria were developed by the International Headache Society relatively recently, i.e. in 1988 (DIB 2008). According to these criteria, migraine is classified as one of the primary (spontaneous) headaches, which include also cluster headaches, tension headaches and other spontaneous headaches (OLESEN, STEINER 2004).

Migraine is characterized by recurrent paroxysmal headaches lasting 4 to 72 hours, usually pulsating, unilateral and most commonly involving the parietal, temporal and periorbital regions. The pain may be provoked by multiple factors. The disorder is exacerbated by emotional stress and physical activity (GOADSBY et al. 2005). The classification proposed by the International Headache Society identifies two types of migraine: migraine without aura and less common migraine with aura, which usually lasts 5 to 60 minutes (GOADSBY et al. 2005). In the case of the latter type, the onset of headache is preceded by focal neurological cerebral or brain stem symptoms.

Despite many years of research, pathophysiology of migraine has not been elucidated. The predominant opinion is that the onset of migraine headaches is associated with cerebral vascular spasms (KURTH 2007, TIETJEN 2007).

THE ROLE OF MAGNESIUM IN MIGRAINE PATHOGENESIS

DEMIRKAYA et al. (2000) demonstrated a relationship between migraine headaches and magnesium levels within human body, pointing out several possible mechanisms for the anti-migraine activity of magnesium. Maintenance of normal plasma levels of magnesium ions is required for proper function of vascular endothelium and proper vascular contractility. Thus, hypomagnesemia is a factor predisposing patients to vascular spasm (GRUBB 2005, SCHRUCKS et al. 2010). The likelihood of cerebral vascular spasm underlying the migraine headache was shown to be proportional to the ratio of calcium and magnesium ion levels in plasma. Magnesium ions are known to modulate the tone of the smooth muscles of cerebral and peripheral vessels. Magnesium acts as a calcium antagonist on vascular smooth muscle tone. (WEINBERGER 2006, BO, PISU 2008).

By acting as NMDA receptor antagonists, magnesium ions prevent propagation of glutamatergic transmission-dependent of cortical depression, which is associated, among others, with the onset of migraine aura. *In vitro* studies showed that low concentration of magnesium within the cerebral tissue leads to earlier onset and easier propagation of cortical depression (LAURITZEN 2001, SUN-EDELSTEIN, MAUSKOP 2009). In addition, hypomagnesemia is associated with induction of platelet aggregation or vascular endothelial function damage (ROMANI 2008, WOLF et al. 2008). Hypomagnesemia is also a factor predisposing patients to increased serotonin secretion, thus potentiating the vasoconstricive action of this biogenic amine. Earlier exposure to magnesium ions was shown to inhibit serotonin-dependent vascular spasm (GRUBB, CARMO JORGE 2000).

Other suggested mechanisms responsible for migraine headaches include the reduction of vasodilatory effect of prostacyclin (KOSEOGLU et al. 2008). Possibility of migraine attacks was pointed out for conditions usually associated with lowered plasma magnesium levels, e.g. during pregnancy, menstruation, stress, abuse of alcohol or as a consequence of chronic intake of certain drugs, such as diuretics (ALTURA 1985).

Although there are papers that question the relationship between hypomagnesemia and migraine, most authors have no doubt that such a relationship exists (SMEETS et al. 1994, MISHIMA et al. 1997). GALLAI et al. (1992) showed that individuals suffering from migraine headaches were characterized by lower plasma and saliva magnesium levels between the attacks compared to the control group. According to RAMADAN et al. (1989), lowered intracellular magnesium levels are also observed in brain structures in this period in migraine patients. Moreover, additional decrease in magnesium levels is observed during the migraine attack in plasma, erythrocytes and mononu-

clear blood cells, as well as in the cerebrospinal fluid (GALLAI et al. 1992, SUN-EDELSTEIN, MAUSKOP 2009). All these observations seem to support the hypothesis proposed in the 1970s by Durlach stating that the increased urinary excretion of magnesium ions during migraine attacks was associated with the loss of magnesium from plasma, thus leading to hypomagnesemia (DURLACH 1976).

Today, hypomagnesemia is also mentioned as a cause of menstrual migraine headaches. The onset of these headaches was previously associated with the estrogen secretion profile which undergoes changes during the menstrual cycle. (SILBERSTEIN, GOLDBERG 2007). It should be noted that IHS classification of 1988 does not include menstrual migraine. However, a relationship between changes in plasma magnesium levels during the menstrual cycle and the attacks is currently proposed. Headaches associated with the menstrual cycle occur in stages in which the lowest magnesium levels, and thus the highest calcium-to-magnesium ratios are recorded (SILBERSTEIN, GOLDBERG 2007, DULLO, VEDI 2008).

Demonstration of the relationship between hypomagnesemia and migraine headaches contributed to the advances in studies aimed at identification of potential uses of magnesium compounds in prevention and treatment of migraine attacks.

THE ROLE OF MAGNESIUM IN PREVENTION OF MIGRAINE ATTACKS

Initiation of prophylactic treatment should be considered in patients with migraine attacks occurring at least twice a week or lasting above 48 hours, or in cases in which earlier treatment had failed (SILBERSTEIN 2000, DIAMOND et al. 2007, DEMAAGD 2008). Prophylactic migraine treatment should last three to twelve months and involve daily administration of selected compounds (SILBERSTEIN 2000, DIAMOND et al. 2007, DEMAAGD 2008).

Magnesium has been classified by the United States Headache Consortium (USHC) as a macroelement recommended in prevention of migraine headaches. It is listed as one of the so-called second line compounds. At the same time, magnesium compounds were pointed out to be relatively safe. Side effects of treatment with magnesium compounds are relatively rare and mild, which is particularly important in prophylactic treatment lasting at least 3 months (D'AMICO, TEPPER 2008). Moreover, magnesium compounds are the only compounds used for migraine prevention falling into the highest FDA pregnancy category, allowing their use by pregnant women (PRINGSHEIM et al. 2010).

Oral magnesium compounds are used for migraine prevention. According to the recommendations of the Canadian Ministry of Health, the maximum dose of magnesium should not exceed 350 mg day^{-1} (PRINGSHEIM et al. 2010). To date, prophylactic administration of magnesium compounds has

been considered in the cases of migraine both associated and not associated with aura (FACCHINETTI et al. 1991, PEIKERT et al. 1996, PFAFFENRATH et al. 1996, WANG et al. 2003). One of the first randomized studies of prophylactic administration of magnesium was carried out by Facchinetti and included a group of 20 women suffering from menstrual migraine. Patients received magnesium pyrrolidinecarboxylate at the dose of 360 mg day^{-1}. The compound was initiated on Day 15 of the menstrual cycle and continued until the first day of bleeding. The regimen was repeated for two subsequent cycles (FACCHINETTI et al. 1991). The method of treatment initiation was strictly correlated with changes in plasma magnesium levels occurring during the menstrual cycle (ALLAIS et al. 2005). The study confirmed a beneficial effect of magnesium compounds in the prevention of menstrual migraine. In addition, magnesium salts were shown to be efficient in reducing the symptoms of the premenstrual syndrome (FACCHINETTI et al. 1991).

Studies conducted by Peikert et al (1996) showed that administration of magnesium citrate for 3 months reduced the incidence of migraine attacks by 41.6%, as compared to a reduction of less than 16% in patients receiving placebo. Pain intensity and duration of an attack were also reduced as compared to the control group, although the differences were not statistically significant (PEIKERT et al. 1996).

However, not all studies support the efficacy of magnesium compounds in migraine prophylaxis. Such efficacy was not demonstrated in studies by PFAFFENRATH et al. (1996), who administered 480 mg of magnesium to a group of 68 patients. Due to these discrepant reports regarding the efficacy of magnesium in migraine prevention, magnesium compounds are classified as category B. (EVANS, TAYLOR 2006, MOODI, LOWDER 2006).

THE ROLE OF MAGNESIUM IN THE TREATMENT OF MIGRAINE ATTACKS

Oral administration of magnesium compounds is rather unhelpful in treating migraine attacks. As evidenced by the available literature, oral application of magnesium has limited efficacy in the treatment of migraine after as long as several months of supplementation (MAUSKOP et al. 1996, DEMIRKAYA et al. 2001). Results of studies of the headache relief efficacy of intravenous administration of magnesium compounds are ambiguous. The efficacy could not be demonstrated in some of the studies, while results of other studies supported the analgesic activity of magnesium compounds.

Studies conducted by MAUSKOP et al. (1998) showed that intravenous administration of magnesium sulfate reduces the severity of migraine, cluster and tension headaches in 80% of patients. As mentioned before, this effect was most probably caused by the interaction between magnesium and serotonin receptors. Besides, potential mechanisms of action of magnesium ions that would determine its analgesic efficacy include the impact on the syn-

thesis and secretion of nitric oxide (which has a vasodilatory effect) and interaction with NMDA receptors, as well as the influence on many other receptors and neurotransmitters important for headache pathogenesis (MAUSKOP, ALTURA 1998).

Studies conducted by BIGAL et al. (2002) showed that intravenous administration of 1 g of magnesium sulfate had no effect on the course of headache attacks in patients suffering from migraine without aura. However, reduction of pain discomfort, photophobia and hypersensitivity to sounds were observed in patients suffering from migraine with aura compared to the placebo group. No reduction in the incidence of nausea accompanying migraine attacks was observed.

The efficacy of magnesium sulfate was reported also by ZIDVERC et al. (2001). A thirty-minute infusion of magnesium sulfate during an acute migraine attack had similar pain relieving efficacy as sumatriptane, a selective 5-HT1D serotonin receptor agonist. Unfortunately, earlier recurrence of headaches was observed in some of the patients receiving magnesium, compared to sumatriptane group.

As mentioned before, some reports do not support sufficient efficacy of magnesium compounds in interrupting migraine attacks. These include, for example, observations by CORBO et al. (2000), who found that intravenous addition of 2 g magnesium sulfate to methoclopramide administered during the attack had no pain relief efficacy, and even reduced the efficacy of the antiemetic drug. Similar results were published by CETE et al. (2005).

Diverse responses of patients to magnesium sulfate are attributed to numerous factors, including low baseline levels of magnesium ions in plasma (MAUSKOP et al. 1995). However, this opinion is not shared by GINDER et al. (2000), who found no relationship between the plasma magnesium levels and the responses to $MgSO_4$ treatment. It cannot be excluded that different responses of patients to intravenous magnesium treatment may be due to individual-specific factors. However, such factors could not be identified to date.

SUMMARY

Magnesium is a cation required for proper course of many important physiological processes, disturbances of which are associated with migraine pathogenesis (SUN-EDELSTEIN, MAUSKOP 2011). Hypomagnesemia is a factor predisposing patients to the onset and propagation of cortical depression and changes in neurotransmitter secretion, intensifying the synthesis and secretion of P substance, increasing platelet aggregation and, most of all, causing vascular spasms (SUN-EDELSTEIN, MAUSKOP 2011). Based on the available knowledge of biological functions of magnesium, at least several feasible mecha-

nisms for prevention or reduction of the intensity of migraine attacks by magnesium ions have been proposed (TAYLOR 2011). Although hypomagnesemia has been associated with migraine pathogenesis for many years, the breakthrough in the research in this field was possible only after ion-selective electrodes were invented. In contrast to earlier methods, the use of ion-selective electrodes allows the measurement of ionized magnesium, i.e. the magnesium pool directly associated with physiological effects of this element (SUN-EDELSTEIN, MAUSKOP 2011).

Migraine is a chronic disorder that occurs with periodical exacerbations or attacks that significantly reduce the quality of life of the patients (LIPTON et al. 2003, PRINGSHEIM 2010). Despite different classifications as well as prevention and treatment standards being introduced and modified, the incidence of migraine is still considered to be underestimated (Antonaci 2010). There are two aspects to the migraine treatment strategy, namely prevention and interruption of migraine attacks (ANTONACI 2010). In this study, attention was drawn to potential use of magnesium compounds in both prevention and interruption of migraine attacks. This approach proved efficient in many cases, for instance in prevention of menstrual migraine (MAUSKOP et al. 2002). With regard to intravenous administration of magnesium compounds in order to interrupt acute pain, results of available studies remain ambiguous (CORBO et al. 2000, BIGAL et al. 2002). However, analysis of available reports may lead to conclusion that reports that confirm at least partially beneficial effect of magnesium are predominant (MAUSKOP et al. 1995, MAUSKOP 2001). Doubtless, the results of these studies justify further efforts to obtain more unambiguous answers to questions regarding the role of individual sensitivity in responses to magnesium compounds, which might allow a more efficient prophylactic and therapeutic treatment. Currently, it is also pointed out that extracellular levels of magnesium ions may be largely genetically determined. This was confirmed by the study of SHUEN et al. (2009), who demonstrated a significant correlation between estrogen receptor gene-1 (ESR1) polymorphism and total magnesium levels in plasma (SHUEN et al. 2009). Perhaps examination of this issue would open a path to understanding the correlation between the use of magnesium compounds and the patient's response to the treatment.

REFERENCES

ABU-AREFEH I., RAZAK S., SIVARAMAN B., GRAHAM C. 2010. Dev. Med. Child. Neurol., 2010. 52(12): 1088-1097. DOI:10.1111/j.1469-8749.2010.03793.x.

ALLAIS G., BUSSONE G., DE LORENZO C., MANA O., BENEDETTO C. 2005. *Advanced strategies of short-term prophylaxis in menstrual migraine: state of the art and prospects.* Neurol. Sci., 26(suppl.2): s125-s129, DOI: 10.1007/s10072-005-0424-7

ALTURA B.M. 1985. *Calcium antagonism properties of magnesium: Implications for antimigraine actions.* Magnesium, 4(4): 169-175.

ANTONACI F. 2010. *A review of current European treatment guidelines for migraine*. J. Headache Pain, 11(1): 13-19. DOI: 10.1007/s10194-009-0179-2

BIGAL M.E., BORDINI C.A., SHEFTELL F.D., SPECIALI J.G., BIGAL J.O. 2002. *Migraine with aura versus migraine without aura: pain intensity and associated symptom intensities after placebo.* Headache, 42(9): 872-877. DOI: 10.1046/j.1526-4610.2002.02205.x

BO S., PISU E. 2008. *Role of dietary magnesium in cardiovascular disease prevention, insulin sensitivity and diabetes.* Curr. Opin. Lipidol. 19(1): 50-56. DOI: 10.1097/MOL.0b013e3282f33ccc

BUSE D.C., RUPNOW M.F.T., LIPTON R.B. 2009. *Assessing and managing all aspects of migraine: migraine attacks, migraine related functional impairment, common comorbidities, and Quality of Life.* Mayo Clin. Proc., 84(5): 422-435. DOI: 10.4065/?84.5.422

CETE Y., DORA B., ERTAN C., OZDEMIR C., OKTAY C. 2005. *A randomized prospective placebo-controlled study of intravenous magnesium sulphate vs. metoclopramide in the management of acute migraine attacks in the emergency department.* Cephalalgia, 25(3): 199--204. DOI: 10.1111/j.1468-2982.2004.00840.x

CORBO J., ESSES D., INNACOME R., BIJUR P., GALLAGHER E.J. 2000. *Randomized clinical trial of intravenous magnesium sulfate as adjunctive medication for ED treatment of migraine headache.* J. Emerg. Med, 38(6): 621-627. DOI: 10.1111/j.1468-2982.2004.00840.x

D'AMICO D., TEPPER S.J. 2008. *Prophylaxis of migraine: general principles and patient acceptance.* Neuropsychiatr. Dis. Treat., 4(6): 1155-1167.

DE FEO M.L. 2009. *Magnesium disorders: clinical experience and review of the literature.* Clin. Cases Miner. Bone. Metab., 6(3): 220-222.

DEMAAGD G. 2008. *The pharmalogical management of migraine.* Part 2. P. T., 33(8): 480-487.

DEMIRKAYA S., DORA B., TOPCUOAGLU M.A., ULAS U.H., VURAL O. 2000. *A comparative study of magnesium, flunarizine and amitriptiline in the prophylaxis of migraine.* J. Headache Pain, 1(3): 179-186. DOI: 10.1007/s101940070041

DEMIRKAYA S., VURAL O., DORA B., TOPÇUOĐLU M.A. 2001. *Efficacy of intravenous magnesium sulphate in the treatment of acute migraine attacks.* Headache, 41(2): 171–177. DOI: 10.1046/j.1526-4610.2001.111006171.x

DIAMOND S., BIGAL M.E., SILBERSTEIN S. 2007. *Patterns of diagnosis and acute and preventive Treatment for Migraine in the United States: Results from the American migraine prevalence and prevention study.* Headache, 47(3): 355–363. DOI: 10.1111/j.1526--4610.2006.00631.x

DIB M. 2008. *Optimizing prophylactic treatment of migraine: Subtypes and patient matching.* Ther. Clin. Risk. Manag., 4(5): 1061-1078. DOI: http://dx.doi.org/10.2147/TCRM.S3983

DRIBBEN W.H., EISENMAN L.N., MENNERICK S. 2010. *Magnesium induces neuronal apoptosis by suppressing excitability.* Cell Death Dis., 1(8): e63 DOI:10.1038/cddis.2010.39

DULLO P., VEDI N. 2008. *Changes in serum calcium, magnesium and inorganic phosphorus levels during different phases of menstrual cycle.* J. Hum. Reprod. Sci., 1(2): 77–80.

DURLACH J. 1976. *Neurological manifestations of magnesium imbalance.* In: *Handbook of clinical neurology.* VINKEN P.J., BRUYN G.W. (eds.) vol.28.

EVANS R.W., TAYLOR F.R. 2006. *Natural or alternative medications for migraine prevention.* Headache, 46(6): 1012-1018. DOI: 10.1111/j.1526-4610.2006.00473.x

FACCHINETTI F., SANCES G., BORELLA P., GENAZZANI A.R., NAPPI G. 1991. *Magnesium prophylaxis of menstrual migraine: effects on intracellular magnesium.* Headache, 31(5): 298--301. DOI: 10.1111/j.1526-4610.1991.hed3105298.x

GALLAI V., SARCHIELLI P., GOATA G., FIRENZE C., MORUCCI P., ABBRITTI G. 1992. *Serum and salivary magnesium levels in migraine. Results in a group of juvenile patients.* Headache, 32(3): 132-135. DOI: 10.1111/j.1526-4610.1992.hed3203132.x

GINDER S., OATMAN B., POLLACK M. 2000. *A prospective study of i.v. magnesium and i.v. prochlorperazine in the treatment of headaches*. J. Emerg. Med., 18(3): 311-315. DOI:10.1016/S0736-4679(99)00220-6

GOADSBY P.J., LIPTON R.B., FERRARI M.D. 2005. *Migraine current understanding and treatment*. N. Engl. J. Med., 346(4): 257-270.

GRUBB B.P., CARMO JORGE S. 2000. *A review of the classification, diagnosis, and management of autonomic dysfunction syndromes associated with orthostatic intolerance*. Arq. Bras. Cardiol., 74(6): 545-552. dx.doi.org/10.1590/S0066-782X2000000600008

GRUBB B.P. 2005. *Neurocardiogenic syncope and related disorders of orthostatic intolerance*. Circulation. 111: 2997-3006. DOI:10.1161/CIRCULATIONAHA.104.482018

HU X.H., MARKSON L.E., LIPTON R.B., STEWART W.F., BERGER M.L. 1999. *Burden of migraine in the United States*. Arch. Intern. Med., 159(8): 813-818.

HU L., YANG H., SHI J., CUI J. 2006. *Effects of multiple metal binding siteson calcium and magnesium-dependent activation of BK channels*. J. Gen. Physiol. 127(1): 35-50. DOI: 10.1085/jgp.200509317

KOSEOGLU E, TALASHOGLU A, GONUL A.S., KULA M. 2008. *The effect of magnesium prophylaxis in migraine without aura*. Magnes. Res, 21(2): 101-108. DOI: 10.1684/mrh.2008.0132 101

KURTH T. 2007. *Migraine and ischaemic vascular events*. Cephalalgia, 27(8): 965-75. Erratum in: Cephalalgia., 27(11): 1290. DOI: 10.1111/j.1468-2982.2007.01405.x

KURTH T., SCHÜRKS M., LOGROSCINO G., BURING J.E. 2009. *Migraine frequency and risk of cardiovascular disease in women*. Neurology, 73(8): 581–588. DOI: 10.1212/WNL. 0b013 e3181ab2c20

LAURITZEN M. 2001. *Cortical spreading depression in migraine*. Cephalalgia, 21(7):757-760. DOI: 10.1111/j.1468-2982.2001.00244.x

LIPTON R.B., LIBERMAN J.N., KOLODNER K.B., BIGAL M.E., DOWSON A., STEWART W.F. 2003. *Migraine headache disability and health-related quality-of-life: a population-based case-control study from England*. Cephalalgia, 23(6): 441-450. DOI: 10.1046/j.1468-2982.2003.00546.x

MAUSKOP A. 2001. *Alternative therapies in headache: is there a role?* Med. Clin. North. Afr., 85(4): 1077-1081. DOI:10.1016/S0025-7125(05)70360-6

MAUSKOP A., ALTURA B.T., ALTURA B.M. 2002. *Serum ionized magnesium levels and serum ionized calcium/ionized magnesium ratios in women with menstrual migraine*. Headache, 42(4): 244-248. DOI: 10.1046/j.1526-4610.2002.02075.x

MAUSKOP A., ALTURA B.T., CRACCO R.Q., ALTURA B.M. 1995. *Intravenous magnesium sulfate relieves cluster headaches in patients with low serum ionized magnesium levels*. Headache, 35(10): 597–600. DOI: 10.1111/j.1526-4610.1995.hed3510597.x

MAUSKOP A., ALTURA B.T., CRACCO R.Q., ALTURA B.M. 1995. *Intravenous magnesium sulphate relieves migraine attacks in patients with low serum ionized magnesium levels: a pilot study*. Clin. Sci. (Colch.), 89(6): 633-636.

MCKEE J.A., BREWER R.P., MACY G.E., PHILIPS-BUTE B., CAMPBELL K.A., BOREL C.O., REYNOLDS J.D., WARNER D.S. 2005. *Analysis of the brain bioavailability of peripherally administered magnesium sulfate: A study in humans with acute brain injury undergoing prolonged induced hypermagnesemia*. Crit. Care Med. 33(3): 661-666. DOI:10.1097/01.CCM.0000156293.35868.B2

MISHIMA K., TAKESHIMA T., SHIMOMURA T., OKADA H., KITANO A., TAKAHASI K., NAKASHIMA K. 1997. *Platelet ionized magnesium, cyclic AMP and cyclic GMP levels in migraine and tension-type headache*. Headache, 37(9): 561-564. DOI: 10.1046/j.1526-4610.1997.3709561.x

MOODI S., LOWDER D.M. 2006. *Medication for migraine prophylaxis*. Am. Fam. Physician. 73(1): 72-78.

Olesen J., Steiner T.J. 2004. *The international classification of headache disorders, 2nd edition (ICHD-II).* J. Neurol. Neurosurg. Psychiatry, 75(6): 808-811 DOI: 10.1136/ /jnnp.2003.031286

Peikert A., Wilimzig C., Köhne-Volland R. 1996. *Prophylaxis of migraine with oral magnesium: results from a prospective, multi-center, placebo-controlled and double-blind randomized study.*, 16(4): 257-63. DOI: 10.1046/j.1468-2982.1996.1604257.x

Pfaffenrath V, Wessely P., Meyer C., Isler H.R., Evers S., Grotemeyer K.H., Taneri Z., Soyka D., Göbel H., Fischer M. 1996. *Magnesium in the prophylaxis of migraine-a double-blind, placebo-controlled study.* Headache, 16(6): 436-440. DOI: 10.1046/j.1468-2982.1996.1606436.x

Pringsheim T., Davenport J.W., Becker W.J. 2010. *Prophylaxis of migraine headache.* CMAJ., 182(7): E269–E276. DOI: 10.1503/cmaj.081657.

Ramadan N.M., Halvorson H., Vande-Linde A., Levine S. R., Helpern J.A., Welch K.M.A. 1989. *Low brain Magnesium in migraine.* Headache, 29(7): 416-419. DOI: 10.1111/j.1526-4610.1989.hed2907416.x

Romani A.M. 2008. *Magnesium homeostasis and alcohol consumption.* Magnes. Res., 21(4): 197-204. DOI: 10.1684/mrh.2008.0152

Safavi M., Honarmand A. 2007. *Admission hypomagnesemia – impact on mortality or morbidity in critically ill patients.* Middle East J.Anesthesiol. 19(3):645-660.

Schrucks M., Buring J.E., Kurth T. 2010. *Migraine, migraine features, and cardiovascular disease.* Headache, 50(6): 1031-1041. DOI: 10.1111/j.1526-4610.2009.01609.x

Shuen A.Y., Wong B.Y.L., Wei C., Liu Z., Li M., Cole D.E.C. 2009. *Genetic determinants of extracellular magnesium concentration: Analysis of multiple candidate genes, and evidence for association with the estrogen receptor á (ESR1) locus.*): 28-32.

Silberstein S.D. 2000. *Practice parameter: Evidence-based guidelines for migraine headache (an evidence-based review). Report of the quality standards Subcommittee of the American Academy of Neurology.* Neurology, 55(6): 754-762.

Silberstein S.D., Goldberg J. 2007. *Menstrually related migraine breaking the cycle in your clinical practice.* J. Reprod. Med.; 52(10): 888-895.

Smeets M.C., Vernoy C.B., Souverijn J.H.M., Ferrari M.D. 1994. *Intracellular and plasma magnesium in familial hemiphlegic migraine and migraine with and without aura.* Cephalalgia, 14(1): 29-32. DOI: 10.1046/j.1468-2982.1994.1401029.x

Sontia B., Touyz R. 2007. *Role of magnesium of hypertension.* Arch. Biochem. Biophys., 458(1):33-9.

Stang P, Cady RK, Batenhorst AS, Hoffman L. 2001. *Workplace productivity: a review of the impact of migraine and its treatment.* Pharmacoeconomics., 19(3): 231-244.

Sun-Edelstein C., Mauskop A. 2011. *Alternative headache treatments: Nutraceuticals, behavioral and physical treatments.* Headache., 5(3): 469-483. DOI: 10.1111/j.1526--4610.2011.01846.x

Sun-Edelstein C., Mauskop A. 2009. *The role of magnesium in the pathogenesis and treatment of migraine.* Expert Rev. Neurother. 9(3): 369-379.

Taylor F. 2011. *Nutraceuticals and Headache: The biological basis.* Headache, 51(3): 484--501. DOI: 10.1111/j.1526-4610.2011.01847.x

Tietjen E.G. 2007. *Migraine and ischaemic heart disease and stroke: potential mechanisms and treatment implications.* Cephalalgia, 27(8): 981-987.

Wang F., Van Den Eeden S.K., Ackerson M.L., Salk S.E., Reince R.H., Elin R.J. 2003. *Oral magnesium oxide prophylaxis of frequent migrainous headache in children: A randomized, double-blind, placebo-controlled Trial.* Headache, 43(6): 601-610. DOI: 10.1046/ /j.1526-4610.2003.03102.x

WEINBERGER J. 2006. *Interactions between migraine and stroke.* Curr. Treat. Option. Neurol. 8(6): 513-517. DOI: 10.1007/s11940-006-0041-7

WHANG R., RYDER K.W. 1990. *Frequency of hypomagnesemia and hypermagnesemia. Requested vs routine.* JAMA, 263(22): 3063-4. DOI:10.1001/jama.1990.03440220087036

WOLF FI, TRAPANI V, CITTADINI A. 2008. *Magnesium and the control of cell proliferation: looking for a needle in a haystack. Review.* Magnes. Res., 21(2): 83-91. DOI: 10.1684/mrh.2008.0138

ZIDVERC-TRAJKOVIĆ J., PAVLOVIĆ A.M., JOVANOVIĆ Z., ŠTERNIĆ N., KOSTIĆ V.S. 2001. *Efficacy of intravenous magnesium sulfate in severe migraine attacks.* J. Headache Pain, 2(2): 79-82. DOI: 10.1007/PL00012190

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