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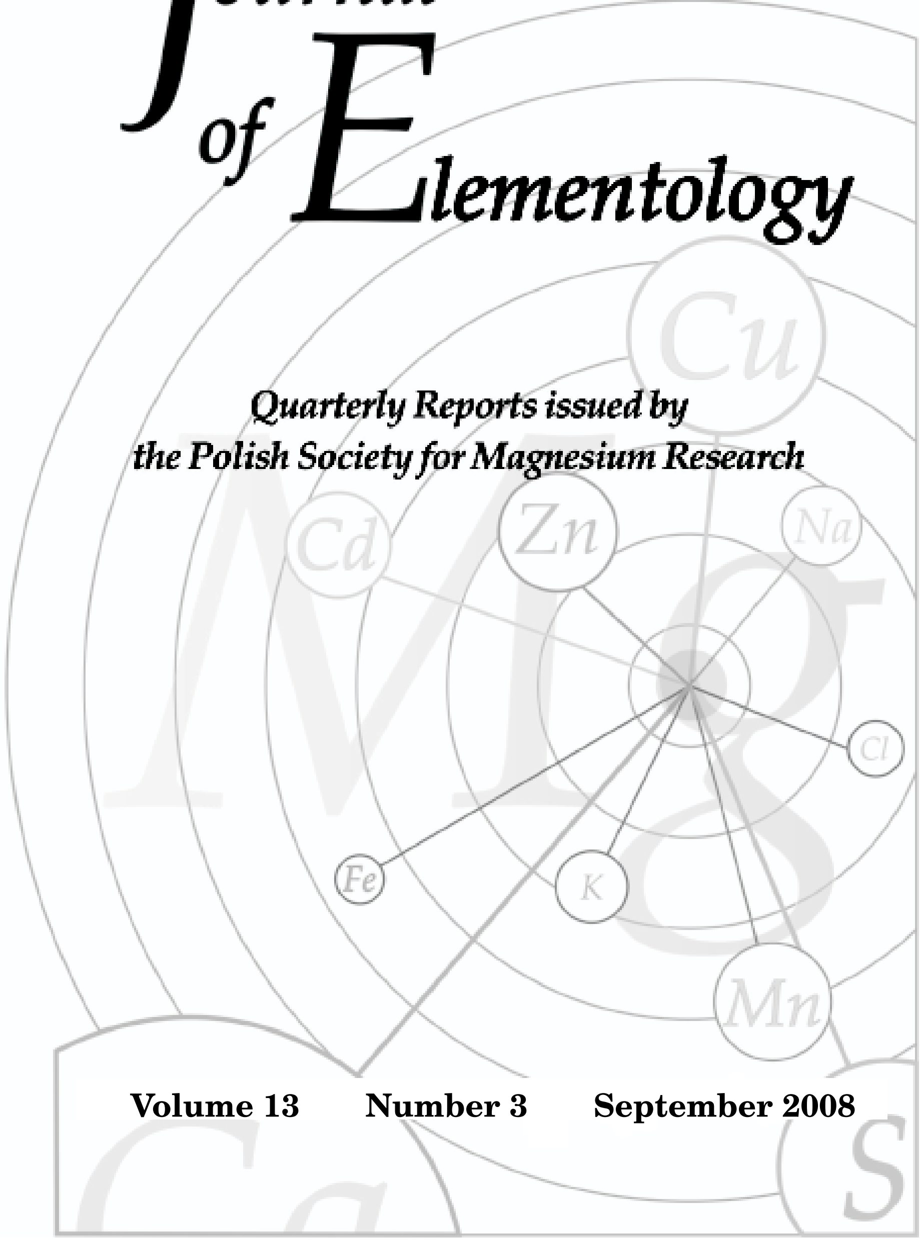

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CONTENTS AND RATIOS OF MINERAL COMPONENTS IN WINTER BARLEY BIOMASS CULTIVATED UNDER CONDITIONS OF DIFFERENT NITROGEN FERTILISATION

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Abstract

In 1999-2002, a strict two-factor field experiment was conducted at the University of Technology and Agriculture in Bydgoszcz to evaluate the effect of different nitrogen doses on the content and mutual ratios of macro-elements in green mass of winter barley in relation to barley growth stage. The experiment was established at the Research Station in Wierzchucinek near Bydgoszcz. The plant material consisted of samples of winter barley biomass taken at five stages of maturity: tillering, shooting (stem elongation), heading, initial grain filling and soft dough phases. The following nitrogen doses in $kg \cdot ha^{-1}$ were applied as ammonium nitrate: 0, 60, 120, 180. The results showed that the content of N, P, K, Ca, Mg and Na in winter barley vegetative mass decreased in the consecutive phenological phases, from tillering to soft dough. The largest decrease in the consecutive growth stages was detected for nitrogen and magnesium. With respect to nitrogen, phosphorus, calcium and sodium, their decrease in winter barley biomass during the growing season was generally higher in objects fertilised with nitrogen than in objects with no nitrogen fertilisation. In general, nitrogen had a positive effect on the content of the assayed macroelements in winter barley vegetative mass in all the growth phases. The ratio of the total content of nitrogen cations was observed to have attained the highest values in the later plant growth phases in the objects with no nitrogen or those fertilised with 60 kg N ha^{-1}.

Key words: winter barley, maturity stages, nitrogen, fertilization, macroelements.

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ZAWARTOŚĆ I PROPORCJE SKŁADNIKÓW MINERALNYCH W BIOMASIE JĘCZMIENIA OZIMEGO UPRAWIANEGO W WARUNKACH ZRÓŻNICOWANEGO NAWOŻENIA AZOTEM

Abstrakt

Materiał badawczy stanowiła biomasa jęczmienia ozimego, pochodząca z czteroletniego doświadczenia polowego, zbierana w następujących fazach wegetacji jęczmienia ozimego: krzewienie, strzelanie w źdźbło, kłoszenie, początek zawiązywania ziarna, dojrzałość mleczno-woskowa ziarna. W doświadczeniu zastosowano azot w formie saletry amonowej w dawkach (kg N·ha^{-1}): 0, 60, 120, 180. Wykazano, że zawartość N, P, K, Ca, Mg i Na w masie wegetatywnej jęczmienia ozimego zmniejszała się w kolejnych fazach rozwoju roślin, począwszy od krzewienia aż do dojrzałości mleczno-woskowej ziarna. Największy spadek zawartości w kolejnych stadiach wegetacji dotyczył azotu i magnezu. W przypadku azotu, fosforu, wapnia i sodu zmniejszanie się zawartości w biomasie jęczmienia ozimego podczas jego wegetacji było z reguły większe na obiektach nawożonych azotem, w porównaniu z obiektami bez azotu. Wykazano na ogół dodatni wpływ nawożenia azotem na zawartość badanych makroskładników w masie wegetatywnej jęczmienia ozimego we wszystkich jego fazach rozwojowych. Stwierdzono, że iloraz sumy zawartości kationów do azotu osiągał najwyższe wartości w późnych fazach rozwoju roślin na obiektach bez azotu lub nawożonych dawką 60 kg N ha^{-1}.

Słowa kluczowe: jęczmień ozimy, fazy dojrzałości, azot, nawożenie, makroelementy.

INTRODUCTION

Maintaining proper contents of macro- and microelements in cultivated plants is essential for their correct growth as well as the amount and quality of the yield. The content of mineral components in a plant, which is a measure of its nutritional condition, depends mainly on a species but environmental factors, agronomic practice as well as the plant's growth phase are important. Although physiological roles of basic nutrients in plants are well recognised, mutual ionic ratios of macro-components in biomass of plants cultivated under conditions of diverse mineral fertilisation are less known.

Winter barley is a cereal plant that may produce a high crop of green mass abundant in protein and mineral salts. With the growth in animal farming, green fodder has become a basic component in ruminants' nutrition. Nitrogen fertilisation is one of the most important factors that shapes the quality of winter barley fodder (SPALDON, HLAVENKOVA 1990, KANDERA 1991, RENZO et al. 1991, HEFNAVY, SAYED 1992, OSCARSSON et al. 1998, BARCZAK 1999). This is not only a results of the growth in the protein content but also a consequence of the changes in the content of microelements in the vegetative mass of crop plants (CZARNOWSKA 1975, BROGOWSKI, CZARNOWSKA 1987, JURKOWSKA et al. 1990, KOBBIA et al. 1992, MOTTALEB et al. 1992, CZAPLA 2000, KRZBIETKE, SIENKIEWICZ 2004, KOŁODZIEJ et al. 2005).

The aim of this research has been to assess the effect of differentiated nitrogen doses on the content and mutual proportion of macro-components in green mass of winter barley, depending on its growth phase.

MATERIAL AND METHODS

In 1999-2002, a strict two-factor field experiment with three replications, designed according to the method of randomised sub-blocks, was conducted at the University of Technology and Agriculture in Bydgoszcz. The experiment was established at the Research Station in Wierzchucinek near Bydgoszcz, on a typical fallow soil belonging to a very good rye complex. The soil, classified as 3b soil in the Polish soil classification system, was slightly acidic in reaction (pH in 1 $mol \cdot dm^{-3}$ KCl – 5.7) and moderately abundant in available forms of nitrogen, phosphorus and magnesium. The soil contained 1.5 g $humus \cdot kg^{-1}$.

The first factor of the experiment included vegetation phases of winter barley (n=5):

- tillering (phase A),
- stem elongation (phase B),
- heading (phase C),
- initial grain-filling (phase D),
- soft dough stage (phase E).

The other factor was made by nitrogen doses (n=4), in kg $N \cdot ha^{-1}$: 0, 60, 120 and 180. Nitrogen fertilisers were sown as 34% ammonium nitrate once in the springtime, when barley vegetation started. Fertilisation with phosphorus and potassium as 60% potash salt (100 kg $K \cdot ha^{-1}$) and magnesium superphosphate (25 kg $P \cdot ha^{-1}$) was applied in the autumn, before sowing. Aerial parts of winter barley plants were used for the assays. Green mass produced by barley in the five growth phases was sampled in order to perform determinations. Samples were taken from randomly chosen 1 m^2 areas over individual 15 m^2 plots. In each year of the experiment, barley was preceded by oats as a forecrop. Cultivar Paweł winter barley, bred at the Plant Breeding Station in Polanowice, was tested.

The plant material was wet mineralised and subjected to the following determinations: total nitrogen by Kjeldahl's method, phosphorus – colorimetrically, using a DR 400 colorimeter, calcium, potassium and sodium – in a flame photometer Flavo 4, and magnesium – using an AAS spectrometer.

The results are reported in milligram equivalents (meq) per kg of winter barley aerial plant dry mass and analysed statistically to assess differences between the means, using Tukey's test at p = 0.05.

RESULTS AND DISCUSSION

Nitrogen was a prevailing macroelement among the components determined in the green mass of the test winter barley, with its content reaching 1,488 meq·kg^{-1} (Table 1). Phosphorus, potassium, calcium and magnesium appeared in considerably lower concentrations – their average content in meq·kg^{-1} was respectively: 129, 422, 120 and 192. Sodium was present in the lowest concentration (17 meq·kg^{-1}). The content in the elements in winter barley biomass was arranged in a decreasing series N>K>Mg>Ca=P>Na, being identical in all the growth phases, regardless of nitrogen fertilisation.

In the consecutive phenological phases, irrespectively of the nitrogen rates, a significant decrease in the content of all the macroelements except phosphorus occurred in the winter barley vegetative mass. A decrease in the content of these elements which appeared in the growing plants grow was a result of a considerable biomass increase, resulting in the so called „dilution effect" (JURKOWSKA et al. 1990, BROGOWSKI, CZARNOWSKA 1987, GEREUDAS, SATTELMACHER 2000). The biggest differences in the content of the macroelements in winter barley biomass between phase A (tillering) and phase E (soft dough) was found for nitrogen and magnesium: 67.5% and 62.2% respectively as four-year means. Sodium was the element whose content declined the least as barley plants grew. In thesoft dough stage, the mean concentration of this element in winter barley biomass was 75.0% of its content in the tillering phase. The analogous ratios for potassium, calcium and magnesium of winter barley biomass in phases E and A were, respectively, 37.0, 36.9 and 62.2%. According to BROGOWSKI et al. (1989), the elements whose contents decrease proportionally to the increase in the growing plants' biomass are absorbed mainly in the early growing season and, to a much smaller degree, in the later growth stages. It is worth noticing that, in general, a decrease in nitrogen, phosphorus, calcium and sodium during the growth of winter barley was significant on these plots where nitrogen fertilisation was applied. This relationship was probably created by what is known as a dilution effect, which appears when winter barley biomass grows larger owing to a better nitrogen supply, a powerful yield forming factor (SPALDON, HLAVENKOVA 1990, HEJNAK et al. 2001, GEREUDAS, SATTELMACHER 2000). A slightly higher decrease in the magnesium content in the objects not fertilized with nitrogen was more difficult to explain.

The results of our research find confirmation in the results achieved by BROGOWSKI and CZARNOWSKA (1987) for winter wheat, BROGOWSKI et al. (1989) for spring barley, JURKOWSKA et al. (1990) for oats, BROGOWSKI et al. (1993) for winter rye and by KRUCZEK (1996) for maize. Some of the authors (e.g. BROGOWSKI et al. 1993), noticed a progressing decrease in the content of calcium, potassium and sodium in aerial parts of plants, but only from the phase of heading and not starting in the tillering stage, as it has been shown in this research.

Table 1

Content of macroelements in biomass of winter barley in different phenological phases (four-year means)

Macro-elements	Doses kg N·ha^{-1}	Phenological phases A	B	C	D	E	Mean	$LSD_{0.05}$
N	0	1582	1195	947	781	673	1036	I fac. – 40.4
	60	2531	1648	1252	905	738	1415	II fac. – 34.2
	120	3004	1968	1532	1005	831	1668	IxII – 76.5
	180	3105	2079	1670	1225	1079	1832	IIxI – 68.4
	x	2556	1723	1350	979	830	1488	
P	0	159	142	132	118	77	126	Ifac. – n.s.
	60	172	149	139	102	83	129	IIfac. – n.s.
	120	182	151	153	89	76	130	IxII – n.s.
	180	176	155	140	108	82	132	IIxI – n.s.
	x	172	149	141	99	80	129	
K^+	0	435	433	385	301	287	368	Ifac. – 15.5
	60	514	507	445	339	307	422	IIfac. – 15.4
	120	531	537	453	339	321	436	IxII – 34.4
	180	534	552	468	372	353	456	IIxI – 30.7
	x	503	507	438	348	317	422	
Ca^{2+}	0	111	100	89	84	75	92	Ifac. –14.3
	60	156	130	113	99	85	117	IIfac. – 12.4
	120	130	164	138	105	98	136	IxII – 27.6
	180	155	151	135	130	119	138	IIxI – 24.7
	x	149	136	119	104	94	120	
Mg^{2+}	0	262	238	201	129	88	184	Ifac. – 15.6
	60	262	233	201	148	107	190	IIfac. – n.s
	120	275	233	204	136	104	190	IxII – n.s.
	180	271	242	229	164	103	202	IIxI – n.s.
	x	267	237	209	144	101	192	
Na^+	0	18	16	16	14	15	16	Ifac. – 0.7
	60	19	18	15	16	15	17	IIfac. – n.s
	120	21	17	16	15	15	17	IxII – n.s.
	180	22	17	17	15	16	17	IIxI – n.s.
	x	20	17	16	15	15	17	

I fac. – phenological stages (A – tillering, B – shooting (stem elongation), C – heading, D – initial grain filling E – soft dough maturity), II fac.–- nitrogen

Table 2

Content of macroelements in biomass of winter barley in different phenological phases of (four-year means) (meq·kg^{-1})

Macro-elements	Doses kg N·ha^{-1}	Phenological phases					Mean	$LSD_{0.05}$
		A	B	C	D	E		
Σcat.	0 60 120 180	826 951 1000 982	787 888 951 962	691 774 811 849	528 602 595 681	465 514 538 591	659 746 779 817	Ifac. – 38.8 IIfac. – 36.6 IxII – 75.3 IIxI – 73.0
	x	939	897	781	602	527	751	
Σcat./N	0 60 120 180	5.2 3.8 3.3 3.2	6.6 5.4 4.8 4.6	7.3 6.2 5.3 5.1	6.8 6.7 5.9 5.6	6.9 7.0 6.5 5.5	6.6 5.8 5.2 4.8	Ifac. – 0.54 IIfac. – 0.48 IxII – 1.07 IIxI – 0.96
	x	3.9	5.3	6.0	6.2	6.5	5.6	
$Ca^{2+}+Mg^{2+}$ $/K^{+}+Na^{+}$	0 60 120 180	8.2 7.8 8.1 7.7	7.5 6.9 7.2 6.9	7.2 6.8 7.3 7.5	6.8 7.0 6.8 7.6	5.4 6.0 6.0 6.0	7.0 6.9 7.1 7.1	Ifac. – 0.11 IIfac. – 0.09 IxII – 0.21 IIxI – 0.19
	x	8.0	7.1	7.2	7.1	5.9	7.0	
Ca^{2+}/P	0 60 120 180	7.0 9.1 9.5 8.8	7.0 8.7 10.9 9.7	6.7 8.1 9.0 9.6	7.1 9.7 11.8 12.0	9.7 10.2 12.9 14.5	7.5 9.2 10.8 10.9	Ifac. – n.i./n.s. IIfac. – 0.47 IxII – 0.68 IIxI – 0.72
	x	8.6	9.1	8.4	10.2	11.8	9.6	

Likewise in other plant species (CZARNOWSKA 1975, BROGOWSKI, CZARNOWSKA 1987, JURKOWSKA et al. 1990, RENZO et al. 1991, MOTTALEB et al. 1992, KRUCZEK 1996, GERENDAS, SATTELMACHER 2000), this study has demonstrated that higher doses of nitrogen generally increased the content of macroelements in winter barley biomass in all the plant growth stages assayed. Nitrogen fertilization had the strongest effect on the content of nitrogen and calcium – the four-year mean increase in the content of these elements resulting from an application of 180 kg N·ha^{-1} and was 76.8 and 54.4% respectively. At the same time, a relatively small effect of nitrogen doses on the content of phosphorus and sodium was shown.

For nitrogen and phosphorus, the effect of increasing doses of nitrogen was the strongest during the tillering phase. In the next growth stages, the variations in the content of these elements in the green mass of winter barleywere much smaller. For univalent cations, changes in their contents under the effect of intensifying nitrogen fertilization depended on the plant

Table 3

Correlation coefficients between macroelements

	P	K	Ca	Mg	Na	Σcat.	Σcat./N	Ca+Mg /K+Na
N	0.847*	0.878*	0.810*	0.841*	0.930*	0.905*	-0.960*	0.687*
P		0.900*	0.690*	0.971*	0.766*	0.934*	-0.744*	0.824*
K			0.870*	0.921*	0.760*	0.991*	-0.821*	0.657*
Ca				0.713*	0.635*	0.859*	-0.857*	0.569*
Mg					0.784*	0.957*	-0.748*	0.853*
Na						0.799*	-0.867*	0.636*
Scat.							-0.846*	0.749*
Σcat./N								0.641*

growth stage, but this relationship was statistically proven to a smaller degree. The total content of cations in the vegetative mass of winter barley (Ca^{2+}+Mg^{2+}+K^{+}+Na^{+}) gradually decreased from the tillering to soft dough phases, which was a consequence of such a direction of changes in the concentrations of individual cations during the vegetative season. The average value for four years of the total sum of cations in the biomass collected in the soft dough phase was 57.7% of their content in the tillering phase. In the literature some authors mention the phenomenon of "ion competition" (Dijkshorn 1972, cit. by Brogowski et al. 1993), which relies on the fact that the total content of basic cations in a given growth phase of a plant is constant, but the values of the components of this sum can vary. This regularity is analogous to the phenomenon of isomorphism in minerals, which comprises a possibility of replacing ions in their crystal lattices. This phenomenon in plants can be observed under deficiency of one of the cations, e.g. under potassium deficiency, when the plant takes up more sodium or calcium. Our field experiment was carried on soil of moderate potassium and magnesium abundance at pH_{KCl} = 5.7, therefore there is no reason to suppose that a phenomenon of ion compensation took place. An increase in the total content of ions resulting from nitrogen fertilisation was observed. The dose of 180 kg $N \cdot ha^{-1}$ resulted in the highest increase in the total ions in the winter barley biomass collected during the soft dough maturity phase (the difference was 27.1% with reference to the plants from the control). The lowest increase occurred in plants in the heading phase (the difference was 13.0%).

It was proven that an average ratio of the content of divalent cations (Ca^{2+}+Mg^{2+}) to the content of univalent cations (K^{+}+Na^{+}) was 0.72. The value of this ratio less than one is characteristic for monocotyledonous plants (Brogowski et al. 1993) and is probably connected with the type of root system of these plants (Mattson 1975). Monocotyledonous plants, such as winter barley, have a root system with a limited replacement capacity. Therefore, this species takes up more K^+ and Na^+ than Ca^{2+} and Mg^{2+}. Quantitative proportions of uni- and divalent cations in a plant can be modified by soil sorption capacity. According to Mattson (1975), the higher sorption capacity of soil with the same sorption capacity of roots, the greater the amounts of univalent cations taken up by a plant and vice versa.

The highest values of the quotient of the content Ca^{2+}+Mg^{2+} and K^++Na^+ were characteristic for the green mass of winter barley in the tillering and heading phases in the objects with no nitrogen fertilization. The values of this quotient tended to decrease in the consecutive phases of the winter barley growth, which means that divalent ions were reduced during vegetation to a higher degree than univalent ones. The above relationships are only partly confirmed in the research on winter wheat (Brogowski and Czarnowska 1987), spring barley and winter rye (Brogowski et al. 1989, 1993), where a constant ratio of divalent to univalent ions in particular plant growth phases was proven.

The increase in the nitrogen dose generally reduced not only the value of the above ratio but also that of the ratio of total cations to nitrogen as well. The highest effect of nitrogen fertilisation on shaping the ratio of total cations to nitrogen was found in plants in the tillering phase, which was a consequence of the highest increase in the nitrogen content in this plant growth stage, under the effect of nitrogen fertilisation.

The largest variation in values of the calcium to phosphorus ratio was demonstrated, in the later growth stages of winter barley, i.e. initial grain-filling and soft dough stages, particularly under conditions of intensive nitrogen fertilisation. Similar results were reported by Jurkowska et al. (1990).

The correlation analysis showed that the content of the individual macroelements in winter barley biomass are strongly correlated. Noticeable is a highly significant correlation of the nitrogen content versus the other elements, and also the values of the calculated ionic ratios.

CONCLUSIONS

1. The content of N, P, K, Ca, Mg and Na in the vegetative mass of winter barley decreased in the consecutive phenological phases, from tillering to soft dough stages. The highest decrease was detected for nitrogen and magnesium.

2. During the vegetation period of winter barley, a decrease in the content of nitrogen, phosphorus, calcium and sodium in barley biomass was generally higher in objects fertilised with nitrogen than in the objects with no nitrogen fertilisation.

3. Nitrogen fertilisation generally produced a positive effect on the content of the analysed macroelements in the vegetative mass of winter barley in all its growth phases. The highest increase in the nitrogen and calcium content was observed under the influence of nitrogen fertilization.

4. The ratio of total cations to nitrogen for winter barley biomass in the consecutive plant growth phases tended to decrease.

5. The highest values of the content ratio of divalent (Ca^{2+}+Mg^{2+}) to univalent ones (K^{+}+Na^{+}) cations in winter barley green mass were observed in the tillering phase.

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INFLUENCE OF LIMING AND MINERAL FERTILIZATION ON THE CONTENT OF MINERAL NITROGEN IN SOIL

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Abstract

Nitrogen is a key factor which shapes the fertility and fecundity of soils. Liming and mineral fertilization significantly modify transformations of nitrogen compounds in soil. The aim of our experiment was to evaluate the influence of liming and ammonium sulphate or calcium nitrate fertilization on the content of total nitrogen and its mineral forms in soil. The study was based on chemical analysis of the soil material obtained from a two-year pot experiment. The design of the experiment comprised 9 treatments in 4 replications on acidic soil and an analogous number of trials on limed soil. The experimental factors were: liming (acidic soil, limed soil), fertilization with ammonium or nitrate nitrogen at two levels (N_1, N_2) as well as fertilization with phosphorus at two levels (P_1, P_2). Liming was applied only once, before setting the experiment. The mineral fertilizers were applied every year before plant sowing in the form of fertilizers: ammonium sulphate, calcium nitrate and triple granulated superphosphate. The test plant was spring barley, which was harvested at its full ripeness. The results indicated that the biggest influence on the $N\text{-}NH_4$ content was produced by liming and fertilization with nitrogen. The application of calcium carbonate as well as calcium nitrate led to a decrease in the ammonium nitrogen content in soil. The content of nitrate nitrogen was higher in objects fertilized with calcium nitrate than in those fertilized with ammonium sulphate. Liming and nitrogen fertilization had the largest effect on the formation of mineral nitrogen content in soil. Liming contributed to decreased mineral nitrogen amounts in soil. A reverse situation was observed after increasing the rates of fertilization. The application of calcium carbonate and nitrate form of nitrogen contributed to a decrease in the total nitrogen content in soil. This fact can be explained by increased yield of spring barley in the objects limed and fertilized with calcium nitrate compared with the barley yield in the non-limed and ammonium sulphate fertilized trials.

Key words: nitrate nitrogen, ammonium nitrogen, liming, soil.

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WPŁYW WAPNOWANIA I NAWOŻENIA MINERALNEGO NA ZAWARTOŚĆ AZOTU MINERALNEGO W GLEBIE

Abstrakt

Azot ma decydujący wpływ na kształtowanie żyzności i urodzajności gleb. Istotnymi czynnikami modyfikującymi przemiany związków azotowych w glebie są wapnowanie i nawożenie mineralne. Celem badań było określenie wpływu wapnowania oraz nawożenia siarczanem amonu lub saletrą wapniową na ogólną zawartość azotu oraz jego mineralnych form w glebie. Badania oparto na analizie chemicznej materiału glebowego otrzymanego z dwuletniego doświadczenia wazonowego. Schemat doświadczenia obejmował 9 kombinacji w 4 powtórzeniach na glebie kwaśnej i wapnowanej. Czynnikami doświadczalnymi były: wapnowanie (gleba kwaśna, gleba wapnowana), nawożenie formą amonową lub azotanową azotu stosowane na dwóch poziomach (N_1, N_2) oraz nawożenie fosforem w dwóch dawkach (P_1, P_2). Wapnowanie zastosowano jednorazowo przed założeniem doświadczenia. Nawozy mineralne stosowano w każdym roku badań przed siewem roślin w postaci nawozów: siarczanu amonu, saletry wapniowej i superfosfatu potrójnego granulowanego. Rośliną testową był jęczmień jary, który zbierano w fazie pełnej dojrzałości. Wykazano, że największy wpływ na zawartość N-NH_4 miało wapnowanie oraz nawożenie azotem. Zastosowanie węglanu wapnia, a także saletry wapniowej prowadziło do zmniejszenia ilości azotu amonowego w glebie. Zawartość azotu azotanowego w obiektach nawożonych saletrą wapniową była większa niż w kombinacjach z siarczanem amonu. Wapnowanie oraz zastosowana dawka azotu miały największy wpływ na kształtowanie zawartości azotu mineralnego w glebie. Wapnowanie przyczyniło się do zmniejszenia ilości azotu mineralnego w glebie. Odmienną sytuację zaobserwowano po zwiększeniu dawek analizowanego składnika. Zastosowanie węglanu wapnia oraz azotanowej formy azotu prowadziło do zmniejszenia ogólnej zawartości azotu w glebie, co można wyjaśnić zwiększeniem plonu jęczmienia jarego w obiektach wapnowanych i nawożonych saletrą wapniową w porównaniu z plonem rośliny testowej w obiektach nie wapnowanych i nawożonych siarczanem amonu.

Słowa kluczowe: azot azotanowy, azot amonowy, wapnowanie, gleba.

INTRODUCTION

Nitrogen is a key factor which shapes the fertility and fecundity of soils. Total nitrogen content in mineral soils varies from 0.02 to 0.35%. Most of the nitrogen in soils is present as organic compounds. Only 1-5% of total nitrogen is in the mineral form, the fact that has principal meaning in plant nutrition. The average content of mineral nitrogen in the Polish soils ranges from 76-90 kg N ha^{-1} in spring and 89-97 kg N ha^{-1} in autumn (FOTYMA et al. 2004). Its content is controlled by a combination of physical, chemical and biological processes: oxidation-reduction and mineralization-immobilization. The direction and range of these transformations depends on the type of soil, moisture, temperature, reaction, content of clay minerals, the amount of organic substance, activity of microorganisms, etc. Another significant factor which modifies transformations of nitrogen compounds in soil is mineral fertilization (ŁOGINOW et al. 1987, DECHNIK, BEDNAREK 1989, MAZUR, CIEĆKO 1998, FOTYMA 2000, KOÓS, NEMETH 2006, SOSULSKI et al. 2006a).

In order to secure high effectiveness of mineral fertilization in the formation of ammonium and nitrate nitrogen in soil, soil reaction needs to be suitably regulated. Liming influences the activity of ammonifying and nitrifying microorganisms, and through that – the amount of available forms of nitrogen in soil (KOZANECKA 1995, SAPEK 1995, MALHI 2002, FOTYMA et al. 2004, SOSULSKI et al. 2006b).

The aim of our study has been to define the influence of liming and ammonium sulphate or calcium nitrate fertilization on the total content of nitrogen and the content of its mineral (ammonium and nitrate) forms in soil.

MATERIAL AND METHODS

A two-year pot experiment has been completed, set up on soil material of grain-size distribution typical of light loamy sand. The soil was characterized by very acidic reaction (pH_{KCl} 4.00), low abundance of available phosphorus and potassium and very low content of available magnesium. The total nitrogen content was 0.73 g kg^{-1}, with 15.02 mg kg^{-1} of mineral nitrogen, 11.82 mg kg^{-1} of $N\text{-}NH_4$, and 3.20 mg kg^{-1} of $N\text{-}NO_3$.

The trials were set in 5 kg pots. Against the background of permanent potassium and magnesium on acid and limed soil, differentiated fertilization with phosphorus and nitrogen was applied: 1) 0; 2) $P_1N_1\text{-}NH_4$; 3) $P_1N_1\text{-}NO_3$; 4) $P_1N_2\text{-}NH_4$; 5) $P_1N_2\text{-}NO_3$; 6) $P_2N_1\text{-}NH_4$; 7) $P_2N_1\text{-}NO_3$; 8) $P_2N_2\text{-}NH_4$; 9) $P_2N_2\text{-}NO_3$.

Liming with $CaCO_3$ in the amount calculated according to 1 Hh was applied once before the establishment of the experiment. Fertilization with nitrogen, potassium and magnesium was applied every year, before sowing plants. Phosphorus was applied in the form of granulated triple superphosphate (20.1% P) in two rates (P_1 – 0.06 g P kg^{-1}, P_2 – 0.12 g P kg^{-1} d.m. of soil); nitrogen was used as ammonium sulphate (20% N) or calcium nitrate (15.5% N) at two levels (N_1 – 0.1 g N kg^{-1}, N_2 – 0.2 g N kg^{-1} d.m. of soil); potassium (0.1 g K kg^{-1} d.m. of soil) was added as potassium high-percentage potash salt (49.8% K) and magnesium (0.025 g Mg kg^{-1} d.m. of soil) was introduced as magnesium sulphate (9.6% Mg). The experimental factors were tested versus the control object. During the vegetation season, constant soil moisture was maintained at 60% field water capacity. The test plant was cv. Bryl spring barley, harvested at full ripeness.

In each year of the studies, after harvest, soil material was collected for chemical analyses, in which total nitrogen content was determined by Kjeldahl method, the ammonium nitrogen content was tested by Nessler method and the content of nitrate nitrogen was determined by the salicylate method (LITYŃSKI et al. 1976). The determinations were performed with the use of a Cecil 2011 photocolorimeter.

The influence of the experimental factors on the formation of N_{total}, Nmin., $N\text{-}NH_4$ and $N\text{-}NO_3$ in soil was determined by variance analysis including Tukey's confidence half-intervals. The results presented in the tables are mean values from a two-year experiment. Only significant LSD values were given.

RESULTS AND DISSCUSION

In the experiment the content of ammonium nitrogen was significantly greater in acidic than in limed soil (Table 1). The reason for that might have been the reduction of the nitrification process at low soil pH values. Another important fact was that the plants cultivated in acidic soil environment took up NO_3^- ions quicker than NH_4^+ ions. Besides, a decrease in the amount of ammonium nitrogen at increased pH may have resulted from losses caused by ammonia disappearing from soil. According to SAPEK (1995), soil acidification and lower pH enhance ammonification and accumulation of $N\text{-}NH_4$ in soil. Lower ammonium nitrogen content coinciding with increased soil pH was also observed by SOSULSKI et al. (2006a). Similarly, KOZANECKA (1995) as well as BEDNAREK and TKACZYK (2003) found that $N\text{-}NH_4$ content was lower in limed than non-limed soil. The application of ammonium sulphate caused a significant increase of the $N\text{-}NH_4$ content as compared to its content in soil fertilized with calcium nitrate. This effect was clearly caused by the application of the ammonium form of nitrogen in the fertilizer. The results are in accord with the observations reported by SOSULSKI et al. (2006a). In our experiment, increasing nitrogen rates were associ-

Table 1

Effect of experimental factors on the content of ammonium nitrogen and nitrate nitrogen in soil (mg N kg^{-1})

Object	Content	$N\text{-}NH_4$				$N\text{-}NO_3$			
	soil	acidic		limed		acidic		limed	
	N form	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
P_1N_1		41.54	12.19	17.89	8.04	9.64	22.11	11.09	28.78
P_1N_2		61.58	13.39	22.18	7.33	9.78	29.71	13.32	32.32
P_2N_1		37.75	11.41	17.59	7.99	9.43	26.85	13.75	28.64
P_2N_2		46.58	12.47	28.18	6.62	12.17	32.03	9.64	31.37
$\bar{x}$ soil		29.61		14.48		18.96		21.11	
$\bar{x}$ form				34.16	9.93			11.10	28.97
LSD (p=0.05)		soil, N dose, N form – 3.75 soil x N form, N dose x N form – 7.03				N form – 4.19			
Control		15.63		7.44		4.86		11.40	

ated with a significant increase in the amount of ammonium nitrogen in combinations with ammonium sulphate. Such a dependence was not observed in the objects fertilized with calcium nitrate. Increased ammonium nitrogen in soil after application of increased rates of nitrogen fertilizer has been reported by other authors (Chmielewska, Dechnik 1987, Kozanecka 1995, Czekała et al. 2002, Bednarek, Tkaczyk 2002, 2003). In conrast, The differentiation of phosphorus rates did not significantly affect the content of $N\text{-}NH_4$ in soil, a finding which is confirmed by the results of studies performed by Bednarek and Tkaczyk (2002).

Nitrate nitrogen is an unstable part of mineral nitrogen. The content of this nitrogen form can indicate a potential threat to groundwater (Fotyma et al. 2004). In the present experiment, the content of $N\text{-}NO_3$ ranged from 4.86 to 32.32 mg kg^{-1} of soil (Table 1). Among the tested factors, statistically proven effect was produced only by this form of nitrogen. Both in very acidic and in limed soils increased amounts of nitrate nitrogen were observed under the influence of calcium nitrate. Significantly more nitrate form of nitrogen after an application of nitrate fertilizer was also found by Sapek (1995) and Sosulski et al. (2006a). The mean values from the objects indicate that calcium carbonate raised the content of $N\text{-}NO_3$, which was most probably caused by favorable conditions for nitrification. The stimulating effect of this procedure on the content of $N\text{-}NO_3$ was also observed by Kozanecka (1995), Bednarek and Tkaczyk (2003) and Sosulski et al. (2006b). In the present experiment, the content of nitrate nitrogen was not significantly related to the rates of nitrogen and phosphorus. However, other authors reported positive influence of increased rates of nitrogen fertilizers on $N\text{-}NO_3$ in soil (Chmielewska, Dechnik 1987, Dechnik, Bednarek 1989, Malhi 2002, Bednarek, Tkaczyk 2002, 2003, Qian et al. 2004, Koós, Nemeth 2006).

The results presented in Table 2 suggest that the content of mineral nitrogen was significantly formed under the influence of liming and the applied rate of nitrogen. Calcium carbonate caused a decrease in the mineral N content in comparison with the content observed in non-limed soil, which was nevertheless higher than in the soil before the experiment was established. The decrease of N_{min} content can be justified by a large, distinct, over two-fold, increase of the test plant yield under the influence of this procedure (Bednarek, Reszka 2007), and, consequently, the increased uptake of nitrogen. The absorption of nitrogen by spring barley being the main cause of depressed mineral nitrogen in soil is also indicated by the results of a study conducted by Bednarek and Tkaczyk (2003). An increased rate of nitrogen, both in the form of ammonium sulphate and calcium nitrate, leads to an increased content of mineral nitrogen. This increase was greater in treatments on non-limed than on limed soil. High rates of nitrogen fertilizers favour the narrowing of C : N ratio and consequently the surplus nitrogen from fertilizers is not used by multiplying microorganisms and the soil accumulates mineral forms of nitrogen. A positive effect of increased rates

Table 2

Effect of experimental factors on the content of mineral nitrogen (mg N kg^{-1}) and total nitrogen (g N kg^{-1}) in soil

Object	Content	$N\text{-}NH_4 + N\text{-}NO_3$				N total			
	soil	acidic		limed		acidic		limed	
	N form	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
P_1N_1		51.18	34.30	28.98	36.81	0.84	0.77	0.73	0.73
P_1N_2		71.36	43.10	35.50	39.65	0.99	0.79	0.79	0.79
P_2N_1		47.17	38.26	31.34	36.64	0.84	0.77	0.74	0.76
P_2N_2		58.74	44.50	37.82	37.99	0.97	0.79	0.81	0.81
$\bar{x}$ soil		48.57		35.59		0.85		0.77	
$\bar{x}$ form				45.26	38.90			0.84	0.78
LSD (p=0.05)		soil, N dose – 7.23 soil x N form – 13.55				soil, N dose, N form – 0.04 soil x N form, N dose x N form – 0.07 soil x N dose x N form – 0.1			
Control		20.49		18.83		0.73		0.70	

of nitrogen on the content of mineral nitrogen in soil is also indicated by the results obtained by CZEKAŁA et al. (2002). Smaller amounts of $N\text{-}NH_4$ + + $N\text{-}NO_3$ were observed in the soil fertilized by the nitrate form as compared to the objects treated with ammonium sulphate, although these differences were not statistically confirmed. This fact can be explained by the increase (69%) of spring barley yield in the objects fertilized with calcium nitrate compared with the yield from those fertilized with ammonium sulphate (BEDNAREK, RESZKA 2007). What was significant, however, was the correlation between the soil series and the form of nitrogen used.

In our own study, liming contributed to a significant decrease of total nitrogen content (Table 2). SOSULSKI et al. (2006a) also observed a decreasing tendency in the total N content in soil as the soil pH increased. The use of calcium nitrate significantly depressed the total amount of nitrogen as compared to its amount in objects fertilized with ammonium sulphate, which was most probably caused by the difference in the amount of this nutrient taken up by plants. Formation of the total N content was also significantly affected by the rate of ammonium sulphate. A significant increase of total nitrogen in soil was observed at higher ammonium sulphate fertilization rates.

CONCLUSIONS

1. Liming and nitrogen fertilization significantly affected the $N\text{-}NH_4$ content in soil. The application of calcium carbonate, as well as calcium nitrate contributed to a significantly lower content of this N form in soil in comparison with ammonium sulphate fertilization.

2. The $N\text{-}NO_3$ content was significantly modified only by fertilization with differentiated forms of nitrogen. Calcium nitrate caused an increase in the nitrate nitrogen content, irrespective of the soil pH.

3. The biggest effect on the content of mineral nitrogen in soil was caused by liming and nitrogen rates. Lower content of N_{min} was found in soil from limed rather than non-limed combinations. Increased nitrogen rates enhanced a statistically proven increase of $N\text{-}NH_4 + N\text{-}NO_3$ content in soil.

4. The total content of nitrogen was significantly related to liming and nitrogen fertilization. The application of calcium carbonate and the nitrate form of nitrogen led to a decrease in the total nitrogen content in soil in comparison with acidic soil and ammonium sulphate fertilization.

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INFLUENCE OF ORGANIC FORMS OF COPPER, MANGANESE AND IRON ON BIOACCUMULATION OF THESE METALS AND ZINC IN LAYING HENS

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Abstract

The paper presents results of research concerning an assessment of bioaccumulation of copper, manganese and zinc in Lohmann Brown layer hens (5 groups of 12 hens in each). Using ICP-MS method the concentration of these elements was determined in the content and shell of eggs, whole blood and in feathers of hens.

Feeding was based on all-mash feed mixture J-297 type with a content of Cu – 21.8, Fe – 200.8, Mn 140.5 $mg \cdot kg^{-1}$, but in particular groups the contribution of organic and inorganic forms of these 3 microelements was different. Content of Zn in the mixture was 86 $mg \cdot kg^{-1}$ (zinc oxide). Microelements in the amounts of: Cu – 10, Fe – 40 and Mn – 80 $mg \cdot kg^{-1}$ were separately introduced to the control and to the test mixtures by using special premixes. In the control version, inorganic forms of these elements were used in a premix (copper sulfate, iron sulfate and manganese oxide), while in the experimental version they appeared in the organic form, i.e. *Saccharomyces cerevisiae* yeasts enriched with the three elements. In the experimental mixtures the contribution of organic forms of microelements was for Cu – 47, Fe – 20 and Mn – 58%. Content of the premix in a mixture was 0.5%. Yeasts contained: Fe – 33.9, Mn – 35.4, and Cu – 22.7 $mg \cdot kg^{-1}$ d.m. Content of yeasts in the mixtures did not exceed 0.4%.

Application of organic forms of copper caused a significant increase in copper concentration in the egg content and shell, in blood and in feathers in the group receiving organic-Cu, which proves better availability of copper from organic forms compared to copper sulfate.

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Introduction of organic forms of iron and manganese to feed did not cause any significant changes in the content of these metals in eggs, blood and feathers of hens, except the organic-Mn group (the level of Mn in feathers was significantly higher in feathers compared to the control group).

Organic forms of copper, manganese and iron did not result in any interactions with respect to Zn although an antagonistic influence of Cu (organic-Cu group) and synergistic of Mn (organic-Mn group) in the egg content was observed.

Key words: copper, iron, manganese, zinc, hen egg, blood, feathers.

WPŁYW ORGANICZNYCH FORM MIEDZI, MANGANU I ŻELAZA NA BIOAKUMULACJĘ TYCH PIERWIASTKÓW ORAZ CYNKU U KUR NIEŚNYCH

Abstrakt

W pracy przedstawiono wyniki badań dotyczących określenia biakumulacji miedzi, manganu i żelaza oraz cynku u kur nieśnych Lohmann Brown (5 grup po 12 kur). Żywienie opierało się na pełnoporcjowej mieszance typu J-297 o zawartości Cu – 21,8, Fe – 200,8, Mn 140,5 mg·kg^{-1}, z tym że w poszczególnych grupach różny był w udział organicznych i nieorganicznych form tych 3 mikroelementów. Zawartość Zn w mieszance wynosiła 86 mg□kg^{-1} (tlenek cynku). Mikroelementy w ilościach: Cu – 10, Fe – 40 i Mn – 80 mg·kg^{-1} wprowadzono osobno do paszy kontrolnej i do pasz doświadczalnych, stosując specjalne premiksy. W wersji kontrolnej w premiksie zastosowano formy nieorganiczne tych pierwiastków (siarczan miedzi, siarczan żelaza oraz tlenek manganu), natomiast w wersji doświadczalnej formy organiczne, tj. drożdże *Saccharomyces cerevisiae* wzbogacone w te trzy pierwiastki. W mieszankach doświadczalnych udział organicznych form mikroelementów wynosił: Cu – 47, Fe – 20 i Mn – 58%. Udział premiksu w mieszance wynosił 0,5%. Drożdże zawierały: Fe – 33,9, Mn – 35,4, Cu – 22,7 mg·kg^{-1} s.m. Udział drożdży w mieszankach nie przekroczył 0,4%.

Zastosowanie organicznych form miedzi spowodowało istotny wzrost stężenia miedzi w treści i skorupach jaj, we krwi i piórach w grupie otrzymującej Cu-org., co świadczy o lepszej przyswajalności miedzi w formach organicznych w porównaniu z siarczanem miedzi.

Wprowadzenie do paszy organicznych form żelaza i manganu nie powodowało istotnej zmiany w kształtowaniu się zawartości tych metali w jajach, krwi i piórach kur, z wyjątkiem grupy otrzymującej Mn-org. (istotnie więcej było Mn w piórach w porównaniu z grupą kontrolną).

Organiczne formy miedzi, manganu i żelaza nie spowodowały interakcji w odniesieniu do Zn, chociaż w treści jaja zaznaczył się antagonistyczny wpływ Cu (grupa Cu-org.) oraz synergistyczny Mn (grupa Mn-org.).

Słowa kluczowe: miedź, żelazo, mangan, cynk, jajo kurze, krew, pióra.

INTRODUCTION

Copper, iron and manganese, beside zinc, belong to the most important, basic microelements that are standardized in poultry feeding (Smulikowska, Rutkowski 2005). They are accumulated in tissues and organs of birds, and also in the content and egg shells in quite different concentrations, dependent on a dose and form of these elements as well as on many other factors, including physiological ones. According to Jamroz (2001) absorption of copper, iron and manganese from commercial mixtures for poultry reaches 10-15% on average (zinc – 30%). In turn, Jongbloed et al. (2002) reveal that in poultry (and other livestock species) a relative biological value for Cu, Fe, Mn or Zn is higher from organic sources (e.g. Metal Amino Acid Complex, Metal Amino Acid Chelate, Metal Proteinate) comparing to oxides or chlorides of these metals.

A good source of organic forms of microelements are *Saccharomyces cerevisiae* yeasts (Dobrzański et al. 2006, Vasudevan et al. 2002). There is a biotechnological possibility of yeasts enrichment with Cu, Fe and Mn and their application in the form of dry feed yeasts in poultry feeding (Dobrzański et al. 2008). Little information is available on how organic forms of these elements influence their bioaccumulation in poultry, mainly in eggs, and if there is any interaction with zinc that plays important metabolic functions in poultry (Park et al. 2004).

The aim of the study was to evaluate the influence of organic forms of copper, iron and manganese from *Saccharomyces cerevisiae* yeasts on the content of Cu, Fe, Mn and Zn in eggs, blood and feathers of laying hens.

MATERIAL AND METHODS

An eight-week experiment was conducted on 60 Lohmann Brown layers, divided into 5 groups and kept in vivarium conditions (cage housing). A standard lighting programme was applied, and thermal-humid conditions were controlled. Birds had constant access to water and feed. The level of egg laying by hens was high and similar in all the groups (95% on average; phase – peak and after a peak of egg laying). Feed consumption was similar per one layer (127.9-130.3 g^{-1} per day) or production of one egg and did not differ significantly between the groups (Dobrzański et al. 2008).

Feeding was based on all-mash standard mixture of J-297 type, with crude protein content of 16.5 % and 11.6 $MJ \cdot kg^{-1}$ of metabolizable energy; an overall content of calcium was 3.65%, of available phosphorus 0.30%, while Cu – 21.8, Fe – 200.8, Mn 140.5 $mg \cdot kg^{-1}$, and in particular groups the contribution of organic and inorganic form of these 3 microelements was different (Table 1). Content of Zn in mixtures was similar: 86 $mg \cdot kg^{-1}$ (zinc

Table 1

Experiment design – content of Cu, Fe and Mn in feed mixture for laying hens ($mg \cdot kg^{-1}$ d.m.)

Micro-element	Source	Group				
		I inorg. Cu+Fe+Mn	II Cu-org.	III Fe-org.	IV Mn-org.	V org. Cu+ Fe+Mn
Cu	feed*	11.2	11.2	11.2	11.2	11.2
	premix**	10	-	10	10	-
	yeast	-	10	-	-	10
Calculated values		21.2	21.2	21.2	21.2	21.2
Determined values		21.8	23.4	22.7	19.9	22.5
Fe	feed *	160.8	160.8	160.8	160.8	160.8
	premix**	40	40	-	40	-
	yeast	-	-	40	-	40
Calculated values		200.8	200.8	200.8	200.8	200.8
Determined values		226.0	215.4	203.2	192.3	198.3
Mn	feed *	60.5	60.5	60.5	60.5	60.5
	premix**	80	80	80	-	-
	yeast	-	-	-	80	80
Calculated values		140.5	140.5	140.5	140.5	140.5
Determined values		138.5	144.6	136.9	143.0	157.8

* feed mixture without premix
** premix contains $CuSO_4$, $FeSO_4$, MnO_2, ZnO providing the following concentrations of elements: Cu – 2000, Fe – 8000, Mn – 16000, Zn – 10 000 $mg \cdot kg^{-1}$

oxide was used in a premixe). The above concentrations of microelements correspond to the feeding recommendations for laying hens (Smulikowska, Rutkowski 2005).

Microelements in the following amounts: Cu – 10, Fe – 40 and Mn – 80 $mg \cdot kg^{-1}$ were separately introduced to the control and experimental mixtures by an application of special premixes. In the control version inorganic forms of these elements were used in the premix (copper sulfate, iron sulfate and manganese oxide), while in the experimental trials they occurred in the organic form, i.e. *Saccharomyces cerevisiae* yeasts enriched with these three elements. The contribution of the organic form of microelements in the experimental mixtures was for Cu – 47, Fe – 20 and Mn – 58%. The share of the premix in the mixture was 0.5%.

Saccharomyces cerevisiae yeast enriched with microelements (Cu, Fe, Mn) was produced on a laboratory scale according to an original technology based on whey (Dolińska et al. 2006). The yeast contained almost 40% of protein, about 1% of crude fat, a little more than 10 MJ·kg^{-1} of metabolizable energy, and the concentrations of the elements were: Fe – 33.9, Mn – 35.4, and Cu – 22.7 mg·kg^{-1} d.m. The yeast share in the mixtures did not exceed 0.4%.

Birds were divided into 5 groups (12 hens in a group), one control and four experimental one, with different contribution of organic and inorganic forms of microelements introduced to the premix:

– I group (control C) – inorganic forms of Cu, Fe and Mn;
– II group – organic forms of Cu and inorganic forms of Fe and Mn;
– III group – organic forms of Fe and inorganic forms of Cu and Mn;
– IV group – organic forms of Mn and inorganic forms of Cu and Fe;
– V group – organic forms of Cu, Fe and Mn.

Eggs for the analysis (12 from each group) were collected three times, i.e. after 25, 40 and 55 days of the experiment. Mean mass of an egg was from 62.4 to 65.0 g. After breaking, the content of an egg was separated from an egg shell. Eggs from the same groups were pooled for 2, thus 18 samples of egg content and 18 samples of egg shell for chemical analysis were obtained in total from each group. On day 55 blood from the wing vain (n=12) and also feathers from the back area (n=12) were collected from hens from each group. Albumen and yolk were carefully mixed, and egg shells (with under-shell membranes) were dried, while feathers were washed with warm water (about 50°C) and detergent, and then rinsed off with distilled water. Egg shells were ground and feathers were crushed. Also samples of the mixtures, premix and yeasts were collected for analysis before the experiment to determine their content of Cu, Mn, Fe and Zn. Mass spectrometry method (ICP-MS) with the use of a Varian Ultramass-700 apparatus was applied. Before the analyses, the samples were mineralized by the microwave method using an MCS-2000 microprocessor station (Górecka et al. 2001).

The results were elaborated statistically using analysis of variance, and significance of differences between particular groups was assessed by Duncan's test (Statgraphics software ver. 5.1).

RESULTS AND DISCUSSION

The content of microelements in eggs, blood or feathers of hens, i.e. in the biological material that may be collected without birds' decapitation, is to a high degree conditioned by feeding factors (chemical content of feed) and by the maintenance system. For instance, Skřivan et al. (2005) using different concentrations of Cu, Fe and Zn in diet of hens, obtained variious concentrations of these elements in eggs' content, liver and droppings of these birds. In turn, Kołacz et al. (2003) reveal different concentrations of Cu and Zn in muscles and liver of hens from small scale and farm husbandry, while DOBRZAŃSKI et al. (2004) demonstrated significant differences in bioaccumulation of these metals in the content of eggs laid by hens from industrialized and agricultural regions.

CONTENT OF MICROELEMENTS IN EGGS

Results of the determinations of the elements in the content and shells of eggs are presented in Tables 2 and 3. Mean concentration of Cu in egg content oscillated within 0.699 – 0.784 $mg \cdot kg^{-1}$ of fresh mass and was significantly higher ($p<0.05$) in group II (organic-Cu supplement) than in the other groups. The mean content of Cu in the egg shell was 2.273--3.034 $mg \cdot kg^{-1}$ d.m. with an increasing tendency in groups II and IV. Thus, copper from Y-Cu was better available to hens than from copper sulfate.

Table 2

Mean concentration of microelement in egg content ($mg \cdot kg^{-1}$ f.m.)

Element	Group				
	I	II	III	IV	V
Cu	0.735^{b}	0.784a	0.703^{b}	0.699^{b}	0.715^{b}
Fe	23.76	20.34	21.43	23.34	22.46
Mn	0.392	0.460	0.413	0.482	0.409
Zn	16.48	15.97^{a}	16.51	16.93^{b}	16.12

a-b, $p<0.05$

Table 3

Mean concentration of microelement in egg shell ($mg \cdot kg^{-1}$ d.m.)

Element	Group				
	I	II	III	IV	V
Cu	2.273^{a}	2.905^{b}	2.384	3.034^{b}	2.368
Fe	2.104	2.464	2.068	1.984	2.187
Mn	1.329	1.776	1.417	1.465	1.384
Zn	3.448	2.914	3.397	2.779	2.792

a-b, $p<0.05$

Quite wide ranges of the concentrations of this element are given in literature. ELMADFA and MUSKAT (2003) report a mean Cu range in the egg content on the level of 0.5-2.3, while UZIĘBŁO et al. (1993) claimed it was 0.49-0.70 $mg \cdot kg^{-1}$ of fresh mass. SKŘIVAN et al. (2006) give Cu values of 1.98-

-2.62 in hens' egg shells, depending on a copper dose in a diet, while DOBRZAŃSKI et al. (2007a) observed 2.2-2.42 mg of Cu·kg^{-1} d.m. in shells of hatching eggs.

The mean concentration of Fe in the egg content oscillated from 20.34 to 23.76 mg·kg^{-1} of fresh mass, and in the egg shell – from 1.984 to 2.464 mg·kg^{-1} d.m. The differences demonstrated between the groups were not statistically significant. Thus, Fe from Y-Fe was no more available than that from iron sulfate.

In literature, ranges of Fe concentration are given in narrow limits. FAKAYODE and OLU-OWOLABI (2003) give the mean concentration iron in the content of eggs from hens maintained in the free range system as 23.2, and according to some American data (*Eggcyclopedia* 1994) – the mean value is 14.4 mg·kg^{-1} of fresh mass. The content of this element in the egg shell given by SKŘIVAN et al. (2005) is between 13.2 and14.7 mg·kg^{-1} d.m. depending on the level of Cu, Fe and Zn in a diet. The latter values are thus a few-fold higher that the results of the present study.

The mean concentration of Mn in the egg ranged from 0.392 to 0.482 mg·kg^{-1} of fresh mass and was the highest in group IV, but the differences with respect to the other groups were not statistically significant. An average content of Mn in the egg shell was within 1.329-1.776 mg·kg^{-1} d.m. Thus, Mn from Y-Mn was not available better than that from manganese oxide although there was a tendency towards higher Mn accumulation in the egg content in group IV (organic-Mn supplement).

Ranges of Mn concentrations given in literature are in quite narrow. ELMADFA and MUSKAT (2003) give a mean concentration of Mn in the egg content of 0.3 mg·kg^{-1} of fresh mass, while DOBRZAŃSKI et al. (2001) demonstrated a 14.2% decrease in the Mn concentration of the egg content (from 0.274 to 0.235 mg·kg^{-1} of fresh mass) following an increased content of iodine and selenium in a feed premix. In shells of hens' eggs (without membranes) the content of this element is 0.4-1.1 mg·kg^{-1} (KONIECZNA 1993) or, according to MABE et al. (2003), 2.74-2.81 mg·kg^{-1} d.m.

The average concentration of Zn in the egg content oscillated within 15.97-16.93 mg·kg^{-1} of fresh mass, and was significantly higher (p<0.05) in group IV comparing to group II (organic-Cu supplement). The mean content of Zn in the egg shell was 2.779-3.448 mg·kg^{-1} d.m. Thus, introduction of organic forms of Cu, Fe and Mn did not cause any interactions with respect to Zn, even though an antagonistic influence of Cu (group II) and synergistic of Mn (group IV) were observed in the egg content.

There are many data concerning this element in literature. For example, depending on a system of hens husbandry, the concentration of Zn in the egg content was from 9.77 to 13.11 mg·kg^{-1} of fresh mass (DOBRZAŃSKI et al. 1999, ŻMUDZKI et al. 1992). ELMADFA and MUSKAT (2003) give an even higher mean concentration: 13.5 mg·kg^{-1} of fresh mass. Values of Zn in hens' egg shell are within a wide range of 4.2-5.47 mg·kg^{-1} of fresh mass (MABE et al. 2003) to 7.8-8.7 mg·kg^{-1} d.m. (SKŘIVAN et al. 2005).

Generally, it should be stated that organic forms of Cu, Fe and Mn applied in the form of enriched *Saccharomyces cerevisiae* yeasts caused exclusively an increase in the copper concentration in the content and shell of eggs. However, the concentration of Fe, Mn and Zn in the egg content and in shells was similar in all groups.

CONTENT OF MICROELEMENTS IN BLOOD

Results of the whole blood analysis of hens are presented in Table 4. The content of copper was 0.32-0.343 mg·l^{-1}, being significantly higher in group II ($p<0.05$) versus groups II, III and IV. The iron concentration was 272.4-305.7, manganese 0.174-0.21, and zinc 8.312-8.931 mg·l^{-1}, without any significant differences between the groups. The organic forms of Cu, Mn and Fe introduced to the hens' diet did not cause any interactions with respect to zinc, an important element in hens' nutrition.

Table 4

Mean concentration of microelements in whole blood of laying hens (mg·l^{-1})

Element	Group				
	I	II	III	IV	V
Cu	0.326^{b}	0.343^{a}	0.317^{b}	0.320^{b}	0.333
Fe	305.7	302.6	280.3	272.4	302.3
Mn	0.181	0.210	0.195	0.174	0.179
Zn	8.534	8.931	8.312	8.770	8.730

a-b, $p<0.05$

Table 5

Mean concentration of microelements in feathers of hens (mg·kg^{-1} d.m.)

Element	Group				
	I	II	III	IV	V
Cu	7.098^{b}	8.349^{a}	6.735^{b}	6.922^{b}	7.750
Fe	89.27	93.03	95.40	87.02	85.66
Mn	54.31^{a}	65.18	66.67	68.92^{b}	59.68
Zn	60.53	64.30	61.19	58.44	59.57

a-b, $p<0.05$

The concentrations of these elements revealed by our tests are difficult to comment on, as there is lack of reference values for poultry in literature. MONDAL et al. (2007) in blood plasma of 42-day-old chickens observed the concentration of Cu of 0.45 or 0.51 $\mu g \cdot l^{-1}$ when the animals were given a supplement of Cu-proteinate or Cu-sulphate, respectively. Other microelements like Zn, Mn and Fe did not undergo any significant changes. In turn, after introduction of organic Mn (Mn-amino acid complex) to a diet of chickens, JI et al. (2006) observed a significant increase in the Mn concentration in blood serum (maximum concentration 26.16 $\mu g \cdot l^{-1}$) compared to manganese sulfate.

Thus, blood of poultry is quite a good indicator of bioavailability of microelements. It is probable that higher doses of organic forms of copper, manganese of iron would give a wider differentiation of results in our present study although the level of these microelements is quite changeable in blood of poultry (SAHIN et al. 2007).

CONTENT OF MICROELEMENTS IN FEATHERS

Results of hens' feathers analysis are presented in Table 5. The content of copper was 6.922-8.349 $mg \cdot kg^{-1}$ d.m., iron concentration was 85.66-95.4 $mg \cdot l^{-1}$, manganese 54.31-68.92, while zinc 58.44-64.3 $mg \cdot kg^{-1}$ d.m. The differences we observed, with the exception of group II (Y-Cu) and group IV (Y-Mn), were not statistically significant between the groups. The organic forms of Cu, Mn and Fe added to the feeds did not cause any interactions with respect to zinc.

In the case of poultry, concentrations of these elements in feathers are important because birds can eat feathers (JENSEN et al. 2006). Moreover, there are technologies which enable us to use feathers for production of feed components (BERTSCH, COELLO 2005). Nevertheless, feather meals in animals feeding are not allowed in EU countries.

In wild birds living in an urban environment, examined in Belgium, the given values of Zn in feathers are 176-244, while these of Cu are 7.6-88 $\mu g \cdot g^{-1}$ (DAUWE et al. 2002). In pigeons in Israel the following contents of elements were observed in feathers: Cu – 5.45 – 11.8, Mn – 0.42 – 36.9, Zn – 28.7--146 $\mu g \cdot g^{-1}$ depending on the region (urban, industrial, agricultural regions) (ADOUT et al. 2007).

It seems, however, that feathers of birds are not a good index of elemental availability in a diet, since they are exposed to a risk of environmental pollution. Besides, their structure is not uniform.

CONCLUSIONS

1. Application of organic forms of copper in nutrition of layer hens caused a significant increase in copper in the content and shell of eggs, in blood and feathers in the group receiving organic-Cu, which proves better availability of organic forms of copper comparing to copper sulfate.

2. Introduction of organic forms of iron and manganese to feed for hens did not cause any significant changes in the concentration of this element in eggs, blood and feather of hens, with the exception of the group receiving organic-Mn (significantly higher content of Mn in feathers relative to the control group).

3. Organic forms of copper, manganese and iron did not cause any interactions with respect to Zn, although an antagonistic influence of Cu (organic-Cu group) and synergistic effect of Mn (organic-Mn group) were observed in the egg content.

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EFFECT OF WEED CONTROL OPERATIONS AND TILLAGE SIMPLIFICATIONS ON IRON CONTENT AND UPTAKE WITH POTATO TUBER YIELD

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Abstract

Potato tubers obtained from a field experiment carried out in 2002-2004 were used for our analysis. The soil grain-size structure was that of light loamy sand. Two soil tillage systems were compared: conventional (ploughing + fall ploughing + harrowing + cultivating + harrowing) and simplified (reversing ploughing + cultivating). The second factor involved seven weeding methods such as application of the following herbicides: Plateen 41.5 WG, Plateen 41.5 WG + Fusilade Forte 150 EC, Plateen 41.5 WG + Fusilade Forte 150 EC + Atpolan 80 EC, Barox 460 SL, Barox 460 SL + Fusilade Forte 150 EC, Barox 460 SL + Fusilade Forte 150 EC + Atpolan 80 EC. Iron content and uptake with tuber yield significantly depended on the tillage practices and weeding methods as well as the weather conditions over the growing period. Conventionally tilled potato tubers had more iron, 65.80 $g\cdot kg^{-1}$ on average, than the tubers whose cultivation was based on simplified tillage, 64.82 $g\cdot kg^{-1}$ on average. The herbicides applied significantly increased the iron content and its uptake with tuber yield by an average 4.2 and 13.8%, respectively.

Key words: potato, iron, content, uptake.

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WPŁYW ZABIEGÓW PIELĘGNACYJNYCH I UPROSZCZENIA UPRAWY NA ZAWARTOŚĆ I POBRANIE ŻELAZA Z PLONEM BULW ZIEMNIAKA

Abstrakt

Pracę oparto na próbach bulw z doświadczenia polowego przeprowadzonego w latach 2002-2004 na glebie o składzie piasku gliniastego lekkiego. Porównywano dwa sposoby uprawy roli – tradycyjną (orka + orka przedzimowa + bronowanie + kultywatorowanie + + bronowanie) i uproszczoną (orka odwrotna + kultywatorowanie) i siedem sposobów pielęgnacji z udziałem herbicydów: Plateen 41,5 WG, Plateen 41,5 WG + Fusilade Forte 150 EC, Plateen 41,5 WG + Fusilade Forte 150 EC + Atpolan 80 EC, Barox 460 SL, Barox 460 SL + Fusilade Forte 150 EC, Barox 460 SL + Fusilade Forte 150 EC + Atpolan 80 EC. Zawartość żelaza i jego pobranie z plonem bulw zależały istotnie od sposobów uprawy i pielęgnacji oraz warunków pogodowych w okresie wegetacji. Więcej żelaza zawierały bulwy ziemniaka z uprawy tradycyjnej – średnio 65,80 $g \cdot kg^{-1}$ w porównaniu z uproszczoną – średnio 64,82 $g \cdot kg^{-1}$. Herbicydy zastosowane w pielęgnacji podwyższały istotnie zawartość żelaza – średnio o 4,2% i jego pobranie z plonem bulw – średnio o 13,8% w porównaniu z obiektem kontrolnym.

Słowa kluczowe: ziemniak, żelazo, zawartość, pobranie.

INTRODUCTION

Due to its low soil and climatic requirements as well as a significant role in crop rotation, potato is a valuable crop plant in Poland. Presently, it is mainly used for consumption purposes and the per capita consumption is as high as 129 kg per year (DZWONKOWSKI et al. 2006). The nutritional value of potato tubers is determined by their chemical composition, including macroelements and microelements, as well as a trace content of non-nutritive compounds (LISIŃSKA 2006). Microelements represent the compounds that are necessary for human and animal metabolism, and the body has to be supplied with them along with food. Iron, being a component of many enzymes and taking part in processes of oxy-reduction and blood formation, is one of the more important microelements and its deficiency results in anaemia. The daily iron requirement of an adult person ranges from 10 to 15 mg (CZAPSKA et al. 2000, GRAJETA 2006). According to GĄSIOR (1996) eating 200-350 g potatoes containing an average of 150 mg $Fe \cdot kg^{-1}$ tuber d.m. can satisfy the daily iron requirement. Many authors believe that the iron content in tubers depends on soil and weather conditions, agronomic practice and cultivar properties. (GĄSIOR 1996, PROŚBA-BIAŁCZYK, MYDLARSKI 2000, RUDZIŃSKA-MĘKAL, MIKOS-BIELAK 2000). In the experiments by MIKOS-BIELAK et al. (1996), which included many cultivars representing different maturity groups, the iron content ranged from 9.5 to 23.9 $mg \cdot kg^{-1}$ tuber dry matter. In turn, PROŚBA-BIAŁCZYK and MYDLARSKI (2000), and GĄSIOR (1996) obtained the respective ranges of 29-74 $mg \cdot kg^{-1}$, and 37-241 $mg \cdot kg^{-1}$ tuber dry matter. There is a paucity of literature on the subject of an impact of plant protection chemicals on microelement content in potato tubers. Thus, it has been attempted to determine the effect of weeding methods, including herbicide application, and soil tillage methods on the iron content in tubers and iron uptake with potato tuber yield.

MATERIAL AND METHODS

The studies was based on a field experiment carried out in 2002-2004 at the Zawady Experimental Farm on soil belonging to a very good rye complex. Selected soil chemical properties prior to the experiment are shown in Table 1. The experiment was in a randomized sub-block design with three replicates each year. The experimental factors were as follows: two soil tillage methods, conventional and simplified tillage, and seven methods of weed control consisting of herbicide application (Table 2).

Table 1

Chemical properties of the soil

Years	pH 1 mol KCl dm^3	Organic matter $(g \cdot kg^{-1})$	Macroelements content $(mg \cdot 100\ g^{-1})$			Microelements content $(mg \cdot kg^{-1})$			
			P	K	Mg	Fe	Mn	Cu	Zn
2002	6.5	11.3	3.88	15.03	7.00	755.0	113.0	6.2	11.0
2003	6.4	11.3	4.30	10.22	15.70	962.0	108.0	4.2	8.4
2004	5.6	11.5	6.25	10.39	15.90	1300.0	120.0	3.8	10.7

Table 2

Experimental factors

Factor I – tillage system
1. Traditional (ploughing + fall ploughing + harrowing + cultivating + harrowing) 2. Simplified (second ploughing + cultivating)
Factor II – weed control methods
1. Control object (mechanical weeding before and after potato plants sprouting) 2. Plateen 41.5 WG 2.0 $kg \cdot ha^{-1}$ 3. Plateen 41.5 WG 2.0 kg ha^{-1} + Fusilade Forte 150 EC 2.5 $dm^3 \cdot ha^{-1}$ (mixture) 4. Plateen 41.5 WG 1.6 kg ha^{-1} + Fusilade Forte 150 EC 2.0 $dm^3 \cdot ha^{-1}$ + Atpolan 80 EC 1.5 $dm^3 \cdot ha^{-1}$ (mixture) 5. Barox 460 SL 3.0 $dm^3 \cdot ha^{-1}$ 6. Barox 460 SL 3.0 dm^3 ha^{-1} + Fusilade Forte 150 EC 2.5 $dm^3 \cdot ha^{-1}$ (mixture) 7. Barox 460 SL 2.4 dm^3 ha^{-1} + Fusilade Forte 150 EC 2.0 dm^3 ha^{-1} + Atpolan 80 EC 1.5 $dm^3 \cdot ha^{-1}$ (mixture)

Farmyard manure fertilization and mineral fertilization amounted to, respectively, 25.0 t·ha^{-1}, and 90 kg N, 32.9 kg P and 112.1 kg K·ha^{-1}. Iron content in the tubers of cultivar Wiking potato was determined by the atomic absorption spectrophotometry method (AAS). The results were subjected to the analysis of variance and conclusions were drawn on the basis of Tukey's test at the significance level of p=0.05. Precipitation sums and average air temperatures during the period of potato growth (April – September) were the following respective percentages of the multi-year mean: 112.7 and 110.2 for 2002, 48.3 and 105.4 in 2003, and 116.6 and 95.9 in 2004.

RESULTS AND DISCUSSION

Iron content in potato tubers. Iron content in the tubers of edible potato cv. Wiking averaged 65.36 mg·kg^{-1} d.m., and was significantly differentiated by the soil tillage systems, weed control methods and weather conditions in the study years (Table 3). Higher iron concentration was found in tubers of conventionally tilled potatoes, compared with the simplified tillage. Żurawski and Sienkiewicz (1981) proved that deeper tillage prior to the cultivation of root and tuber crops positively influenced yields of basic plants, as well as the content and uptake of nutrients. Nowak et al. (2004) stress that potato, due to its poorly developed and shallow root system, takes up nutrients mainly from the plough layer of the soil, which should be well worked.

Table 3

Content of iron in potato tubers (mg·kg^{-1} d.m.)

Weed control methods	Tillage systems		Years			Mean
	traditional	simplified	2002	2003	2004	
1.*	63.45	62.68	63.88	64.16	61.17	63.07
2.	67.02	65.64	66.25	69.83	62.92	66.33
3.	67.75	65.36	66.89	67.33	65.47	66.56
4.	65.18	65.33	64.30	68.50	62.99	65.26
5.	66.09	65.87	64.59	70.83	62.53	65.98
6.	65.49	64.82	65.50	66.67	63.30	65.16
7.	65.62	64.72	64.84	67.33	63.35	65.17
Mean	65.80	64.92	65.18	67.81	63.10	65.36
Mean for 2-7	66.19	65.29	65.40	68.42	63.43	65.75
$LSD_{0.05}$ for:						
tillage systems						0.24
weed control methods						1.28
years						0.37
interaction:						
tillage systems x weed control methods						1.19
weed control methods x years						2.21

* designations as in Table 2

Herbicides applied in potato cultivation increased iron concentration in tubers by 4.2%, on average. The highest iron concentration was found in the tubers of potato sprayed with a mixture of Plateen 41.5 WG + Fusilad Forte 150 EC, and herbicide Plateen 41.5 WG – 66.33 and 66.56 $mg \cdot kg^{-1}$, respectively. Similar changes were observed by ZARZECKA (2004), a significant increase in iron concentration occurring only in two treatments where plant protection agents were applied as mixtures or as post-emergence chemicals sprayed twice. Increased iron concentration in tubers of some cultivars was also observed by RUDZIŃSKA-MĘKAL and MIKOS-BIELAK (2000), who applied the growth regulator Mival.

Analysis of the effect of weather conditions over the study years showed that tubers harvested in the warm and dry year 2003 were the highest in iron. Distinct differentiation in the iron content in the study years was also reflected in the works by BOLIGŁOWA (1996), GĄSIOR (1996), and ZARZECKA and GUGAŁA (2005).

The iron content in tubers was close to the literature values (RUDZIŃSKA-MĘKAL, MIKOS-BIELAK 2000, PROŚBA-BIAŁCZYK, MYDLARSKI 2000). KUCHARZEWSKI et al. (2002) believe that iron concentration below the average value, ranging from 21 to 58 $mg \cdot kg^{-1}$ d.m., is not harmful because plants take up the element in varying amounts (200-2500 g per 1 ha) and, in general, show a marked tolerance to high Fe concentration in tissues. Under the Regulation of the Ministry for Health of April 30, 2004, on permissible levels of food chemical and biological pollution, limiting copper, zinc, iron and manganese contents in potato tubers has been abandoned due to the present deficiencies of these elements in diets (WOJCIECHOWSKA-MAZUREK et al. 2003). However, the maximum threshold levels of lead, cadmium, mercury and arsenic are maintained (*Regulation* 2004). Also GEMBARZEWSKI (2000) in his studies on basic crops found a decreased uptake of Cu, Mn and Fe with potato tuber yield in the years 1966-1970.

Iron uptake with tuber yield. In the yield of tubers, averaging 31.4 $t \cdot ha^{-1}$, potatoes took up and accumulated an average of 532.6 g iron per 1 ha (Table 4). Cultivation operations performed in the experiment and moisture and thermal conditions in the study yields significantly affected the iron uptake with potato tuber yield. Conventionally-tilled potatoes took up over 8% more iron than the potatoes whose cultivation was based on simplified tillage. Moreover, the chemically-protected potatoes took up 9.6 to 21.0% more iron than the potatoes grown in the control treatment, where weeds were mechanically controlled. In addition, the present research has shown that iron uptake is to a large extent conditioned by the level of yields, which was reflected in the study years, which were different as far as the weather was concerned. The highest iron uptake was recorded in the wet and warm year 2002. The lowest uptake was found in 2003, when precipitation was twice as low as in the multi-year period.

Table 4

Uptake of iron with the yield of potato tubers ($g \cdot h^{-1}$)

Weed control methods	Tillage systems		Years			Mean
	traditional	simplified	2002	2003	2004	
1.*	494.0	458.8	626.6	399.7	402.9	476.4
2.	543.9	511.8	669.6	471.6	452.5	527.9
3.	571.9	532.3	707.0	452.2	497.1	552.1
4.	602.1	550.7	719.6	470.4	539.2	576.4
5.	541.6	502.7	620.7	497.1	448.7	522.2
6.	549.0	500.0	624.8	474.4	457.7	525.0
7.	576.2	520.8	650.1	509.7	485.7	548.5
Mean	554.1	511.1	659.8	466.5	471.7	532.6
Mean for 2-7	564.1	519.9	665.3	477.6	480.2	542.0
$LSD_{0.05}$ for:						
tillage systems						6.5
weed control methods						29.2
years						10.0
interaction:						
tillage systems x weed control methods						n.s.
weed control methods x years						50.5

* designations as in Table 2
n.s. – not significant

CONCLUSIONS

1. Simplifications of soil tillage created poorer conditions for iron accumulation in potato tubers compared with the conventional tillage.

2. When potatoes were cultivated using herbicide weed control, the iron content in their tubers was higher than in the tubers of potatoes whose cultivation included mechanical weed control.

3. Iron uptake with tuber yield was higher under the conditions of conventional than simplified tillage, and when intense chemical protection of crop was applied compared with mechanical control of weeds.

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MAGNESIUM CONCENTRATIONS IN THE WATERS OF RE-NATURISED RESERVOIRS IN RURAL AREAS*

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Abstract

The objective of the study has been to determine magnesium concentrations and their seasonal changes in waters of re-naturised reservoirs situated in a rural area, 25 years after their re-creation. The study included 3 small, non-flow-through reservoirs, situated close to the village of Sętal, in the commune of Dywity. Water samples for analysis were collected once a month in 2005 and 2006. They were examined in respect of the magnesium level and such physicochemical parameters as temperature, pH, oxygenation, dissolved oxygen, electrolyte conduction and water depth.

The results prove that the environment of the surface waters in this area is poor in magnesium (5.2 mg·dm^{-3} - 6.6 mg·dm^{-3} on average), which is characteristic for postglacial regions. The re-created water reservoirs can be listed in water quality class I in terms of their magnesium content.

The waters of these small re-created lakes were characterised by huge seasonal changes of magnesium concentrations. However, the fluctuations of Mg^{+2} concentrations were often larger within particular sites than between the examined reservoirs. The highest average seasonal magnesium concentration of 6.6 mg·dm^{-3}, varying from 3.9 mg·dm^{-3} to 10.0 mg·dm^{-3}, was determined in the waters of a reservoir whose whole catchment had for many years been used for agriculture.

The lowest magnesium concentrations in the waters of the reservoirs occurred in springtime, with a slight increase in early summer and an equally slight decline afterwards. No significant increase in magnesium amounts was found until autumn, before they reached their peaks in wintertime. Such a course of fluctuations was caused by the maximum magnesium biosorption in spring and releasing internal reserves (green matter and

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bottom deposits) of the reservoirs in autumn and winter. Decrease and increase in the Mg^{+2} ion concentration in the waters of the reservoirs occurred in two 'pulses': in the springtime and wintertime, respectively.

Key words: re-naturisation of water reservoirs, magnesium concentrations, non-flow--through reservoirs.

STĘŻENIA MAGNEZU W WODACH ZBIORNIKÓW ZRENATURYZOWANYCH NA OBSZARACH WIEJSKICH

Abstrakt

Celem pracy było określenie stężenia magnezu oraz jego sezonowych zmian w wodach zrenaturyzowanych zbiorników wodnych zlokalizowanych na terenach wiejskich, po 25 latach od ich odtworzenia. Badaniami objęto 3 małe bezodpływowe zbiorniki wodne, położone w sąsiedztwie miejscowości Sętal, w gminie Dywity. Próbki wody do analiz laboratoryjnych pobierano 1 raz w miesiącu w latach 2005-2006 i oznaczano w nich magnez oraz parametry fizykochemiczne: temperaturę, pH, nasycenie tlenem, tlen rozpuszczony, przewodnictwo elektrolityczne oraz głębokość wód.

Wykazano, że środowisko występowania wód powierzchniowych na tym obszarze jest ubogie w magnez (średnio 5,2 $mg \cdot dm^{-3}$ - 6,6 $mg \cdot dm^{-3}$), co jest charakterystyczne dla terenów polodowcowych. Odtworzone zbiorniki wodne pod względem zawartości magnezu można zaliczyć do I klasy jakości wód.

W wodzie niewielkich, odtworzonych jezior objętych badaniami stwierdzono dużą sezonową zmienność stężeń magnezu. Jednak wahania stężeń Mg^{+2} często były większe w obrębie poszczególnych obiektów niż między obiektami badań. Najwyższe średnie sezonowe stężenie magnezu – 6,6 $mg \cdot dm^{-3}$, z wahaniami od 3,9 $mg \cdot dm^{-3}$ do 10,0 $mg \cdot dm^{-3}$, odnotowano w wodach zbiornika, którego zlewnia w całości jest użytkowana rolniczo od wielu lat.

Najniższe stężenia magnezu w wodach zbiorników występowały wiosną, niewielki ich wzrost obserwowano na początku lata, a następnie niewielki spadek. Wyraźny przyrost ilości magnezu stwierdzono dopiero jesienią, aż do osiągnięcia maksimum zimą. Taki przebieg był spowodowany maksymalną biosorpcją magnezu wiosną i uruchamianiem jesienią oraz zimą z zasobów wewnętrznych zbiorników (masa roślinna i osady denne). Spadek oraz wzrost stężenia jonów Mg^{+2} w wodach zbiorników odbywał się więc w postaci dwóch „pulsów" odpowiednio wiosną i jesienią.

Słowa kluczowe: renaturyzacja zbiorników wodnych, stężenia magnezu, zbiorniki bezodpływowe.

INTRODUCTION

Field water reservoirs play many roles in ecology and economy, primarily because they are an important element in the water balance of microcatchments and, secondly, they are of some influence on water relations in soils (DRWAL, LANGE 1985). Organic and mineral substances flowing in water enter biological cycling and become accumulated in small ponds and their shore zones (SZYPEREK 2005). However, such reservoirs continually collect organic and mineral residues, which diminishes them and finally causes their disappearance (Ostendorp et al. 1995, Pieńkowski 1996, Mitraki et al. 2004). Water reservoirs play various functions in the environment, some of which are impossible to replace, therefore it is sometimes necessary to re-cultivate or re-naturise natural water bodies, depending on the direction and progress of changes they undergo.

In the recent years, as a result of the new economic and social conditions in Poland, it has become quite common to abandon farming in some areas of the country. This, in combination with the staggering water management, has made such areas vulnerable to rapid changes. Neglected drainage systems gradually fail to operate properly. Setting aside fallows initiates the growth of weeds and brambles. One possible way to use former farmland rationally re-naturisation, which in terms of water bodies means re-creating water reservoirs. Re-naturisation deserves particular attention because rural areas often hold some enclaves of natural hydrophilic flora and clumps of trees, which may serve as a source of propagating local species native of a given area (Jędryka, Kamińska 2003).

Calcium and magnesium concentrations in water depend mostly on their influx from a catchment area and on sedimentation processes. High levels of these elements in surface waters result from their intensive leaching from soils, which is a natural and inevitable process (Kajak 2001, Glińska-Lewczuk 2005).

The objective of the present study was to analyse magnesium concentrations and their seasonal changes in the waters of small field water reservoirs, situated in the areas used for agriculture, 25 years after they had been re-created. Our attention was paid to magnesium because it is one of the elements essential for the proper development of plants and animals, and its circulation in the environment depends on the processes which occur in water reservoirs.

MATERIAL AND METHODS

The three water reservoirs we examined are located near the village Sętal in the commune of Dywity, which lies in the central part of the Olsztyn Lake District (Pojezierze Olsztyńskie) – Figure 1. Geologically, the commune is situated in the Mazovian Basin (Niecka Mazowiecka), which lies on Cretaceous formations (Lewicka, Tomkiewicz 1994). The dominant earth types are clays, glacial gravels, and sands together with fluvioglacial formations, mostly covered with forests and forest-like plant assemblages. The landscape is highly diverse, with characteristic erratic blocks, moraine uplands and hills, typical of a postglacial area. The area of the commune we examined only slightly forested, mostly occupied by farmland and dominated by brown soils (GUS 2003).

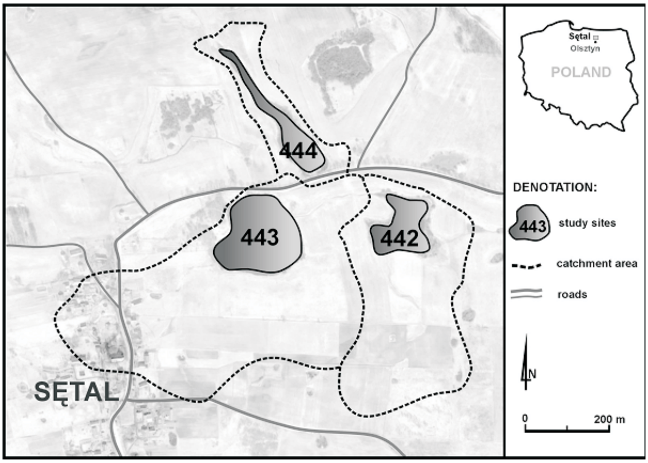

Fig. 1. Location of the study area and sites

The study has included three small, non-flow-through water reservoirs marked as nos 442, 443 and 444, of the maximum depth of 2.5 m. Reservoir 442 has the smallest area of 1.49 ha. Until 2004, only the western part of the catchment covering 16.7 ha was used for agriculture whereas the land in the east was fallow. It was reintroduced to farming in 2005. Reservoir 443, of an area of 3.61 ha, has the largest catchment among the three study sites: 29.2 ha, of which 60% is arable land and the remaining land consists of wastelands and bushes. The area of Reservoir 444 is 1.65 ha and its catchment is the smallest one, just 4.9 ha, all of which is used for agricultural purposes. The arable lands in all these catchments were used for plantations of rape in 2005 and winter triticale in 2006.

The above reservoirs were dried out in the late 19th century by a complex drainage system. end of the 19th century. In 1980, following some regulation of the water conditions in this area, Reservoir 443 was re-created and a few years later the other two ponds spontaneously filled with water as a result of an elevated groundwater level in the catchment.

The following physical and chemical parameters were recorded: temperature – using a thermometer integrated with oxygen probe, oxygen and oxygenation – using a WTW Multiline P3 meter, electrolytic conductivity – using a Hanna conductometer, and pH reaction – with a potentiometer. The water depth was measured once a month based on the watermark readings. Water samples for laboratory analyses were collected once a month in 2005 and 2006, from the central depth of every reservoir. Their magnesium concentration was determined colorimetrically with titanium yellow (HERMANOWICZ et al. 1999). Species composition of the flora was identified in the filed using the key by RUTKOWSKI (1998).

Monthly means of total precipitation were calculated from the data interpolated from the meteorological stations in Lidzbark Warmiński and in Olsztyn – Dajtki, which were obtained from the Institute of Meteorology and Water Management.

Statistical calculations of the results were performed with Statistica 7 programme, and the coefficient of variation was calculated according to the formula: standard deviation/arithmetic mean x 100%. Seasonal changes in the magnesium concentration in water were calculated according to the following groups of samples: winter (January – March), spring (April – June), summer (July – September), autumn (October – December). This division was created on the basis of the air and water temperatures during the sample collection.

RESULTS AND DISCUSSION

Chemical composition of water is very complex and closely related to its migration and the environment it originates from. Chemical properties of waters in agricultural and forested areas are dependent on soil's physical, chemical and biological properties as well as on the method of land management, intensity of agronomic practices, draining processes and climatic conditions (BOROWIEC, PIEŃKOWSKI 1993, KOC, SZYMCZYK 2003).

Our results (low concentrations of Mg^{+2}) indicate that the water environment in the study area is poor in magnesium, which is characteristic for postglacial areas (MACIOSZCZYK 1987). The re-created water reservoirs can be listed in water quality class I in respect of their magnesium content (*Rozporządzenie MŚ* 2004).

The three sites we have examined are classified as polytrophic reservoirs with richly developed rush flora. In the reservoirs, significant changes of physical and chemical properties were observed (Table 1). The changes in the Mg^{+2} concentration were influenced by meteorological conditions (the seasons). In the summertime, over-oxygenation was noticed, caused by an intensive flora growth. Electrolytic conductivity of the waters, which indicates water-dissolved ions, also changed significantly. The waters of the small, re-created lakes subjected to our study were characterised by huge seasonal changes of magnesium concentrations (Figures 2 and 3). However, the fluctuations were often larger within the particular sites than between them.

Slightly different meteorological conditions in the time of the study (2005 and 2006) triggered various trends in magnesium fluctuations in the analysed waters. For each site, the highest concentrations were noticed in the winter months (January – March), particularly in 2006, when the reservoirs had a 30-centimetre-thick ice cover. At that time, the amount of oxygen dissolved in the waters was at its lowest, and the electrolyte conduction was the highest for whole study period. Such conditions favoured a release of mineral components, including magnesium, from the bottom deposits (Table 1).

Seasonal changes of Mg^{+2} concentrations were characterised by a decrease in springtime, which was connected with an intensive absorption of this element by the developing rush flora (reed canary grass, common

Table 1

Seasonal changes of physical and chemical parameters in the waters of the reservoirs

Parameter	Season*	Study site (Reservoir)					
		442		443		444	
		2005	2006	2005	2006	2005	2006
Dissolved oxygen ($mg\ O_2 \cdot dm^{-3}$)	WI	4.6	2.9	4.9	5.5	4.8	2.6
	SP	10.4	12.0	7.8	9.0	9.7	8.5
	SU	10.2	11.1	11.8	9.1	9.5	9.2
	AU	12.2	8.6	11.7	9.3	13.1	8.1
	YM	9.3	8.6	9.0	8.2	9.3	7.1
Oxygenation (%)	WI	34.5	20.1	37.1	36.9	36.6	17.6
	SP	105.8	126.8	80.6	91.9	99.8	90.7
	SU	122.6	123.8	141.6	107.3	116.3	149.1
	AU	97.1	76.0	90.8	80.1	100.8	69.4
	YM	90.0	86.7	87.5	79.0	84.6	81.7
Temperature (°C)	WI	1.56	0.3	1.4	0.3	1.7	0.1
	SP	15.9	17.5	16.1	17.0	15.9	17.6
	SU	24.2	23.0	23.2	24.1	23.9	23.7
	AU	4.8	6.4	5.0	6.4	4.8	5.4
	YM	11.6	11.8	11.4	11.9	11.6	11.7
pH	WI	8.9	7.3	8.1	7.3	8.1	7.2
	SP	7.8	7.7	7.9	7.7	7.7	7.6
	SU	8.5	8.2	8.7	8.2	8.2	7.8
	AU	8.3	7.8	7.9	7.9	8.0	7.8
	YM	8.4	7.7	8.2	7.8	8.0	7.6
Conduction ($\mu S \cdot cm^{-1}$)	WI	250	522	283	375	246	406
	SP	209	200	299	256	307	266
	SU	206	268	328	268	322	251
	AU	222	260	308	267	299	265
	YM	222	313	251	292	294	297
Water depth (cm)	WI	205	140	166	161	173	164
	SP	211	148	165	159	174	164
	SU	185	117	155	156	142	142
	AU	134	122	152	160	130	176
	YM	184	132	160	159	155	162

* WI – winter; SP – spring; SU – summer; AU – autumn; YM – yearly mean

reed, water horsetail, broadleaf cattail), the event which is also confirmed by SZYPEREK (2003). The lowest Mg^{+2} concentration (1.6 mg·dm^{-3}) during the two years was noticed in Reservoir 442 in June 2005. The results support the opinion that low magnesium concentrations in spring are characteristic for small non-flow-through ponds with intensively developed flora (KOC 1999). When compared to spring, slight increases in the magnesium concentrations were noticed in summer, apart from Reservoir 443 in 2006, in which the Mg^{+2} concentration continued to decrease. The increased amount of Mg^{+2} may originate from its production in the processes of mineral and organic matter decomposition as well as its release from bottom deposits. Movements of plants (caused by waving generated by winds), thermal circulation and activities of organisms disturb and disperse bottom deposits, which accelerates their decomposition (SKONIECZEK et al. 2004, SKORBIŁOWICZ 2005). In summertime, the water level was lowered by evapotranspiration, which additionally increased magnesium concentrations (Table 1 and Figure 2.).

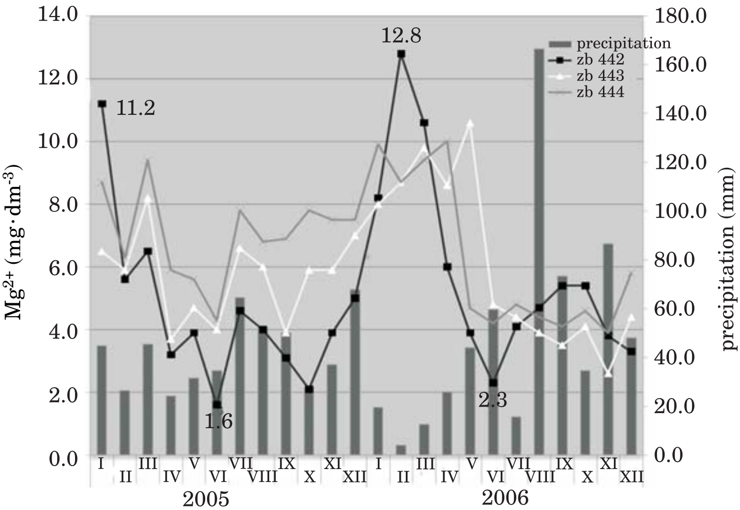

Fig. 2. Seasonal changes of magnesium concentrations (mg·dm^{-3}) in the waters of the reservoirs in 2005 and 2006 in relation with the meteorological conditions

A distinct increase in the Mg^{+2} concentrations in the waters of all the reservoirs was observed from autumn 2005. This trend lasted in Reservoir 442 until the winter 2005, and in Reservoirs 443 and 444 until the early spring of the following year. It could have been caused the atmospheric precipitations occurring outside the growing season (limited phytosorption) which may have caused extraction of Mg^{+2} from arable lands (SZYMCZYK, CYMES 2005) as well as its release from the deposits (Figure 2).

When analysing the dynamics of Mg^{+2} concentrations in the waters of the reservoirs, it should be noticed that the biggest fluctuations occurred in Reservoir 442: from 1.6 to 11.2 mg·dm^{-3} (CV = 56.5%, SD = 2.6) in 2005 and from 2.3 to 12.8 mg·dm^{-3} (CV = 55%, SD = 3.3) in 2006. Most likely, it was the effect of the most disadvantageous ratio of the catchment to the reservoir area (16.7 ha : 1.49 ha) as compared to the other basins (Table 2 and Figure 1). Such substantial fluctuations may have also been the result of reintroducing farming in the eastern part of the catchment, thus accelerating the release of Mg^{+2} ions from the soils. The increase in magnesium concentration observed from autumn to winter in the water of this basin may have been caused by a large influx of groundwater, rich in magnesium. In these two seasons of the year, the leaching of elements from soil is facilitated by ploughing and temporary absence of plant cover (FALKOWSKI et al. 1996).

Table 2

Magnesium concentrations in the waters of the reservoirs in 2005 and 2006

Study site	2005			2006		
	mean X / range of values	SD	CV (%)	mean X / range of values	SD	CV (%)
442	4.6 / 1.6 - 11.2	2.6	56.5	5.9 / 2.3 - 12.8	3.3	55.0
443	5.6 / 3.7 - 8.2	1.4	24.5	6.1 / 2.6 - 10.2	2.8	44.4
444	7.1 / 4.3 - 9.4	1.5	21.1	6.2 / 3.9 - 10.0	2.5	39.6

SD – standard deviation, CV – coefficient of variation

A large increase in the concentration of this element was also highly dependent on the water level fluctuations. Diminishing water volume in the reservoirs in spring accelerated the heating of the whole system, including its deposits, and this in turn accelerated the changes which occurred in the system. Additionally, exposition of some part of the deposits as well as their transformations under aerobic conditions and higher temperatures may have speeded up mineralisation to the simplest forms. Autumn atmospheric precipitations made magnesium return to the reservoir's waters (Table 1). Smaller fluctuations of magnesium concentrations were observed in Reservoirs 443 (CV = 24.5, SD = 1.4) and 444 (CV = 21.1, SD = 1.5) in 2005 and higher in 2006, when the coefficient of variation and the standard deviation were almost two-fold higher (Table 2). This could have been caused by different atmospheric conditions, suggesting that the dynamics of magnesium concentrations in the waters of the reservoirs was influenced more by the anaerobic decomposition of their deposits under the ice cover (in 2006) than by the mixing of ice-free waters by wind.

An analysis of correlations between magnesium concentration and physicochemical parameters of the waters in the reservoirs has shown statistically significant correlations (at $p \geq 0.05$) between the above parameters occurred only in Reservoir 442 (Table 3). The concentration of magnesium

Table 3

Magnesium concentrations in the waters of the reservoirs in 2005 and 2006

Physical parameters / Reservoir number	Temperature	pH	Conductivity	Oxygenation
442	- 0.57*	-0.46*	0.73*	-0.66*
443	-0.39*	-0.25	0.24	-0.23
444	-0.53*	-0.06	0.11	-0.47*

*correlations significant at $p \leq 0.05$

positively correlated with conductivity (r = 0.75). As for thethe other relationships, the correlation coefficients were negative: temperature (r = -0.57), pH (r = -0.46), oxygenation (r = -0.66). However, in Reservoir 444, the Mg^{+2} concentration was significantly correlated only with temperature and oxygenation, and in Reservoir 443 it was significantly correlated, with the correlation coefficients being negative. This suggests that the Mg^{+2} concentration in waters of small reservoirs is highly variable and depend on a wider range of parameters than those analysed in this study.

In the two years of the study, in all the lakes the seasonal means for magnesium concentrations were at their highest in the wintertime (Fig. 3). The magnesium concentrations began to rise as early as autumn, which to some extent was caused by the presence of flora, which participates not only in accumulation of elements but also in their release. This phenomenon generally happens in two ‘pulses’ – in summer and in winter (Wojciechowska, Solis 1999).

The study has proved that during the analysed time average magnesium concentrations in the reservoirs were varied: from 5.2 mg□dm^{-3} (lake no 442), 5.8 mg·dm^{-3} (443) to 6.6 mg·dm^{-3} (444) – Figure 3. Thus, the variability ranged from from 10% to 26%. The highest average magnesium concen-

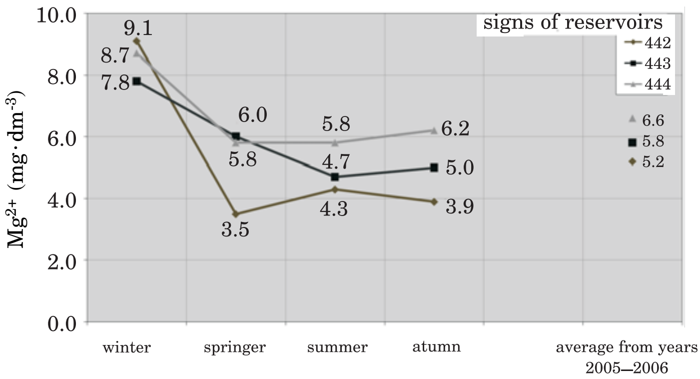

Fig. 3. Average seasonal magnesium concentrations (mg·dm^{-3}) in 2005 and 2006 in the waters of the reservoirs

tration in the water of Reservoir 444 was closely related to the agricultural character of its catchment, which for many years had been used agriculturally. The influx of elements from the catchment is mostly conditioned by the landscape features, its flora, soil compactness, intensity of agricultural production and type of land use (Koc et al. 2002, Banach, Szczurowska 2007). The results of our study clearly demonstrate that reservoirs situated on arable lands are most vulnerable to contamination with substances flowing out of their catchments (e.g. excess of unused mineral fertilizers).

CONCLUSIONS

1. The water reservoirs re-created 25 years before our study were characterised by low values of average magnesium concentrations (from 5.2 mg·dm^{-3} to 6,6 mg·dm^{-3}), which is characteristic for postglacial areas, such as the area where the study sites are situated.

2. The highest average seasonal magnesium concentration of 6.6 mg·dm^{-3} oscillating from 3.9 mg·dm^{-3} to 10.0 mg·dm^{-3} was noticed in the water of the reservoir whose whole catchment had been used for agriculture for many years.

3. The seasonal changes of Mg^{+2} concentrations were evidently influenced by meteorological conditions, which affect the circulation of substances in the catchments. The reservoirs displayed a distinctly cyclic nature of magnesium concentration changes connected with a decrease in magnesium levels in spring (when magnesium is intensively absorbed by plants) and its increase in autumn and winter due to depressed or absent bioaccumulation of Mg^{+2} released from the catchments and deposits of the lakes.

4. The highest amplitude of Mg^{+2} ionic concentrations in water was noticed in winter, and the lowest one – in spring, in the reservoir whose catchment was partly covered by fallows. Reintroducing the fallow land into farming, in addition to the disadvantageous ratio of the catchment to the lake's surface area and depth resulted in such a high coefficient of magnesium fluctuations.

5. The analysis of the changes in magnesium concentrations and their relationship with the physical and chemical parameters of the water has proved that in small water bodies magnesium levels are a highly variable feature, only partly connected with other properties of water. Magnesium concentrations negatively correlate with the water temperature and oxygenation.

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INFLUENCE OF DIFFERENT FORMS OF NITROGEN FERTILIZATION ON THE CONTENT OF MACROLEMENTS (K, Na) IN MEADOW SWARD
Part I

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Abstract

Present intensification of plant production rises a need to search for new solutions, such as novel fertilization technologies which reduce environmental pollution.

The aim of this work has been to examine principles of rational fertilization of permanent meadows, including delivery of nitrogen doses applied in various forms. The investigations were led in the region of Siedlce in 1999-2001. Basic fertilization was applied once during the growing season, in spring, and consisted of N–60 kg·ha^{-1}, P–60 kg·ha^{-1}, K–60 kg·ha^{-1} applied to soil. The fertilizers were used in two forms: multiple (Polifoska 15) [P] and a mix of fertilizers (ammonium nitrate, superphosphate, potassium salt) [M]. Nitrogen fertilization of the second and third cuts was conduced as various foliar (20%, 30%, 40% urea solution) or soil (ammonium nitrate). The doses of nitrogen in respective variants were 27.6 kg·ha^{-1} (N_1), 41.4 kg·ha^{-1} (N_2) and 55.2 kg·ha^{-1} (N_3). Every year three cuts for determination of potassium and sodium were collected.

Fertilization of the experimental objects with the multiple fertilizer resulted in increasing plants' potassium content whereas the mixture of fertilizers increased the quantity of sodium in plants. Foliar fertilization with nitrogen in the form of urea solution gave a higher increase in the plants' content of potassium and sodium than fertilization to roots with ammonium nitrate (independently on the basic fertilization).

By analysing the content of potassium in plants in dependence of the doses as well as the kind of supplementary nitrogen fertilization, it has been verified that most of this component was determined in plants treated with foliar fertilization consisting of 55.2 kg N·ha^{-1} (N_3), the lowest potassium concentration occurred in plants receiving foliar fertilization with dose 41.4 kg N·ha^{-1} (N_2).

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The analysis of the sodium content in plants in dependence of the doses and type of supplementary nitrogen fertilization showed that most potassium was in plants produced on the plots receiving foliar fertilization with a nitrogen dose of 27.6 kg·ha^{-1} (N_1), and the least potassium was determined in plants nourished with 41.4 kg N·ha^{-1} (N_2) applied to soil.

Key words: meadow sward, nitrogen dose, mineral fertilization, foliar fertilization, potassium, sodium.

WPŁYW FORMY NAWOŻENIA AZOTEM NA ZAWARTOŚĆ MAKROELEMENTÓW (K, NA) W RUNI Z ŁĄKI TRWAŁEJ

Abstrakt

Celem pracy było porównanie wpływu nawożenia azotem użytków zielonych na zawartość makroelementów w runi łąkowej. Badania prowadzono w latach 1999-2001 na łące trwałej. Każdego roku po ruszeniu wegetacji stosowano nawożenie podstawowe pogłównie, wnosząc do gleby odpowiednio: N–60 kg·ha^{-1}, P–60 kg·ha^{-1}, K–60 kg·ha^{-1}. Zastosowano dwie formy nawozów: wieloskładnikowy (Polifoska 15) [P] i mieszaninę nawozów jednoskładnikowych [M] (saletra amonowa, superfosfat pojedynczy, sól potasowa). W drugim i trzecim odroście stosowano nawożenie azotem w formie dolistnej (20%, 30%, 40% roztwór mocznika) i dokorzeniowej (saletra amonowa). Dawki azotu wynosiły: 27,6 kg·ha^{-1} (N_1), 41,4 kg·ha^{-1} (N_2), 55,2 kg·ha^{-1} (N_3). W każdym roku badań zebrano po trzy pokosy. W runi łąkowej określono zawartość potasu i sodu. Nawożenie nawozem wieloskładnikowym powodowało wzrost zawartości potasu w roślinach, a nawożenie mieszaniną nawozów jednoskładnikowych przyczyniło się do wzrostu ilości sodu. Dolistne dokarmianie roślin azotem w formie roztworu mocznika spowodowało większy wzrost zawartości potasu i sodu w porównaniu z nawożeniem dokorzeniowym saletrą amonową (niezależnie od zastosowanego rodzaju nawożenia podstawowego).

Analizując zawartość potasu w roślinach w zależności od dawek i rodzaju nawożenia uzupełniającego azotem, stwierdzono, że najwięcej tego składnika było w roślinach nawożonych dolistnie dawką 55,2 kg·ha^{-1} azotu (N_3), natomiast najmniej w przypadku zastosowania dolistnie dawki 41,4 kg·ha^{-1} (N_2).

Analiza zawartości sodu w roślinach w zależności od dawek i rodzaju nawożenia uzupełniającego azotem wykazała, że najwięcej tego składnika było w roślinach z poletek nawożonych dolistnie dawką azotu 27,6 kg·ha^{-1} (N_1), natomiast najmniej w przypadku dawki 41,4 kg·ha^{-1} (N_2) zastosowanej dokorzeniowo.

Słowa kluczowe: ruń łąkowa, dawka azotu, forma azotu, dokarmianie dolistne, nawożenie mineralne, potas, sód.

INTRODUCTION

According to many authors (DOBOSZYŃSKI 1994, WINNICKA, BOBRECKA-JAMRO 1996, WASILEWSKI, SUTKOWSKA 2001), mineral fertilization is one of the basic treatments influencing the botanical composition of meadow sward. Mineral fertilization influences the height and quality of crops. In order to obtain high yields of crops, it is necessary to apply a suitable mineral fertilization regime (Czuba 1996, Wasilewski 1999, Jodełka et. al. 2000). However, for mineral fertilization to be an economically profitable practice, one must establish optimum doses of fertilizers to be applied on grasslands.

Fertilization of grasses most often consists of traditional fertilizers applied in the solid form as top-dressing. However, in a period of drought such fertilizers stay on the surface of soil for a long time and their effect becomes delayed. In contrast, during excessive precipitations the fertilizers can be washed out to deeper layers of soil, where they are inaccessible to shallow roots of grasses. Present intensification of plant production rise a need to search for various solutions, such as new fertilization technologies which reduce environmental pollution. Such technologies allow combination of fertilizing components and improved utilization of nitrogen by plants. It has been empirically confirmed that foliar fertilization of meadow sward is beneficial (Jankowski et. al. 1999, Jodełka et. al. 1999, 2000, Jodełka, Jankowski 2001), which has encouraged us to study the reaction of fodder grasses to this fertilization method in terms of their content of mineral compounds. Thus, the aim of this work has been to examine the influence of nitrogen fertilization applied in different forms on the content of potassium and sodium in meadow sward.

MATERIAL AND METHODS

A three-year experiment was established in spring 1999 on permanent meadow in the region of Siedlce.

The trial plots were set out on gley proper soil created from light loamy sand on medium silt clay. The soil was slightly alkaline in reaction, both in KCl solution and in H_2O (pH in 1 n KCl 7.15); it had a high content of total nitrogen (0.45%), manganese (450 $mg \cdot kg^{-1}$) and iron (1700 $mg \cdot kg^{-1}$), an average content of magnesium (0.31 $mg \cdot kg^{-1}$) and a very low content of phosphorus (0.15 $mg \cdot kg^{-1}$) and potassium (0.25 $mg \cdot kg^{-1}$).

The meteorological measurements (temperature and precipitations) used for this report were obtained from the meteorological station in Siedlce. The meteorological conditions in the particular years of the investigations were different (Table 1). The average air temperature in the growing period (April – September) in the successive years was higher than the long-term average (4.1, 3.9 and 3.8°C, respectively). Also the total precipitation in the growing seasons analyzed exceeded the long-term average (about 87.3 mm in 1999, about 74.5 mm in 2000 and about 46 mm in 2001).

Two forms of fertilizer were used for basic fertilization: multiple (Polifoska 15) [P] and a mixture of single-element fertilizers (ammonium nitrate, superphosphate, potassium salt) [M]. Both introduced to the soil 60 kg $N \cdot ha^{-1}$, 60 kg $P \cdot ha^{-1}$ and 60 kg $K \cdot ha^{-1}$. Additionally, the second and third cut were

Table 1

Meteorological conditions 1999-2001, data provided by the meteorological station in Siedlce

Year	Mean monthly air temperatures (°C)						Mean in growing season (Apr-Sept)
	Apr	May	June	July	Aug	Sept	
1999	9.9	12.9	20.5	21.8	18.7	16.1	16.7
2000	12.9	16.4	19.5	19.0	19.1	11.8	16.3
2001	8.7	15.5	17.1	23.8	20.6	12.1	14.7
Long-term mean (1987-1999)	7.8	12.5	17.2	19.2	18.5	13.1	14.7
Year	monthly precipitations (mm)						Sum in growing season (Apr-Sept)
	Apr	May	June	July	Aug	Sept	
1999	87.5	26.4	121.7	21.9	77.4	27.8	362.5
2000	47.5	24.6	17.0	155.9	43.6	61.1	349.7
2001	69.8	28.0	36.0	55.4	24.0	108.0	321.2
Long-term mean (1987-1999)	38.6	44.1	52.4	49.8	43.0	47.3	275.2

fertilized with nitrogen applied as foliar treatments (20%, 30%, 40% urea solution) or in the solid form (ammonium nitrate) to soil. Both fertilization treatments introduced identical amounts of nitrogen: N_1– 27.6 $kg \cdot ha^{-1}$, N_2– 41.4 $kg \cdot ha^{-1}$ and N_3– 55.2 $kg \cdot ha^{-1}$.

Three cuts were harvested in the vegetation period. The chemical analysis of the plant material was performed by absorption atomic spectrophotometry (ACE) for the following macronutrients: K and Na.

Mathematical models proposed for this type of experiments by TRĘTOWSKI and WÓJCIK (1991) were applied. Significance of differences between means of the experimental factors was determined with Tukey's test at the level of significance $P<0.05$.

RESULTS AND DISCUSSION

The results confirmed that spring application of two fertilization (Table 2) significantly differentiated the content of potassium in plants. Significantly more potassium was found in meadow sward from plots fertilized with the multiple fertilizer. However, these contents were lower than an optimum potassium concentration suggested by other authors, for example 17 $g \cdot kg^{-1}$ K of d.m (FALKOWSKI et. al. 1990), about 20 $g \cdot kg^{-1}$ d.m. (Nowak 1992), and from 10 to 30 $g \cdot kg^{-1}$ d.m. (PREŚ 1984).

The content of potassium determined in our study was also variable depending on the type of basic fertilization, supplementary nitrogen fertilization method and doses of nitrogen (Table 2). The highest content of potassium, between 16.2 and 16.0 $g \cdot kg^{-1}$ of d.m., appeared in plants which had received foliar fertilization with urea solution in the fertilizer variants N_1 and N_3 with the multiple fertilizer for basic fertiliztaion. This potassium level was significantly higher in comparison with the variants of identical additional fertilization and basic fertilization consisting of a mixture of single-component fertilizers.

The present experiment has demonstrated (Figure 1) that most potassium was in plants from the first re-growth. Other authors, e.g. BARRYŁA (1992) and JODEŁKA (1998), also reported higher potassium content in the first regrowth on meadows. Our comparison of the results produced by additional

Table 2

Content of K and Na in plants ($g \cdot kg^{-1}$ d.m.) depending on fertilization technique (soil and foliar) and nitrogen dose (mean for the years of the experiment

Basic fertilization	Nitrogen dose	Potassium additional fertilization foliar	Potassium additional fertilization soil	$\bar{x}$	Sodium additional fertilization foliar	Sodium additional fertilization soil	$\bar{x}$
Polifoska [P]	N_1	16.2	13.9	15.1	2.4	2.1	2.3
	N_2	13.8	15.1	14.5	2.5	2.5	2.5
	N_3	16.0	15.3	15.7	2.1	2.2	2.2
	$\bar{x}$	15.3	14.8	15.1	2.3	2.3	2.3
Mix of fertilizers [M]	N_1	14.5	14.6	14.6	2.7	2.0	2.4
	N_2	13.9	14.8	14.4	2.5	2.3	2.4
	N_3	15.4	14.5	15.0	2.6	2.2	2.4
	$\bar{x}$	14.6	14.6	14.6	2.6	2.2	2.4
Mean [PM]	N_1	15.4	14.3	14.9	2.6	2.1	2.4
	N_2	13.9	15.0	14.5	2.5	2.4	2.5
	N_3	15.7	14.9	15.4	2.4	2.2	2.3
	$\bar{x}$	15.0	14.7	14.9	2.5	2.3	2.4
n.s. – non-significant		$LSD_{0.05}$ additional fertilization (A) – n.i. nitrogen dose (B) – 0.4 basic fertilization (C) – 0.4 interaction: A x B – 1.0 A x C – 0.5 B x C – 0.5 A x B x C – 0.6			$LSD_{0.05}$ additional fertilization (A) – 0.22 nitrogen dose (B) – n.s. basic fertilization (C) – n.s. interaction: A x B – 0.30 A x C – 0.27 B x C – 0.25 A x B x C – 0.36		

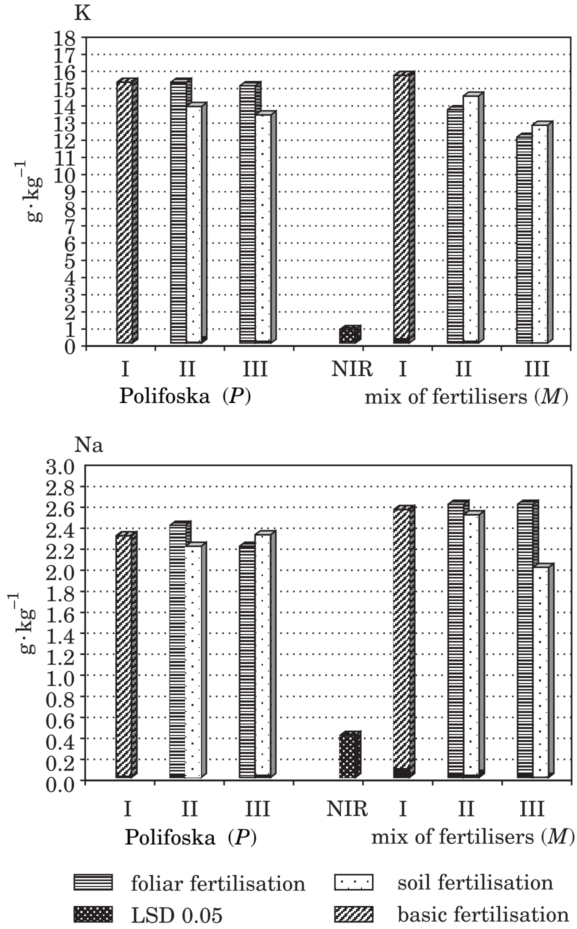

Fig. 1. Content of K and Na in plants (g·kg^{-1}) depending on basic fertilization type, additional fertilization to soil or foliar and nitrogen dose and cuts (means for the years of the experiment)

fertilization showed that, against the background of the multiple fertilizer basic nutrition, a significantly higher content of potassium in the second regrowth occurred in the sward which had received foliar fertilization. However, when a mixture of single-component fertilizers had been used, the results were contrary – most potassium was found in the sward fertilized to the soil. When analyzing the content of potassium in plants in dependence of the nitrogen doses and method of supplementary nitrogen fertilization (Table 2), it was found out that most potassium was in plants from the plots receiving 55 kg·ha^{-1} of nitrogen in foliar fertilization (N_3). The smallest potassium concentration occurred in plants which had received 41.4 kg N·ha^{-1} applied as foliar fertilization (N_2).

Sodium is another important component animal nutrition, which is often deficient in grasses according to nutritional needs of animals. FALKOWSKI et. al. (1990) claim that animal fodder should contain 1.5 – 2.5 $g \cdot kg^{-1}$ Na in d.m. However, NOWAK (1981) suggests that sodium in fodder above 1.8 $g \cdot kg^{-1}$ d.m. fully satisfies the alimentary needs of animals.

In our investigations (Table 2), a slightly higher content of sodium in plants (average 2.4 $g \cdot kg^{-1}$ d.m.) occurred in plots fertilized with a mixture of single-component fertilizers in the comparison with plots fertilized with the multiple fertilizer (2.3 $g \cdot kg^{-1}$ d.m.).

The content of sodium was also differentiated by the nitrogen doses as well as the form of nitrogen fertilization and the type of basic fertilization (Table 2). The highest content of sodium (2.7 $g \cdot kg^{-1}$ d.m.) was determined in sward harvested from plots receiving foliar fertilization with the lowest nitrogen dose 27.6 $kg \cdot ha^{-1}$ (variant N_1) against the background of a mixture of single-component fertilizers. It was significantly higher than its concentration in the fodder fertilized with the same dose of nitrogen but applied to the soil (2.0 $g \cdot kg^{-1}$ d.m.).

The statistical analysis of the interaction between the type of basic fertilization and the way supplementary nitrogen fertilization was applied on the successive cuts (Figure 1) showed that significantly more sodium was present only in the fodder from second and third re-growths which had received foliar fertilization in the comparison with nitrogen fertilization applied to the soil.

The analysis of sodium in plants in dependence of the doses and methods of supplementary nitrogen fertilization (Table 2) showed that most sodium was in plants taken from plots where 27.6 kg $N \cdot ha^{-1}$ was introduced in foliar applications (N_1), whereas the lowest sodium concentration occurred in plants which had received 41.4 kg $N \cdot ha^{-1}$ applied to the soil (N_2). In this experiment, no statistically significant differences were revealed in sodium content in plants depending on the nitrogen doses irrespective of the basic and supplementary fertilization regimes.

CONCLUSIONS

1. The form of fertilizers significantly differentiated the content of mineral components in meadow sward. Fertilization with a multiple fertilizer resulted in an increase in the potassium content, whereas application of a mixture of single- component fertilizers increased the quantity of sodium.

2. More potassium was contained in meadow sward fertilized with a multiple fertilizer and additionally top-dressing fertilized as well as in grasses fertilized with a mixture of single-component fertilizers but receiving additional nitrogen fertilization to the soil.

3. The highest sodium content was determined in meadow sward additionally fertilized with nitrogen in the form of foliar application; the lowest sodium concentration was found in plants receiving the lowest nitrogen dose under a fertilization regime consisting of a mixture of single-component fertilizers.

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INFLUENCE OF DIFFERENT FORMS OF NITROGEN FERTILIZATION ON THE CONTENT OF MACROLEMENTS (Ca, Mg) IN MEADOW SWARD
Part II

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Abstract

Foliar fertilization is not a new application method, especially with respect to field crops. However, little information is available regarding foliar fertilization of grasses.

This paper contains a presentation of guidelines for rational fertilization of permanent meadow through delivery of nitrogen doses applied in various forms. The study was conducted near Siedlce in 1999-2001. Basic fertilization was applied once in the growing period, in the spring, introducing to the soil the following quantities of nutrients: N–60 $kg \cdot ha^{-1}$, P–60 $kg \cdot ha^{-1}$, K–60 $kg \cdot ha^{-1}$. The fertilizers were used in two forms: multiple (Polifoska 15) [P] and a mix of fertilizers (ammonium nitrate, superphosfate, potasic salt) [M]. Under the second and third cut of grass, additional nitrogen nutrition was applied on the experiment plots, as foliar or soil fertilization treatments. The following nitrogen doses were used (in $kg \cdot ha^{-1}$): 27.6 (N_1), 41.4 (N_2), 55.2 (N_3). Every year, three cuts were collected for determination of the content of calcium and magnesium.

The fertilization variants modified the content of calcium in sward. The concentration of calcium increased from 8.4 to 9.0 g kg^{-1} d.m. only under the influence of increasing doses of nitrogen applied in the liquid form against the background of the multiple fertilizer.

While analysing the content of calcium in plants in dependence of the applied doses and method of supplementary nitrogen fertilization, it was found out that most calcium was in plants from plots foliar fertilized with a nitrogen dose of 55.2 $kg \cdot ha^{-1}$ (N_3). The lowest calcium level was in grass fertilized with 41.4 kg $N \cdot ha^{-1}$ (N_2) applied to soil.

The content of magnesium in sward was high: on average 3.0 $g \cdot kg^{-1}$ d.m. in grass fertilized with the multiple fertilizer and 3.1 $g \cdot kg^{-1}$ d.m. in grass receiving a mixture of single-component fertilizers.

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Key words: meadow sward, nitrogen dose, mineral fertilization, foliar fertilization, calcium, magnesium.

WPŁYW FORMY NAWOŻENIA AZOTEM NA ZAWARTOŚĆ MAKROELEMENTÓW (Ca, Mg) W RUNI Z ŁĄKI TRWAŁEJ

Abstrakt

Obecnie intensyfikacja produkcji roślinnej wymusza konieczność szukania rozwiązań ograniczających m.in. zanieczyszczenie środowiska. Są nimi np. nowe technologie nawożeniowe umożiwiające łączenie stosowanych składników, co wpływa na lepsze wykorzystanie azotu przez rośliny.

Celem pracy było porównanie wpływu nawożenia użytków zielonych azotem na zawartość makroelementów w runi łąkowej. Badania prowadzono w latach 1999-2001 na łące trwałej. Każdego roku po ruszeniu wegetacji stosowano nawożenie podstawowe pogłównie, wnosząc do gleby odpowiednio: N–60 $kg \cdot ha^{-1}$, P–60 $kg \cdot ha^{-1}$, K–60 $kg \cdot ha^{-1}$. Zastosowano dwie formy nawozów: wieloskładnikowy (Polifoska 15) [P] i mieszaninę nawozów jednoskładnikowych [M] (saletra amonowa, superfosfat pojedynczy, sól potasowa). W drugim i trzecim odroście stosowano nawożenie azotem w formie dolistnej (20%, 30%, 40% roztwór mocznika) i dokorzeniowej (saletra amonowa). Dawki azotu wynosiły: 27,6 $kg \cdot ha^{-1}$ (N_1); 41,4 $kg \cdot ha^{-1}$ (N_2); 55,2 $kg \cdot ha^{-1}$ (N_3). W każdym roku badań zebrano po trzy pokosy. W runi łąkowej określono zawartość wapnia i magnezu.

Analizując zawartość wapnia w roślinach w zależności od zastosowanych dawek i rodzaju nawożenia uzupełniającego azotem, stwierdzono, że najwięcej tego składnika było w roślinach z poletek nawożonych dolistnie dawką 55,2 $kg \cdot ha^{-1}$ azotu (N_3), natomiast najmniej po zastosowaniu dokorzeniowo dawki 41,4 $kg \cdot ha^{-1}$ (N_2).

Zawartość magnezu w badanej runi była wysoka i wynosiła średnio 3,0 $g \cdot kg^{-1}$ s.m. w przypadku nawożenia nawozem wieloskładnikowym i 3,1 $g \cdot kg^{-1}$ s.m. w przypadku nawozów jednoskładnikowych.

Słowa kluczowe: ruń łąkowa, dawka azotu, forma azotu, dokarmianie dolistne, nawożenie mineralne, wapń, magnez.

INTRODUCTION

High fertilization of grasslands often has negative consequences, such as a worse chemical composition of the fodder produced from grass, disappearance of some bird or insect species, or unfavourable changes in the content of macroelements in soil (Czuba 1996, Doboszynski 1994, Winnicka, Bobrecka-Jamro 1996). Currently, less fertilizers are being used on meadows or pastures. Limiting fertilization can help to maintain the ecological equilibrium of natural grasslands by improving their biodiversity (Spatz, Buchgraber 2003, Wasilewski-Sutkowska 2001). It is, however, necessary to look for other solutions which would, for example, reduce environmental contamination. Foliar fertilization could be an answer, as it can deliver small amounts of nutrients very effectively (Jankowski et al. 1999, Jodełka et al.

2001). Empirically tested profitability of foliar fertilization of meadow sward (JANKOWSKI et. al. 1999, JODEŁKA et. al. 1999, 2000, JODEŁKA, JANKOWSKI 2001) has encouraged us to test the reaction of fodder grasses to this fertilization method in relation to the content of mineral compounds in grasses. Foliar fertilization is not a new application method, especially with respect to field crops. However, little information is available regarding foliar fertilization of grasses.

The present study is part of an attempt to formulate guidelines for fertilization of grasslands by testing supplementary nitrogen fertilization in two forms (soil and foliar).

Thus, the aim of this work has been to evaluate the influence of nitrogen fertilization applied in the two forms on the content of calcium and magnesium in meadow sward.

MATERIAL AND METHODS

A detailed description of the soil and meteorological conditions prevailing during the trials can be found in part one of this paper (KOLCZAREK et al. 2008).

Chemical analyses of the plant material for determination of Ca and Mg were performed by the method of absorption atomic spectrophotometry.

RESULTS AND DISCUSION

The content of calcium in plants ranged from 8.2 $g \cdot kg^{-1}$ d.m. to 9.2 $g \cdot kg^{-1}$ d.m. A comparison of the influence of the type of basic fertilization (Table 1) showed that the plants from objects fertilized with the mixture of single-component fertilizers contained more calcium (9.0 $g \cdot kg^{-1}$ d.m.). FALKOWSKI et. al. (1990) as well as TRĄBA and WOLAŃSKI (1999) suggested that such levels of calcium in sward was sufficient to satisfy animals' alimentary needs. Our results proved that the fertilization variants modified the content of calcium in sward. The content of calcium rose from 8.4 to 9.0 $g \cdot kg^{-1}$ d.m. only under the influence of an increasing doses of nitrogen applied in the liquid form in conjunction with the multiple fertilizer. Moreover, plants from plots fertilized with the mixture of single-component fertilizers contained more calcium irrespective of the method of supplementary nitrogen fertilization.

With regard to the influence of basic and supplementary fertilization methods on the cuts (Figure 1), a decrease of the calcium content was demonstrated in the successive re-growths when nitrogen was applied in the solid form, independently of the type of basic fertilization. While analysing

Table 1

Content of Mg and Ca in plants ($g \cdot kg^{-1}$ d.m.) depending on fertilization method (soil and foliar) and nitrogen dose (mean for the years)

Basic fertilization	Nitrogen dose	Magnesium: Additional fertilization: foliar	Magnesium: Additional fertilization: soil	Magnesium: $\bar{x}$	Calcium: Additional fertilization: foliar	Calcium: Additional fertilization: soil	Calcium: $\bar{x}$
Polifoska [P]	N_1	2.9	2.9	2.9	8.4	8.4	8.4
	N_2	3.0	2.8	2.9	8.6	8.3	8.5
	N_3	3.0	3.0	3.0	9.0	8.2	8.6
	$\bar{x}$	3.0	2.9	3.0	8.7	8.3	8.5
Mix of fertilizers [M]	N_1	3.1	3.0	3.1	8.8	8.9	8.9
	N_2	3.1	3.1	3.1	9.2	8.7	9.0
	N_3	3.0	3.0	3.0	9.1	9.2	9.2
	$\bar{x}$	3.1	3.0	3.1	9.0	8.9	9.0
$\bar{x}$ Mean [PM]	N_1	3.0	3.0	3.0	8.6	8.7	8.7
	N_2	3.1	3.0	3.0	8.9	8.5	8.8
	N_3	3.0	3.0	3.0	9.1	8.7	8.9
	$\bar{x}$	3.1	3.0	3.1	8.9	8.6	8.8
n.s. non-significant		$LSD_{0.05}$ additional fertilization (A) – n.s. nitrogen dose (B) – n.s. basic fertilization (C) – n.s. interaction: A x B – n.s. A x C – n.s. B x C – n.s. A x B x C – n.s.			$LSD_{0.05}$ additional fertilization (A) – n.s. nitrogen dose (B) – n.s. basic fertilization (C) – 0.5 interaction: A x B – 0.6 A x C – 0.6 B x C – 0.6 A x B x C – 0.8		

the content of calcium in plants in dependence of the applied doses and method of supplementary nitrogen fertilization, it was found out that most calcium was in plants from plots foliar fertilized with a nitrogen dose of 55 $kg \cdot ha^{-1}$ (N_3). The lowest calcium level was in grass fertilized with 41.4 kg $N \cdot ha^{-1}$ (N_2) applied to soil.

Moreover, the highest content of calcium in the whole period of investigations was found in the sward taken from plots receiving foliar fertilization with nitrogen (8.9 $g \cdot kg^{-1}$ d.m.).

Magnesium is another important element in animals nourishment (BARYŁA 1992, NOWAK 1992, PREŚ 1984) and its threshold level in grasses should be 2 $g \cdot kg^{-1}$ d.m. (FALKOWSKI et. al. 1990). The content of magnesium in the

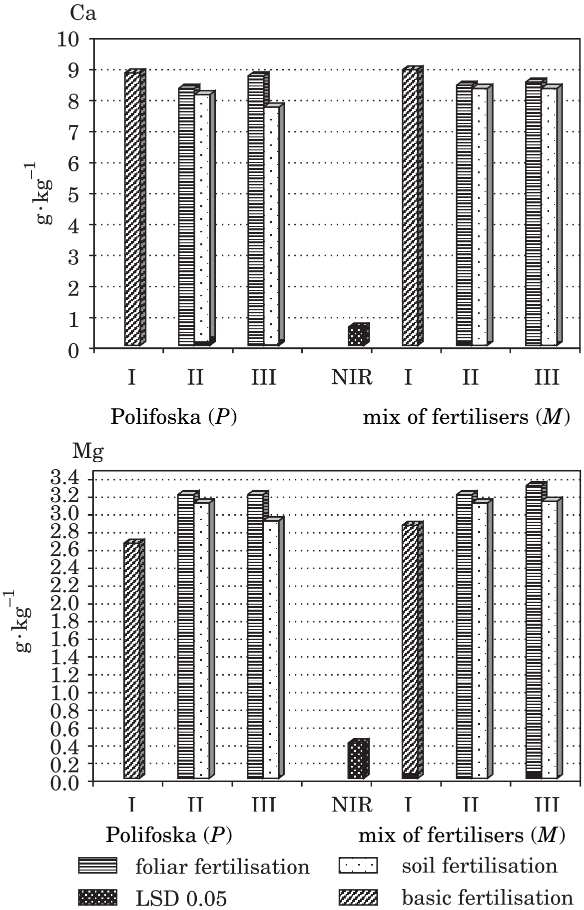

Fig. 1. Content of Ca and Mg in plants (g□kg^{-1}) depending on basic fertilization type, additional soil or foliar fertilization, nitrogen dose and cuts (means for the years)

sward we studied was high (Table 1): an average 3.0 g·kg^{-1} d.m. in grass fertilized with the multiple fertilizer and 3.1 g·kg^{-1} d.m. in sward receiving the mixture of single-component fertilizers. A comparison of the influence of the interaction between the basic and supplementary fertilization as well as individual nitrogen doses on the content of magnesium in plants (Table 1) showed this effect was not statistically significant as the values obtained ranged 2.8 to 3.1 g·kg^{-1} d.m.

However, our analysis of the influence of the type of basic fertilization and the method of introducing supplementary nitrogen fertilization to successive cuts (Figure 1) showed that the content of magnesium in meadow sward increased together with the increasing nitrogen dose applied in the foliar form, independently of the type of basic fertilization. Similar results have been presented by Ciepiela (2004) and Jodełka et al. (2000).

Besides, the analysis of the magnesium content in plants in dependence of the doses and method of supplementary nitrogen fertilization (Table 1) showed that most magnesium appeared in plants from plots receiving 41.4 kg $N \cdot ha^{-1}$ in foliar fertilization (N_2). The other doses in conjunction with either way of supplementary fertilization did not differentiate significantly the content of magnesium.

CONCLUSIONS

1. The form of fertilizers significantly differentiated the content of mineral components in meadow sward.

2. Meadow fertilization with a mixture of single-component fertilizers resulted in an larger increase in the quantity of calcium and magnesium than a multiple fertilizer.

3. The content of magnesium in meadow sward increased together with an increase in the does of nitrogen dose applied in the foliar form, independently of the type of basic fertilization.

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BIOLOGICAL PROPERTIES OF SOIL CONTAMINATED WITH THE HERBICIDE APYROS 75 WG

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Abstract

The objective of the study has been to determine the effect of the herbicide Apyros 75 WG on counts of various aerobic microorganisms, activity of soil enzymes and yields of spring wheat.

For this purpose, a pot experiment was carried out in a greenhouse. Samples of soil used for the trials represented loamy sand. Having mixed the soil samples with mineral fertilizers, doses of the herbicide were added and the soil was placed in plastic pots. The lowest herbicide dose was the optimum dose recommended by the producer, and the two other doses were 10- and 100-fold higher. The experiment was conducted in two series: I – unsown soil, and II – soil under spring wheat.

It has been determined that Apyros 75 WG disturbs soil's homeostasis, as it disrupts multiplication of some microbial groups, inhibits the activity of soil enzymes and depresses the yield of spring wheat, even if applied in a recommended dose. Among the soil enzymes, dehydrogenases and urease were the least tolerant to the effect of the herbicide, whereas alkaline phosphatase proved to be the most tolerant one. The vulnerability of microorganisms to soil pollution with the herbicide can be arranged in the following decreasing order: ammonifying bacteria > *Pseudomonas* > copiotrophic bacteria > oligotrophic bacteria > nitrogen binding bacteria > spore-forming oligotrophic bacteria > *Arthrobacter* > cellulolytic bacteria > *Actinomyces* > fungi. Growing spring wheat had a positive effect on the counts of microorganisms and activity of soil enzymes.

Key words: Apyros 75 WG, herbicide, activity of enzymes, soil microorganisms.

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BIOLOGICZNE WŁAŚCIWOŚCI GLEBY ZANIECZYSZCZONEJ HERBICYDEM APYROS 75 WG

Abstrakt

Celem badań było określenie wpływu herbicydu Apyros 75 WG na liczebność różnych grup drobnoustrojów tlenowych, aktywność enzymów glebowych oraz plonowanie pszenicy jarej. Badania przeprowadzono w wazonach w hali wegetacyjnej. W doświadczeniu wykorzystano próbki gleby o składzie granulometrycznym piasku gliniastego. Glebę po wymieszaniu z nawozami mineralnymi zanieczyszczono herbicydem i umieszczono w plastikowych wazonach. Najniższa dawka herbicydu była dawką optymalną, zalecaną do stosowania przez producenta, a kolejne były 10 i 100-krotnie wyższe. Badania prowadzono w dwóch seriach: I – gleba nieobsiana i II – gleba obsiana pszenicą jarą.

Stwierdzono, że Apyros 75 WG narusza homeostazę gleby, gdyż nawet w dawce zalecanej przez producenta zakłóca namnażanie niektórych grup drobnoustrojów, hamuje aktywność enzymów glebowych oraz zmniejsza plon pszenicy jarej. Najmniej odpornymi enzymami na działanie herbicydu są dehydrogenazy oraz ureaza, a najbardziej odporna jest fosfataza alkaliczna. Wrażliwość drobnoustrojów na zanieczyszczenie gleby herbicydem Apyros 75 WG jest następująca: bakterie amonifikacyjne > *Pseudomonas* > bakterie kopiotroficzne > bakterie oligotroficzne > bakterie immobilizujące azot > bakterie oligotroficzne przetrwalnikujące > *Arthrobacter* > bakterie celulolityczne > promieniowce > grzyby. Na liczebność drobnoustrojów oraz aktywność enzymów korzystnie wpływa uprawa pszenicy jarej.

Słowa kluczowe: Apyros 75 WG, herbicyd, aktywność enzymów, drobnoustroje glebowe.

INTRODUCTION

Plant protection chemicals improve and protect crop yields but they can also have an adverse effect on many ecosystems (CUPPLES, SIMS 2007, SHEN et al. 2005, SŘRENSEN et al. 2003, WG et al. 2005). Their effect on soil environment depends mainly on the type of active substance, application rates as well as the oxidation-reduction potential of soil, soil content of organic substance, physicochemical properties of soil, temperature, moisture, pH, sorptive capacity, grain-size distribution, and counts of bacteria, *Actinomyces* and fungi (AWASTHI et al. 2000, SÁNCHEZ et al. 2004). Irrespective of the above factors, biocides can migrate from soil to groundwater and foodstuffs, posing threat to humans and animals (MCDONALD et. al. 1999, ZHANG et al. 2006). Widespread use of pesticides and herbicides in agriculture makes it necessary to investigate their influence on natural environments. Thus, monitoring the effects produced by plant protection chemicals, which may involve inhibition of a series of biological processes and consequent disorders in the enzymatic activity of soil or multiplication of microorganisms, can provide us with a reliable measure of the unwanted side-effects (BENDING et al. 2006, BRASCHI et al. 2000, KUCHARSKI et al. 2006, WYSZKOWSKA 2002a, 2002b, WYSZKOWSKA, KUCHARSKI 2004).

Apyros 75 WG belongs to a new generation of herbicides, and its effect on soil environment has not been thoroughly investigated or discussed yet.

Therefore, this study's aim has been to determine how Apyros 75 WG affects counts of many groups of aerobic bacteria, activity of soil enzymes and yields of spring wheat.

MATERIAL AND METHODS

The experiment with 5 replicates was conducted in a greenhouse at the University of Warmia and Mazury in Olsztyn. Samples of soil used for the laboratory trials were collected from proper brown soil created from light loamy sand possessing the following properties: 5.60 pH in 1 mol KCl^{-3}, 13.05 mmol(H^+) kg^{-1} hydrolytic acidity, 5.00 $g \cdot kg^{-1}$ C_{org}, 57.06 mmol(+) kg^{-1} total exchangeable base cations, 70.11 mmol(+)kg^{-1} sorptive complex exchangeable capacity, 81.39% saturation with base cations. Prior to placing in the pots, soil was fertilized with the following macro- and microelements, in $mg \cdot kg^{-1}$ soil (expressed as pure component): 100 N [$CO(NH_2)_2$], 44 P [K_2HPO_4], 83 K [KH_2PO_4 + KCl], 20 Mg [$MgSO_4 \cdot 7H_2O$], 5 Zn [$ZnCl_2$], 5 Cu [$CuSO_4 \cdot 5H_2O$], 5 Mn [$MnCl_2 \cdot 4H_2O$], 5 Mo [$Na_2MoO_4 \cdot 2H_2O$] and 0.33 B [H_3BO_3]. All the mineral fertilizers, except urea, were introduced to soil once before sowing wheat. Urea was added in doses: 1/2 N dose before sowing and 1/2 N dose in the wheat tillering stage.

The experiment's variables were:

1) rates of the herbicide Apyros 75 WG in μm kg^{-1} of soil: 0, 8.9, 89.0 and 890.0;
2) application of the herbicide: soil application (the herbicide was mixed with a whole portion of soil used to fill up one pot prior to placing it in the pot), top-dressing (the herbicide was applied over the surface of soil during the tillering of wheat plants);
3) soil use: unsown soil and soil sown with spring wheat;
4) day of analysis: 14 days after placing the soil in pots (on the day when spring wheat was sown) and on day 73 of the trials (on harvest day).

The lowest rate of the herbicide was the optimum dose recommended by the producer, and the following rates were 10- and 100-fold higher.

Before setting up the experiment, a portion of soil to fill up one pot (3.2 kg) was mixed with the mineral fertilizers and with the herbicide Apyros 75 WG where appropriate. The active substance in Apyros 75 Wg is 75% sulfosulfuron 1-(4,6-dimethoxypyrimidin-2-yl)-3-(2-ethylsulfonylimidazo[1,2-*a*]pyridin-3-ylsulfonyl)urea. Apyros 75 WG is used to control loose silkybent (*Apera spica-venti*) and broad-leaved weeds in winter wheat, spring wheat, winter triticale and potato. This herbicide does not represent a significant risk to human health (class IV).

Having placed the soil in the pots, its moisture content was brought up to 60% of capillary water capacity and maintained at this level over the whole experiment (73 days). During the first 14 days soil was not sown. On day 14 samples of soils were collected for microbiological and biochemical assays, after which cv. Sakwa spring wheat (12 plants per pot) was sown. The experiment was conducted in two series: I – unsown soil, II – soil under spring wheat. Wheat plants were harvested in the inflorescence stage (on day 59 of the growing season). On the same day soil samples were collected for the second time to perform analyses.

The microbiological assays consisted of determination of counts of the following microorganisms: copiotrophic and spore-forming copiotrophic bacteria on ONTA-HATTORI medium (1983), *Arthrobacter*, *Pseudomonas*, nitrogen binding, ammonifying and cellulolytic bacteria on the medium described in the paper by WYSZKOWSKA et al. (2007), *Actynomyces* on Kuster and Williams medium with nystatin and actidione (PARKINSON et al. 1971) and fungi – on a medium proposed by MARTIN (1950). The microorganisms were grown on Petri plates at 28°C for 2 (*Azotobacter*) to 21 days (oligotrophic bacteria). Spores of oligotrophic and copiotrophic bacteria were determined on material which was pasteurised at 85°C for 15 minutes. The count of colony forming units (cfu) was determined using a colony-count method.

The biochemical tests included determination of the activity of: dehydrogenases using a TTC substratum (ÖHLINGER 1996), urease – according to ALEF and NANNPIERI (1998) and acid and alkaline phosphatase – by the method described by ALEF et al. (1998). Activity of dehydrogenases was expressed in cm^3 of H necessary to reduce TTC to TFP; urease – in mg N-NH_4 produced from hydrolysed urea, the phosphatases – in nmol of *p*-nitrophenol (PNP) produced from sodium 4-nitrophenyl phosphate.

In addition, physiochemical analyses were performed, involving the determination of hydrolytic acidity (Hh) and total base exchangeable cations (S) using Kappen method (MOCEK et al. 1997). From these two values total exchangeable capacity (T) and saturation with base cations (V) were computed using the formulas: $T = S + Hh$ and $V = S \cdot T^{-1}$ 100%. Because the physicochemical properties of soil were not significantly correlated with the day of sampling, method of herbicide application or soil use, the results of the above determinations are presented in the paper as means for two days of sampling and independently from the method Apyros 75 WG was applied.

The results were processed statistically by Duncan's multiple-range test, using two- and four-factorial analysis of variance (StatSoft, Inc. ... 2006).

RESULTS AND DISCUSSION

The research proves that soil contamination with Apyros 75 Wg disturbs the microbiological equilibrium of soil (Tables 1-3), although the actual disorders in soil's homeostasis depend on a rate of the herbicide, time period of the herbicide present in soil, soil use, herbicide application method and

Table 1

Count of oligotrophic and copiotrophic bacteria (in 1 kg d.m. of soil)

Herbicide dose Apyros 75 WP	Before spring wheat sowing	Day of analysis		
		after harvest		
		soil use		
		sown		unsown
		g	l	
Copiotrophic total bacteria (cfu 10^{10})				
0	123.7 ± 8.8	124.4 ± 8.8	124.4 ± 1.6	91.0 ± 1.6
Optimum	97.5 ± 1.2	128.3 ± 5.6	102.2 ± 9.7	106.5 ± 4.7
10 x	95.0 ± 7.0	103.2 ± 2.2	92.8 ± 1.6	77.8 ± 4.3
100 x	97.1 ± 4.8	59.1 ± 5.4	86.0 ± 5.4	40.1 ± 2.2
Average	103.3 ± 5.4	103.8 ± 3.4	101.3 ± 3.1	78.9 ± 1.7
r	-0.358	-0.965	-0.666	-0.937
LSD*	a – 3.1; b – 2.2; c – 2.2; d – 2.2; a· b – 4.4; a · c – 4.4; a · d – 4.4; b · c – 3.1; b · d – n.s.; c · d – n.s.; a · b · c – 6.3; a · b · d – 6.3; a · c · d – n.s.; b · c · d – n.s.; a · b · c · d – 8.9			
Sporulating copiotrophic bacteria (cfu 10^{7})				
0	54.5 ± 1.6	19.4 ± 1.1	19.4 ± 1.1	27.6 ± 2.2
Optimum	56.3 ± 4.5	18.6 ± 2.2	21.5 ± 1.9	29.0 ± 2.2
10 x	66.7 ± 2.2	14.3 ± 0.6	15.1 ± 1.1	28.7 ± 2.2
100 x	69.2 ± 4.1	15.1 ± 2.2	17.2 ± 1.1	25.4 ± 1.6
Average	61.6 ± 1.3	16.8 ± 0.4	18.3 ± 0.5	27.7 ± 0.5
r	0.747	-0.554	-0.339	-0.909
LSD*	a – 1.5; b – 1.1; c – 1.1; d – 1.1; a · b – 2.2; a · c – 2.2; a · d – 2.2; b · c – 1.5;b · d – n.s.; c · d – n.s.; a · b · c – n.s.; a · b · d – n.s.; a · c · d – 2.2; b · c · d – n.s.; a · b · c · d – 4.3			
Total oligotrophic bacteria (cfu 10^{8})				
0	30.1 ± 2.2	40.9 ± 1.1	40.9 ± 1.1	15.8 ± 2.2
Optimum	26.9 ± 2.8	44.8 ± 2.2	38.4 ± 2.7	15.1 ± 1.9
10 x	22.9 ± 0.6	25.8 ± 2.2	33.0 ± 2.7	16.1 ± 1.1
100 x	20.1 ± 1.2	16.8 ± 1.2	16.5 ± 0.6	16.5 ± 1.2
Average	25.0 ± 1.4	32.1 ± 0.8	32.2 ± 0.6	15.9 ± 0.7
r	-0.801	-0.831	-0.977	0.727
LSD*	a – 1.2; b – 0.9; c – 0.9; d – n.s.; a · b – 1.7; a · c – 1.7; a · d – n.s.; b · c – 1.; b · d – 1.2; c · d – n.s.; a · b · c – 2.4; a · b · d – 2.4; a · c · d – 1.7; b · c · d – n.s.; a · b · c · d – 3.5			

Sporulating oligotrophic bacteria (cfu 10^7)				
0	17.9 ± 0.6	17.2 ± 1.1	17.2 ± 1.1	19.7 ± 1.6
Optimum	13.3 ± 1.2	16.8 ± 1.6	18.3 ± 1.9	17.2 ± 1.1
10 x	13.3 ± 1.2	15.8 ± 1.2	11.5 ± 0.6	16.8 ± 0.6
100 x	7.9 ± 0.6	12.2 ± 0.6	11.1 ± 0.6	16.1 ± 1.1
Average	13.1 ± 0.6	15.5 ± 0.3	14.5 ± 0.9	17.5 ± 0.3
r	-0.870	-0.984	-0.674	-0.623
LSD*	a – 0.6; b – 0.4; c – 0.4; d – 0.4; a · b – 0.9; a · c – 0.9; a · d – n.s.; b · c – 0.6; b · d – 0.6; c · d – 1.2; a · b · c – 1.2; a · b · d – n.s.; a · c · d – 0.9; b · c · d – n.s.; a · b · c · d – 1.8			

LSD for: a – Apyros 75 WG dose; b– day of analysis; c – soil use; d – herbicide application method; g – herbicide applied to soil before sowing plants; l – herbicide applied to soil surface in the tillering phase of spring wheat

group of microorganisms. Noteworthy is the fact that the herbicide caused changes in the biological properties of soil even when applied in the recommended dose. This effect is implied by modifications in the counts of total copiotrophic, spore-forming oligotrophic (Table 1), ammonifying (Table 2), cellulolytic and *Pseudomonas* bacteria as well as *Actinomyces* (Table 3). The remaining groups of microorganisms (total oligotrophic, nitrogen immobilising, *Arthrobacter*, spore-forming copiotrophic bacteria and fungi) were affected by Apyros 75 WG only when it was applied in the rates 10- and 100-fold higher than the recommended dosage.

The values of the correlation coefficients indicate that the above soil microorganisms differed in their tolerance to the herbicide in soil. According to their vulnerability to the highest herbicide rate, the microorganisms can be ordered as follows: ammonifying bacteria (counts depressed by 49%) > > *Pseudomonas* (by 43%) > copiotrophic bacteria (by 42%) > oligotrophic bacteria (by 38%) > nitrogen immobilising bacteria (by 35%) > spore-forming oligotrophic bacteria (by 34%) > *Arthobacter* (by 32%) > cellulolytic bacteria (by 31%) > *Actinomyces* (by 27%) > fungi (by 5%).

Counts of soil microorganisms were determined by a degree of soil pollution with the herbicide as well as by the time it persisted in soil (Tables 1-3). In soil not sown with wheat, the average number of copiotrophic bacteria and their spores, oligotrophic, ammonifying, nitrogen immobilising bacteria, *Arthrobacter*, *Pseudomonas*, *Actinomyces* and fungi was higher on day 14 of the trials. On day 73 of the experiment, spore-forming oligotrophic and cellulolytic bacteria occurred in higher counts than earlier.

DAS et al. (2003) claim that modifications observed as a result of application of herbicides concern mainly changes in the qualitative and quantitative composition of microorganisms and biochemical activity. As a rule, when herbicides are used in compliance with the manufacturer's recommendations,

Table 2

Number of ammonifying, nitrogen immobilizing bacteria and *Arthrobacter* (in 1 kg d.m. of soil)

Herbicide dose Apyros 75 WP	Before spring wheat sowing	Day of analysis		
		after harvest		
		soil use		
		sown		unsown
		g	l	
Ammonifying bacteria (cfu 10^8)				
0	117.2 ± 3.2	169.2 ± 2.7	169.2 ± 2.7	72.4 ± 2.7
Optimum	99.6 ± 5.5	162.0 ± 6.2	150.5 ± 4.3	76.3 ± 4.3
10 x	82.4 ± 5.1	84.2 ± 6.2	140.5 ± 9.6	61.6 ± 4.8
100 x	78.5 ± 4.9	69.5 ± 4.1	139.4 ± 9.1	36.2 ± 3.5
Average	94.4 ± 1.4	121.2 ± 3.9	149.9 ± 4.9	61.6 ± 3.2
r	-0.664	-0.734	-0.571	-0.965
LSD*	a – 2.9; b – 2.0; c – 2.0; d – 2.0; a · b – 4.1; a · c – 4.1; a · d – 4.1; b · c – 2.9; b · d – 2.9; c · d – 2.9; a · b · c – 5.7; a · b · d – 5.7; a · c · d – 5.7; b · c · d – 4.0; a · b · c · d – 8.1			
Nitrogen immobilizing bacteria (cfu 10^8)				
0	70.3 ± 0.6	127.6 ± 8.4	127.6 ± 8.4	16.5 ± 1.6
Optimum	71.3 ± 5.3	138.0 ± 8.6	91.0 ± 5.0	16.5 ± 1.2
10 x	65.9 ± 5.9	68.5 ± 4.3	69.2 ± 2.2	12.5 ± 1.6
100 x	63.1 ± 6.6	62.7 ± 8.4	55.2 ± 4.8	14.0 ± 1.1
Average	67.7 ± 1.3	99.2 ± 2.8	85.8 ± 3.3	14.9 ± 1.1
r	-0.846	-0.690	-0.702	-0.393
LSD*	a – 2.9; b – 2.0; c – 2.0; d – 2.0; a · b – 4.1; a · c – 4.1; a · d – 4.1; b · c – 2.9; b · d – 2.9; c · d – 2.9; a · b · c – 5.8; a · b · d – 5.8; a · c · d – 5.8; b · c · d – 4.1; a · b · c · d – 8.1			
Arthrobacter spp. (cfu 10^9)				
0	48.2 ± 0.8	22.2 ± 2.0	22.2 ± 2.0	8.4 ± 0.3
Optimum	48.0 ± 1.6	21.1 ± 1.4	28.9 ± 1.1	9.7 ± 0.5
10 x	26.3 ± 0.9	22.6 ± 1.4	26.7 ± 1.9	9.3 ± 0.8
100 x	23.7 ± 1.9	22.0 ± 1.6	24.4 ± 2.4	8.4 ± 0.8
Average	36.6 ± 0.4	22.0 ± 0.7	25.5 ± 0.5	9.0 ± 0.3
r	-0.711	0.107	-0.244	-0.534
LSD*	a – 0.8; b – 0.6; c – 0.6; d – 0.6; a · b – 1.1; a · c – n.s.; a · d – n.s.; b · c – 0.8; b · d – 0.8; c · d – 0.8; a · b · c – n.s.; a · b · d – n.s.; a · c · d – 1.6; b · c · d – 1.1; a · b · c · d – 2.2			

* explanations under Table 1

Table 3

Number of *Pseudomonas* and cellulolytic bacteria, *Actinomyces* and fungi
(in 1 kg d.m. of soil)

Herbicide dose Apyros 75 WP	Before spring wheat sowing	Day of analysis		
		after harvest		
		soil use		
		sown		unsown
		g	l	
Pseudomonas spp. (cfu 10^9)				
0	28.1 ± 1.6	28.5 ± 1.4	28.5 ± 1.4	23.3 ± 1.4
Optimum	26.0 ± 0.8	33.2 ± 2.2	27.2 ± 1.6	15.2 ± 0.3
10 x	24.7 ± 1.6	28.5 ± 1.4	19.9 ± 0.5	15.6 ± 0.5
100 x	18.6 ± 1.2	14.7 ± 0.8	16.3 ± 0.8	11.8 ± 1.9
Average	24.4 ± 0.7	26.2 ± 0.9	23.0 ± 0.7	16.5 ± 0.5
r	-0.961	-0.968	-0.818	-0.677
LSD*	a – 0.8; b – 0.5; c – 0.5; d – 0.5; a · b – 1.1; a · c – 1.1; a · d – 1.1; b · c – 0.8; b · d – 0.8; c · d – 0.8; a · b · c – 1.5; a · b · d – 1.5; a · c · d – 1.5; b · c · d – 1.1; a · b · c · d – 2.2			
Cellulolytic bacteria (cfu 10^6)				
0	11.8 ± 1.1	39.8 ± 2.8	39.8 ± 2.8	84.9 ± 1.1
Optimum	11.8 ± 1.1	33.0 ± 2.7	39.4 ± 2.2	48.4 ± 1.9
10 x	10.8 ± 1.1	21.5 ± 2.2	17.6 ± 0.6	50.2 ± 1.2
100 x	5.7 ± 0.6	18.6 ± 1.2	13.3 ± 0.6	41.9 ± 2.8
Average	10.0 ± 0.4	28.2 ± 1.2	27.5 ± 0.2	56.4 ± 1.1
r	-0.997	-0.712	-0.740	-0.538
LSD*	a – 0.9; b – 0.6; c – 0.6; d – n.s.; a · b – 1.3; a · c – 1.3; a · d – 1.3; b · c - n.s.; b · d – 0.9; c · d – n.s.; a · b · c – 1.8; a · b · d – 1.8; a · c · d – 1.8; b · c · d – 1.3; a · b · c · d – 2.6			
Actinomyces (cfu 10^8)				
0	52.3 ± 3.8	112.2 ± 5.5	112.2 ± 5.5	29.7 ± 1.6
Optimum	41.9 ± 2.8	91.0 ± 4.8	100.0 ± 2.8	34.4 ± 1.9
10 x	39.4 ± 2.5	78.1 ± 2.2	90.7 ± 3.3	33.3 ± 3.2
100 x	41.2 ± 0.6	68.8 ± 4.9	68.8 ± 7.1	31.2 ± 1.1
Average	43.7 ± 1.1	87.5 ± 1.2	92.9 ± 1.6	32.2 ± 1.3
r	-0.349	-0.720	-0.912	-0.278
LSD*	a – 6.5; b – 4.6; c – 4.6; d – n.s.; a · b – 9.2; a · c – n.s.; a · d – n.s.; b · c – 6.5; b · d – 6.5; c · d – 6.5; a · b · c – n.s.; a · b · d – n.s.; a · c · d – n.s.; b · c · d – 9.2; a · b · c · d – 18.4			

Fungi (cfu 10^6)				
0	43.4 ± 5.9	17.9 ± 0.6	17.9 ± 0.6	23.7 ± 1.9
Optimum	45.9 ± 2.7	17.6 ± 2.2	17.2 ± 1.1	26.9 ± 3.1
10 x	45.9 ± 5.1	15.8 ± 1.6	15.4 ± 0.6	18.6 ± 1.6
100 x	46.2 ± 3.2	15.8 ± 0.6	13.3 ± 0.6	18.6 ± 1.6
Average	45.3 ± 1.8	16.8 ± 0.7	15.9 ± 0.3	22.0 ± 0.6
r	0.497	-0.646	-0.904	-0.612
LSD*	a – n.s.; b – 1.8; c – 1.8; d – n.s.; a · b – 3.6; a · c – 3.6; a · d – n.s.; b · c – 2.5; b · d – 2.5; c · d – 2.5; a · b · c – n.s.; a · b · d – n.s.; a · c · d – n.s.; b · c · d – 3.6; a · b · c · d – 7.2			

* explanations under Table 1

they do not produce any significant influence on the counts of microorganisms or the activity of soil enzymes (WYSZKOWSKA 2002a, WYSZKOWSKA 2004). However, when misused, herbicides can disturb the biological balance of soil, with the disorders being ever more severe as herbicides are resistant to microbial decompositions (BERGER 1998, JOHNSEN et al. 2001, SŘRENSEN et al. 2003, WYSZKOWSKA 2002b, WYSZKOWSKA, KUCHARSKI 2004). This observation has not been fully verified in our study on Apyros 75 WG, which, when applied according to the manufacturer's recommendations (8.9 μm kg^{-1}), depressed significantly counts of 6 out of 11 analysed groups of microorganisms. This means that the herbicide should be applied with great caution. ARAÚJO et al. (2003) proved that soil pollution with glyphosate increased populations of fungi and *Actinomyces* while depressing counts of bacteria. In the authors' own study it has been discovered that Apyros 75 WG had a stimulating effect only on fungi and only in the objects treated with the optimum dose of the herbicide. In contrast, Apyros 75 WG had a negative effect on *Actinomyces* in all variants of the experiment.

The results of our study prove that the biological activity of soil is conditioned not only by microbial counts but also by the activity of soil enzymes. The herbicide modified the activity of all the analysed soil enzymes (Table 4). The influence of this biocide on the soil's enzymatic activity depended on all the variable factors tested in the experiment. In general, Apyros 74 WG had an inactivating effect on dehydrogenases, urease, acid and alkaline phosphatases. The activity of dehydrogenases, urease, acid and alkaline phosphatases, both in soil analysed before sowing spring wheat and after the harvest, was significantly negatively correlated with the concentration of the herbicide, which is confirmed by Pearson's simple regression coefficients between doses of Apyros 74 WG and the enzymatic activity of soil. Dehdyrogenases and urease proved to be the least tolerant to the herbicide. The highest dose of Apyros 74 WG (100-fold higher than recommended by the manufacturer) depressed the activity of dehydrogenases in soil

Table 4

Activity of enzymes (in 1 kg d.m. of soil)

Herbicide dose Apyros 75 WP	Before spring wheat sowing	Day of analysis		
		after harvest		
		soil use		
		sown		unsown
		g	l	
Dehydrogenases (cm^3 H_2 · d^{-1})				
0	5.34 ± 0.05	14.24 ± 0.19	14.24 ± 0.19	1.35 ± 0.10
Optimum	5.24 ± 0.05	10.87 ± 0.10	8.37 ± 0.10	1.01 ± 0.05
10 x	5.24 ± 0.05	5.29 ± 0.10	2.65 ± 0.05	0.53 ± 0.05
100 x	4.66 ± 0.05	1.88 ± 0.05	2.16 ± 0.05	0.43 ± 0.05
Average	5.12 ± 0.05	8.07 ± 0.12	6.85 ± 0.07	0.83 ± 0.02
r	5.34	14.24	14.24	0.43
LSD*	a – 0.04; b – 0.03; c – 0.03; d – 0.03; a · b – 0.05; a · c – 0.05; a · d – 0.05; b · c – 0.04; b · d – 0.04; c · d – 0.04; a · b · c – 0.07; a · b · d – 0.07; a · c · d – 0.07; b · c · d – 0.05; a · b · c · d – 0.11			
Urease (mg N-NH_4 · h^{-1})				
0	11.52 ± 0.48	10.08 ± 0.48	10.08 ± 0.48	2.16 ± 0.24
Optimum	9.60 ± 0.48	8.40 ± 0.24	9.36 ± 0.24	2.64 ± 0.24
10 x	11.28 ± 0.24	7.44 ± 0.24	7.44 ± 0.24	1.68 ± 0.24
100 x	10.80 ± 0.24	2.16 ± 0.24	7.44 ± 0.24	0.72 ± 0.24
Average	10.80 ± 0.17	7.02 ± 0.25	8.58 ± 0.25	1.80 ± 0.10
r	0.029	-0.968	-0.637	-0.911
LSD*	a – 0.20; b – 0.14; c – 0.14; d – 0.14; a · b – 0.28; a · c – 0.28; a · d – 0.28; b · c – 0.20; b · d – 0.20; c · d – 0.20; a · b · c – 0.39; a · b · d – 0.39; a · c · d – 0.39; b · c · d – 0.28; a · b · c · d – 0.56			
Acid phosphatase (mmol PNP · h^{-1})				
0	1.85 ± 0.07	3.28 ± 0.07	3.28 ± 0.07	2.02 ± 0.03
Optimum	1.95 ± 0.03	3.06 ± 0.02	3.25 ± 0.09	2.14 ±0.02
10 x	1.93 ± 0.02	2.62 ± 0.02	3.08 ± 0.07	2.09 ± 0.03
100 x	1.91 ± 0.01	2.24 ± 0.02	2.92 ± 0.02	1.90 ± 0.02
Average	1.91 ± 0.04	2.80 ± 0.03	3.13 ± 0.07	2.03 ± 0.02
r	0.104	-0.853	-0.888	-0.869
LSD*	a – 0.02; b – 0.01; c – 0.01; d – 0.01; a · b – 0.03; a · c – 0.03; a · d – 0.03; b · c – 0.02; b · d – 0.02; c · d – 0.02; a b · c – 0.04; a · b · d – 0.04; a · c · d – 0.04; b · c · d – 0.03; a · b · c · d – 0.06			

Alkaline phosphatase (mmol PNP· h^{-1})				
0	0.66 ± 0.01	0.59 ± 0.01	0.59 ± 0.01	0.31 ± 0.02
Optimum	0.63 ± 0.02	0.66 ± 0.01	0.57 ± 0.03	0.28 ± 0.01
10 x	0.62 ± 0.03	0.52 ± 0.01	0.52 ± 0.01	0.25 ± 0.01
100 x	0.58 ± 0.03	0.43 ± 0.02	0.49 ± 0.02	0.21 ± 0.02
Average	0.62 ± 0.01	0.55 ± 0.01	0.54 ± 0.01	0.26 ± 0.02
r	-0.919	-0.863	-0.840	-0.863
LSD*	a – 0.01; b – 0.01; c – 0.01; d – 0.01; a · b – 0.02; a · c – 0.02; a · d – 0.02; b · c – 0.01; b · d – 0.01; c · d – 0.01; a · b · c – 0.03; a · b · d – 0.03; a · c · d – 0.03; b · c · d – 0.02; a · b · c · d – 0.04			

* explanations under Table 1

prior to wheat sowing by 12.6% and after the harvest by up to 86.8% in the variant where the herbicide was applied to soil (mixed with the whole mass of soil in a pot) and by 84.8% in the series where it was applied top dressing during the tillering phase. The herbicide produced the strongest effect on the activity of urease in soil under spring wheat (78.6% decrease) and unsown soil (66.7% decrease) when it was mixed with a whole portion of soil to fill up one pot.

Apyros 75 WG also had an adverse effect on acid and alkaline phosphatases. Its negative influence on these enzymes was particularly evident after the harvest in the objects where it was applied to soil. There, the activity of acid phosphatase under the effect of the highest herbicide rate was 31.8% lower. An analogous decrease in the activity of alkaline phosphatase was 27.5%.

More severe disturbance of the biochemical balance in soil occurred in the objects in which Apyros 75 WG was applied by mixing with the whole mass of soil to fill up one pot than in the variants where top dressing application was performed. In the former case, the optimum dose of the herbicide decreased the activity of dehydrogenases by 23.6%, urease by 16.7%, alkaline phosphatase by 11.6% and acid phosphatase by 6.8%. In the latter series, the analogous percentages were 41.2, 7.1, 2.9 and 1.0.

With respect to the next variable, i.e. the time period over which the herbicide was present in soil, it has been found that the average activity of dehydrogenases and urease was higher on day 14 of the experiment (before sowing spring wheat) whereas the activity of acid phosphatase and alkaline phosphatase was higher on day 73 of the tests (in the series without wheat). The largest differences occurred for dehydrogenases and urease, whose activity before wheat sowing was, respectively, 6.2- and 6.0-fold higher after the harvest. Increased activity of these enzymes can be attributed to a close relationship between the number of live bacterial cells in soil and the rate of biological decomposition of sulfosulfrone. This conclusion is drawn on the basis of the counts of microorganisms, where up to 80% of the analysed groups of microorganisms were more numerous on day 14 of the experiment.

Negative influence on excessive doses of herbicides on the activity of soil enzymes find confirmation in several reports (MICHALCEWICZ 2004, WYSZKOWSKA 2002a, WYSZKOWSKA, KUCHARSKI 2004, SUKUL 2006, YAO et al. 2006). Significant depression in counts of soil microorganisms and in the soil's enzymatic activity which occurred under the effect of excessively high rates of Apyros 75 WG could have been caused by the fact that herbicide decomposition products can be more toxic than the initial compound (ACCINELEI et al. 2005, WALKER et al. 2001). Such products permeate through plant tissues and destroy cellular structures, causing disorders in metabolism of cells (JOHNSEN et al. 2001, SŘRENSEN et al. 2003). Negative effects produced by Apyros 75 WG on the soil's biological life could also be associated with an indirect influence it exerted on soil organisms by modifying soil's physicochemical properties (Table 5), especially by increasing the soil's acidity.

Table 5

Some properties of soil contaminated with herbicide Apyros 75 WG

Herbicide dose Apyros 75 WP	(g·C kg^{-1} of soil)	pH (1 mol KCl dm^{-3})	Hh	S	T	V (%)
			(mmol(+) kg^{-1} of soil)			
0	5.2 ± 0.1	5.8 ± 0.1	11.8 ± 0.3	54.7 ± 0.7	66.4 ± 0.5	82.3 ± 0.3
Optimum	5.3 ± 0.1	5.7 ± 0.1	12.0 ± 0.3	54.8 ± 0.8	66.8 ± 1.0	82.1 ± 0.3
10 x	5.1 ± 0.1	5.6 ± 0.1	12.7 ± 0.3	54.7 ± 1.3	67.4 ± 1.4	81.2 ± 0.5
100 x	5.2 ± 0.1	5.5 ± 0.1	12.5 ± 0.3	54.1 ± 1.6	66.6 ± 1.7	81.2 ± 0.5
Average	-0.380	-0.995	0.792	-0.892	0.123	-0.880
LSD*	n.s.	0.02	0.3	1.2	1.5	0.4

C – organic carbon content, Hh – hydrolytic acidity, S – total base exchangeable cations, T – total sorptive capacity, V – saturation with base cations, n.s. – non-significant

Furthermore, the unfavourable effect of excessive amounts of pesticides is not restricted to changes in counts of microorganisms or activity of enzymes. Plant protection preparations can also lead to disorders in the growth and development of crops (WYSZKOWSKA, KUCHARSKI 2004, MARTINS et al. 2007). The present study shows that plants can be a good indicator of changes occurring in microbiological and biochemical properties of soil as the test spring wheat proved to be susceptible to high concentrations of Apyros 75 WG (Figure 1). The extent of its toxic impact depended on its concentration in soil and method of application. Although producers of pesticides and herbicides claim that Apyros 75 WG is a selective herbicide producing a system-

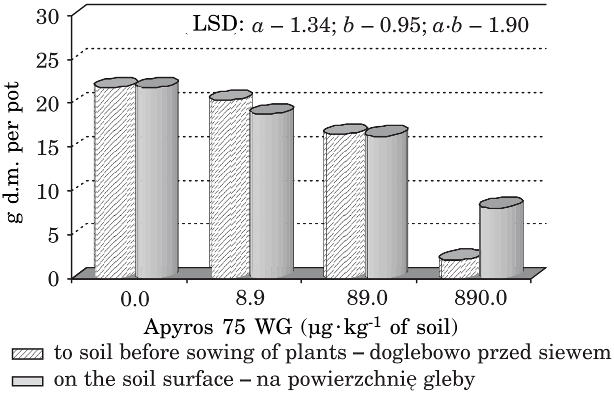

Fig. 1. Spring wheat yield (g d.m. per pot)

ic effect, taken up by leaves and roots of plants and easily transported within whole plants, and that crops are able to easily decompose this herbicide, our tests showed that only optimum doses of Apyros 74 WG, recommended by the manufacturer, did not produce a significant negative effect on the growth and development of wheat plants. Higher rates of this herbicide had a toxic effect on plants, regardless the method of application. Toxic symptoms, such as the browning of leaf blades, chlorosis of new leaves, lost of turgor and, in extreme cases, necrosis of plants, were clearly observable in the objects treated with the highest rate of the herbicide (100-fold above the recommended dosage). When such high doses of Apyros were mixed with soil, necrosis of wheat seedlings occurred in some cases. Phytotoxicity of herbicides has also been indicated by other researchers (URBAN 2000, MARTINS et al. 2007, SUKUL 2006).

CONCLUSIONS

1. Apyros 75 WG disturbs soil's homeostasis as it as it disrupts multiplication of some microbial groups, inhibits the activity of soil enzymes and depresses the yield of spring wheat, even if applied in a recommended dose.

2. Among the soil enzymes, dehydrogenases and urease were the least tolerant to the effect of the herbicide, whereas alkaline phosphatase proved to be the most tolerant one.

3. The tolerance of the microorganisms to the effect produced by the herbicide Apyros 75 WG was as follows: ammonifying bacteria > *Pseudomonas* > copiotrophic bacteria > oligotrophic bacteria > nitrogen immobilising bacteria > spore-forming oligotrophic bacteria > *Arthobacter* > cellulolytic bacteria > *Actinomyces* > fungi.

4. Counts of microorganisms and activity of enzymes are beneficially affected by growing spring wheat on the herbicide polluted soil.

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THE LEVELS OF Ca, Mg AND PHYTATE PHOSPHORUS PRESENT IN SOME X TRITICOSECALE WITTMACK WITH AEGILOPS SP. HYBRIDS AND THEIR TRITICALE PARENTAL FORMS TRITICALE PARENTAL FORMS

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Abstract

The investigations aimed at the determination of total and phytate phosphorus content as well as concentration of Ca and Mg bound in the phytic acid complexes in hybrid kernels of triticale forms with *Aegilops* sp. and of triticale parental components. The research objects consisted of kernels of 4 strains, of which 2 had *Aegilops* sp. as a maternal form and triticale: *Ae. crassa* 4x x(Panda x Dańkowskie Złote) and *Ae. juvenalis* 6x x [(Lanca x L 506/79)x CZR 142/79] as a paternal form, whereas 2 other strains were obtained by reciprocal crossbreeding:[(Jana x Tempo) x Jana]x *Ae. juvenalis* 6x. The highest total phosphorus content occurred in triticale kernels of (Jana × Tempo) × Jana as well as in two hybrid strains created on the basis of this form (strains No 6 and 7); higher level was found in a strain of the previous generation. The percentage of phytic acid phosphorus in the total phosphorus contained in the kernels varied from 32.8 up to 69.4%. Among the parental components we compared, triticale (Lanca x L 506/79) x CZR 142/79 was characterized by the highest phytate phosphorus percentage in the total phosphorus, which was not confirmed in the hybrid kernels. The phytate Mg share in kernels of the hybrid strains appeared higher compared to the parental components, except no 5 cross combination *Ae. juvenalis* 6x x [(Lanca x L 506/79)x CZR 142/79]. As for Ca, the kernels of the strains were characterized by a lower content of this element in the phytate complexes compared with the parental forms. Among the strains analyzed, no 4, a cross-combination of *Ae. Cras-*

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sa 4x × (Panda × Dańkowskie Złote) and No 6 – [(Jana × Tempo) × Jana] × *Ae. Juvenalis* 6x, deserves special attention as its kernels contained higher Ca and Mg content, lower phytate phosphorus level and more advantageous Ca/P tot and Mg/P tot ratios.

Key words: X *Triticosecale* Wittmack, X *Triticosecale* Wittmack with *Aegilops* sp. hybrids, phytate phosphorus, phytate complexes calcium and magnesium.

POZIOM Ca, Mg I FOSFORU FITYNOWEGO W WYBRANYCH MIESZAŃCACH PSZENŻYTA Z KOZIEŃCAMI I ICH PSZENŻYTNICH FORMACH RODZICIELSKICH

Abstrakt

W badaniach określono zawartość fosforu ogólnego, fitynowego oraz poziom Ca i Mg związanego w kompleksach fitynowych w ziarniakach mieszańcowych form pszenżyta z kozieńcami oraz pszenżytnich komponentów rodzicielskich. Obiektem badań były ziarniaki 4 rodów, w tym 2, w których formą mateczną był kozieniec, a ojcowską pszenżyto: *Ae. crassa* 4x x (Panda x Dańkowskie Złote) i *Ae. juvenalis* 6x x [(Lanca x L 506/79)x CZR 142/79] oraz 2 rody otrzymane w wyniku krzyżowania odwrotnego: [(Jana x Tempo)x Jana]x *Ae. juvenalis* 6x. Największą zawartość fosforu ogólnego stwierdzono w ziarniakach pszenżyta (Jana x Tempo) x Jana i dwóch rodach mieszańcowych powstałych w oparciu o tę formę (rodach nr 6 i 7); wyższy poziom wykazano w rodzie wcześniejszej generacji. Udział fosforu fitynowego w całkowitym fosforze zawartym w badanych ziarniakach był zróżnicowany od 32,8 do 69,4%. Z porównywanych komponentów rodzicielskich pszenżyto (Lanca x L 506/79)x CZR 142/79 odznaczało się największym udziałem fosforu fitynowego w odniesieniu do całkowitego, co nie znalazło potwierdzenia w ziarniakach mieszańcowych. Udział Mg fitynowego w ziarniakach badanych rodów mieszańcowych był większy w porównaniu z komponentami rodzicielskimi, z wyjątkiem nr 5 kombinacji krzyżówkowej *Ae. juvenalis* 6x x [(Lanca x L 506/79)x CZR 142/79]. W ziarniakach badanych rodów, w porównaniu z formami rodzicielskimi, stwierdzono mniejszy udział Ca w kompleksach fitynowych. Spośród analizowanych rodów na uwagę zasługują nr 4 kombinacji krzyżówkowej *Ae. crassa* 4x x (Panda x Dańkowskie Złote) i nr 6 [(Jana x Tempo)x Jana]x *Ae. juvenalis* 6x], w których ziarnie stwierdzono większą zawartość Ca i Mg oraz mniejszy udział fosforu fitynowego, a także korzystniejsze wskaźniki Ca/P całkowitego i Mg/P całkowitego.

Słowa kluczowe: pszenżyto, mieszańce pszenżyta z kozieńcami, fosfor fitynowy, kompleksy fitynowe wapnia i magnezu.

INTRODUCTION

In cereal grains and seeds of leguminous crops or oil plants, 2/3 of total phosphorus is made up of phytates, (phytic acid salts) (myo-inositol hexakisphosphate IP6), which are accumulated chiefly in the aleuron grains and globoids to be utilized by plants during germination as a source of phosphorus and some other elements, such as Mg, K, Ca, Mn, Fe and Zn (HARALD, MORIS 1995, TROSZYŃSKA et al. 1992).

Phytate complexes are poorly available to monogastric animals and possess several antimetabolic properties. Owing to its chemical structure, phytic acid demonstrates high affinity for polyvalent cations. Being a potent chelator of macro- and microelements, this acid substantially reduces the availability of nutrients to monogastric animals, thus effectively limiting their resorption from the digestive tract (HURRELL 2003, LOPEZ et al. 2000). Phytic acid strongly binds to important mineral nutrients such as iron and zinc, forming salts which are largely excreted. Phytic acid-derived P in animal waste can contribute to water pollution, a significant problem in the world (SHARPLEY et al. 1994). Because phytic acid is a chelator of various chemical components and inhibits some digestive enzymes, it lowers the availability of protein, starch and minerals essential for the proper functioning of the animal organism (HARALD, MORIS 1995, HEINDL 2000, HURRELL 2003). With respect to poultry and swine production, since much of grain P is phytic acid P and as such it is excreted, in order to provide for animals' nutritional requirement for P and sustain optimal productivity, animal feeds must be supplemented with either an available form of P or the enzyme phytase (HEINDL 2000, RUTHERFURD et al. 2004, TROSZYŃSKA et al. 1992). As one possible approach to solving the problems associated with seed-derived dietary phytic acid, the U.S. Department of Agriculture and other institutions have isolated cereal and legume low-phytic acid mutations and have used them to breed first-generation low-phytate hybrids, cultivars and lines. Seed phytic acid is reduced in these crops by 50-95% (LARSON et al. 2000, RABOY et al. 2000, RABOY 2002).

The genetic breeding research conducted on triticale hybrids with *Aegilops* sp. aim at obtaining genotypes of a far wider economic importance than the initial forms. Some new forms developed through transfer of beneficial genes from *Aegilops* sp. to triticale and selection may improve yield components, technological value, plant resistance to take-all and root diseases, rusts and powdery mildew as well as their adaptability to soil acidity (ACHREMOWICZ et al. 2002, ARSENIUK et al.1998, GRUSZECKA et al. 1996a,b, GRUSZECKA et al. 2004, MASŁOWSKI et al. 1997, STRZEMBICKA, GRUSZECKA 1997).

Introduction of new triticale forms to crop production requires performing qualitative analyses of grains, including the content of phytate phosphorus and phytates, the compounds considered as antinutritional ones.

The objective of the present research was to determine the phytic acid content and the concentrations of Ca, Mg bound in the phytate complexes in relation to the total content of these minerals in hybrid kernels of the triticale strains with *Aegilops* sp. and their triticale parental components.

MATERIAL AND METHODS

The research material comprised kernels of 4 hybrid strains obtained through distant or wide hybridization of triticale with *Aegilops* sp. and their triticale parental components. The experiments were performed on two strains of cross combinations *Ae. crassa* 4x x (Panda x Dańkowskie Złote) (No 4) and *Ae. juvenalis* 6x [(Lanca x L 506/79) x CZR 142/79] (No 5) with *Aegilops* as a maternal form, and triticale as a paternal one, and two other strains of different generations of a combination [(Jana x Tempo) x Jana] x *Ae. juvenalis* 6x (nos 6 and 7) obtained through a reciprocal cross (Table 1). The hybrids strains were developed by two- or threefold back pollination of F1 hybrids with respective triticale parental form (B_2, B_3 or B_4) to be afterwards four- or fivefold reproduced by self-pollination (F_4 or F_5, respectively). In the subsequent generations, positive selection was performed including resistance to pathogens and environmental stressors followed by negative selection regarding some unfavorable traits of wild-*Aegilops* forms.

Table 1

Research material

Sample No	Forms	Generation
	triticale	
1	Panda x Da□kowskie Z□ote	-
2	(Lanca x L 506/79) x CZR 142/79	-
3	(Jana x Tempo) x Jana triticale hybrids with *Aegilops* sp:	-
4	*Ae. crassa* 4x x (Panda x Da□kowskie Z□ote)	B_4/F_5
5	*Ae. juvenalis* 6x x [(Lanca x L 506/79) x CZR 142/79]	B_4/F_5
6	[(Jana x Tempo) x Jana] x *Ae. juvenalis* 6x [1]	B_2/F_4
7	[(Jana x Tempo) x Jana] x *Ae. juvenalis* 6x [2]	B_3/F_5

After the selection, the kernels were examined for the total phosphorus content by the spectrophotometric method (λ=365 nm) (Shimadzu Corp. 160 A) after the phosphorus and ammonium molybdate complex color had been developed in the presence of sulfuric acid.

For determination of phytic acid, the samples were extracted with trichloroacetic acid (TCA) and prepared for determination of the Fe content according to the method of LATTA, ESKIN (1980).

The level of minerals (Mg, Ca) in phytates complexes after extraction with trichloro acetic acid was determined by the atomic absorption spectrometry (AAS) method (Unicam 939).

All the data were expressed on the dry weight basis.

RESULTS AND DISCUSSION

Our previous studies on triticale hybrids with *Aegilops* had demonstrated that an exogenous phytase additive to the investigated triticale forms, under *in vitro* conditions, increased a level of phosphorus released from the phytate complexes. The most favourable dephosphorilation effects were obtained during 5-hour incubation at pH 3 (MAKARSKA, GRUSZECKA 2001).

In the present research, the highest total phosphorus content was determined in the kernels of triticale (Jana x Tempo)x Jana and two hybrid strains (No 6 and 7) formed on the basis of this triticale. It is noteworthy that a higher phosphorus concentration was recorded in strain no 6 of the previous generation as compared to strain no 7 (Table 2).

Table 2

Content of total and phytate phosphorus in kernels of triticale hybrids with *Aegilops* sp. and their triticale parental forms

Sample No	Forms	Phosphorus total (%)	Phosphorus phytate (mg·g-1)	Phosphorus phytate/ /Phosphorus total (%)
	triticale			
1	Panda x Dańkowskie Złote	0.287±0.004	1.429±0.012	49.79
2	(Lanca x L 506/79) x CZR 142/79	0.234±0.008	1.396±0.009	59.65
3	(Jana x Tempo) x Jana triticale hybrids with *Aegilops* sp	0.377±0.008	1.336±0.005	35.43
4	*Ae. crassa* 4x x (Panda x Dańkowskie Złote)	0.217±0.010	1.507±0.011	69.44
5	*Ae. juvenalis* 6x x [(Lanca x L 506/79) x CZR 142/79]	0.358±0.007	1.512±0.007	42.23
6	[(Jana x Tempo) x Jana] x *Ae. juvenalis* 6x [1]	0.375±0.005	1.232±0.005	32.85
7	[(Jana x Tempo) x Jana] x *Ae. juvenalis* 6x [2]	0.360±0.004	1.468±0.003	40.78

The content of phytate in the grain of the test triticale strains ranged from 1.232 $mg \cdot g^{-1}$ (sample 6) to 1.512 $mg \cdot g^{-1}$ (sample 5). In comparison with phytate in wheat grain, these results were nearly fivefold lower (HERNANDEZ et al. 2003).

The percentage of phytate phosphorus in total phosphorus varied in kernels of the test forms from 32.85 up to 69.44% (Table 2). The values were lower or close to those reported by MATYKA et al. (1993), who studied numerous triticale cultivars. Among the parental components we compared, Lanca x L 506/79) x CZR 142/79 triticale distinguished itself by the highest phytate phosphorus content in total phosphorus. However, this result was not confirmed for the hybrid strain kernels. In hybrid strains nos 6 and 7, the phytate phosphorus percentage was close to a level recorded in the triticale parental form. It should be emphasized that a higher phytate content was determined in the kernels of the later generation (strains nos 6 and 7), whereas in grain of strain no 4 (*Ae. crassa* 4x x (Panda x Dańkowskie Złote) a substantially higher phytate phosphorus level was noticed versus the parental form: Panda x Dańkowskie Złote.

Evaluation of the mineral components in the examined triticale hybrid strains, presented in our previous paper, indicated a beneficial influence of the wild forms of *Ae. crassa* 4x and *Ae. juvenalis* 6x on their level and univalent/divalent cations ratio shown in the hybrid strains (MAKARSKA, GRUSZECKA 2003).

Generally, the phytate Mg content in kernels of the strains researched in this work proved higher as compared to the respective triticale parental components (except strain no 5) – Table 3, while the Ca concentration was usually lower in the phytate complexes of the strains investigated than in their triticale parental components (except strain no 5).

Among the strains analyzed, attention should be given to strains nos 4 and 6, whose grains contained more Ca and Mg but less phytate phosphorus and had superior indices of Ca/P total and Mg/P total compared to the respective triticale parental forms (Tables 3 and 4).

Table 3

Content of Ca and Mg in phytate complexes in kernels of triticale hybrids with *Aegilops* sp. and their triticale parental forms

Sample No	Ca ($g \cdot kg^{-1}$)	Phytate Ca ($mg \cdot g^{-1}$)	Phytate Ca /Total Ca (%)	Mg ($g \cdot kg^{-1}$)	Phytate Mg ($mg \cdot g^{-1}$)	Phytate Mg /Total Mg (%)
1	0.22	0.0254	11.54	1.32	34.04	13.33
2	0.24	0.0258	10.75	1.42	42.59	15.35
3	0.27	0.0259	9.59	1.60	31.70	10.25
4	0.25	0.0219	8.75	1.41	41.17	14.96
5	*0.24*	0.0252	9.00	1.49	33.84	11.74
6	0.31	0.0283	9.13	1.74	35.37	10.49
7	0.25	0.0248	9.93	1.50	35.87	12.33

Table 4

Percentage of Ca and Mg to total and inorganic phosphorus in kernels of triticale with *Aegilops* sp. hybrids and their triticale parental forms

Sample No	P inorganic/P total (%)	Ca/P total	Ca/P inorganic	Mg/ P total	Mg/P inorganic
1	50.17	0.07	0.15	0.46	0.92
2	40.17	0.10	0.26	0.61	1.51
3	64.46	0.07	0.19	0.42	0.66
4	30.41	0.12	0.38	0.65	2.13
5	57.82	0.07	0.12	0.42	0.72
6	59.73	0.08	0.14	0.46	0.78
7	59.17	0.07	0.12	0.42	0.70

CONCLUSION

1. Strains of triticale with *Aegilops* sp. hybrids, as compared to parental components, were usually characterized by increased Ca and Mg content in grain as well as lower Ca and higher Mg concentration in phytate complexes.

2. Kernels of cross combinations between *Ae. crassa* 4x x (Panda x Dańkowskie Złote) [B_4/F_5] and [(Jana x Tempo)x Jana] x *Ae. juvenalis* 6x [B_2/F_4] distinguished themselves by a higher Ca and Mg content alongside lower phytate phosphorus and more favourable Ca/P total and Mg/P total ratio.

3. Distant or wide hybridization of triticale and *Aegilops* sp.may yield hybrids of more beneficial nutritional parameters compared to their triticale parental components.

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SOIL CONCENTRATION OF C AND N SHAPED BY LONG-TERM UNIDIRECTIONAL FERTILIZATION VERSUS NOXIOUS SOIL MACROFAUNA

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Abstract

Unidirectional fertilization, if applied for many years, has a strong and sometimes negative effect on soil and natural environment. Such fertilization can cause unfavourable processes including humus degradation, the leaching of nutrients (mainly nitrogen), soil erosion as well as accumulation of weed seeds, pathogens and plant pests. In the last ten years threats caused to agricultural habitats by noxious soil macrofauna, particularly polyphagous insects representing *Agrotinae, Elateridae*, *Scarabaeidae* and *Tipilidae*, have become more explicit. Long-term unidirectional organic or mineral fertilization applied in a long-term static experiment established in 1972 on grey-brown pozdolic soil caused elevated concentrations of organic carbon and total nitrogen in soil. The highest increase was observed when farmyard manure had been used. Unidirectional application of organic fertilizers in rates balanced for the amount of nitrogen added to soil (rate I of liquid manure and FYM), when carried out for many years, caused a significant increase in the density of soil macrofauna. Fertilization with liquid manure balanced with FYM in terms of organic carbon added to soil as well as with mineral fertilizers did not favour presence of macrofauna.

Key words: fertilization, soil macrofauna, polyphages, *Agrotinae*, *Elateridae, Scarabaeidae, Tipulidae.*

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ZAWARTOŚĆ C ORAZ N W GLEBIE UKSZTAŁTOWANA JEDNOSTRONNYM WIELOLETNIM NAWOŻENIEM A SZKODLIWA MAKROFAUNA GLEBOWA

Abstrakt

Jednostronne nawożenie stosowane przez wiele lat ma znaczący, nie zawsze pozytywny, wpływ na środowisko glebowe, a także przyrodnicze. W wyniku takiego systemu nawożenia mogą zachodzić w glebie procesy polegające na degradacji próchnicy, wymywaniu składników pokarmowych, głównie azotu, erozji oraz nagromadzaniu się nasion chwastów, patogenów i szkodników. W ostatnim dziesięcioleciu szczególnie uwidoczniły się w agrocenozach zagrożenia ze strony szkodliwej makrofauny glebowej, zwłaszcza polifagów reprezentujących *Agrotinae*, *Elateridae, Scarabaeidae* i *Tipulidae.* Wieloletnie jednostronne nawożenie organiczne i mineralne stosowane w doświadczeniu statycznym założonym w 1972 r. na glebie płowej spowodowało wzrost zawartości węgla organicznego i azotu ogólnego w glebie, najwyższy w przypadku stosowania obornika. Stwierdzono istotny dodatni wpływ zawartości węgla organicznego oraz dodatni wpływ azotu ogółem na zagęszczenie potencjalnie szkodliwej makrofauny glebowej.

Słowa kluczowe: nawożenie, makrofauna glebowa, polifagi, *Agrotinae*, *Elateridae, Scarabaeidae, Tipulidae.*

INTRODUCTION

At present there are two types of farms that dominate in Polish agriculture. One group consists of plant production farms, where mainly mineral fertilization is applied. The other group of farms is engaged in animal production and uses high organic fertilization in the form of natural fertilizers (liquid manure, farmyard manure).

Unidirectional fertilization, if carried out for many years, has a strong and sometimes negative effect on soil and natural environment. Such fertilization can cause unfavourable processes including humus degradation, the leaching of nutrients (mainly nitrogen), soil erosion as well as accumulation of weed seeds, pathogens and plant pests.

In the last ten years threats caused to agricultural habitats by noxious soil macrofauna, particularly polyphagous insects representing *Agrotinae, Elateridae*, *Scarabaeidae* and *Tipilidae* (MRÓWCZYŃSKI, SOBKOWIAK 1999, WALCZAK, JAKUBOWSKA 2001, KOWALSKA, WIERZBOWSKI 2002, SĄDEJ et al. 2003, TREPASHKO, PURANOK 2006) have become more explicit. Species which belong to *Elateridae* and *Scarabaeidae* are especially dangerous due to their numerous occurrence compounded by the fact that their larvae forage on plants for several vegetative seasons.

The research presented in this paper was based on the hypothesis that a unidirectional fertilization system carried out for thirty years could not have left the soil macrofauna unaffected. The research objectives were: to determine the concentrations of carbon and nitrogen in soil, to assess numbers of individuals belonging to several groups of macrofauna and dominant species and to test correlations between the above factors and densities of some groups of the zooedaphon.

MATERIAL AND METHODS

The research, conducted at the Chair of Phytopathology and Entomology of the University of Warmia and Mazury in Olsztyn, encompassed the years 2002-2004 and was based on a long-term static field experiment established by the Chair of Environmental Chemistry of the UWM in 1972. The experiment was set up on grey-brown pozdolic soil formed from medium boulder clay lying on light loam, which was rated as good wheat complex class IIIb soil in the Polish soil class system. The following crops are grown in a 7-year crop rotation system: potato, spring barley, winter oilseed rape, winter wheat + winter rye aftercrop, maize, spring barley, winter wheat. In 2002 the fifth crop rotation series began. Objects selected for our study were fertilized exclusively with liquid manure, farmyard manure or mineral fertilizers. An unfertilized object served as the control (Table 1).

Table 1

Design of the static experiment together with the rates and quantities of nutrients introduced to soil with fertilizers (annual means for 1972-2004)

Experimental objects	Rates ($t \cdot ha^{-1}$)	N	P	K
		($kg \cdot ha^{-1}$)		
Control	-	-	-	-
FYM	22.4	111	37	98
Slurry rate I	39.6	111	42	125
Slurry rate II	73.0	211	76	227
NPK	-	111	38	106

The experiment was performed in four replications and the size of plots for crop harvest was 52.5 m^2. The objects selected for the study are representative of the tendencies dominating in Polish agriculture, which was signalled in the Introduction to this paper. Before application, chemical composition of the fertilizers was analysed. Liquid manure was applied at two rates: rate I equalled FYM in the amount of total nitrogen introduced to soil; rate II was balanced with FYM in the amount of organic carbon added to soil. The amount of nitrogen added to soil in the form of mineral fertilizers equalled the dose of this component in rate I of liquid manure.

Each year soil samples for chemical analyses were collected after harvest from the soil layer at 0-25 cm depth. The samples were dried and passed through a 0.25 mm mesh sieve. Organic carbon was determined by Tiurin's method whereas total nitrogen was assayed by Kjeldahl's method.

In order to quantify the soil macrofauna, soil samples were collected from each trial plot six times during each vegetative season (May – September) at 3-week intervals. On each occasion 24 samples were taken from each object. The samples were cut out using a cylinder which was 95 mm in diameter and 160 mm in height. The choice of the sampling tool was conditioned by the fact that distances between rows of plants grown in particular seasons differed. Another reason was the specific character of a strict experiment. The samples were passed in the field through a 100 mm mesh sieve and then transferred to a laboratory to be placed in Tullgren's apparatuses. Insects were chased out by the dynamic method for 7-8 days (Górny, Grüm 1981). The soil fauna thus obtained was identified with an aid of entomological keys.

The statistical calculations consisting of analyses of variance and Duncan's test were performed using Statistica software, version 6 (StatSoft, Inc., 2003). In addition, Pearson's correlation coefficients were computed.

RESULTS AND DISCUSSION

Organic carbon concentrations in the arable layer of soil were found to vary greatly between particular research objects: from 7.05 $g \cdot kg^{-1}$ soil in the unfertilized object to 12.14 $g \cdot kg^{-1}$ soil in the plots fertilized exclusively with FYM (Table 2).

Table 2

Mean concentrations of organic carbon and total nitrogen ($g \cdot kg^{-1}$) in soil in 2002-2004

Year	No fertilization	Slurry rate I	Slurry rate II	FYM	NPK
	organic (C $g \cdot kg^{-1}$ of soil)				
2002	7.10	9.36	10.99	12.14	8.48
2003	7.05	9.99	10.01	10.67	7.74
2004	7.15	8.85	9.75	11.80	7.50
Mean	7.10	9.40	10.25	11.54	7.91
Total N ($g \cdot kg^{-1}$ of soil)					
2002	0.61	0.70	0.87	0.94	0.72
2003	0.62	0.79	0.89	0.75	0.74
2004	0.56	0.70	0.80	0.91	0.68
Mean	0.60	0.73	0.85	0.87	0.71

Fertilization caused an evident rise in the amount of organic carbon compared to the control. This increase ranged from 0.81 to 4.44 $g \cdot kg^{-1}$ soil.

Out of all the fertilization variants analysed, FYM had the most beneficial effect on organic carbon concentration in soil. Liquid manure applied at rate II, equal to FYM in the amount of organic carbon introduced to soil, had a much weaker influence on the accumulation of this component in soil than solid manure.

The content of total N varied from 0.56 $g \cdot kg^{-1}$ soil in the unfertilized object to 0.94 $g \cdot kg^{-1}$ soil in the object fertilized with FYM. Larger increase in the total nitrogen in soil was obtained when soil was fertilized with farmyard manure than liquid manure or mineral fertilizers.

The macrofauna potentially harmful to crops consisted of arthropods *Arthropoda* representing three orders of insects: *Coleoptera* beetles, *Lepidoptera* butterflies, *Diptera* dipterans as well as the order of centipedes *Diplopoda*, which are designated as members of the phylum *Myriapoda*.

Five families were determined among the *Coleoptera* beetles: click beetles *Elateridae* (which made up 31% of the whole group) with the dominant dark elaterid beetle *Agriotes obscurus* L.), scarab beetles *Scarabaeidae* (11%) dominated by cockchafer *Melolontha melolontha* L., ground beetles *Tenebrionidae* (5%), chrysomelid beetles *Chrysomelidae* (3%) and burying beetles *Silphidae* (2%). The latter family was represented by single species (Figure 1).

The butterflies *Lepidoptera* were represented mainly by caterpillars of turnip moth *Agrotis segetum* L., of the family *Agrotinae*, which constituted 3% of the total population. The dipterans obtained from the soil samples, of which most were larvae, belonged to two families: crane flies *Tipulidae* with the species *Tipula scripta* L (11%) and March flies *Bibionidae* with the spe-

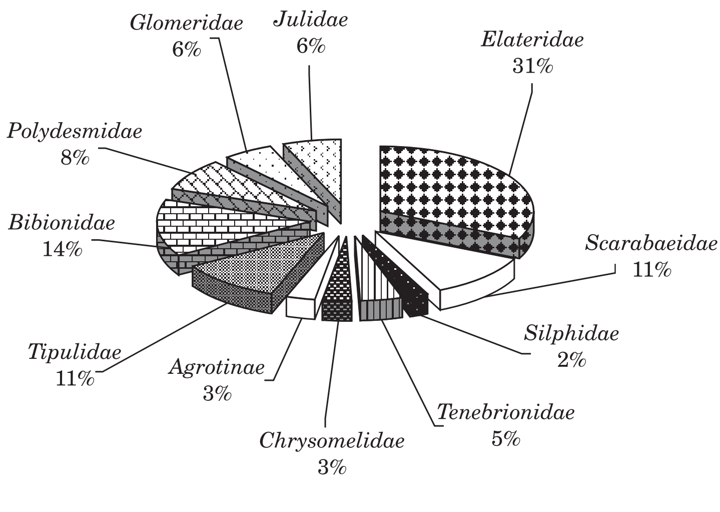

Fig. 1. Percentages of families in the assemblage of potentially noxious macrofauna

cies *Bibio pomonae* L. (14%). The centipedes *Diplopoda* were dominated by adult individuals (14% of all collected individuals of potentially noxious macrofauna) whereas larvae were in minority (4.9%).

The density of the macrofauna varied both between the vegetative seasons and between the research objects (Figure 2). The lowest density (2.4 individuals per 1 m^2) was observed on the control object, which had not been fertilized for 34 years. Significant differences occurred between the objects which were treated with organic fertilizers (rate I of liquid manure and FYM) and the remaining combinations. Low mean densities also appeared on the objects fertilized with mineral fertilizers and rate II of liquid manure, which added to the soil the same quantity of organic carbon as FYM. The respective densities were 2.5 and 3.1 individuals per 1 m^2. The relatively low density of macrofauna noticed on the object fertilized with rate II of liquid manure was most probably caused by high accumulation of nitrates (III and IV), which are noxious to soil organisms – the fact mentioned by Wolender (1988). This dose of liquid manure (rate II) added nearly twice as much nitrogen as the other fertilization treatments. Besides, over 60% of nitrogen in liquid manure occurs in easily dissolvable forms, which encourages the accumulation of large amounts of nitrogen in soil.

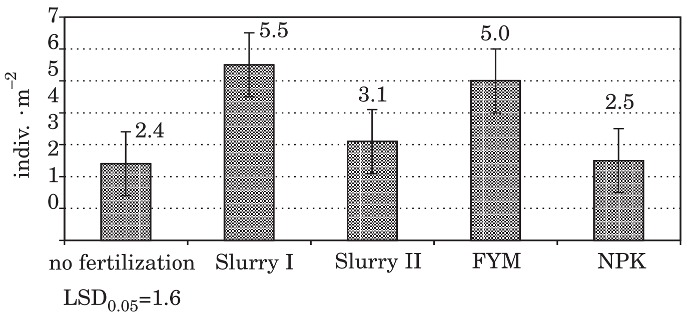

Fig. 2. Average macrofauna density on the experimental objects (individuals · m^{-2})

The diverse densities of noxious macrofauna on particular test objects were obviously conditioned by specific requirements of the analysed insects regarding the soil environment they inhabit. The lowest density, which occurred on the unfertilized object, was a consequence of small annual growths of plant biomass, which in turn resulted in small amounts of post-harvest leftovers.

While comparing the results of the soil chemical analyses and fauna investigations, it was found out that organic carbon concentrations produced strong influence on the density of noxious macrofauna (Table 2). An increase in organic carbon was accompanied by a higher density of the analysed group of zoodaphon. This is evidenced by the positive correlation coefficient $r = 0.61$ (Figure 3).

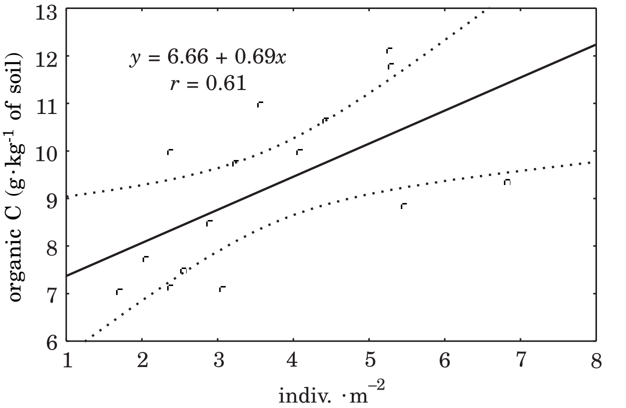

Fig. 3. Correlation between number of noxious macrofauna and organic carbon concentration in soil

In the soil of the objects which were a uniform group according to the highest macrofauna density, the concentration of organic carbon was relatively low. However, there was one exception – the object fertilized with rate I of liquid manure, where the macrofauna density was significantly lower. This was most probably caused by very high rates of nitrogen introduced to the soil together with rate II of liquid manure, and in particular its high concentration of mineral nitrogen. Similar and low densities of macrofauna were determined in the object fertilized each year with mineral fertilizers. As mentioned previously, high concentration of nitrogen in soil, and especially that of nitrate nitrogen, may be harmful to certain soil organisms, particularly young stages of larval soil insects (Górny, Grüm 1981). The correlation between the content of organic carbon and number of noxious soil insects showed a significant relationship between these two parameters. Organic carbon is the basic component of soil humus and the fact that most soil dwelling organisms prefer habitats rich in organic matter and nutrients may explain the positive effect of carbon on the size of soil macrofauna population. The results of the authors' own studies confirm the relationships described by Kowalska, Wierzbowski (2002), who reported that nutrient rich habitats comprised more numerous populations of soil agrophagous organisms than sites poor in nutrients.

The content of total nitrogen in soil and density of harmful macrofauna were also determined to be correlated. The objects which were found to contain the highest densities of macrofauna were the ones which possessed the highest concentrations of nitrogen. On the other hand, the objects with the lowest densities of harmful insects had the lowest nitrogen concentrations in soil (Table 2). Again, there was one exception, namely the object treated with rate II of liquid manure, where the density of noxious macrofauna was very low but the level of nitrogen in soil turned out to be high.

One may presume that this was due to the toxic effect of liquid manure, which added much more nutrients to soil than the other fertilizers. Such high concentration of mineral salts introduced every year with liquid manure must have contributed to the deterioration of biological properties of soil. Positive correlation (r = 0.34) was determined between the mean density of noxious entomofauna and total nitrogen concentration (Figure 4).

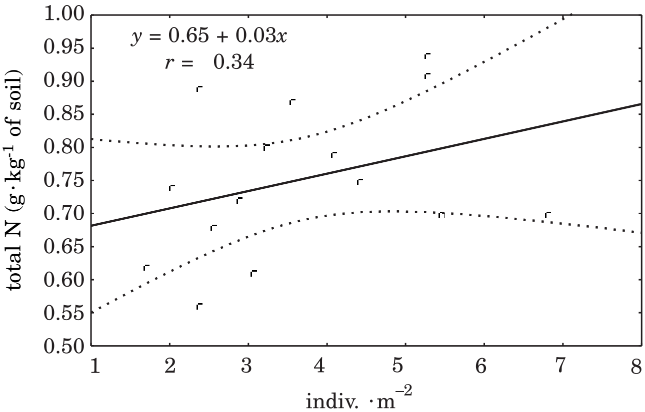

Fig. 4. Correlation between number of noxious macrofauna and total nitrogen concentration in soil

CONCLUSIONS

1. Long-term unidirectional organic and mineral fertilization has led to increased levels of organic carbon and total nitrogen in soil. The highest increase in those components appeared under the influence of farmyard manure fertilization.

2. The following composition of soil macrofauna potentially harmful to cultivated crops was determined: click beetles *Elateridae* with the dominant dark elaterid beetle *Agriotes obscurus* L, March flies *Bibionidae* with the dominant species *Bibio pomonae* L., scarab beetles *Scarabaeidae* with European June beetle *Amphimallus solstitialis* L., and ground beetles *Tenebrionidae* which were dominated by *Tipula scripta* Mig.

3. Unidirectional application of organic fertilizers in rates balanced for the amount of nitrogen added to soil (rate I of liquid manure and FYM), when carried out for many years, caused a significant increase in the density of soil macrofauna.

4. Fertilization with liquid manure balanced with FYM in terms of organic carbon added to soil as well as with mineral fertilizers did not favour presence of soil macrofauna.

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CALCIUM AND MAGNESIUM IN UNDERGROUND WATERS AROUND A COMMUNAL WASTE DUMP

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Abstract

Contaminations carried by effluents from communal waste dumps are a serious threat to underground waters. The effluents may contain solutes washed away by precipitation and also organic and mineral substances that arise during anaerobic and aerobic waste decomposition. Aside of substances that are known to be harmful to the human health (heavy metals), the effluents may contain a large number of common elements which are not regarded harmful at natural concentrations. In effluents from "young" dumps, where the waste undergoes acidic fermentation mainly, calcium concentration may exceed 3,000 $mg \cdot dm^{-3}$ and that of magnesium reach 1,500 $mg \cdot dm^{-3}$. Effluents from "old" dumps, where methane fermentation dominates, most often contain up to 400 $mg \cdot dm^{-3}$ of calcium and 200 $mg \cdot dm^{-3}$ magnesium.

The aim of the work was to elucidtyate the character and dynamics of changes in concentration of the elements studied in effluents from a municipal waste dump at Maślice near the city of Wrocław, and in underground waters of the adjacent land. Deposition of waste in this area began in the late 1960s. The ground conditions provide for an easy contact between underground water and dumped waste, and transport of the washed-out pollutants. Only part of the dump has sealing and drainage that conducts the effluents to a reservoir, where samples for this study were taken. At the turn of 1999 and 2000 the utilization of the dump was terminated and its reclamation began. Thus, the slopes of the refuse heap were fortified with reinforced ground, the cap sealed with synthetic-mineral material, and from the side of underground water inflow a shield was made (in 2002) that reached down to the impermeable ground layer in order to stop the inflowing waters. In 2004 the reservoir for effluents was filled in.

The results, presented in this report, on the content of calcium and magnesium in underground waters flowing into the dump did not show any other extra contamination. In dump effluents the relations between calcium and magnesium concentration remained on similar levels. Like for other dumps, in the first years of study calcium concentration

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prevailed, whereas effluents from older dumps contained greater amounts of magnesium. Increased contents of calcium and magnesium in underground waters flowing under the unsealed part of the dump indicated at continuous inflow of contaminations, that were not eliminated by the technical means applied during reclamation of the installation. Variations in the properties of waters flowing out of the dump depended mainly on the composition of the inflowing waters (max. concentrations occurred concurrently) and the amount of contaminants emitted into the ground from the dump (relations between mean contents of calcium and magnesium varied in them like in the dump effluents).

Key words: municipal waste dump, underground waters, dump effluents, calcium, magnesium.

WAPŃ I MAGNEZ W WODACH PODZIEMNYCH NA TERENACH OTACZAJĄCYCH SKŁADOWISKO ODPADÓW KOMUNALNYCH

Abstrakt

Zanieczyszczenia przenoszone przez odcieki ze składowisk odpadów komunalnych są poważnym zagrożeniem dla wód podziemnych. Mogą zawierać substancje rozpuszczone, wymywane przez opady atmosferyczne, a także substancje organiczne i mineralne powstające w trakcie beztlenowego rozkładu odpadów. Oprócz substancji uznawanych za szkodliwe dla środowiska (jak np. metale ciężkie), odcieki mogą zawierać duże ilości pierwiastków występujących powszechnie w przyrodzie, które w naturalnych stężeniach nie są uznawane za niebezpieczne. W odciekach z „młodych" składowisk, na których odpady podlegają głównie procesom fermentacji kwaśnej, stężenie wapnia może przekraczać 3000 $mg \cdot m^{-3}$, a magnezu dochodzić do 1500 $mg \cdot dm^{-3}$. Odcieki ze „starych" składowisk, z dominacją procesów fermentacji metanowej, najczęściej zawierają do 400 $mg \cdot dm^{-3}$ wapnia i do 200 $mg \cdot dm^{-3}$ magnezu.

Celem pracy było wykazanie charakteru i dynamiki zmian stężeń badanych pierwiastków w odciekach ze składowiska odpadów komunalnych „Maślice" we Wrocławiu oraz w wodach podziemnych terenów przyległych. Deponowanie odpadów na tym terenie rozpoczęto pod koniec lat sześćdziesiątych XX wieku. Warunki gruntowe ułatwiają kontakt wód podziemnych ze składowanymi odpadami oraz transport wymywanych zanieczyszczeń. Tylko część składowiska ma uszczelnienie i drenaż odprowadzający odcieki do zbiornika, z którego pobierano próby do badań. Na przełomie lat 1999-2000 zakończono eksploatację składowiska i rozpoczęto jego rekultywację. W ramach tego procesu wzmocniono zbocza hałdy odpadów gruntem zbrojonym, czaszę uszczelniono materiałem syntetyczno-mineralnym, a od strony dopływu wód podziemnych (w 2002 r.) wykonano ekran sięgający do warstwy nieprzepuszczalnej, mający zatrzymywać dopływające wody podziemne. W 2004 r. zasypano zbiornik na odcieki.

Analizując zawartości wapnia i magnezu w wodach podziemnych dopływających do składowiska, nie wykazano innego źródła zanieczyszczenia. W odciekach składowiskowych proporcje między stężeniami wapnia i magnezu utrzymywały się na zbliżonym poziomie. Podobnie jak na innych składowiskach, w pierwszych latach badań wykazano większe stężenia wapnia, odcieki z dłużej składowanych odpadów zawierały większe ilości magnezu. Podwyższone zawartości wapnia i magnezu w wodach podziemnych przepływających pod nieuszczelnioną częścią składowiska wskazywały na ciągły dopływ zanieczyszczeń, nie zahamowany przez zabiegi techniczne zastosowane w trakcie rekultywacji obiektu. Zmiany właściwości wód odpływających za składowiskiem zależały głównie od składu wód dopływających do obiektu (maksymalne stężenia wystąpiły w podobnym czasie) oraz ilości zanieczyszczeń emitowanych do podłoża ze złoża odpadów (proporcje między średnimi zawartościami wapnia i magnezu zmieniały się w nich podobnie jak w odciekach ze składowiska).

Słowa kluczowe: składowisko odpadów komunalnych, wody podziemne, odcieki składowiskowe, wapń, magnez.

INTRODUCTION

Calcium and magnesium are two elements which are chemically similar and common in the environment. In underground waters calcium concentrations do not usually exceed 100 mg·dm^{-3}, and those of magnesium 50 mg·dm^{-3}. The ratio Ca/Mg in slightly mineralized waters has most often the value 2-6 (MACIOSZCZYK, DOBRZYŃSKI 2002).

Contaminations carried by effluents from communal waste dumps are a serious danger to underground waters. They can contain solutes washed away by atmospheric precipitation, and also organic and mineral substances that are produced in aerobic decomposition of waste. The greatest amounts of contaminants are found in effluents that appear during the first few years of dumping. With oncoming stabilization of the decomposition processes, concentrations of most contaminants in effluents decrease. Aside of substances known to be harmful to the environment (e.g., heavy metals), effluents may contain large amounts of elements commonly occurring in nature, which are regarded not dangerous at natural concentrations. In effluents from "young" dumps, where the waste undergoes acidic fermentation mainly, calcium concentrations may exceed 3,000 mg·dm^{-3} and that of magnesium up to 1,500 mg·dm^{-3}. Effluents from "old" dumps, where methane fermentation dominates, most often contain up to 400 mg·dm^{-3} calcium and 200 mg·dm^{-3} magnesium (THORNTON et al. 2005, PAPADOPOPULOU et al. 2007).

The aim of the work was to find out the character and dynamics of changes in concentrations of the elements studied in effluents from a communal waste dump at Maślice near Wrocław and in underground waters in its vicinity.

MATERIAL AND METHODS

The investigations were carried out in the area around the communal waste dump at Maslice near Wrocław. Dumping of waste on this spot began in the late 1960s, utilizing for that purpose an excavation (of 7 ha area) that remained after sand exploitation. The ground under the dump is formed of sand-gravel formations, and the underground water surface is found at 1-2 m above the dump bottom. Such conditions facilitate contact between underground waters and the dumped waste, and transport of washed-out contaminants. The direction of underground water flow is from south-west to north-east towards the Odra river (SZYMAŃSKA-PULIKOWSKA 2001). Only part of the dump has sealing and drainage that directs the effluents to a reservoir, where the study samples were taken. At the turn of 1999-2000 the dump exploitation was terminated and its reclamation started. Consequently, the slopes of the refuse heap were fortified with reinforced ground, the cap sealed with synthetic-mineral material, and from the side of underground water inflow a shield was made (in 2002) that reached down to the impermeable ground layer in order to stop the inflowing waters. In 2004 the reservoir for effluents was filled in (SZYMAŃSKA-PULIKOWSKA 2005).

The paper presents the results of research on the content of calcium and magnesium in underground waters flowing into the dump, in dump effluents and in underground waters flowing out of the dump. The investigation was conduced in 1995-2007, while the investigations on the dump effluents were terminated when the reservoir was filled in. The samples were taken 3 times a year – the effluents from the reservoir and underground waters from four piezometers localized on the inflow (2) and outflow sides (2). Before sampling the water stagnant in the piezometric well was pumped out twice. Calcium and magnesium contents in the samples taken were determined according to methods described in the literature (NAMIEŚNIK et al. 1998, HERMANOWICZ et al. 1999). Statistical analysis of the results was conducted using the Statistica 7.1 program.

RESULTS AND DISCUSSION

Table 1 presents the characteristic values (mean, standard deviation, variance coefficient) and mean calcium to magnesium ratios of concentration in successive years of investigation on underground waters flowing into the dump.

Calcium content in the samples assayed exhibited great variation, being in the range 4.803-154.3 $mg \cdot dm^{-3}$ and with maximum in the years 1998-2000. Magnesium contents varied similarly, the mean being in the range 6.683-87.20 $mg \cdot dm^{-3}$ and culminating in 1998-2000. Large differentiation was also shown by the relation between mean calcium and magnesium concentrations (from 0.435 to 7.260), exceeding slightly the range determined for natural conditions. In spite of the large variation, the contents of calcium and magnesium observed in waters flowing into the dump did not diverge (aside of 1998-2000) from values that were regarded natural (MACIOSZCZYK, DOBRZYŃSKI 2002).

Table 2 shows the characteristic values (mean, standard deviation, variance coefficient) and means calcium to magnesium ratios of concentrations in successive years of investigation on the dump effluents at Maślice (the years 1995-2003, without 1998).

The highest values occurred in the beginning of the study period in 1995. The effluents then contained, on average, 480.7 $mg \cdot dm^{-3}$ calcium and 280.0 $mg \cdot dm^{-3}$ magnesium. In the successive years the concentrations did not reach such high values. Since 2000 the mean contents of both elements

Table 1

Characteristic values and mean calcium to magnesium ratios in waters coming to the dump

Years		μ ($mg \cdot dm^{-3}$)	Ca/Mg	δ	ν (%)
1995	Ca	35.58	3.881	16.71	46.95
	Mg	9.167		2.251	24.56
1996	Ca	31.58	4.725	17.13	54.24
	Mg	6.683		3.543	53.01
1997	Ca	79.13	7.260	47.21	59.66
	Mg	10.90		7.034	64.54
1998	Ca	129.9	1.881	39.58	30.48
	Mg	69.05		73.53	106.5
1999	Ca	117.8	1.351	76.92	65.33
	Mg	87.20		91.01	104.4
2000	Ca	154.3	6.674	55.53	36.00
	Mg	23.12		28.47	123.1
2001	Ca	55.83	1.794	22.60	40.47
	Mg	31.12		43.37	139.3
2002	Ca	38.57	4.838	30.55	79.21
	Mg	7.973		6.905	86.60
2003	Ca	18.22	2.240	13.98	76.73
	Mg	8.133		4.955	60.92
2004	Ca	19.57	2.082	14.42	73.68
	Mg	9.400		6.232	66.30
2005	Ca	4.803	0.435	2.854	59.43
	Mg	11.05		7.656	69.29
2006	Ca	74.98	4.403	73.71	98.30
	Mg	17.03		18.00	105.7
2007	Ca	77.38	3.726	44.30	57.25
	Mg	20.77		10.59	51.00

Explanations: μ – mean, δ – standard deviation, ν – variance coefficient

systematically decreased, which may indicate a depletion of the "resources" accumulated in the dump. Studies conducted on Dyer Boulevard Landfill (Florida, USA) found that effluent samples (taken after dump closure) contained 176.13 $mg \cdot dm^{-3}$ Ca and 53.75 $mg \cdot dm^{-3}$ Mg (STATOM et al. 2004) – values only slightly higher than those for the Maślice dump after closure.

Table 2

Characteristic values and mean calcium to magnesium ratios in dump run-offs

Years		μ (mg·dm^{-3})	Ca/Mg	δ	ν (%)
1995	Ca	480.7	1.717	160.1	33.29
	Mg	280.0		20.00	7.143
1996	Ca	131.7	1.289	100.1	75.99
	Mg	102.2		76.24	74.59
1997	Ca	117.8	1.758	40.51	34.38
	Mg	67.00		24.62	36.75
1999	Ca	121.1	0.590	130.6	107.9
	Mg	205.3		15.14	7.375
2000	Ca	137.1	1.023	49.81	36.32
	Mg	134.6		95.02	70.57
2001	Ca	93.33	1.150	11.55	12.37
	Mg	81.17		52.98	65.27
2002	Ca	66.43	0.840	16.99	25.57
	Mg	79.00		40.95	51.84
2003	Ca	60.67	0.851	44.76	73.78
	Mg	71.33		44.24	62.02

Explanations: μ – mean, δ – standard deviation, ν – variance coefficient

Relations between calcium and magnesium concentration in the dump effluents varied from 0.590 to 1.758, departing markedly form the values found in waters flowing to the dump. During all the study period the values observed showed large variation. In the first years of the study calcium concentrations prevailed. After the dump closure the concentrations of both the elements reached a similar level, with magnesium concentration even starting to prevail. This tendency could be confirmed by the research done by WILLIAMS (2002). He reported that effluents arising in the phase of acidic fermentation may contain 270-6240 (151 on average) mg Ca·dm^{-3} and 25--820 (384 on average) mg Mg·dm^{-3}; whereas concentration ranges for the phase of methane fermentation are: 20-501 (151 on average) mg Ca·dm^{-3} and 40-1580 (250 on average) mg Mg·dm^{-3}. A similar trend in the changes of calcium and magnesium concentrations in dump effluents was shown in an investigation conducted on a dump in Sieraków (MELLER et al. 2001). The highest concentrations of calcium and magnesium were found in effluents from the youngest quarter of the dump, with calcium concentration prevailing. Effluents from older quarters contained concentrations of the elements that were several times lower, with magnesium concentration prevailing.

Table 3 shows the characteristic values (mean, standard deviation, variance coefficient) and ratio of mean concentrations of calcium and magnesium in successive years of research on underground waters flowing out of the dump. Contents of calcium and magnesium were markedly higher than those in waters flowing into the dump, with large variance. Mean calcium concen-

Table 3

Characteristic values and mean calcium to magnesium ratios in waters flowing from the dump

Years		μ ($mg \cdot dm^{-3}$)	Ca/Mg	δ	ν (%)
1995	Ca	177.0	5.836	37.26	21.06
	Mg	30.33		7.250	23.90
1996	Ca	122.6	8.212	72.15	58.86
	Mg	14.93		3.000	20.10
1997	Ca	201.1	4.428	84.05	41.79
	Mg	45.42		29.38	64.70
1998	Ca	254.8	0.879	96.43	37.85
	Mg	290.0		171.5	59.15
1999	Ca	415.7	1.488	106.6	25.64
	Mg	279.3		78.28	28.03
2000	Ca	624.3	3.315	205.3	32.86
	Mg	188.3		179.9	95.54
2001	Ca	422.5	2.809	293.5	69.46
	Mg	150.4		73.86	49.10
2002	Ca	156.4	1.185	79.24	50.67
	Mg	132.0		23.26	17.62
2003	Ca	204.8	1.476	166.0	81.11
	Mg	138.8		34.46	24.84
2004	Ca	212.9	1.476	141.9	66.64
	Mg	144.2		49.18	34.11
2005	Ca	49.61	0.404	33.53	67.59
	Mg	122.8		70.72	57.61
2006	Ca	293.9	3.016	271.8	92.50
	Mg	97.43		62.71	64.50
2007	Ca	118.4	1.611	72.15	60.95
	Mg	73.48		61.19	83.27

Explanations: μ – mean, δ – standard deviation, ν – variance coefficient trations were from 49.61 to 624.3 mg□dm^{-3}, and those of magnesium 14.93 to 290.0 mg□dm^{-3}. The highest contents of the elements discussed occurred in underground waters flowing out of the dump in the years 1998-2001. At the beginning of the study period (1995-1997) in the waters analyzed calcium prevailed, in later years the prevalence was not so distinct. A high and distinct differentiation in concentrations of the elements was also noticed in studies on a dump in Radiowo (Pachuta, Koda 2001). Underground waters around the dump contained from 68.25 to 329.75 mg Ca$\cdot$dm^{-3}, and from 14.02 to 193.87 mg Mg$\cdot$dm^{-3}. Similar results were obtained in investigations in the vicinity of an unsealed communal dump in Gazipur (Delhi). Underground waters contained from 43 to 447 mg Ca$\cdot$dm^{-3} and up to 220 mg Mg$\cdot$dm^{-3} (Mor et al. 2006). On the other hand, investigation of underground waters from dug wells near a communal waste dump situated in the Green Region of Poland (Tałałaj 2001) showed lower calcium concentrations (73.4 mg$\cdot$dm^{-3} on average) and that of magnesium (34.1 mg$\cdot$dm^{-3} on average), with high variations.

Increased contents of calcium and magnesium in underground waters under the unsealed part of the communal waste dump indicated a continuous inflow of contaminants, not eliminated by the technical means applied during the reclamation of the site. Changes in the composition of waters flowing out of the dump depended mainly on two factors: composition of incoming waters (max. concentrations occurred concurrently) and contaminants emitted into the ground from the dump (relations between mean contents of calcium and magnesium varied in them like in effluents from the dump).

CONCLUSIONS

1. Calcium and magnesium contents in underground waters flowing out of the dump did not indicate other sources of contamination.

2. In spite of the variation in mean concentrations of calcium and magnesium in the dump effluents in the successive years of study, relations between contents of the elements remained at a similar level (the ratio Ca/Mg was from 0.590 to 1.758). Like in other dumps, in the first years of study calcium concentrations prevailed, whereas effluents from old dumps contained more magnesium.

3. The content of calcium and magnesium in underground waters flowing out of dump depended on both quality of incoming waters and composition of effluents to the ground.

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EFFECT OF SOIL SALINITY ON ACTIVITY OF ANTIOXIDANT ENZYMES AND CONTENT OF ASCORBIC ACID AND PHENOLS IN BEAN (*PHASEOLUS VULGARIS* L.) PLANTS

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Abstract

Soil salinity is the one of the most important abiotic factors influencing the growth, development and yields of crops. However, it is difficult to determine exact concentrations of salt which cause soil salinity. Salinity threshold levels depend on a crop species, variety, developmental stage and environmental factors. This paper presents the results of an experiment on the effect of different soil concentrations of NaCl soil on several oxidation stress parameters, such as catalase and peoxidase activity, content of ascorbic acid, phenols and flavonoids in bean plants.

A laboratory pot experiment was carried out on samples of light silty loam containing 1.2% of humus. Pots were filled with 1 kg soil samples each, to which NaCl solution was added in doses 10, 30 and 50 mM kg^{-1}. Each pot was seeded with 7 seeds of cv. Aura bean. The plants grown in soil without NaCl were the control. On days 14, 21 and 28 green parts of plants were collected for determinations of catalase and peroxidase activity by colorometry as well as the content and flavonoids, phenols, ascorbic acid and chloride concentration by Mohr's method.

The results show that chloride concentration in bean plants increased at higher of NaCl concentration in soil. The activity of the antyoxidative enzymes such as catalase and peroxidase in bean plants, on sampling days, was higher as the chloride concentration in plants increased. Non-enzymatic antioxidants: flavonoids, phenols and ascorbic acid content during the experiment showed different changes with relation to the chloride content, but in all the trials ascorbic acid content was significantly positively correlated whereas the content of phenols was significantly negatively correlated with the chloride content in plant tissues.

Key words: salinity, oxidative stress, bean, flavonoids, phenols.

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WPŁYW ZASOLENIA GLEBY NA AKTYWNOŚĆ ENYZMÓW ANTYOKSYDACYJNYCH ORAZ ZAWARTOŚĆ KWASU ASKORBINOWEGO I FENOLI W ROŚLINACH FASOLI (*PHASEOLUS VULGARIS* L.)

Abstrakt

Jednym z ważniejszych abiotycznych czynników wpływających na wzrost, rozwój i produktywność roślin jest zasolenie podłoża. Trudno jest jednak określić, w przypadku jakiego stężenia soli mówi się o zasoleniu podłoża. Graniczna jego wartość jest uzależniona od gatunku, a w nawet odmiany, etapu rozwoju rośliny oraz od wielu towarzyszących czynników środowiska. W pracy zaprezentowano wyniki doświadczeń mających na celu określenie, w jaki sposób dodatek do gleby NaCl o różnych stężeniach oddziałuje na wybrane parametry stresu oksydacyjnego: aktywność katalazy i peroksydazy oraz zawartość kwasu askorbinowego, fenoli i flawonoidów w roślinach fasoli.

Doświadczenie wazonowe w warunkach laboratoryjnych przeprowadzono na próbkach gliny pylastej lekkiej o zawartości próchnicy 1,2%. Glebę podzielono na 1 kg naważki, którymi, po wcześniejszym dodaniu wodnych roztworów NaCl w dawkach 10, 30 i 50 mM kg^{-1}, napełniono wazony. Do każdego wazonu wysiano po 7 nasion fasoli odmiany Aura. Próbę kontrolną stanowiły rośliny rosnące w glebie bez dodatku soli. W 14., 21. i 28. dniu doświadczenia pobrano zielone części roślin i oznaczono w nich kolorymetrycznie aktywność katalazy i peroksydazy oraz zawartość flawonoidów, fenoli i kwasu askorbinowego, a także zawartość chlorków miareczkową metodą Mohra.

Stwierdzono, że zawartość chlorków w roślinach fasoli wzrastała wraz ze zwiększaniem stężenia NaCl w podłożu. Aktywność enzymów antyoksydacyjnych: katalazy i peroksydazy w roślinach fasoli, w poszczególnych terminach pomiarów, wzrastała wraz ze zwiększaniem się w nich ilości chlorków. Zawartość nieenzymatycznych antyutleniaczy w roślinach fasoli: kwasu askorbinowego, flawonoidów i fenoli wykazywała w trakcie trwania doświadczenia zmienne zależności w stosunku do ilości w nich chlorków, jednakże w całym doświadczeniu zawartość kwasu askorbinowego była istotnie dodatnio, a fenoli istotnie ujemnie skorelowana z ilością chlorków w tkankach roślinnych.

Słowa kluczowe: zasolenie, stres oksydacyjny, fasola, flawonoidy, fenole.

INTRODUCTION

Soil and water salinity have an increasing importance in agriculture (SUDHAKER et al. 2001). Salinity is one of the most important abiotic factors influencing the growth, development and yield of plants (CHAPARZADEH et al. 2004, RAHNAMA, EBRAHIMZADEH 2005) and causes its considerable losses in crops (SMIRNOFF 1998). The influence of high concentrations of salt in soil on the growth and metabolism of plants often manifests as deformations and decay of leaves. The excessive content of salt in soil solution also impedes germination, particularly in case of sensitive plants.

However, it is difficult to state precisely what concentration of salt causes soil salinity. Salinity thresholds depend on the plant's species, cultivar, development stage and environmental factors.

One of the biochemical responses of plants to biotic and abiotic stresses is the production of reactive oxygen forms (RAHNAMA, EBRAHIMZADEH 2005). Reactive oxygen forms also occur during the physiological metabolic activity of plants (photosynthesis, respiration) and are under control of a complex antioxidative system (DIXON, PAIVA 1995, YAMASAKI et al. 1997). This system consists of enzymes, e.g. superoxide dismutase, catalase, peroxidases, and low-molecular compounds, e.g. ascorbate, glutathione, β-carotene, α-tocopherol, or phenolic compounds (MALENĆIĆ et al. 2003).

The aim of the present work has been to establish how soil salinity affects some oxidative stress parameters, such as catalase and peroxidase activity and the content of ascorbate, phenols and flavonoids in bean plants.

MATERIALS AND METHODS

A laboratory pot experiment was carried out on black earth from the Gumieńce Plain. In the arable-humus horizon this soil is light silty loam with 1.2% humus content, highly abundant in available phosphorus and moderately to highly abundant in available potassium and magnesium. Soil collected from a field was passed through a 2 mm sieve and brought to 60% maximum water capacity. Then it was divided into 1 kg weighed samples, which received aqueous NaCl solutions in the doses given in table 1 and put in the pots. In each pot, 7 seeds of cv. Aura bean (produced by CNOS-VILMORIN from Poznań) were seeded. The control trial consisted of bean plants growing on soil with an addition of bitter salt.

During the experiment the plants were illuminated by a sodium lamp Son-T Agro 400W (Philips) at the radiation intensity measured on level of the soil equal 90 $\mu E \cdot m^2 \cdot s^{-1}$ PAR (photosynthetic active radiation). The photoperiod was set as 12 hours of light and 12 hours of darkness.

In days 14, 21 and 28 of the experiment, green parts of the bean plants were collected for assays of the activity of catalase and peroxidase as well as the content of flavonoids, phenols and ascorbic acid. For this purpose, the plant samples were homogenized with an appropriate buffer for antioxidant enzymes, 80% acetone for phenols, 80% methanol for phenols and 2% oxalic acid for ascorbic acid. Afterwards, the samples were centrifuged at 14800 rpm. The supernatants thus obtained were tested for the selected biochemical parameters. Methods elabotrated by the following researchers were applied: LÜCK (1963) for catalase activity, CHANCE and MACHLY (1955) for peroxidase activity, WOISKY and SALATINO (1998) for the content of flavonoids, and SINGLETON and SAMUEL-RAVENTOS (1999) for the content of phenols content. The content of ascorbic acid was evaluated according to Polish Norm PN-90/A-75101/11. All the analyses were performed without any changes or modifications to the above procedures. In addition, the content of chlorides in plant tissues was determined using Mohr's titration method (KREŁOWSKA-KUŁAS 1993).

The experiment was established in a completely randomized design with three replications. The results were processed statistically using ANOVA. The least significance differences (LSD) were determined by Tukey's test at α=0.05. The statistical analyses were performed executed independently for every sampling day and experimental variants.

Correlation coefficients were calculated between the content of chlorides versus the activity of catalase and peroxidase and the content of phenols, flavonoids and ascorbate. Non-linear quadratic regression curves whose determination coefficients were above 0.850 were plotted to assess how on the subsequent sampling dates the stress parameters changes according to the increased Cl^- content in plants.

RESULTS AND DISCUSSION

Bean plants growing in soil with an addition of 10 mM $NaCl \cdot kg^{-1}$ soil were similar to the control plants except their height. The development of plants growing in soil with larger NaCl concentrations was distinctly inhibited. Plants growing in soil with 50 mM $NaCl \cdot kg^{-1}$ soil developed so poorly that on day 28 of the experiment it was impossible to obtain any plant material for analyses (Table 1).

Table 1

Doses NaCl added into soil

Concentration	NaCl	
	$(mM \cdot kg^{-1})$	$(g \cdot kg^{-1})$
I	10.00	0.585
II	30.00	1.755
III	50.00	2.925

The average Cl^- ionic content in the tissues of bean plants growing in soil without addition of salt for the whole experiment ranged from 2.000 to 2.632 $\mu g \cdot g^{-1}$ f.w.

The content of chlorides tissues of bean plants (Table 2) increased alongside an increasing NaCl concentration in the soil and on the subsequent sampling days. The largest accumulation of Cl^- ions, about fourfold more than the control, was observed on day 28 of the experiment at 50 mM NaCl. This finding finds confirmation in the literature, where increasing concentration of chlorides due to higher NaCl doses in soil have been observed in *Thellungiella halophilla* (M'RAH et al. 2006) and *Catharanthus roseus* (ELKAHOUI et al. 2005).

Table 2

Chloride concentration in bean plants growing in soil with different amounts of NaCl (μg Cl^- g^{-1}f.w. plant)

Dose of NaCl added into soil (mM kg^{-1})	14^{th} day	21^{st} day	28^{th} day
0	2.000±0.095	2.632±0.110	2.580±0.087
10	3.372±0.101	4.639±0.114	5.577±0.116
30	4.308±0.150	8.067±0.189	8.901±0.131
50	-	-	9.820±0.270

In most cases, introduction of NaCl to soil caused significant changes occurring during the experiment in the activity of the enzymes analysed as well as in the content of the compounds determined in bean plants (Table 3).

While analysing dependences among the Cl^- content in plants and the assayed oxidative stress parameters, it was discovered that on day 14 an increased Cl^- content in bean plants coincided with a growing activity of the enzymes, especially peroxidase, as well as a higher total content of flavonoids (Figure 1). This observation was confirmed by the correlation coefficients, which proved that activity of catalase and peroxidase as well as the content of flavonoids were significantly positively correlated with chlorides in plants (Table 4). With respect to peroxidase, this correlation persisted in the subsequent days of the experiment, which is in agreement with the report by Jebary et al. (2005). A rise in the peroxidase activity as a result of soil salinity has also been verified in *Morus alba* (SUDHAKER et al. 2001), *Glicyne max* (GHORBANLI et al. 2004) and *Lycopersicon esculentum* (RAHNAMA, EBRAHIMZADEH 2005). However, the activity of catalase on days 21 and 28 of the experiment fluctuated, first increasing and then declining as the ionic concentration Cl^- content in bean plants rose. Besides, a significantly positive correlation between the catalase activity and chlorides in bean plants appeared on day 28. CHAPARZADEH et al. (2004) suggested a stimulating influence of NaCl on the catalase activity in green parts of *Calandula officinalis*. Also RAHNAMA, EBRAHIMZADEH (2005) observed a rise in the catalase activity in *Lycopersicon esculentum* plants.

The content of flavonoids in bean plants was stable on day 21, but on day 28 it was found to decrease as the content of Cl^- ions in plants rose, thus being negatively correlated with the content of chlorides. A similar tendency was observed for the total content of phenols. On day 14 the total phenolic content slightly fell as the concentration of Cl^- in plants increased. Moreover, on all sampling days, the total content of phenols and the content of chlorides in plants were significantly negatively correlated. The fact

Table 3

Changes of biochemical parameters in bean plants growing in soil with different amounts of NaCl

Parameter	Dose of NaCl added into soil ($mM\ kg^{-1}$)	14^{th} day	21^{st} day	28^{th} day
$\mu M\ H_2O_2 \cdot (g\ f.w.\ plant \cdot min)^{-1}$				
Catalase	0	1.087	2.590	2.252
	10	1.649	1.557	2.078
	30	2.732	2.581	2.066
	50	-	-	3.656
	$LSD_{0.05}$	0.211	0.321	0.125
μM purpurogaline $\cdot$ $(g\ f.w.\ plant \cdot 4\ min)^{-1}$				
Peroxidase	0	0.463	0.659	0.610
	10	1.267	0.516	0.574
	30	2.915	1.385	1.129
	50	-	-	1.274
	$LSD_{0.05}$	0.133	0.129	0.137
mg quercetine $\cdot$ g^{-1} f.w. plant				
Flavonoids	0	0.168	0.564	1.098
	10	0.283	0.470	1.200
	30	0.351	0.557	0.978
	50	-	-	0.235
	$LSD_{0.05}$	0.054	0.043	0.032
mg gallate $\cdot$ g^{-1} f.w. plant				
Phenols	0	0.076	0.079	0.148
	10	0.073	0.074	0.099
	30	0.060	0.064	0.084
	50	-	-	0.058
	$LSD_{0.05}$	0.004	0.003	0.002
mg ascorbate $\cdot$ g^{-1} f.w. plant				
Ascorbate	0	0.297	0.205	0.249
	10	0.281	0.315	0.388
	30	0.287	0.468	0.291
	50	-	-	0.361
	$LSD_{0.05}$	0.041	0.029	0.021

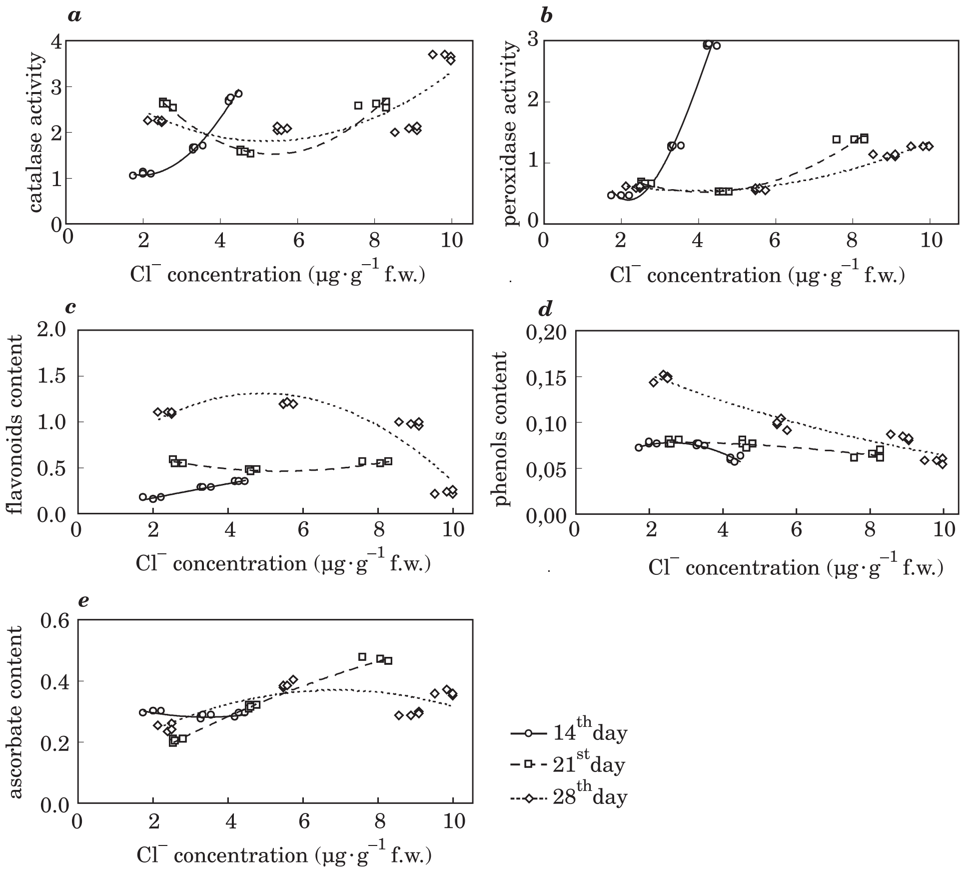

Fig. 1. Relationships between chloride concentration and biochemical parameters in bean plants

Table 4

Correlation coefficients between chloride concentration and biochemical parameters in bean plants

Day of experiment	Catalase	Peroxidase	Flavonoids	Phenols	Ascorbate
14th	0.954*	0.944*	0.984*	-0.769*	-0.506
21st	0.147	0.861*	0.071	-0.878*	0.993*
28th	0.536*	0.903*	-0.694*	-0.963*	0.450
Whole experiment	0.574*	0.237	0.079	-0.510*	0.627*

*significant $p = 0.05$

that the content of phenols in bean plants becomes depressed under soil salinity has also been reported by VERGEER et al. (1995), while NAVARRO et al. (2006) have observed such a relationship in *Capsicum annum* and VERMA and MISHRA (2005) noticed this in *Brassica juncea*.

On days 14 and 28 of the experiment, the content of ascorbate in bean plants remained relatively stable under an increasing Cl^- content in plants, while rising on day 21, when it was significantly positively correlated with the content of chlorides in plants. Increasing concentrations of ascorbate in *Calandula officinalis* caused by soil salinity was noticed by CHAPARZADEH et al. (2004), while SAIRAM et al. (2005) found a decrease in the ascorbate content in *Triticum aestivum* growing in soil with NaCl.

The correlation coefficients computed for the data from the whole experiment revealed that the content of chlorides in bean plants was significantly negatively correlated only with phenols, being significantly positively correlated with the content of ascorbate and the activity of catalase.

CONCLUSION

Growing NaCl concentration in soil caused an increase in the activity of catalase and peroxidase as well as in the content of ascorbate content. In contrast, it depressed the concentration of phenols in bean plants.

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EFFECT OF GROWTH REGULATORS APPLIED TOGETHER WITH DIFFERENT PHOSPHORUS FERTILIZATION LEVELS ON THE CONTENT AND ACCUMULATION OF POTASSIUM, MAGNESIUM AND CALCIUM IN SPRING WHEAT

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Abstract

The objective of this study has been to trace the effect of endogenous growth regulators and different phosphorus fertilization levels on the content and accumulation of potassium, magnesium and calcium in spring wheat. The research was based on the results of a pot experiment established on soil of grain size distribution of light loamy sand. The soil was slightly acidic and moderately abundant in available phosphorus, potassium and magnesium. Against the background of stable NKMg fertilization (1.5 g N, 1.5 g K and 0.25 g Mg), increasing phosphorus rates (0.0 to 1.02 g P per pot) were tested. In order to compare the effect produced by growth regulators, applied in conjunction with rising doses of phosphorus, the pots were divided into 4 groups according to the sprays: distilled water (control), kinetin, gibberellin and auxin.

Kinetin and auxin increased the content of potassium in wheat grain, whereas gibberellin stimulate the removal of this element. Increasing P rates depressed the concentration of potassium in wheat except the oldest leaves. The highest uptake of potassium was observed following an application of 0.68 g P per pot. Gibberellin increased the content of Mg in leaves, glumes, oldest leaves and grain, whereas kinetin and auxin resulted in lower Mg levels. The highest Mg uptake occurred after using 0.68 g P per pot. The plant hormones raised the content of ca in wheat grain. Gibberellin and auxin increased the uptake of calcium by P non-fertilized wheat. Phosphorus fertilization broadened slightly the Ca:P ratio in grain, glumes and stems. Kinetin and auxin narrowed the Ca:P ratio in leaves. Higher phosphoru rates, especially in vegetative organs, narrowed the Ca:P ratio. Gibberellin narrowed the K:(Mg+Ca) ratio in grain and vegetative organs, which in con-

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trast was broadened by kinetin. Auxin broadened these proportions in wheat grain and leaves, while narrowing them in glumes and stems. The lowest and the highest phosphorus rates narrowed the K:(Mg+Ca) ratios in grain and glumes.

Key words: potassium, calcium, magnesium, phosphorus fertilization, growth regulators.

WPŁYW REGULATORÓW WZROSTU STOSOWANYCH W WARUNKACH ZRÓŻNICOWANEGO NAWOŻENIA FOSFOREM NA ZAWARTOŚĆ I AKUMULACJĘ POTASU, MAGNEZU I WAPNIA W PSZENICY JAREJ

Abstrakt

Celem pracy było prześledzenie wpływu endogennych regulatorów wzrostu i zróżnicowanego nawożenia fosforem na zawartość i akumulację potasu, magnezu i wapnia w pszenicy jarej. Podstawą badań było doświadczenie wazonowe założone na glebie o składzie granulometrycznym piasku gliniastego lekkiego, na której uprawiano pszenicę jarą odmiany Jasna. Gleba charakteryzowała się lekko kwaśnym odczynem i średnią zasobnością w przyswajalny fosfor, potas i magnez. Na tle stałego nawożenia NKMg (1,5 g N, 1,5 g K i 0,25 g Mg) zastosowano wzrastające dawki fosforu (0,0-1,02 g P na wazon). W celu porównania działania regulatorów wzrostu, stosowanych w warunkach wzrastających dawek fosforu, wazony podzielono na 4 grupy, w zależności od stosowanych oprysków: woda destylowana (kontrola), kinetyna, giberelina i auksyna.

Kinetyna i auksyna zwiększyły zawartość potasu w ziarnie pszenicy, natomiast giberelina stymulowała wynos tego pierwiastka. Wzrastające dawki P powodowały zmniejszenie koncentracji potasu w pszenicy, z wyjątkiem najstarszych liści. Najwyższe pobranie potasu uzyskano po zastosowaniu 0,68 g P na wazon. Giberelina zwiększyła zawartość Mg źdźble, plewach i najstarszych liściach oraz ziarnie, a kinetyna i auksyna zmniejszały jego koncentrację. Regulatory wzrostu zwiększyły też pobranie magnezu przez pszenicę nienawożoną fosforem. Najwyższe pobranie Mg uzyskano po zastosowaniu 0,68 g P na wazon. Regulatory wzrostu zwiększyły zawartość Ca w ziarnie pszenicy. Giberelina i auksyna zwiększyły pobranie wapnia przez pszenicę nienawożoną P. Nawożenie fosforem tylko w niewielkim stopniu wpłynęło na akumulację Ca w roślinach. Regulatory wzrostu nieznacznie rozszerzyły proporcje molowe Ca:P w ziarnie, plewach i źdźble. Kinetyna i auksyna zacieśniły stosunek Ca:P w liściach. Wzrastające dawki fosforu, głównie w organach wegetatywnych, zawężały proporcje Ca:P. Giberelina zawęziła, a kinetyna rozszerzyła proporcje K:(Mg+Ca) w ziarnie i organach wegetatywnych. Auksyna rozszerzyła te proporcje w ziarnie i liściach, a jednocześnie zawęziła w plewach i źdźble. Najniższe i najwyższe dawki fosforu spowodowały zawężenie proporcji K:(Mg+Ca) w ziarnie i plewach.

Słowa kluczowe: potas, wapń, magnez, nawożenie fosforem, regulatory wzrostu.

INTRODUCTION

Suitable supply of all nutrients throughout the whole growing season is a necessary condition for producing high and good quality wheat grain yields. Not only nitrogen fertilization but also phosphorus, potassium or magnesium nutrition is important. Phosphorus has a stronger effect on the development of generative rather than vegetative parts, and with respect to cereal crops, it is an essential nutrient for a proper growth of the root system and good tillering (SANDER et al. 1991, VALIZADEH et al. 2002).

Potassium is one of the cations which are absorbed by plants in advance to their biomass growth. Among cereals, the most rapid uptake of potassium coincides with the shooting stage and continues until the heading stage. Accumulation of potassium in wheat goes on until the flowering stage, after which it fell by half compared to the maximum uptake (WIERZBOWSKA, NOWAK 2000, PREZZ DU BENNIE 1991, LÁSZTITY 1988 a,b). During the grain filling, only a small fraction of magnesium present in grain originates directly from soil. Some of magnesium found in vegetative organs, mainly in the flag leaf, undergoes remobilisation and is transferred to kernels. However, excessive depletion of magnesium in leaves depressed photosynthesis and leads to lower yields (GRIMME 1987). Good magnesium supply favours higher content of nitrogen and proteins. Being a co-factor of enzymes responsible for synthesis and transport of carbohydrates, magnesium contributes to a larger mass of kernels, which in turn means higher grain yield (CHWIL 2001, GRZEBISZ 1999).

For farming and economic results, biologically active substances which act as growth regulators have a positive influence on metabolic reactions in crop plants. However, their effect is not reproducible. They can regulate the uptake of nutrients by plants and their further transport; they can also affect the remobilisation of nutrients during the formation of grains. These effects are associated with a better, stronger growth of the root system, especially root hairs, and consequently a more intensive uptake of mineral components from soil and fertilizers. The response of plants to plant hormones is varied, depending on the age and physiological state of a plant, environmental conditions as well as synergic or antagonistic reactions between endogenous and exogenous phytohormones.

The aim of this research has been to trace the effect of endogenous growth regulators and different phosphorus fertilization rates on the content of accumulation of potassium, calcium and magnesium in spring wheat.

METHODS

A two-factorial experiment, with 4 replications, was set up in Mitscherlich pots filled with 6.5 kg of light loamy sand, slightly acidic (6.4 pH in 1 mol $KCl \cdot dm^{-3}$) and moderately abundant in phosphorus, potassium and magnesium. Using a stable NKMg fertilization regime: 1.5 g N [NH_4NO_3], 1.5 g K [KCl and K_2SO_4 at a 1 : 1 ratio], and], 0.25 g Mg [$MgSO_4 \cdot 7H_2O$] per pot, increasing rates of phosphorus (0.0, 0.17, 0.34, 0.51, 0.68, 0.95 and 1.02 g P per pot in the form of [$Ca(H_2PO_4O_2 \cdot H_2)$]),were tested. All of the P and Mg rates and half the doses of N and K were added to soil before sowing wheat. The remaining portions of the nitrogen and potassium fertilizers were applied in 2 equal doses – at the early inflorescence and the ear shooting stages. Twenty cv. Jasna wheat plants were grown in each pot. In order to compare the results produced by growth regulators under the effect of increasing phosphorus fertilization rates, the pots were divided into 4 groups (Table 1). Each spraying treatment consisted of an application of 0.5 dm^3 of a liquid containing 50 $mg \cdot dm^{-3}$ of a growth regulator.

Table 1

Design of the application of the plant growth regulators

Experiment variant	Spraying time and plant growth applied	
	beginning of tillering	beginning of flowering
I – control (C)	aqua destillata	aqua destillata
II – kinetin (K)	BAP (benzylaminopurine)	FAP (phurphurilaminopurine)
III – gibberellin (G)	GA_3 (gibberellic acid)	GA_3 (gibberellic acid)
IV – auxine (A)	IAA (indole-3-acetic acid)	NAA (naphthaleneacetic acid)

Wheat was harvested at the full maturity stage. Following the biometrical measurements, wheat plants were dissected into organs: grains, glumes including rachises, stems, flag leaf, penultimate leaf and other leaves. The plant material was ground and digested with concentrated sulphuric acid supplemented with hydrogen dioxide as an oxidizer. Next, potassium an calcium were determined by the emission atomic spectrophotometry (ESA) and magnesium – by the absorption atomic spectrophotometry (ASA).

RESULTS

The content of mineral components in cereal grains varies depending on the climatic factors and plant cultivation conditions (Makarska, Michalik 2003). In the present study as well as in the previous reports by Wierzbowska (2006a, b) and Wierzbowska and Nowak (2000, 2002), growth regulators had a more evident influence on the content of potassium, calcium and magnesium in wheat vegetative parts and grains than mineral fertilization.

Kinetin increased by nearly 13.8% the content of potassium in wheat grain and all vegetative organs except the flag leaf (Table 2). Auxin caused a nearly 11% increase in the concentration of potassium in grain; it also raised its levels in the penultimate leaf and in the other leaves, depressing the content of potassium in glumes (by 6.9%) and stems. The slightest effect on the concentration of potassium in wheat, compared to the control plants, occurred in the treatments involving gibberellin. Higher doses of phosphorus, except for the oldest leaves, depressed the content of potassium, which was confirmed by relatively high correlation coefficients.

Table 2

Potassium content ($g \cdot kg^{-1}$ d.m.)

Specification	Part of wheat					
	grain	glume	stem	flag leaf	penultima te leaf	remaining leaves
Mean for growth regulators						
Control	8.31	28.59	43.86	31.93	33.20	43.39
Kinetin	9.46	30.27	45.73	31.26	33.70	45.17
Gibberellin	8.57	27.46	44.91	30.64	31.83	42.67
Auxine	9.21	26.61	42.81	31.79	34.64	44.53
Mean for dose P						
0.00	9.35	30.33	45.35	32.60	34.20	43.40
0.17	9.03	28.58	45.10	31.60	33.75	44.63
0.35	9.43	29.55	45.88	31.13	34.35	44.60
0.51	9.28	28.60	44.38	31.60	33.58	44.60
0.68	9.03	28.60	43.80	30.93	32.90	43.63
0.85	8.45	26.60	43.65	31.03	32.58	44.33
1.02	7.68	25.38	42.15	30.95	32.05	42.40
r	0.61	0.54	0.56	0.48	0.54	0.37

r - correlation coefficient

The growth regulators to some extent stimulated the uptake of potassium by wheat plants (Figure 1). The strongest effect was produced by gibberellin (on average 79.14 mg k per plant) by stimulating the development of vegetative mass, particularly stems (WIERZBOWSKA, SIENKIEWICZ 2004). Phosphorus fertilization, and the rate of 0.68 g P per pot, improved the uptake of potassium by wheat.

Gibberellin sprays contributed to increased content of magnesium in wheat organs except the flag and penultimate leaves (Table 3). The highest increment of magnesium (by 36%) was observed in glumes, whereas the grains contained just 5.4% more magnesium than the control. The other plant hormones tended to depress the level of Mg in particular organs of wheat. Increasing doses of phosphorus decreased the content of magnesium in grain (r=0.37) and in the penultimate leaf (r=0.49). In the flag leaf this dependence followed a parabolic course – the content of magnesium continued to increase following the application of small P doses, but declined after the highest phosphorus rates were used (r=0.63). Phosphorus fertilization, in the whole range of the test doses, increased the content of Mg in the other leaves.

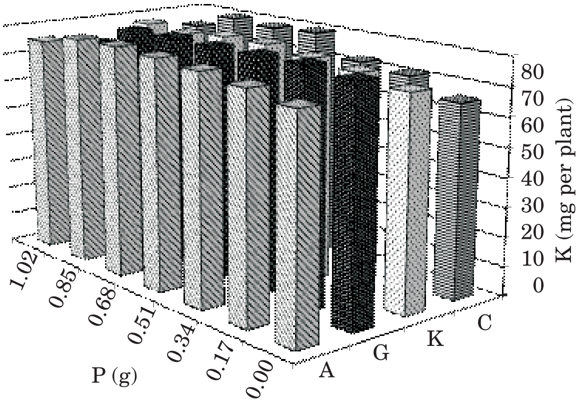

Fig. 1. Potassium uptake: C – control, K – kinetin, G – gibberellin, A – auxine

Table 3

Magnesium content ($g \cdot kg^{-1}$ d.m.)

Specification	Part of wheat					
	grain	glume	stem	flag leaf	penultima te leaf	remaining leaves
Mean for growth regulators						
Control	1.67	0.61	0.43	2.20	2.83	3.47
Kinetin	1.69	0.53	0.40	2.01	2.69	3.53
Gibberellin	1.76	0.83	0.47	1.96	2.81	3.79
Auxine	1.63	0.66	0.46	2.03	2.53	3.40
Mean for dose P						
0.00	1.75	0.65	0.48	2.03	2.95	3.43
0.17	1.68	0.68	0.40	2.13	2.83	3.43
0.35	1.75	0.63	0.45	2.18	2.68	3.58
0.51	1.68	0.60	0.40	2.15	2.70	3.73
0.68	1.65	0.60	0.45	2.15	2.73	3.53
0.85	1.68	0.55	0.45	1.95	2.68	3.60
1.02	1.63	0.90	0.45	1.78	2.45	3.55
r	0.37	0.33	0.00	0.63	0.49	0.32

r - correlation coefficient

The growth regulators sprayed over wheat plants grown without phosphorus fertilization improved the uptake of magnesium by 11% (kinetin) up to 23% (gibberellin) compared to the control plants (Figure 2). Phosphorus fertilization levelled the stimulating influence of the phytohormones on the uptake of magnesium, although wheat sprayed with gibberellin absorbed over 5% more Mg than the control plants. On average, the highest magnesium uptake (79.66 mg Mg per plant) was obtained when 0.68 P per pot was applied.

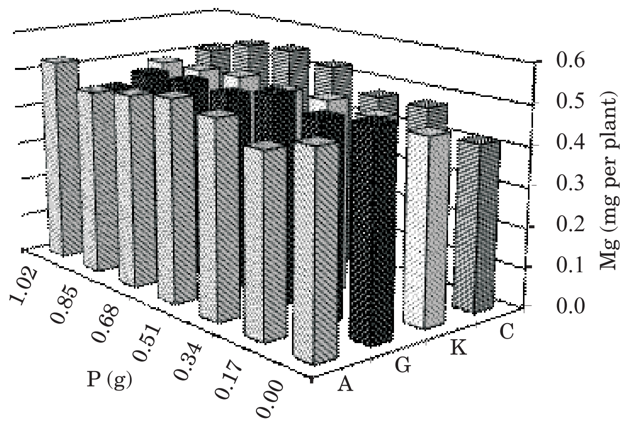

Fig. 2. Magnesium uptake (legends as Fig. 1)

Kinetin and gibberellin increased by over 20% the content of calcium in grain and glumes of wheat (Table 4). Under the influence of gibberellin the concentration of calcium also went up in the other vegetative organs, whereas kinetin depressed by 15% the content of Ca in the flag and penultimate leaves, decreasing it albeit less severely in the other leaves. In contrast, auxin had a very weak influence of the concentration of calcium in particular wheat organs. It was only when very low phosphorus rates had been applied that the content of calcium in grain, glumes and stems of wheat slightly increased (r=0.53). For al the range of the P rates tested, the concentration of calcium in the penultimate leaf increased (r=0.40), increasing in the other leaves (r-0.51).

Gibberellin and auxin sprayed over wheat grown without phosphorus nutrition increased by about 40% the uptake of calcium compared to the control plants. An analogous effect produced by kinetin reached only 20% (Figure 3). Likewise magnesium, phosphorus fertilization reduced the stimulating effect of the growth regulators on the uptake of calcium, but when analysing the means for each plant hormone it was discovered that the uptake of ca rose by a few per cent under the effect of gibberellin and auxin. Phosphorus fertilization only weakly improved the accumulation of calcium in wheat.

Our previous experiments showed that growth regulators modified the content of potassium, calcium and magnesium in wheat grain and vegetative parts to a higher degree than phosphorus fertilization (WIERZBOWSKA

Table 4

Calcium content ($g \cdot kg^{-1}$ d.m.)

Specification	Part of wheat					
	grain	glume	stem	flag leaf	penultima te leaf	remaining leaves
Mean for growth regulators						
Control	0.49	2.13	2.39	9.64	11.36	14.30
Kinetin	0.60	2.54	2.39	8.20	9.70	13.57
Gibberellin	0.61	2.61	2.56	9.63	13.00	15.84
Auxine	0.51	2.20	2.53	9.16	11.16	14.70
Mean for dose P						
0.00	0.58	2.50	2.53	8.85	12.85	14.10
0.17	0.65	2.55	2.55	8.63	11.68	14.38
0.35	0.58	2.43	2.65	10.08	10.70	14.55
0.51	0.55	2.23	2.40	8.93	10.78	14.53
0.68	0.50	2.40	2.55	10.13	10.83	14.63
0.85	0.48	2.20	2.30	8.58	11.28	15.80
1.02	0.55	2.30	2.28	8.93	11.03	14.25
r	0.32	0.35	0.53	0.28	0.40	0.21

r – correlation coefficient

2006a,b). According to NIEMYSKA and STARCK (1988), exogenous gibberellin can produce an effect of increased remobilization of K^+ ions from aging plant organs, which means that potassium ions are used more efficiently under nutrient deficiency conditions. The influence of exogenous plant hormones (IAA, GA_3) on the uptake and transport of ions can be diverse and depends on many factors including dates of application (CHOŁUJ 1988). Transport of ions can be regulated by IAA or ABA via their effect on the opening and closing of ionic channels in cellular membranes (BLATT 1993).

The growth regulators we tested slightly broadened the molar ratios of calcium to phosphorus in wheat grain, glumes and stems (Figure 4), mainly by increasing the amounts of calcium in plants. Kinetin and auxin narrowed the Ca:P ratio in leaves. Increasing rates of phosphorus, however, did not play any major role in shaping the Ca:P molar proportions in grain, although, due to decreasing calcium concentrations (glumes, the penultimate leaf) or increasing phosphorus content (the stem, flag leaf and other leaves), these two plant hormones narrowed the Ca:P ratio in the vegetative organs of wheat.

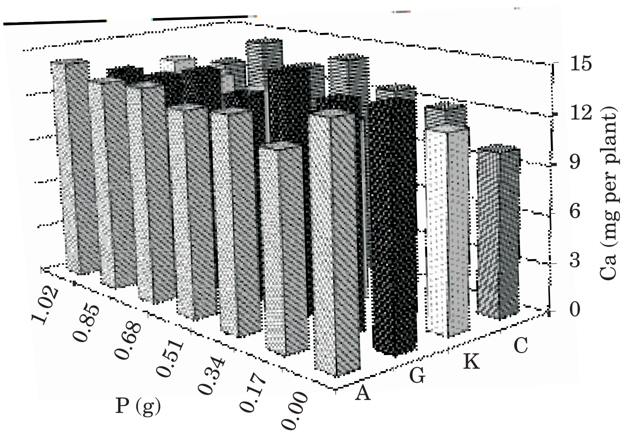

Fig. 3. Calcium uptake (legends as Fig. 1)

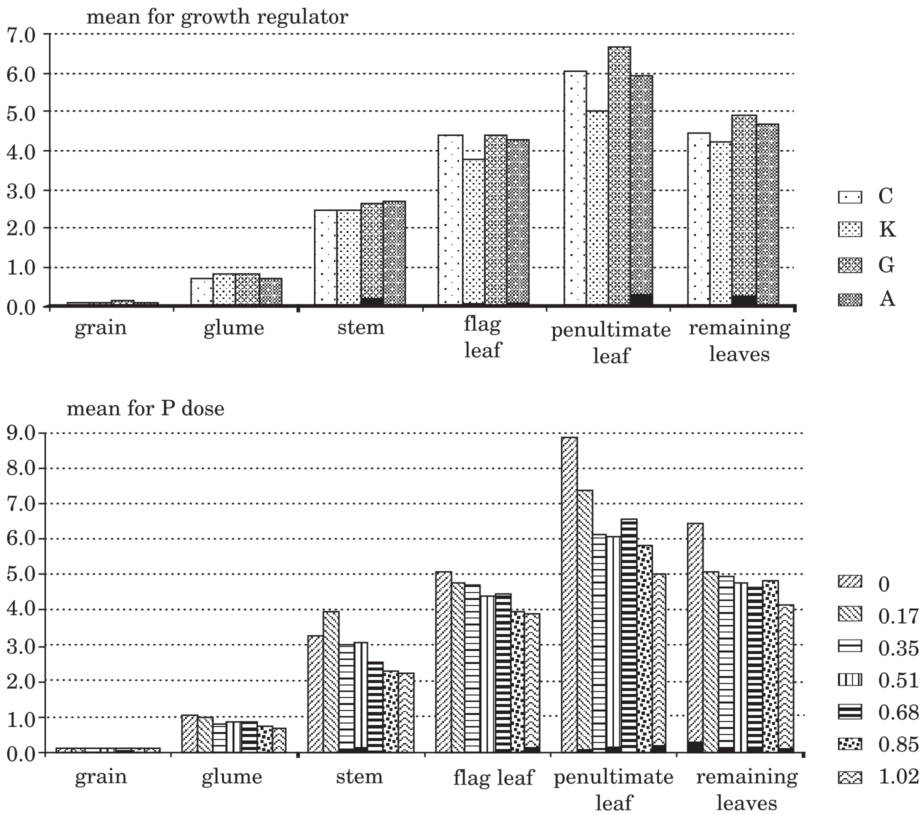

Fig. 4. Ca:P molar relations (legend as. Fig. 1)

Both mineral fertilization and growth regulators exert a more powerful effect on the modification of K:(Mg+Ca) ratios in vegetative organs than in grains of cereals (WIERZBOWSKA 2006b, MAKARSKA and MICHALIK 2003). In the present study, gibberellin, which depressed the content of potassium while rising that of calcium and magnesium in wheat, narrowed the K:(Mg+Ca) ionic ratio in wheat vegetative organs and grain (Figure 5). Wheat treated with kinetin responded differently, which meant that the proportions between the above ions were broader in grain and leaves but narrower in glumes and stems. The lowest and the highest doses of phosphorus resulted in the narrowing of the K:(Mg+Ca) ratio in grains and glumes. In the remaining parts of wheat plants, the phosphorus fertilization rates produced an ambiguous effect on the size of the K:(Mg+Ca) ratio.

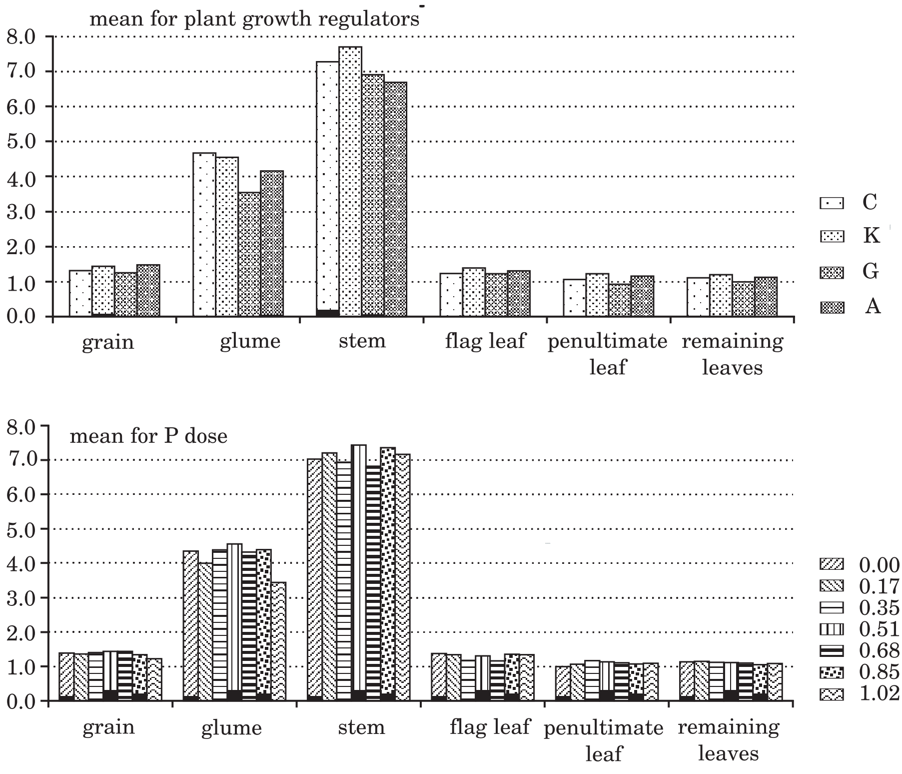

Fig. 5. K : (Ca+Mg) ionic relations (legends as. Fig. 1)

CONCLUSIONS

1. The growth regulators increased the content of potassium and calcium in wheat grain, with gibberellin raising also the concentration of magnesium in wheat grain. Increasing rates of phosphorus fertilization depressed the content of K, Mg and Ca in wheat grain and vegetative organs, except for the oldest leaves.

2. In general, the phyrohormones improved the uptake of potassium, calcium and magnesium by wheat. The highest uptake of potassium and magnesium occurred after the application of 0.68 g P per pot. Phosphorus fertilization had only a weak effect on improved Ca accumulation in wheat.

3. The growth regulators slightly broadened the Ca:P molar ratios in wheat grain, glumes and stems. Kinetin narrowed the Ca:P ratio in leaves. Increasing phosphorus rates did not produce any stronger effect on the Ca:P proportions in grains, but they narrowed the ratios between these two elements in wheat vegetative organs.

4. Gibberellin narrowed the K:(Mg+Ca) proportions in wheat grain and vegetative organs, in contrast to kinetin, which caused their narrowing. Auxin broadened these ratios in grain and leaves, while narrowing them in glumes and stems. The lowest and the highest phosphorus rates made the K:(Mg+Ca) ratios in grain and glumes narrower.

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INFLUENCE OF GROWTH REGULATORS AND PHOSPHORUS FERTILIZATION RATES ON NITROGEN BALANCE IN SPRING WHEAT

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Abstract

A pot experiment was carried out on cv. Jasna spring wheat grown on light loamy sand soil. The soil was slightly acidic in reaction and moderately abundant in available phosphorus, potassium and magnesium. Consistent NKMg fertilization rates (1.5 g N, 1.5 g K and 0.25 g Mg) were accompanied by increasingly high doses of phosphorus (0.0 to 1.02 g P per pot). In order to compare the effect produced by growth regulators, applied in conjunction with the growing phosphorus doses, the pots were split into four groups, depending on the sprays applied: distilled water (control), kinetin, gibberellin and auxin. The target was to determine the influence of plant hormones and phosphorus fertilization on nitrogen balance in spring wheat. The content of nitrogen in wheat grain depended mainly on the phosphorus fertilization level, the relationship which became particularly evident following auxin and kinetin application. The highest N concentration occurred when 0.85 g P per pot was used. The growth regulators, auxin and gibberellin in particular, depressed the concentration of nitrogen in grain. The level of nitrogen in glumes and stems was only slightly dependent on the level of phosphorus nutrition. In leaves, it was negatively correlated with the doses of phosphorus. The phytohormones depressed the concentration of nitrogen in stems, oldest leaves and in the flag leaf, raising it in the penultimate leaf. The uptake and accumulation of nitrogen in grain tended to increase up to the phosphorus rate of 0.68 g P per pot. The growth regulators inhibited the accumulation of nitrogen in aerial organs of wheat, especially in grain. Gibberellin, in turn, increased the accumulation of nitrogen in glumes and stems. The contribution of grain in nitrogen accumulation ranged from 62% (without P fertilization) to 68% when 0.68 g P per pot was applied. The growth regulators, especially gibberellin, decreased the ratio of nitrogen accumulated in grain. Protein yield per plant, except wheat sprayed with gibberellin, increased proportionately to the rate of phosphorus.

Key words: spring wheat, phosphorus fertilization, nitrogen, growth regulators.

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WPŁYW REGULATORÓW WZROSTU I POZIOMU NAWOŻENIA FOSFOREM NA GOSPODARKĘ AZOTEM W ROŚLINACH PSZENICY JAREJ

Abstrakt

W doświadczeniu wazonowym, na glebie o składzie granulometrycznym piasku gliniastego lekkiego, uprawiano pszenicę jarą odmiany Jasna. Gleba charakteryzowała się lekko kwaśnym odczynem i średnią zasobnością w przyswajalny fosfor, potas i magnez. Na tle stałego nawożenia NKMg (1,5 g N, 1,5 g K i 0,25 g Mg) zastosowano wzrastające fosforu (0,0-1,02 g P na wazon). W celu porównania działania regulatorów wzrostu, stosowanych w warunkach wzrastających dawek fosforu, wazony podzielono na 4 grupy, w zależności od stosowanych oprysków: woda destylowana (kontrola), kinetyna, giberelina i auksyna. Celem pracy było określenie wpływu regulatorów wzrostu i poziomu nawożenia fosforem na gospodarkę azotem w roślinach pszenicy jarej. Zawartość azotu w ziarnie zależała od poziomu nawożenia fosforem, zwłaszcza po oprysku auksyną i kinetyną. Najwyższą koncentrację N uzyskano po zastosowaniu 0,85 g P na wazon. Regulatory wzrostu, zwłaszcza auksyna i giberelina, zmniejszyły koncentrację azotu w ziarnie. Zawartość azotu w plewach i źdźble w niewielkim stopniu zależała od poziomu nawożenia fosforem, natomiast w liściach była ujemnie skorelowana z dawką P. Fitohormony zmniejszyły koncentrację azotu w źdźble, najstarszych liściach i liściu flagowym, a zwiększyły w podflagowym. Pobranie i akumulacja azotu w ziarnie wzrastały do dawki 0,68 g P na wazon. Regulatory wzrostu zmniejszyły akumulację azotu w nadziemnych organach pszenicy, szczególnie w ziarnie, a pod wpływem gibereliny zwiększyło się nagromadzenie tego składnika w plewach i źdźble. Udział ziarna w gromadzeniu azotu wahał się od 62% (bez nawożenia P) do 68% po zastosowaniu 0,68 g P na wazon. Regulatory wzrostu, zwłaszcza giberelina, zmniejszyły udział ziarna w gromadzeniu tego składnika. Plon białka z rośliny, z wyjątkiem pszenicy opryskiwanej gibereliną, wzrastał proporcjonalnie do dawki fosforu.

Słowa kluczowe: pszenica jara, nawożenie fosforem, azot, regulatory wzrostu.

INTRODUCTION

Spring wheat constitutes about 5% of all sown cereals. The nutritional value of spring wheat grain is often superior to that of winter wheat grain (Kaczyński 2002). Quality cultivars of spring wheat should produce grain containing at least 12.5% protein and over 26% gluten, which guarantees good baking quality of wheat flour. Fertilization is one of the major factors which determine the volume and quality of yields. In order to obtain high, good quality grain yield, it is necessary to provide wheat with all essential nutrient throughout the whole growing season. It is crucial that wheat plants receive good nitrogen fertilization, which stimulates high yields, but it is of equal importance that wheat is well supplied with phosphorus, potassium and magnesium. Wheat plants which receive enough phosphorus form larger grains and mature earlier. Phosphorus has beneficial influence on the synthesis and quality of proteins and carbohydrates. It can also alleviate negative effects of excessive nitrogen fertilization. In cereal crops, phosphorus conditions proper root system growth and tillering (Valizadeh et al. 2002, Sander et al. 1991).

Biologically active substances which act as growth regulators can modify metabolic responses of plants in a way that is economically and agriculturally profitable, but without producing a reproducible effect. Growth regulators can indirectly affect the uptake of nutrients by plants, their further transport and remobilization during the formation of grains. This indirect effect is a consequence of the root system, particularly root hairs, being better developed, which means that the plants absorb mineral components from soil and fertilizers more effectively. The response of plants to phytohormones is highly diverse and depends on the age and physiological state of a plant, environmental conditions as well as synergic or antagonistic reactions between endogenous and exogenous phytohormones.

The aim of the present study has been to determine the effect of growth regulators and phosphorus fertilization doses on the nitrogen balance in spring wheat.

METHODS

The experiment, with four replications, was established in Mitscherlich pots filled with 6.5 kg of light loamy sand, whose reaction was 6.4 pH in 1mol KCL·dm^{-3} and which was moderately abundant in available phosphorus, potassium and magnesium. The following mineral fertilization regime was applied: 1.5 g N [NH_4NO_3]; 0.0 – 1.02 g P[$Ca(H_2PO_4O_2 \cdot H_2)$], 1.5 g K [KCl and K_2SO_4 at a 1 : 1 ratio], 0.25 g Mg [$MgSO_4 \cdot 7H_2O$] per pot. All of the P and Mg rates and half the doses of N and K were added to soil before sowing wheat. The remaining portions of the nitrogen and potassium fertilizers were applied in 2 equal doses – at the early inflorescence and the ear shooting stages. Twenty cv. Jasna wheat plants were grown in each pot. In order to compare the results produced by growth regulators under the effect of increasing phosphorus fertilization rates, the pots were divided into 4 groups (Table 1). Each spraying treatment consisted of an application of 0.5 dm^3 of a liquid containing 50 mg·dm^{-3} of a growth regulator.

Wheat was harvested at the full maturity stage. Following the biometrical measurements, wheat plants were dissected into organs: grains, glumes including rachises, stems, flag leaf, penultimate leaf and other leaves. The plant material was ground and digested with concentrated sulphuric acid supplemented with hydrogen dioxide as an oxidizer. Finally, nitrogen was determined with Kjeldahl method.

Table 1

Design of the application of the plant growth regulators

Experiment variant	Spraying time and plant growth applied	
	beginning of tillering	beginning of flowering
I – control (C)	aqua destillata	aqua destillata
II – kinetin (K)	BAP (benzylaminopurine)	FAP (phurphurilaminopurine)
III – gibberellin (G)	GA_3 (gibberellic acid)	GA_3 (gibberellic acid)
IV – auxine (A)	IAA (indole-3-acetic acid)	NAA (naphthaleneacetic acid)

RESULTS

The up-to-date results of experiments indicate that plant hormones can raise concentration of nitrogen in grains (WIERZBOWSKA et al. 2006, WIERZBOWSKA et al. 2002, WIERZBOWSKA, NOWAK 1999). HARMS and NOWAK (1990a, b) suggest that this effect is an outcome of inhibited aging of plants, particularly the flag leaf, and induced activity of enzymes responsible for remobilization of nitrogen compounds in vegetative organs of plants. As a result, the content of proteins in grains rises at the cost of decreasing amounts of nitrogen in straw.

However, the present research has revealed contrary effects. The growth regulators tested depressed the content of nitrogen in wheat grain compared to the control (Table 2). Auxin and gibberellin in particular proved to produce adverse influence, depressing the concentration of nitrogen in grain by 21.6 and 15.6%, respectively. The content of nitrogen in wheat grain depended on the phosphorus fertilization doses, especially in the case of wheat sprayed with auxin (r=0.94) and kinetin (r=0.68), but also – to a somewhat lesser degree – the control plants (r=0.50). On average, the highest nitrogen concentration was obtained using 0.85 g P per pot. The highest phosphorus dose depressed the concentration of nitrogen in grain of all wheat plants except these treated with kinetin. Application of gibberellin caused an unprecedented decline in grain concentration of nitrogen, below that found in the grain of wheat not fertilized with phosphorus.

Gibberellin and auxin raised the concentration of nitrogen in glumes by 13.4 ad 6.7%, respectively. The growth regulators, and auxin in particular (a 26.4% decrease) depressed the content of nitrogen in wheat stems. Both in wheat glumes and stems, the content of nitrogen was only weakly dependent on the level of phosphorus nutrition. Gibberellin and auxin depressed the content of nitrogen in the flag leaf by about 17%, while raising it, along with kinetin, in the penultimate leaf. The concentration of nitrogen in the flag and penultimate leaves was largely dependent on the phosphorus dose, typically decreasing as the rate of phosphorus went up, except

Table 2

Nitrogen content (g N · kg^{-1} d.m.)

Plant growth regulators	Dose P (g per pot)							Mean	r
	0.00	0.17	0.34	0.51	0.68	0.85	1.02		
Grain									
C	28.00	30.60	29.80	28.90	29.80	30.60	28.90	29.51	0.50
K	26.80	27.10	28.00	28.90	31.50	32.40	28.40	29.01	0.68
G	24.50	21.90	25.40	26.30	26.30	26.30	23.60	24.90	0.32
A	21.90	21.90	21.90	23.60	23.60	24.50	24.50	23.13	0.94
Mean	25.30	25.38	26.28	26.93	27.80	28.45	26.35	-	0.25
Glume									
C	8.80	10.50	12.20	12.20	8.80	7.00	12.20	10.24	-0.14
K	14.00	8.80	7.00	8.80	10.50	9.60	12.20	10.13	-0.01
G	10.50	12.20	12.20	10.50	14.00	10.50	11.40	11.61	-0.30
A	10.50	14.00	12.30	7.90	12.30	9.00	10.50	10.93	-0.36
Mean	10.95	11.38	10.93	9.85	11.40	9.03	11.58	-	0.13
Stem									
C	5.30	3.50	6.10	6.10	5.30	3.50	3.50	4.76	-0.39
K	5.00	3.50	3.50	5.30	3.50	3.50	5.30	4.22	0.00
G	5.30	3.50	5.30	5.30	3.50	5.30	3.50	4.53	-0.29
A	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	0.00
Mean	4.78	3.50	4.60	5.05	3.95	3.95	3.95	-	-0.21
Flag leaf									
C	17.50	17.50	15.80	19.30	14.00	15.80	13.70	16.22	-0.85
K	15.80	22.80	19.30	17.50	15.80	12.30	10.50	16.29	-0.75
G	15.80	17.50	12.30	12.30	10.50	15.80	10.50	13.53	-0.58
A	12.30	15.80	12.30	16.60	15.80	10.50	10.50	13.40	-0.37
Mean	15.35	18.40	14.93	16.43	14.03	13.60	11.30	-	-0.65
Penultimate leaf									
C	19.30	17.50	15.80	15.80	21.00	15.80	15.80	17.29	-0.32
K	22.80	22.80	22.80	20.10	15.70	15.70	17.50	19.63	-0.87
G	19.30	21.00	22.80	21.00	17.50	19.30	17.50	19.77	-0.56
A	14.00	12.30	20.50	22.80	17.50	19.30	17.50	17.70	0.46
Mean	18.85	18.40	20.48	19.93	17.93	17.53	17.08	-	-0.32
Remaining leaves									
C	10.50	12.30	12.30	10.50	8.80	8.80	8.80	10.29	-0.77
K	8.80	7.00	8.80	12.30	8.80	7.00	7.00	8.53	-0.22
G	5.30	7.00	7.00	10.50	7.00	7.00	10.50	7.76	0.61
A	7.00	5.30	7.00	5.30	7.30	7.00	10.50	7.06	0.64
Mean	7.90	7.90	8.78	9.65	7.98	7.45	9.20	-	0.08

C – control, K – kinetin, G – gibberellin, A – auxine, *r* – correlation coefficient when wheat was treated with auxin. In the other leaves, the plant hormones considerably depressed (16-31%) the content of nitrogen. In the oldest leaves of the control plants (r=-0.77) and plants sprayed with kinetin, the concentration of nitrogen decreased; in plants treated with gibberellin (r=-0.61) and auxin (r=-0.64), the nitrogen concentration in the oldest leaves increased in proportion to the doses of phosphorus.

The uptake of nitrogen by wheat plants and accumulation of this nutrient in grain tended to be larger up to the rate of 0.68 g P per pot, and the nitrogen increment obtained versus the wheat plants not fertilized with phosphorus was 38 and 24%, respectively (Figure 1). The growth regulators, due to depressed yields and lower N concentrations, had a negative effect on the uptake and accumulation of nitrogen in wheat grain. Gibberellin and auxin sprays depressed the nitrogen uptake by plant by about 18%; the accumulation of nitrogen in wheat plants was about 25% lower versus the control.

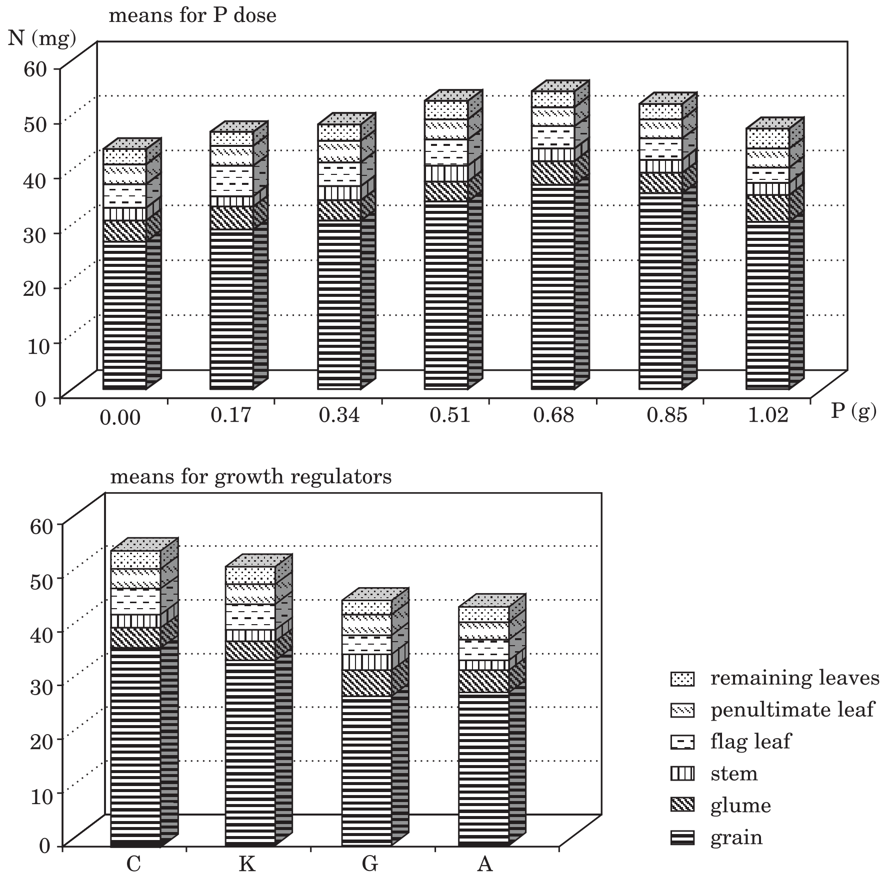

Fig. 1. Nitrogen accumulation (designations as in Table 2)

Kinetin produced the weakest effect, as it depressed the nitrogen uptake and accumulation in grain by just about 6%.

Increasing phosphorus fertilization from 0 to 0.68 g P per pot resulted in a larger participation of wheat grain in nitrogen accumulation (from 61.6 to 68.2%, Figure 2). The highest P doses depressed the contribution of grain to the total nitrogen accumulation while the percentage of nitrogen accumulated in glumes rose. Out of the three tested plant hormones, gibberellin produced the least desirable effect. Under the influence of gibberellin, compared to the control, the percentage of nitrogen accumulated in grain declined by 6% while its concentration in glumes and stems increased.

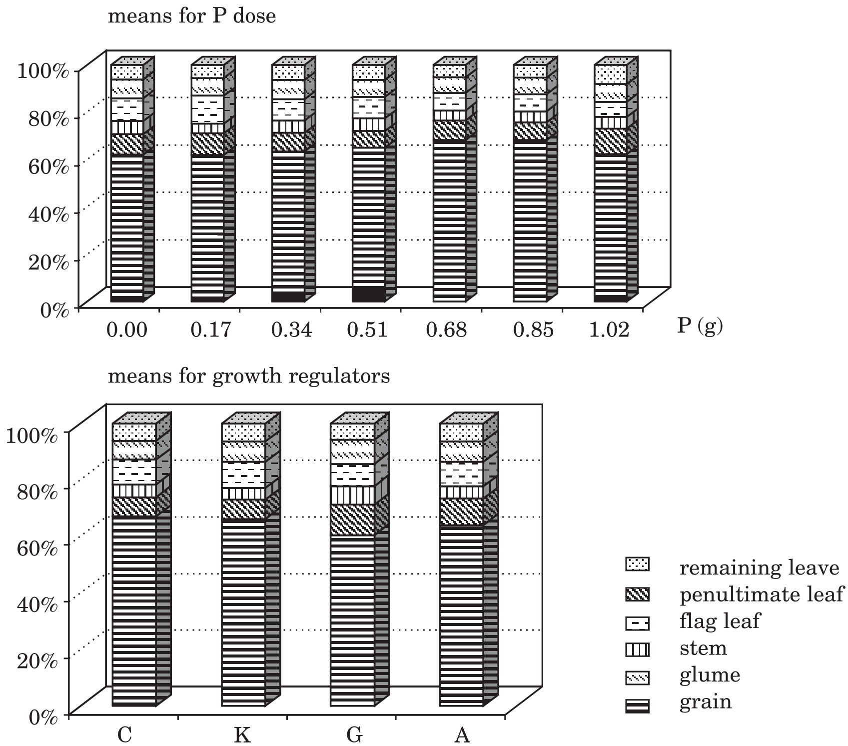

Fig. 2. Nitrogen distribution (designations as in Table 2)

Protein yield produced by the control (r=0.91) and auxin- (r=0.97) or kinetin-treated (r=0.62) wheat plants depended on the level of phosphorus fertilization. According to RAGASTIS et al. (1999), high rates of nitrogen and phosphorus improve wheat grain quality parameters, especially the content of gluten and farinogram properties. The major criterion used for assessment of baking quality of flour is how much good quality protein it contains (CYGANKIEWICZ 1997). The effect of phosphorus fertilization on total protein

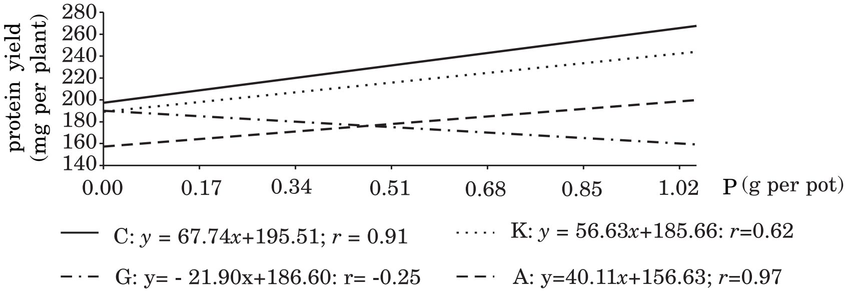

Fig. 3. Protein yield (designations as in Table 2)

and gluten concentration in wheat grain and, consequently, on the baking value of flour produced from this grain is not unilateral. It is often claimed that good baking quality of wheat flour can be obtained by using high nitrogen fertilization rates in combination with proper phosphorus and potassium nutrition. Others claim that on soils which are rich in phosphorus and potassium it is possible to reduce fertilization with these nutrients without any negative effect on yield and baking quality of wheat flour (KLUPCZYŃSKI et al. 2000), whereas increasing phosphorus and potassium rates applied together with constant nitrogen fertilization does not improve the baking quality of flour (KLUPCZYŃSKI et al. 2001). Likewise, studies on other cereal crops suggest that phosphorus nutrition produces a rather weak effect on nitrogen concentration in plants. CIEĆKO et al. (2006) found out that only high NK fertilization rates could slightly improve the total nitrogen and protein nitrogen concentration. On the other hand, as the doses of phosphorus went up, the content of exogenous amino acids declined. Increasing phosphorus fertilization had a very weak and variable effect on the concentration of total and protein nitrogen in grain (WIERZBOWSKA 2006).

CONCLUSIONS

1. The growth regulators, especially auxin and gibberellin, reduced the concentration of nitrogen in wheat grain. The content of nitrogen in wheat grain depended on the level of phosphorus fertilization, particularly in wheat plants treated with auxin and kinetin. The highest content of nitrogen was determined in the plants which received 0.85 g P per pot.

2. The phytohormones depressed the concentration of nitrogen in wheat stems, oldest leaves and in the flag leaf, while raising it in the penultimate leaf. The content of nitrogen in glumes and stems was only very weakly correlated with the rate of phosphorus nutrition. In leaves, the correlation between nitrogen concentration and P fertilization was negative.

3. The uptake and accumulation of nitrogen in wheat grain continued to rise up to 0.68 g P per pot. The plant hormones depressed the accumulation of nitrogen in aerial organs of wheat, especially in wheat grains. Gibberellin, however, favoured the accumulation of nitrogen in wheat glumes and stems.

4. The percentage of nitrogen accumulated in grain versus the whole wheat plant varied from 62% (no phosphorus fertilization) to 68% (0.68 g P per pot). The growth regulators, especially gibberellin, depressed the percentage of nitrogen accumulated in grains.

5. Yields of protein in the control, kinetin and auxin treated wheat plants increased proportionately to phosphorus rates.

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DETERMINING THE CONTENT OF SOME MINERALS IN FRUIT AND VEGETABLE BABY JUICES

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Abstract

Food is the main source of mineral elements but some are also provided with drinking water and supplements. Juices, for example, are an important source of mineral elements. In infants' diet minerals are provided mainly by fruit and vegetable purée juices. Infants and young children should be given juices labelled as "special purpose food". Pasteurised juices are recommended for infants, as they are free of bacteria and toxins, a condition that cannot be completely fulfilled while making juices at home.

The aim of the present work was to determine the content of some minerals in fruit and vegetable juices for infants and children under three years of age. The research material consisted of juices, all before their use-by date, purchased in grocer shops in Lublin in January 2006. In total 20 juices were examined, 8 of which were labelled as 'special purpose food', 2 were recommended by the National Food and Nutrition Institute and ten juices were labelled as 'food for young children'. Juices make an important source of minerals in the diet of infants and young children. The most valuable ones are the fruit and vegetable purée juices, as they provide significant amounts of dry mass which includes fibre, minerals and vitamins. Differences in the content of particular mineral elements in juices result primarily from their composition. Although juices for infants and young children should not contain any additives, products without certificates must be treated with caution as it cannot be excluded that some may contain prohibited compounds, e.g. calcium ascorbate or calcium chloride.

Keywords: mineral components, fruit and vegetable juices, children, infants.

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ZAWARTOŚĆ WYBRANYCH SKŁADNIKÓW MINERALNYCH W SOKACH WARZYWNO-OWOCOWYCH PRZEZNACZONYCH DLA NIEMOWLĄT I MAŁYCH DZIECI

Abstrakt

Źródłem poszczególnych pierwiastków jest głównie pożywienie, niektórych dostarczają woda pitna i suplementy. Ważnym źródłem składników mineralnych w diecie dziecka są soki. Niemowlęta i małe dzieci powinny otrzymywać soki oznaczone jako „żywność specjalnego przeznaczenia żywieniowego". Dla małych dzieci rekomendowane są soki pasteryzowane, ponieważ są one wolne od bakterii i toksyn, a tych warunków mogą nie spełniać soki wytwarzane w domu.

Celem pracy było określenie zawartości wybranych składników mineralnych w sokach warzywno-owocowych przeznaczonych dla niemowląt i dzieci do lat 3. Materiał do badań stanowiły soki zakupione w sklepach spożywczych na terenie Lublina w styczniu 2006 r., w okresie ich przydatności do spożycia. Osiem zakupionych soków miało adnotację, że jest to „środek specjalnego przeznaczenia żywieniowego", 2 były rekomendowane przez Instytut Żywności i Żywienia, natomiast 10 soków nie miało żadnych adnotacji o przeznaczeniu do żywienia niemowląt i małych dzieci. Soki są ważnym źródłem składników mineralnych w diecie niemowląt i małych dzieci. Najcenniejsze są soki przecierowe owocowo-warzywne, ponieważ dostarczają znacznych ilości suchej masy, w skład której wchodzą m.in. błonnik, składniki mineralne i witaminy. Różnice w zawartości poszczególnych składników mineralnych w sokach wynikają przede wszystkim z ich składu komponentowego. Soki dla niemowląt i małych dzieci nie powinny zawierać żadnych substancji dodatkowych, należy jednak traktować z rezerwą produkty bez atestów, nie można bowiem w nich wykluczyć obecności dodatków niedozwolonych, np. askorbianianu wapnia czy chlorku wapnia.

Słowa kluczowe: składniki mineralne, soki owocowo-warzywne, dzieci, niemowlęta.

INTRODUCTION

In the first year of life, the main type of nutrition for a baby is milk. A baby's body requires many nutrients in order to grow into a healthy child.

Determining the demand for mineral elements in infants is based on the knowledge of the composition of human breast milk, which is regarded as the best food in the first period of an infant's life. The concentration of minerals in woman's milk is low and the total content of ash amounts to 0.2 $g \cdot dl^{-1}$. The content of sodium, potassium and chlorine is threefold lower than in cow's milk (these are elements responsible for the osmotic pressure in the kidneys). Iron present in mother's milk is characterised by very high bioavailability, *ca* 50% (LYNCH, STOLTZFUS 2003). By comparison, bioavailability of iron from milk mixtures reaches about ca. 5%.

Food is the main source of particular chemical elements, but some are provided with drinking water and supplements. An infant immediately after birth lacks properly functioning regulatory mechanisms, while the absorption of minerals, including toxic elements, is more intensive than in older children and adults. Mineral elements are usually absorbed more effectively in the periods of intensive growth and pregnancy (DROBNIK, LATOUR 2006).

Juices are an important source of mineral elements. In infants' diet these elements are provided mainly by fruit and vegetable purée juices. Infants and young children should be given juices labelled as 'special purpose foods'. Making juices for infants at home is not recommended as it is impossible to pasteurize juice under such conditions and this means that some bacteria or toxins may contaminate juice. Pasteurization of fruit juices helps to eliminate bacteria occurring at levels which may cause food poisoning (GILLIAND et al. 2003). Juices that are 100% fruit juice will contain vitamin C, foliate, vitamin B6, iron, potassium and magnesium nutrients, which are essential in a baby's diet.

The aim of this study was to determine crude ash and mineral components (Ca, Mg, Na, K, Fe, Zn, Cu, Mn) in the fruit and vegetable juices for infants and children under three years of age.

MATERIAL AND METHODS

The experimental material included samples of fruit and vegetable juice for babies, all before their use-by date, purchased in Lublin shops in January 2006 (Table 1). In total 20 juices were examined, 8 recommended as 'special purpose food' (A-1, A-2, A-3, A-4, B-1, B-2, B-3, B-4), 2 recommended by the National Food and Nutrition Institute (F-1, F-2) and ten labelled as 'food for young children'. All the products were available in small bottles (175-330 ml) with brightly coloured labels. Most consumers are convinced that these juices are for infants and young children, although not all of them are classified as 'special purpose food'.

The content of dry mass and raw ash in the samples was determined with the AOAC methods (1990). The content of minerals (Ca, Mg, Na, K, Mn, Cu, Fe and Zn) was assayed by means of the AAS flame technique, using a Unicam 939 apparatus (AA Spectrometer Unicam). The determinations were carried out as follows: 10ml of 6N HCl was added to mineralised samples; next the solution was filtered into measuring flasks and filled up to 50 ml with distilled water.

All the analyses were made in three replications.

Table 1

Trade mark and ingrediends in analysed baby juices

Trade mark	Ingredients	Annotation
A-1	apple, carrot	*
A-2	apple, carrot, peach	*
A-3	apple, orange, carrot	*
A-4	apple, apricot, pumpkin	*
B-1	apple, banana, carrot	*
B-2	apple, pear, dog rose	*
B-3	apple, apricot, carrot	*
B-4	carrot, apple, pumpkin, grape	*
C	carrot, apple, strawberry	***
D-1	carrot, apple, banana	***
D-2	carrot, apple, raspberry	***
E	apple, carrot, lemon	***
F-1	carrot, pear, apple	**
F-2	multivitamin	**
G-1	carrot, apple, strawberry	***
G-2	carrot, apple, banana	***
H	carrot, apple, orange	***
I	apple, carrot, strawberry	***
J	carrot, banana	***
K	multivitamin	**

* foods for special purposes
** recommended by the Polish National Food and Nutrition Institute
*** no annotation

RESULTS AND DISCUSSION

Table 2 presents the content of dry mass, crude ash and minerals in the juices.

The content of dry mass was 7.35% to 14.40%. The highest content of dry matter was found in F-2, A-4 and F-1 juices. The lowest amount of dry mass was observed in the juice labelled as J. This value was significantly

Table 2

Dry matter (%), crude ash (%) and some minerals levels in the fruit-vegetable baby juices (n=3)

Trade mark	Dry matter (%)	Crude ash (%)	Macroelements ($mg \cdot g^{-1}$)				Microelements ($\mu g \cdot g^{-1}$)			
			Ca	Mg	Na	K	Fe	Zn	Cu	Mn
A-1	11.50	0.29	0.06	0.06	0.11	0.97	2.60	2.20	0.45	1.07
A-2	11.00	0.34	0.06	0.06	0.10	0.69	3.25	1.97	0.87	1.37
A-3	11.20	0.39	0.05	0.06	0.06	0.75	2.40	1.40	0.45	0.91
A-4	12.50	0.37	0.06	0.15	0.03	1.17	3.52	1.10	0.51	0.37
B-1	11.80	0.41	0.05	0.09	0.07	1.16	4.57	3.00	0.82	1.24
B-2	10.90	0.28	0.05	0.11	0.02	0.74	3.35	1.53	0.89	2.59
B-3	14.40	0.45	0.06	0.08	0.08	1.38	3.72	1.21	0.72	0.89
B-4	10.40	0.40	0.07	0.07	0.13	1.10	4.50	3.28	0.84	1.40
C	11.44	0.14	0.03	0.04	0.15	0.45	2.98	2.23	0.60	1.25
D-1	10.25	0.31	0.08	0.05	0.17	0.54	2.47	3.85	0.48	0.81
D-2	10.46	0.37	0.04	0.04	0.16	0.59	4.20	1.58	0.42	0.47
E	11.85	0.23	0.06	0.05	0.05	0.48	2.37	1.69	0.50	0.68
F-1	12.49	0.25	0.04	0.04	0.06	0.63	1.19	2.00	0.23	1.24
F-2	12.5	0.20	0.04	0.05	0.03	0.55	0.36	1.66	0.09	0.72
G-1	11.73	0.14	0.06	0.04	0.05	0.48	0.82	1.49	0.14	1.60
G-2	11.91	0.16	0.06	0.05	0.07	0.41	1.06	1.95	0.32	0.60
H	10.31	0.24	0.04	0.04	0.15	0.40	2.54	1.55	0.33	1.22
I	12.20	0.23	0.04	0.04	0.16	0.48	3.61	1.97	0.92	1.46
J	7.35	0.19	0.09	0.05	0.12	0.44	2.93	2.10	0.34	1.30
K	11.18	0.19	0.03	0.05	0.01	0.52	4.06	1.45	0.29	0.34

different from the remaining results. The juice contained carrots and bananas, which are characterised by a low content of water (KUNACHOWICZ et al. 1998). This, together with the low content of dry matter we determined proves that the juice was highly diluted, which is very bad because dry mass includes fibre, minerals and vitamins. In infants' diet these elements are provided mainly by fruit and vegetable purée juices. Juice J contained very little ash and few minerals in comparison with the other juices. It should be noticed that no information concerning any certificates could be found on the label of this juice.

The level of calcium in juices ranged from 0.025 $mg \cdot g^{-1}$ to 0.084 $mg \cdot g^{-1}$. The highest content of calcium was in juice J; its lowest amount occurred in juice C. The labels did not offer any information concerning the percentage of these two ingredients. The content of mineral elements in plants depends to a high degree on the soil's abundance, including the intensity of fertilisation (KRUCZEK 2005). Increased presence of calcium in juices may occur as a result of using acidity regulators during the production process, e.g. calcium ascorbate or calcium chloride. These substances are used to prevent enzymatic browning or to enrich the products in vitamin C, to prevent changes in the smell of juices and as antioxidants and acidity regulators (GUZ et al. 2007, MAO et al. 2007, ZHU et al. 2007). Although juices for infants and young children are not supposed to contain any such substances, products without any certificates should be treated with caution. Since the label on juice J does not give a detailed description of its composition, the presence of prohibited additives cannot be excluded. Some concern can arise because this juice is extremely popular among consumers, which was observed by the authors, and its chemical composition may suggest some harmful effect on very young organisms. Most probably its popularity results from its price. The lowest amount of calcium in juice C was probably caused by its composition. Carrots and apples contain relatively little calcium and, apart from these ingredients, only wild strawberry aroma was used for the production.

Calcium makes the major element of bones and teeth. It also participates in muscle contractions, conduction of nerve impulses and cell membrane permeability, blood coagulation. Moreover, calcium is a co-factor of numerous enzymes, e.g. those active in glycogenesis. Because of rapidly increasing mass of the body, including bones, a particularly high demand for calcium and phosphorus occurs between the ages of 1-3 and 10-15 years. The recommended calcium/phosphorus ratio in the diet of a child between 1 and 3 years of age should be 1 : 1 (2:1 would be ideal) (SZOTOWA et al. 1996). Calcium bioavailability with food is 25-50% and is depressed by aninutrient substances (oxalic acid, phytic acid) but raised by some amino acids, lactose and vitamin D (WAINE 2001, LYNCH, STOLTZFUS 2003). Protein has positive and negative effects on calcium balance. Dietary protein stimulates the production of insulin-like growth factor-1, a factor that promotes osteoblast-mediated bone formation. On the other hand, protein increases urinary calcium losses (KERSTETTER 2003). In children, pregnant women and during lactation the level of absorption rises (WAINE 2001). Shortage of calcium in children is manifested by rickets and inadequate growth.

The content of magnesium in juices ranged from 0.035 $mg \cdot g^{-1}$ to 0.145 $mg \cdot g^{-1}$. The highest level of magnesium was observed in juice A-4, which could result from its high content of pumpkin (20%) and low volume of water in the product. Pumpkin contains 14 mg of magnesium per 100 g of the raw product (KUNACHOWICZ et al. 1998). The lowest amount of magnesium was in juice C. Its main components were carrots and apples, which are poor in magnesium. The authors' own studies revealed that juice C had the lowest amounts of calcium and magnesium among all the juices. Little magnesium was also noted in juice H (0.036 mg·g^{-1}). The composition of this juice revealed that it was made of large amounts of apple juice and apple Cremogen, and apples contain small amounts of magnesium. The content of Mg in apples is 3 mg per 100 g of the raw product (KUNACHOWICZ et al. 1998). According to MARZEC et al. (2007) fruit juices for infants contained 12.1-76 mg of magnesium per 1 g.

The highest level of sodium was determined in juices D-1 (0.168 mg·g^{-1}), D-2 (0.162 mg·g^{-1}) and I (0.164 mg·g^{-1}), whereas its lowest presence was noted in juice K (0.013 mg·g^{-1}). High content of sodium probably meant a high share of carrots in the juices, yet the labels did not give any information concerning the percentage composition of the product. Carrots contain 82 mg of sodium per 100 g of the raw product (KUNACHOWICZ et al. 1998).

The highest amount of potassium was detected in juice B-3 (1.380 mg·g^{-1}), and its lowest level was observed in juice H (0.398 mg·g^{-1}). Little potassium was also found in juice G-2 (0.408 mg·g^{-1}). High content of potassium in juice B-3 results from a high content of this element in carrots and other fruit used to produce this juice. According to KUNACHOWICZ et al. (1998) the content of potassium is 282 mg 100·g^{-1} in carrots and from 118 mg 100·g^{-1} (pears) to 395 mg 100·g^{-1} (bananas) in other fruit. The lowest content of potassium was noted in juice G-2 made from carrots, apples and bananas.

The highest content of iron was revealed in juice B-1 (4.57 μg·g^{-1}), slightly lower values were determined in juices B-4 (4.50 μg·g^{-1}) and D-2 (4.200 μg·g^{-1}). The lowest amount of iron occurred in juices F-2 (0.36 μg·g^{-1}) and G-1 (0.82 μg·g^{-1}). The content of iron in juices depends on the percentage composition of raw materials. Studies by MARZEC et al. (2007) revealed that fruit juices for infants contained from 0.76 to 2.92 μg of Fe in 1 g. About 10% of iron is absorbed from an average food ration. The presence of calcium reduces the absorption of iron, whereas the presence of copper increases its absorption (HALLBERG et al. 1993, SZOTOWA et al. 1996, BEARD, TOBIN 2000). Iron absorption is also stimulated by ascorbate acid (CARPENTER, MAHONEY 1992, DAVIS et al. 1992). Fruit juice is marketed as a healthy, natural source of vitamin C. However, vitamin C in fresh fruits has greater bioavailability than vitamin C added to fortified juices.

Zinc was present most abundantly in juice D-1 (3.85 μg·g^{-1}), which resulted from the ingredients: carrots, apples and bananas, which are all rich in zinc. The least zinc was found in juice A-4 (1.10 μg·g^{-1}), despite the fact that pumpkin, an ingredient of this juice, contains the largest amount of zinc, and juice B-3 (1.21 μg·g^{-1}). The content of zinc in the authors' own studies was higher than that reported by MARZEC et al. (2005), where its average content in fruit juices was 0.51 μg·g^{-1}.

Following the Polish norm PN-A-75048:1994, the content of zinc as an element harmful for human health in fruit and vegetable juices for babies must not exceed 15.0 $\mu g \cdot g^{-1}$ of the product. Zinc plays an important role in human growth as it is active in more than 300 enzymes by participating in their structure and catalytic and regulatory reactions. It is closely related to bone metabolism and therefore plays a positive role in the growth and development (FESTA et al. 1985, BRANDAO-NETO et al. 1995). Zinc insufficiency may lead to inhibiting the growth in children and to changes in their appetite, taste, smell and body weight loss (BRANDAO-NETO et al. 1995, BLACK et al. 2004).

The largest content of copper was observed in juice I (0.915 $\mu g \cdot g^{-1}$). This most probably resulted from a large share of copper in raspberries. The lowest content of this element was found in juice F-2 (0.090 $\mu g \cdot g^{-1}$). In the studies performed by MARZEC et al. (2005) the determined value of copper in fruit juices was on average 0.31 $\mu g \cdot g^{-1}$. This value was slightly lower than the results obtained in the authors' own studies. Little information could be found in the available references concerning the toxicity of copper in infants' organisms, which suggests copper homeostasis in young organisms. Similarly, copper insufficiency is rare and the information in the literature usually concerns infants fed with cow's milk for a long time (LONNERDAL 2005). According to the Polish norm PN-A-75048:1994, the content of copper in vegetable and fruit juices for children must not exceed 4.0 $\mu g \cdot g^{-1}$ of the product.

Most manganese was present in juice B-2 (2.59 $\mu g \cdot g^{-1}$) and this value was significantly different from the other results. This was most probably caused by the presence of wild rose fruit as an ingredient of the juice. The lowest amounts of manganese were detected in juices K (0.339 $\mu g \cdot g^{-1}$) and A-4 (0.370 $\mu g \cdot g^{-1}$). The studies by MARZEC et al. (2007) revealed the presence of 0.21-1.80 mg of manganese in 1kg of fruit juice for infants. The symptoms of manganese insufficiency are rare as manganese is in large abundance in food products. If it insufficiency occurs, it may lead to disorders of the growth and development in children (LJUNG, VAHTER 2007).

No specific data could be found in the available bibliography which would refer to the content of mineral elements in juices for children and their maximum values of these minerals. It is known that the content of minerals should not be higher than the recommended daily nutritional norms. Food products for infants and young children should be labelled as far as the content of minerals is concerned. The packaging of the product is supposed to include the information regarding the content of such ingredients if they amount to no less than 5% of daily intake (SZPONAR, MOJSKA 1996). No such information was found on any packaging.

The chemical composition of food products for infants and young children must be continuously monitored. Many authors (MARZEC, ZARĘBA 2003, WINIARSKA-MIECZAN, GIL 2007, WINIARSKA-MIECZAN, KWIECIEŃ 2007) have shown that foods for special purposes (baby juices and baby foodstuff) are not always safe in respect of lead level.

WINIARSKA-MIECZAN and GIL (2007) demonstrated that, unlike juices, convenience foods for infants are not very popular in Poland. The authors' own survey studies have revealed that more than 90% of parents of young children declared a wish to purchase fruit and vegetable juices (WINIARSKA-MIECZAN, KWIECIEŃ 2007). The respondents stated that the most vital characteristic of juices was their nutritional value, and as many as 86% of the respondents were satisfied with the quality of juices.

CONCLUSION

Juices are an important source of mineral elements in the diet of infants and young children. The most valuable juices are those produced from fruit and vegetables in the form of purée, since they offer large amounts of dry mass, which contains fibre, minerals and vitamins. The differences in the content of particular minerals in juices result primarily from their ingredients.

Although juices for infants and young children should not contain any additives, products with no certificates should be treated with caution as the presence of prohibited additives, such as calcium ascorbate or calcium chloride in their composition, cannot be excluded.

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RESPONSE OF BACTERIA TO SOIL CONTAMINATION WITH HEAVY METALS

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Abstract

The effect of contamination of loamy sand with single heavy metals (Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}) and with their mixtures on the number of copiotrophic, ammonifying, nitrogen immobilising, cellulolytic bacteria and bacteria of the *Arthrobacter* and *Pseudomonas* genera was examined in a pot experiment. The research was performed in two series: with soil sown with oat and unsown soil.

It was found that the sensitivity of bacteria to Cd^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+} is a specific characteristic related to the content of these metals in soil and to the method of soil use. The development of the bacteria of *Arthrobacter* and *Pseudomonas* was most strongly inhibited in the soil sown with oat, while ammonifying, nitrogen immobilising, and cellulolytic bacteria were most inhibited in the unsown soil. Copiotrophic, cellulolytic, nitrogen immobilising and ammonifying bacteria proved to be more resistant to this contamination than bacteria of *Arthrobacter* and *Pseudomonas* genera. Increasing the number of heavy metals simultaneously contaminating the soil to two (Cd^{2+} and Cu^{2+}; Cd^{2+} and Zn^{2+}; Cd^{2+} and Pb^{2+}) and to three (Cd^{2+}, Cu^{2+} and Zn^{2+}; Cd^{2+}, Cu^{2+}, and Pb^{2+}; Cd^{2+}, Pb^{2+} and Zn^{2+}) generally did not increase the intensity of their effect on the examined bacteria. Changes brought about by these mixtures were usually similar to changes caused by individual heavy metals.

Key words: heavy metals in soil, bacteria count, copper, zinc, cadmium, lead.

REAKCJA BAKTERII NA ZANIECZYSZCZENIE GLEBY METALAMI CIĘŻKIMI

Abstrakt

W doświadczeniu wazonowym badano wpływ zanieczyszczenia piasku gliniastego pojedynczymi metalami ciężkimi (Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}) i ich mieszaninami na liczebność bakterii kopiotroficznych, amonifikacyjnych, immobilizujących azot, celulolitycznych oraz bak-

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terii z rodzaju *Arthrobacter* i *Pseudomonas*. Badania wykonano w dwóch seriach: z glebą obsianą owsem i nieobsianą.

Stwierdzono, że wrażliwość bakterii na Cd^{2+}, Cu^{2+}, Zn^{2+} i Pb^{2+} jest cechą specyficzną związaną z zawartością tych metali w glebie oraz sposobem jej użytkowania. Rozwój bakterii z rodzaju *Arthrobacter* oraz *Pseudomonas* był intensywniej hamowany w glebie obsianej owsem, natomiast bakterii amonifikacyjnych, immobilizujących azot oraz celulolitycznych – w glebie nieobsianej. Bardziej odporne na te zanieczyszczenia okazały się bakterie kopiotroficzne, celulolityczne, immobilizujace azot i amonifikacyjne niż bakterie z rodzaju *Arthrobacter* i *Pseudomonas*. Zwiększenie liczby metali ciężkich jednocześnie zanieczyszczających glebę do dwóch (Cd^{2+} i Cu^{2+}; Cd^{2+} i Zn^{2+}; Cd^{2+} i Pb^{2+}) i trzech (Cd^{2+}, Cu^{2+} i Zn^{2+}; Cd^{2+}, Cu^{2+} i Pb^{2+}, Cd^{2+}, Pb^{2+} i Zn^{2+}) z reguły nie zwiększało intensywności ich oddziaływania na badane bakterie. Zmiany wywołane przez te mieszaniny były zazwyczaj zbliżone do zmian powodowanych przez pojedyncze metale ciężkie.

Słowa kluczowe: metale ciężkie w glebie, liczebność bakterii, miedź, cynk, kadm, ołów.

INTRODUCTION

Heavy metals present in soil are a serious threat to human and animal health. Neither are they neutral to plants (BELYAEVA et al. 2005) or microorganisms (WYSZKOWSKA et al. 2007). They can have an inhibitory effect on the development of bacteria, fungi and actinomycetes (BOROS et al. 2007, LUGAUSKAS 2005). Heavy metals reduce biomass of microorganisms and lower their soil activity (WYSZKOWSKA et al. 2008a, MIN et al. 2005), and even if they do not reduce their number, they depress their biodiversity (MOFFETT 2003).

The mechanism of heavy metals affecting the environment in the soil-plant relation has not been completely clarified. On the one hand, soil bacteria immobilize heavy metals. On the other hand, they contribute to higher mobility of heavy metals, which is mainly due to metabolites they produce (KUFFNER et al. 2008). It is particularly difficult to establish mutual relations when soil is simultaneously contaminated with a number of various heavy metals. Under such conditions, the task of establishing which heavy metal predominantly destroys soil microbiological properties becomes complicated, since the joint influence of several metals may not be the sum of individual effects.

The aim of the research was to establish the effect of soil contamination with single heavy metals: cadmium, zinc, copper and lead, and with their mixtures on the count of bacteria of *Arthrobacter* and *Pseudomonas* genera, as well as on copiotrophic, ammonifying, nitrogen immobilising and cellulolytic bacteria.

MATERIALS AND METHODS

The research was carried out in a pot experiment, in four replications. The subject of the research was the soil of the granulometric composition of loamy sand (pH_{KCl} – 5.60, $C_{org.}$: N ratio – 11.8), uncontaminated and contaminated with individual heavy metals (Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}) and with their mixtures containing two metals (Cd^{2+} and Cu^{2+}; $Cd^{2+}$and Zn^{2+}; $Cd^{2+}$and Pb^{2+}), three metals (Cd^{2+}, Cu^{2+} and Zn^{2+}; Cd^{2+}, Cu^{2+}, and Pb^{2+}; Cd^{2+}, Pb^{2+} and Zn^{2+}) and all the heavy metals (Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}). The soil was contaminated with the following doses of heavy metals in mg kg^{-1} of soil: Cd^{2+} – 4 and 12; Cu^{2+} – 150 and 450; Pb^{2+} – 100 and 300; Zn^{2+} – 300 and 900. These elements were introduced to the soil in the form of the following compounds: cadmium – $CdCl_2 \cdot 2''H_2O$, copper – $CuSO_4 \cdot 5H_2O$, lead – $PbCl_2$ and zinc – $ZnCl_2$. After mixing the soil with compounds containing heavy metals and macroelements satisfying alimentary requirements of the experimental plant (oat), soil portions of 3 kg each were placed in plastic pots. The same mineral fertilization as in the previous research (WYSZKOWSKA et al. 2006) was applied. The soil in pots was brought to a moisture content corresponding to 60% capillary water capacity using distilled water and left for 14 days unsown. On day fourteen, soil samples were collected, microbiological analyses were performed, and cv. Borowik oat was sown in a series with four replications (after plant germination, 12 plants were left in each pot), while the other series, which was also carried out with four replications, the soil was left unsown. On the harvest day (in the inflorescence phase), soil samples were collected from both series of experiments and microbiological analysis were conducted. Their scope included determination of the number of the following bacteria: *Arthrobacter, Pseudomonas*, ammonifying, nitrogen immobilising, cellulolytic and copiotrophic, using the colony-count method. A detailed procedure of determining these microorganisms has been provided in WYSZKOWSKA at al. (2008b).

The results of microbiological analysis were statistically analysed using Duncan's multiple range test, applying a three factor variance analysis. The statistical analysis was accomplished with Statistica software (StatSoft, Inc....2006).

RESULTS AND DISCUSSION

The number of copiotrophic bacteria in soil sown with oat was 1.25-fold higher than in the unsown soil (Table 1). It was also influenced by the level and type of heavy metal contamination. In the soil contaminated with smaller doses of metals, no negative effects of cadmium, copper, lead or zinc on these bacteria were found. Neither were they affected by cadmium with an

Table 1

Number of copiotrophic bacteria (cfu 10^8 kg^{-1} of d.m. soil)

Object	Contamination level			
	I		II	
	soil use			
	unsown	sown	unsown	sown
0	88 ± 3	110 ± 6	88 ± 3	110 ± 6
Cd	87 ± 7	129 ± 5	68 ± 3	97 ± 4
Cu	87 ± 5	129 ± 6	64 ± 4	90 ± 4
Pb	85 ± 5	126 ± 5	69 ± 6	93 ± 8
Zn	85 ± 6	126 ± 7	86 ± 4	111 ± 7
CdCu	88 ± 7	104 ± 4	85 ± 7	109 ± 8
CdPb	88 ± 5	104 ± 6	60 ± 6	92 ± 9
CdZn	85 ± 5	104 ± 4	82 ± 3	117 ± 5
CdCuPb	85 ± 6	104 ± 7	57 ± 3	78 ± 4
CdCuZn	98 ± 5	140 ± 8	72 ± 6	100 ± 8
CdPbZn	98 ± 5	140 ± 7	71 ± 3	99 ± 5
CdCuPbZn	97 ± 4	111 ± 5	33 ± 2	74 ± 5
Mean	89 ±1	119 ± 2	70 ± 1	97 ± 2
LSD	a – 3.3, b – 1.4, c – 1.4, a · b – 4.7, a · c – 4.7, b · c – 1.9; a · b · c – 6.7			

LSD for: a – kind of contamination; b – contamination level; c – soil utilization, d – analysis term
I contamination level in mg per kg d.m. of soil: Cd – 4; Cu – 150; Pb – 100; Zn – 300
II contamination level in mg per kg d.m. of soil: Cd – 12; Cu – 450; Pb – 300; Zn – 900

addition of copper, lead or zinc, or by cadmium with an addition of copper and lead. Mixtures of cadmium and copper, zinc and lead as well as of copper, zinc and lead caused a significant increase in the number of copiotrophic bacteria. In the soil under oat, at this level of contamination, single metals stimulated bacterial growth. Cadmium with one additional metal or with the three other metals had no significant influence on microorganisms, but when applied with copper and zinc or with zinc and lead, it produced a stimulating effect,

In the soil contaminated by higher doses of heavy metals, regardless of the soil use, cadmium, copper and lead significantly reduced the count of copiotrophic bacteria. However, zinc had no such effect. Cadmium reduced total contamination with copper or zinc, but it increased the additional contamination with copper and lead. The bacterial count was most severely reduced as a result of the joint effect of all the four metals, in the unsown soil – by 63%, and in the sown soil – by 33%.

The number of ammonifying bacteria depended both on the method of soil use and on the type and extent of contamination with heavy metals (Table 2). In the soil sown with oat but uncontaminated with metals, it was 13% higher than in the unsown soil, and in the contaminated soil it was higher by up to 47% on average. A stronger, unfavourable effect on these bacteria was produced by metals in the unsown soil. Lead and zinc reduced their count by 22% - 23% on average, while cadmium and copper – by 13% and 16%, respectively. Cadmium applied with zinc lowered their number by as much as 43%, but applied with copper – by 18%, and with lead – by 22%, the same as lead alone. The joint effect of cadmium and zinc was mitigated by lead. The introduction of lead into the soil contaminated with cadmium and zinc reduced the results of the negative effect of those two elements from 43% to 29%, although it depended the negative effect of contaminating soil simultaneously with cadmium and copper. The highest inhibition of ammonifying bacteria in the unsown soil was found in the sample of soil con-

Table 2

Number of ammonifying bacteria (cfu 10^8 kg^{-1} of d.m. soil)

Object	Contamination level*			
	I		II	
	soil use			
	unsown	sown	unsown	sown
0	137 ± 8	162 ± 9	137 ± 12	162 ± 12
Cd	123 ± 5	155 ± 10	115 ± 9	150 ± 10
Cu	123 ± 8	155 ± 7	106 ± 5	153 ± 9
Pb	106 ± 2	134 ± 5	109 ± 8	138 ± 9
Zn	106 ± 7	134 ± 10	105 ± 9	146 ±14
CdCu	111 ± 5	169 ± 10	114 ± 5	158 ± 7
CdPb	111 ± 7	169 ± 8	103 ± 5	159 ± 5
CdZn	90 ± 7	122 ± 7	65 ± 4	125 ± 5
CdCuPb	90 ± 6	122 ± 6	98 ± 4	158 ± 5
CdCuZn	114 ± 6	180 ± 7	88 ± 6	149 ±6
CdPbZn	114 ± 7	180 ±11	80 ± 4	143 ± 9
CdCuPbZn	102 ± 5	162 ±9	50 ± 2	115 ± 5
Mean	111 ± 1	153 ±2	97 ± 2	146 ± 2
LSD	a – 4.5, b – 1.8, c – 1.8, a · b – 6.3, a · c – 6.3, b · c – 2.6, a · b · c · 8.9			

* designations under Table 1

taminated by the four heavy metals at the same time. The count was reduced under the influence of the metal mixture by 45%, on average. It was observed for the soil of the second degree of contamination to a higher extent (bz 64% than for the first degre (by 26%). In the soil sown with oat, the highest reduction of ammonifying bacteria was found as a result of lead contamination (by 16%) and zinc contamination (by 14%), as well as resulting from joint contamination with cadmium and zinc (by 24%). An increasing number of metals in the soil contaminating pool produced a more intense effect on the number of bacteria in unsown soil in comparison with soil under oat.

The count of nitrogen immobilising bacteria in the soil sown with oat and free from heavy metals was significantly higher than in the unsown soil, although the difference was much smaller than in the case of other groups of bacteria (Table 3). In contaminated soil, these differences grew in favour of sown soil, as in the case of ammonifying bacteria. It proves that heavy metals have a more negative effect on ammonifying bacteria deprived of plant cover. However, in the soil contaminated with the lowest rates of

Table 3

Number of nitrogen immobilizing bacteria (cfu 10^8 kg^{-1} of d.m. soil)

Object	Contamination level*			
	I		II	
	soil use			
	unsown	sown	unsown	sown
0	58 ± 3	66 ± 4	58 ± 4	66 ± 5
Cd	56 ± 2	100 ± 2	57 ± 4	71 ± 4
Cu	56 ± 4	100 ± 7	51 ± 3	60 ± 3
Pb	58 ± 5	111 ± 5	41 ± 2	69 ± 6
Zn	58 ± 4	111 ± 6	33 ± 2	56 ± 3
CdCu	37 ± 1	45 ± 3	53 ± 2	43 ± 4
CdPb	37 ± 2	45 ± 4	63 ± 2	68 ± 3
CdZn	46 ± 3	63 ± 3	43 ± 1	47 ± 3
CdCuPb	46 ± 5	63 ± 3	53 ± 4	64 ± 5
CdCuZn	52 ± 4	72 ± 4	26 ± 3	40 ± 3
CdPbZn	52 ± 2	72 ± 2	34 ± 3	47 ± 3
CdCuPbZn	37 ± 3	57 ± 4	20 ± 2	47 ± 3
Mean	49 ± 1	76 ± 2	44 ± 1	57 ± 1
LSD	a – 2.2, b – 0.9, c – 0.9, a · b – 3.1, a · c – 3.1, b · c – 1.3, a · b · c – 4.5			

* designations under Table 1

single heavy metals, there was no reduction in the count of ammonifying bacteria, while a higher concentration of lead and zinc significantly reduced their number, particularly as regards unsown soil. Zinc proved to be most toxic to this group of bacteria. It reduced the number of ammonia forming bacteria by as much as 43%, while cadmium had no toxic effect on these microbes. On the other hand, excessive quantities of heavy metals in soil, but only if present in twos, had an explicit, negative effect on ammonia forming bacteria. Cd^{2+} with Cu^{2+} inhibited their development by 28%, and Cd^{2+} with Zn^{2+} by 20% and Cd^{2+} with Pb^{2+} by 14%, on average. Having more metals in the contaminating pool did result in any further reduction in bacterial counts. It was only a joint contamination with Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+} that caused an average reduction by 36% as regards the population of nitrogen immobilising bacteria, which in the unsown soil was reduced by up to 51%.

The count of cellulolytic bacteria, in contrast to the other groups and types, was significantly higher in the unsown soil than in the sown soil (Table 4). The effect of heavy metals on these bacteria was the weakest.

Table 4

Number of cellulolytic bacteria (cfu 10^6 kg^{-1} of d.m. soil)

Object	Contamination level*			
	I		II	
	soil use			
	unsown	sown	unsown	sown
0	44 ± 2	24 ± 1	44 ± 3	24 ± 2
Cd	35 ± 2	22 ± 1	42 ± 3	24 ± 2
Cu	35 ± 4	22 ± 2	38 ± 2	22 ± 2
Pb	41 ± 3	18 ± 1	37 ± 3	24 ± 1
Zn	41 ± 3	18 ± 2	39 ± 2	22 ± 2
CdCu	44 ± 2	23 ± 2	41 ± 3	22 ± 2
CdPb	44 ± 2	23 ± 1	43 ± 1	20 ± 1
CdZn	32 ± 3	21 ± 3	36 ± 3	22 ± 2
CdCuPb	32 ± 3	21 ± 2	35 ± 1	24 ± 2
CdCuZn	37 ± 2	23 ± 2	36 ± 2	23 ± 2
CdPbZn	37 ± 3	23 ± 2	38 ± 3	26 ± 2
CdCuPbZn	38 ± 2	24 ± 1	33 ± 3	19 ± 2
Mean	39 ± 1	22 ± 1	38 ± 1	23 ± 1
LSD	a – 1.4, b – n.s.; c – 0.6, a · b – 2.0, a · c – 2.0, b · c – n.s.; a · b · c – 2.8			

* designations under Table 1

The reduction in the number of these bacteria ranged on average from 5% in the sample jointly contaminated with Cd^{2+} and Cu^{2+} to 18% in the soil contaminated with four heavy metals. In addition, heavy metals in the soil deprived of plant cover revealed a stronger effect on these bacteria.

The number of bacteria of the genus *Arthrobacter* was over three-fold higher in the soil sown with oat than in the unsown soil (Table 5). All the heavy metals had a stronger inhibitory effect on the development of these bacteria in the unsown soil. An average reduction in their count in the sown soil resulting from individual metals ranged from 30% (Pb^{2+}) to 51% (Zn^{2+}), while in the unsown soil, cadmium and copper had no effect on these bacteria, and zinc and lead reduced their number by 5% – 18%.

While examining the effects of heavy metals, disregarding the method of soil usage, it is clear that their effect on the bacteria of *Arthrobacter* genus was not directly proportional to the number of metals used in the contamination pool. The highest changes were caused by cadmium, lead, copper and zinc when they individually contaminated the soil. As a result of their activity, the count was reduced by 21%-28%. In those samples, in cas-

Table 5

Number of *Arthrobacter* spp. (cfu 10^6 kg^{-1} of d.m. soil)

Object	Contamination level*			
	I		II	
	soil use			
	unsown	sown	unsown	sown
0	39 ± 3	122 ± 8	39 ± 2	122 ± 7
Cd	34 ± 3	73 ± 6	45 ± 3	64 ± 3
Cu	39 ± 4	61 ± 4	43 ± 3	62 ± 3
Pb	29 ± 3	104 ± 8	35 ± 2	67 ± 4
Zn	37 ± 2	62 ± 4	37 ± 2	58 ± 3
CdCu	31 ± 3	75 ± 5	44 ± 3	53 ± 4
CdPb	35 ± 4	71 ± 6	43 ± 3	65 ± 4
CdZn	42 ± 4	79 ± 8	28 ± 2	58 ± 3
CdCuPb	34 ± 2	66 ± 3	36 ± 2	63 ± 5
CdCuZn	36 ± 4	56 ± 5	32 ± 2	42 ± 5
CdPbZn	44 ± 3	63 ± 5	42 ± 3	66 ± 4
CdCuPbZn	27 ± 3	59 ± 4	26 ± 3	44 ± 3
Mean	36 ± 1	74 ± 1	38 ± 1	64 ± 1
LSD	a – 2.5, b – 1.0, c – 1.0, a · b – 3.6, a · c – 3.6, b · c – 1.45, a · b · c – 5.0			

* designations under Table 1

es when cadmium was applied together with copper, lead or zinc, the reduction of the count was similar and amounted to 22% - 26%. It was by about 10% higher when cadmium was applied together with copper and zinc, but it did not increase in the result of joint activity of cadmium with copper and lead or cadmium with lead and zinc. It was only under the influence of the contamination of soil with all four heavy metals (Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}) that the reduction by 45% was observed as regards the number of these bacteria.

The count of bacteria of the *Pseudomonas* genus was 1.5-fold higher in the soil sown with oat than in the unsown soil, while heavy metals in soil under oat reduced their number to a higher extent (Table 6). Therefore, they had a similar effect on these bacteria as on the bacteria of the *Arthrobacter* genus. Irrespective of the level of contamination, as regards the soil covered with oat, the highest negative effects on *Pseudomonas* was generated by cadmium and zinc. These metals reduced the number of *Pseudomonas* by 49% and 43%, respectively. A weaker effect was found for copper and lead, which reduced the number of the bacteria by 35% and 29%. In the

Table 6

Number of *Pseudomonas* spp. (cfu 10^6 kg^{-1} of d.m. soil)

Object	Contamination level*			
	I		II	
	soil use			
	unsown	sown	unsown	sown
0	107 ± 5	162 ± 3	107 ± 4	162 ± 4
Cd	72 ± 3	85 ± 4	77 ± 5	79 ± 3
Cu	120 ± 8	93 ± 5	126 ± 6	117 ± 6
Pb	127 ± 10	101 ± 5	113 ± 9	129 ± 7
Zn	92 ± 6	91 ± 5	106 ± 8	95 ± 6
CdCu	82 ± 7	59 ± 4	85 ± 6	78 ± 6
CdPb	88 ± 8	98 ± 5	125 ± 6	120 ± 5
CdZn	104 ± 9	77 ± 6	63 ± 7	55 ± 4
CdCuPb	104 ± 8	82 ± 6	92 ± 8	73 ± 5
CdCuZn	91 ± 6	113 ± 7	85 ± 6	89 ± 4
CdPbZn	128 ± 6	129 ± 7	73 ± 3	84 ± 5
CdCuPbZn	89 ± 5	103 ± 3	48 ± 3	55 ± 4
Mean	100 ± 2	99 ± 2	92 ± 2	95 ± 1
LSD	a – 3.6, b – 1.5, c – n.s., a · b – 5.2, a · c – 5.2, b · c – 2.1, a · b · c – 7.3			

* designations under Table 1

unsown soil, cadmium reduced the count of *Pseudomonas*, by 30% reduction and zinc only by 7%; in contrast, copper and lead increased the amount of these bacteria by 5% - 12%. In the soil sown with oat, a negative effect of cadmium was intensified by a simultaneous contamination with copper and zinc, while lead contamination lowered this effect. Copper resulted in a further 9% and zinc to a 10% reduction in the number of *Pseudomonas* as compared to cadmium alone, while lead mitigated the effect of cadmium by 16%. On the other hand, copper and zinc, and lead and zinc applied together with cadmium revealed a less toxic effect on bacteria of the genus *Pseudomonas*, as compared to cadmium alone, while joint contamination with the four heavy metals (Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}) had a similar effect as the contamination with cadmium alone.

The heavy metals demonstrated a clearly negative effect on soil bacteria, which agrees with other research (Boros et al. 2007, Lugauskas 2005, Oliveira, Pampulha 2006). They had a stronger effect on bacteria of the *Arthrobacter* and *Pseudomonas* genera than on individual physiological groups. This is primarily related to a higher tolerance to heavy metals possessed by qualitatively richer groups of microbes in comparison to individual genera and types (Loc, Janssen 2005, Mertens et al. 2007). An interesting and unexplained phenomenon is the reciprocal neutralization of the effects of metals on bacteria when they are applied in a mixture. Tolerance of bacteria to individual metals was often higher than in the case of their mixtures. One exception was the mixture of all the four metals, which resulted in the most unfavourable changes in the bacterial count, which was caused by the joint effect of the highest number of metals in the soil-contaminating pool as well as the higher contamination rate, being the sum of contamination with individual metals.

CONCLUSIONS

1. The sensitivity of bacteria to Cd^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+} is a specific characteristic, related to the content of those metals in soil and the method of its use. The development of the bacteria of *Arthrobacter* and *Pseudomonas* spp. was most intensively inhibited in the soil sown with oat, while for ammonifying, nitrogen immobilising, cellulolytic bacteria it was in the unsown soil. Copiotrophic, cellulolytic, nitrogen immobilising and ammonifying bacteria proved to be more resistant to these contaminations than bacteria of the *Arthrobacter* and *Pseudomonas* genera.

2. Copper had the strongest effect on copiotrophic bacteria; lead and zinc – on ammonifying bacteria; zinc – on nitrogen immobilising bacteria; while *Arthrobacter* was most strongly affected by zinc and *Pseudomonas* – by cadmium and zinc.

3. Increasing the number of heavy metals simultaneously contaminating the soil to two (Cd^{2+} and Cu^{2+}; Cd^{2+} and Zn^{2+}; Cd^{2+} and Pb^{2+}) and to three (Cd^{2+}, Cu^{2+} and Zn^{2+}; Cd^{2+}, Cu^{2+}, and Pb^{2+}; Cd^{2+}, Pb^{2+} and Zn^{2+}) generally did not increase the intensity of their effect on the examined bacteria. Changes caused by these mixtures were usually similar than changes caused by individual heavy metals.

4. The quality and quantity threshold of soil contamination in relation to bacteria was evidently exceeded by a joint application of the four heavy metals (Cd^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+}).

5. Further studies are required in order to determine the mechanism of mutual neutralization of the effect of heavy metals on bacteria.

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ACCUMULATION OF MACROELEMENTS IN PLANTS ON NEWLY ESTABLISHED FALLOWS

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Abstract

In spring 1996, four types of fallows were established on good wheat complex soil (classified as IIIa in the Polish soil classification system): sown with oriental goat's rue (*Galega orientalis*, Lam.), traditional fallow, seeded with a mixture of oriental goat's rue (*Galega orientalis* Lam) and smooth brome (*Bromus inermis*) and under smooth brome (*Bromus inermis*). This paper documents the results obtained in 2000-2004. Plant samples (4 x 1 m^2) were taken from the fallows once every year at the same plant growth and development stage. The content of macroelements in the plant material was determined using conventional methods. The statistical elaboration of the results was based on the analysis of regression and correlation.

The results proved that fallow under perennial plants is superior in activating and cycling nutrients to traditional fallow, overgrown with wild plants. On the other hand, when fallow soil is covered exclusively with a papilonaceous plant (e.g. oriental goat's rue), it is more likely to experience transfer of nitrates (V) to ground waters. A good solution to this problem could be sowing fields which are set aside as fallows sown with a mixture of oriental goat's rue and smooth brome. Soil protected by these two plants remains fertile and does not create an ecological risk caused by migrating N-NO_3.

Key words: fallow, oriental goat's rue, smooth brome, N, P, K, Mg.

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AKUMULACJA MAKROELEMENTÓW W ROŚLINNOŚCI NA ZAINICJOWANYCH ODŁOGACH

Abstrakt

Wiosną 1996 r. na glebie kompleksu pszennego dobrego, klasy bonitacyjnej IIIa zainicjowano odłogi: obsiany rutwicą wschodnią (Galega orientalis Lam.), odłóg klasyczny, obsiany mieszanką rutwicy wschodniej (Galega orientalis Lam.) ze stokłosą bezostną (Bromus inermis), obsiany stokłosą bezostną (Bromus inermis). W niniejszej pracy przedstawiono wyniki uzyskane w latach 2000-2004. Z obiektów pokrytych roślinnością pobierano próbki (4 1 m2) raz w roku w jednakowej fazie wzrostu i rozwoju roślin. Zawartość makroskładników w materiale roślinnym oznaczono konwencjonalnymi metodami. Opracowanie statystyczne wyników wykonano w oparciu o analizę regresji i korelacji.

W badaniach wykazano, że odłóg obsiany roślinami wieloletnimi w większym stopniu uruchamia składniki pokarmowe i włącza je do obiegu niż roślinność naturalna odłogu klasycznego. Jednak roślina motylkowata (rutwica wschodnia) pokrywająca wyłącznie glebę może stwarzać ryzyko przemieszczania azotanów (V) do wód gruntowych. Dobrym rozwiązaniem może być utrzymywanie pól wyłączonych z produkcji w formie odłogu obsianego mieszanką rutwicy wschodniej i stokłosy bezostnej. Tak zabezpieczona gleba pozwala na utrzymanie żyzności i jednocześnie nie stwarza zagrożenia ekologicznego związanego z przemieszczaniem N-NO3.

Słowa kluczowe: odłóg, rutwica wschodnia, stokłosa bezostna, N, P, K, Mg.

INTRODUCTION

In order to adjust fertilization to soil conditions, it is necessary to determine the uptake of nutrients by plants. For agricultural reasons as well as ecological demands it is more common in Poland as well as in many other countries to prepare nitrogen, phosphorus and potassium balance calculations, which enable farmers to apply biogenic substances with maximum safety (Bach and Frede 1998, Fotyma 2000). Fallow land is not fertilized, which means that the nutrients accumulated by plants growing on fallow soil provide us with the information on the potential stocks of nutrients in the soil – plant – soil cycle. The fertilization value of the biomass produced on fallow depends on the type of plants, the amount of organic matter produced and its chemical composition (Wilczewski and Skinder 2005). Organic matter is an important link in the process of capturing elements via biological sorpotion (Żarczyński and Sienkiewicz 2007). According to Stupnicka-Rodzynkiewicz et al. (1996), weeds tend to accumulate more nutrients than crops. Thus, it seems reasonable to determine potential accumulation of nutrients by plants growing on fallow land. The objective of our study has been to trace the amounts of nitrogen, phosphorus, potassium and magnesium in plants growing on different types of fallows.

MATERIAL AND METHODS

In spring 1996 a field experiment was set up on good wheat complex soil, classified as IIIa in the Polish soil classification system. The fallows, covering 1600 m^2 each, were established on a field set apart for the experiment and were covered with the following plants:

1) oriental goat's rue (*Galega orientalis* Lam.);
2) traditional fallow;
3) oriental goat's rue (*Galega orientalis* Lam.) and smooth brome (*Bromus inermis*);
4) smooth brome (*Bromus inermis*).

No agronomic treatments were carried out while maintaining the fallows. This paper presents the results obtained in 2000-2004. Plant samples (4 x 1 m^2) were taken from the fallows in order to determine the biomass and chemical composition. Samples of the plants were collected once each year at the same growth and development stage, i.e. at the early inflorescence stage of goat's rue, when the plants had reached their maximum weight. The content of the macroelements in the plant material, following wet digestion, was determined using the following methods: Kjeldhal's method for nitrogen, vanadium-molybdenum method for phosphorus, ESA method for potassium and ASA method for magnesium. The results underwent statistical processing using analysis of regression and correlation.

RESULTS AND DISCUSSION

The highest nitrogen accumulation occurred in oriental goat's rue Table 1). Compared to the goat's rue monoculture, the uptake of nitrogen from the fallow sown with the mixture of goat's rue and smooth brome was 40% lower. The accumulation of nitrogen on the fallow covered with smooth brome alone – compared to the fallow sown with goat's rue – was nearly three-fold lower. The lowest potential accumulation of nitrogen was found for plants growing on the traditional fallow. This was due to a very small biomass produced on this fallow and the smallest concentration of nitrogen in the plant dry matter. Similar results on nitrogen uptake be weeds were reported by STINNER et al. (1997).

As large amounts of nitrogen collected in biomass which stays on a field can pose a risk of contaminating waters with nitrates, we compared the amounts of nitrogen accumulated in plants with the concentrations of mineral nitrogen in soil. It turned out that the nitrogen accumulated in the biomass was strongly correlated with the concentration of nitrates (V) in soil in the autumn: September and November (Figure 1). It is worth notic-

Table 1

Accumulation of macroelements in plants (kg·ha^{-1})

Object	Year				
	2000	2001	2002	2003	2004
N					
Goat's rue	345.9	343.6	259.1	360.6	311.6
Goat's rue + brome grass	222.4	238.9	196.7	267.8	223.1
Brome grass	128.3	133.1	93.4	141.0	117.3
Traditional fallow	74.1	61.7	54.0	66.6	61.2
P					
Goat's rue	39.9	39.4	32.3	44.2	36.6
Goat's rue + brome grass	30.7	31.3	23.5	32.3	28.8
Brome grass	17.3	16.5	13.3	16.4	14.1
Traditional fallow	13.2	12.0	10.3	9.9	10.8
K					
Goat's rue	299.6	348.8	250.9	328.6	317.0
Goat's rue + brome grass	277.3	322.3	233.0	337.0	274.0
Brome grass	149.5	169.7	122.1	168.8	144.5
Traditional fallow	148.8	122.9	106.6	125.9	111.9
Mg					
Goat's rue	21.2	21.8	13.8	23.9	19.1
Goat's rue + brome grass	11.4	13.1	8.9	13.4	11.1
Brome grass	5.8	5.3	2.8	4.3	3.3
Traditional fallow	3.6	3.4	2.7	2.8	2.1

ing here that the biomass left on the fields for the winter season, which never exceeded 250-270 kg N·ha^{-1}, did not create a potential threat to the environment (Figure 1). Some higher nitrogen uptake occurred only in the case of oriental goat's rue. The organic matter left on the fields was a perfect substrate for microorganisms, which released the nitrogen it contained. Further transformation of nitrogen (nitrification) caused increased accumulation of N-NO_3 in soil. It is hardly possible for nitrate nitrogen (V) present in large amounts in soil during the autumn to remain there until spring (Koc et al. 2002).

In our experiment, the amount of P accumulated in goat's rue was slightly larger than reported by Ignaczak and Wojciechowska (1992), who assessed the phosphorus accumulation potential of goat's rue at 25-33 kg P·ha^{-1}. We found out that goat's rue growing over nearly all the surface area of fallow

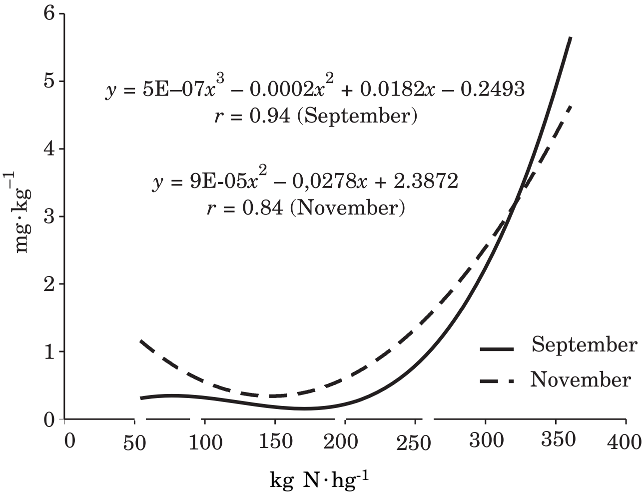

Fig. 1. Influence of the amount of N accumulated in plants (kg N·ha^{-1}) on the N-NO_3 content in soil during the autumn season

land accumulated from 32.2 to 44.2 kg P·ha^{-1} (Table 1), which is similar to the results obtained by Chylińska (2003), who studied bird's foot which received mineral fertilization. In our own research, the mixture of goat's rue and smooth brome accumulated on average 30% more phosphorus than the monoculture of goat's rue. The traditional fallow as well as smooth brome growing alone accumulated much less phosphorus. The uptake of phosphorus by smooth brome was nearly two-fold lower than that by smooth brome and goat's rue growing together. The grass, however, collected over 38% more phosphorus than the plants growing on the traditional fallow.

The quantities of phosphorus stocked in plants were largely dependent on the amounts of available phosphorus in soil (Figure 2). However, it needs to be said here that this relationship could just as well have been reverse. Having been first mineralized, the phosphorus accumulated in biomass would most readily returned to soil as plant available forms.

The weather is one of the factors which significantly affected the accumulation of potassium. In the present study, the lowest K uptake occurred in the driest year, 2002 (Table 1). The shortage of rainfall limited the transfer of potassium to the plant roots, which made it more difficult for the plants to absorb this element; the effect was also related to a much lower amount of biomass produced in that year. Goat's rue as well as its mixture with smooth brome often collected over 300 kg K·ha^{-1}, whereas the amounts of potassium found in the plants sampled from the traditional and smooth brome fallows did not exceed 150 and 170 kg·ha^{-1}, respectively. Nonetheless, the above quantities were much higher than those reported by Ignaczak and Wojciechowska (1992), who concluded that goat's rue could potentially

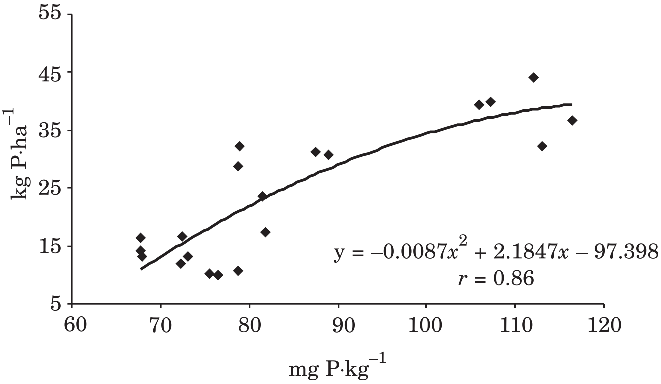

Fig. 2. Dependence of P uptake (kg·ha^{-1}) on the content of available phosphorus in soil (mg·ha^{-1})

accumulate about 130 kg K per ha^{-1}. SZYMANOWICZ and KALEMBASA (2005) determined even lower accumulation potential of this plant. They suggested that goat's rue was capable of accumulating just 65 kg·ha^{-1}. In contrast, SZOSZKIEWICZ et al. (1992) determined that the uptake of potassium by mixtures of meadow grasses was between 229 and 654 kg·ha^{-1}.

Among all the macroelements examined, magnesium proved to be most variable in the uptake by plants – the ratio between the highest uptake (by goat's rue in 2003) and the lowest one (traditional fallow in 2004) was over 11-fold (Table 1). The lowest magnesium accumulation occurred on the traditional fallow. The plants growing on this fallow accumulated on average just 2.9 kg·ha^{-1} magnesium. This was 6.5-fold less than the amount of Mg collected in goat's rue. On the other hand, the amount of magnesium accumulated by plants on the traditional fallow was comparable to that reported by CHYLIŃSKA (2003) for mineral fertilized bird's foot. Another interesting fact was that the quantities of Mg removed from soil to aerial parts of plants tended to decrease during the experiment.

Magnesium is more strongly bound in live organisms than potassium. It is also more slowly released from organic matter to soil solution than potassium. The results reported in this paper enabled us to conclude that the stock of available magnesium in soil was rather strongly correlated with the amount of this element accumulated in the plants which were left on the field (Figure 3). This is yet another piece of evidence suggesting that the availability of elements to plants is largely conditioned by their amounts which enter the matter cycling every year. This should also be taken into consideration when planning and managing fallows. Biological accumulation of elements can prevent their leaching inasmuch as improve stocks of their plant available forms in soil.

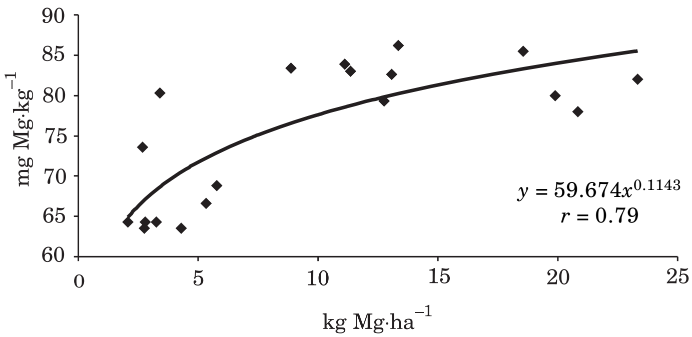

Fig. 3. Influence of Mg accumulated in plants (kg Mg $\cdot$ ha^{-1}) on the content of available Mg in soil (mg Mg $\cdot$ kg^{-1})

CONCLUSIONS

1. The plants sown on newly established fallows, particularly oriental goat's rue, are better at releasing and cycling nutrients than weeds growing on traditional fallow.

2. Goat's rue growing on land set aside from agricultural use can create a risk of excessive accumulation of nitrates (V) in soil and their transfer to ground waters.

3. Maintaining fields temporarily excluded from farming in the form of fallows sown with a mixture of goat's rue and smooth brome can be the best solution as this preserves soil fertility and protects the environment.

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