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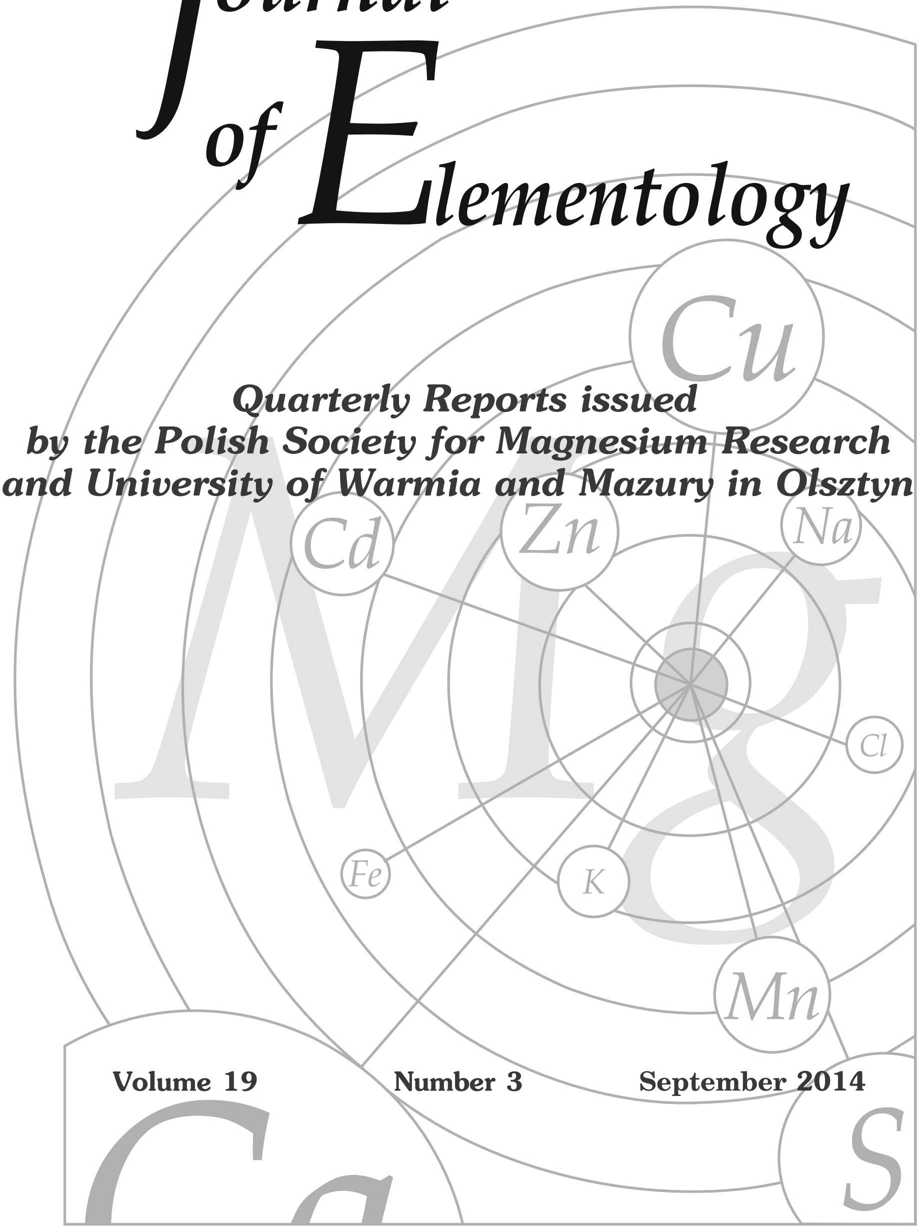

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ORIGINAL PAPERS

LEAD CONCENTRATION AND THE CONTENT OF SELECTED MACROELEMENTS IN LAKE SEDIMENTS IN POLAND

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Abstract

Lead has been used extensively for thousands of years. Once introduced into the environment, like any other heavy metal, lead accumulates in soil and sediments. High lead concentrations in river and lake sediments can be harmful to aquatic organisms. At present, uncontaminated water sediments in the northern and central parts of Poland contain below 9 mg of lead per kg. The lakes located within the following lake districts: Greater Poland, Pomeranian and Masurian, provided 409 samples of surface sediments from deep spots (the profundal zone). All the samples were tested for the content of Pb and other selected macroelements. The content of Ca, Mg, Fe, K, Mn, Na, P, Pb and S was determined by ICP-OES and the total organic carbon (TOC) was evaluated by the coulometric titration method. The observed range of lead concentration was from below 3 to 222 mg kg^{-1}. The average content was 37 mg kg^{-1}, the geometric mean 30 mg kg^{-1}, and the median 33 mg kg^{-1}. In the majority of samples, the lead concentration was higher than the geochemical background. Only in 5.77% of the samples, the lead content was lower than 10 mg kg^{-1}. The lead concentration in the sediments was relatively well correlated with the total organic carbon (r = 0.59), aluminium (r = 0.45) and sulphur (r = 0.47), moderately correlated with iron (r = 0.26) and potassium (r = 0.28), very weakly correlated with phosphorus (r = 0.12) and negatively correlated with the calcium concentration (r = -0.28). No correlation was observed for manganese (r = -0.05), magnesium (r = 0.07) and sodium (r = -0.07). Factor analysis revealed the presence of two factors that together accounted for nearly 45% of variation. The first factor included aluminium, potassium and magnesium, and the second one - sul-

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phur and organic carbon. The lead share was low in the first factor (0.343), but very high in the second factor (0.757). Based on the results of the factor analysis, it can be assumed that lead in the organic matter-rich sediments of the profundal zone is deposited mainly in the form of sulphides. Lead concentration varied among the sediments obtained from various lake districts; it was lower in the lakes located within the Pomeranian Lake District than in those from Greater Poland and Masurian Lakes.

Key words: lake sediments, lead, macroelements.

ZALEŻNOŚĆ MIĘDZY ZAWARTOŚCIĄ OŁOWIU A ZAWARTOŚCIĄ WYBRANYCH PIERWIASTKÓW GŁÓWNYCH W OSADACH JEZIOR POLSKI

Abstrakt

Ołów jest wykorzystywany powszechnie od tysięcy lat. Uruchomiony do środowiska, podobnie jak inne metale ciężkie, podlega akumulacji w glebach i osadach wodnych. W osadach rzecznych i jeziornych duża zawartość Pb może szkodliwie oddziaływać na organizmy wodne. We współczesnych niezanieczyszczonych osadach wodnych na obszarze północnej i środkowej części Polski zawartość ołowiu wynosi poniżej 9 mg kg^{-1}. Z jezior Pojezierzy: Wielkopolskiego, Pomorskiego i Mazurskiego pobrano 409 próbek powierzchniowych osadów z głęboczków jezior (strefa profundalna). We wszystkich próbkach określono zawartość Pb oraz wybranych pierwiastków głównych. Zawartość Ca, Mg, Fe, K, Mn, Na, P, Pb i S określono metodą ICP-OES, a zawartość węgla organicznego (TOC) – metodą kulometrycznego miareczkowania. Stężenie ołowiu wynosiło od <3 do 222 mg kg^{-1}, średnia zawartość – 37 mg kg^{-1}, średnia geometryczna – 30 mg kg^{-1}, a mediana – 33 mg kg^{-1}. W większości zbadanych próbek zawartość ołowiu była podwyższona w stosunku do wartości tła geochemicznego. Jedynie w 5,77% próbek zawartość ołowiu była niższa niż 10 mg kg^{-1}. Stężenie ołowiu w osadach wykazuje stosunkowo wysoką korelację z zawartością węgla organicznego (r = 0,59), glinu (r = 0,45) i siarki (r = 0,47), słabą – ze stężeniem żelaza (r = 0,26) i potasu (r = 0,28), bardzo słabą – ze stężeniem fosforu (r = 0,12), ujemną – z zawartością wapnia (r = -0,28) oraz brak korelacji ze stężeniem manganu (r = -0,05), magnezu (r = 0,07) i sodu (r = -0,07). Analiza czynnikowa wykazała obecność 2 czynników, które łącznie wyjaśniają blisko 45% zmienności. Pierwszy czynnik grupuje glin, potas i magnez, drugi – siarkę i węgiel organiczny. Udział ołowiu w czynniku pierwszym jest niski (0,343), a w drugim – bardzo wysoki – 0,757. Na podstawie wyników analizy czynnikowej można przyjąć, że w bogatych w materię organiczną osadach strefy profundalnej jezior ołów występuje przede wszystkim w formie siarczków. Zaobserwowano zróżnicowanie w zawartości ołowiu w osadach różnych pojezierzy; osady jezior Pojezierza Pomorskiego zawierają mniej Pb niż osady jezior Pojezierzy Wielkopolskiego i Mazurskiego.

Słowa kluczowe: osady jeziorne, ołów, pierwiastki główne.

INTRODUCTION

Having been introduced into the environment, lead - similarly to other heavy metals - accumulates in soils and water sediments. Lead mobility in hypergenic environment is controlled through sorption of clay minerals, organic matter, secondary compounds of aluminium, iron and manganese and poorly soluble lead compounds such as galena – PbS, anglesite ($PbSO_4$), cerussit ($PbCO_3$) and pyromorphite ($Pb_5[Cl/(PO_4)_3]$). Lead is released into the environment from many natural and anthropogenic sources. Its natural content in soils depends on the content in the bedrock, and its concentration in contemporary river and lake sediments is conditioned by the catchment area’s lithology. Lead is a chalcophylic element and can be found in several minerals, the most important of which are galena, cerussite and anglesite. As a trace element, lead is distributed in other minerals, especially in orthoclase, plagioclases, micas, zircon and magnetite. The average lead abundance in the Earth’s crust is 15 mg kg^{-1}; it is low in ultramafic rocks (1 mg kg^{-1}) and much higher in acidic rocks, i.e., granites (15-19 mg kg^{-1}). The lead concentration in sedimentary rocks is determined by the presence of detrital minerals - feldspars, micas and sulphides, and is controlled by clay minerals and organic matter. Limestone contains approximately 5 mg kg^{-1} of lead, sandstone about 10 mg kg^{-1}, loess about 13 mg kg^{-1}, and shales 23 mg kg^{-1}. The lead concentration in the oldest, pre-industrial lake sediments was 2-5 mg kg^{-1} (Pearson et al. 2010). In contemporary, uncontaminated stream sediments in the northern and central part of Poland, the lead content is below 9 mg kg^{-1}, which is due to the presence of quaternary deposits, characterized by a relatively low content of this element (de Vos et al. 2006). The average content of lead in sediments of the littoral zone calculated for 993 lakes was estimated at 7 mg kg^{-1} (Lis, Pasieczna 1995). A significantly higher lead content was recorded in lacustrine deposits of the profundal zone. Geometric means of the concentrations of lead in lake sediments in the area of the Regional Water Management Authority in Gdańsk, Poznań, Szczecin and Warsaw range from 27 to 33 mg kg^{-1} (Bojakowska et al. 2,006). The lead concentration in lake sediments depends significantly on the lithology of the lake catchment. Lakes formed in areas of till occurrence are characterized by a higher content of Pb than sediments of lakes located in areas of sand occurrence (Bojakowska, Gliwicz 2009). Previous studies of lake deposits in Poland showed the presence of a very high content of lead in sediments of some lakes such as Czarnówek (292 mg kg^{-1}), Moczydło (189 mg kg^{-1}), Arkońskie (141.7 mg kg^{-1}), Piaseczno Małe (136.7 mg kg^{-1}) (Bojakowska et al. 2006, Cieślewicz, Różański 2010) An extremely high lead content, such as 469 mg kg^{-1}, was recorded in the sediments of Lake Wigry in the estuary of the Czarna Hańcza River (Alexander-Kwaterczak, Kostka 2011).

Owing to its relatively wide distribution, easy extraction from ores and processing, lead has been widely used for thousands of years. Straightforward mining and extraction, as well as very good malleability contributed to a very early use of lead in the manufacture of various products (mainly dishes, ornaments and water pipes). Half of the current lead production is used for manufacturing batteries, ammunition, sailboat ballast, for lining chambers, towers and tanks for sulphuric acid production, covering cables, obtaining metal alloys and soft solders or stained glass windows. Numerous lead compounds are applied in ceramic and glass industry, in the production of paints and varnishes (PbO, PbO_2 $2PbO$, $PbCO_3$, $PbCrO_4$, $PbNCN$), primers ($Pb(N_3)_{2,}$ - initiating material) or plastics ($3PbO$ $PbSO_4$ H_2O - stabilizing agent). Tetraethyllead ($Pb(C_2H_5)_4$) used to be added to gasoline fuels as an antiknock agent, and sodium arsenate was applied as an insecticide in orchards. The fact that lead has been used for thousand of years, and extensively in the last 150 years, is reflected in lake sediments (Bränvall et al. 2001, Yang, Rose 2005, Cooke et al. 2009, Pompeani et al. 2013).

High levels of lead in the sediments may be harmful for aquatic organisms (Míguez et al. 2012). Considering the potential toxicity of lead to the organisms living in the sediments, it was assumed that Pb content below 36 mg kg^{-1} (TEC – threshold effect concentration) does not affect negatively these organisms, but Pb concentration above 130 mg kg^{-1} (PEC – probable effect concentration) may produce adverse effects in aquatic organisms (MacDonald et al. 2000)

SCOPE AND METHODS

The paper is based on the results of research on water sediments in Poland carried out within the State Environmental Monitoring (SEM) program - Monitoring the quality of inland surface waters, and aims to determine the content of selected heavy metals and harmful organic compounds in the contemporary river and lake sediments in Poland. The analyses have been performed by the Polish Geological Institute - National Research Institute since 1990. The research program is supervised directly by the Monitoring Department in the Chief Inspectorate of Environmental Protection. Every year, about 130 samples of lake sediments taken from lakes in Poland are investigated. Lake sediments have been collected from the selected lakes belonging to the regional monitoring network and 23 reference lakes belonging to the national lake monitoring network. The regional network includes 1,032 lakes with a surface area greater than 50 hectares, and smaller lakes of ecological and economic importance. Analyses of sediments in the regional network lakes are carried out every few years, usually five, and the sediments from the reference lakes are scrutinized every 2 years.

In 2010-2012, the lakes from the Greater Poland, Pomeranian and Masurian Lake Districts were the sources of 409 samples of surface sediments collected from deep sites (profundal zone). All the samples were tested for the content of lead and other elements such as calcium, magnesium, iron, potassium, manganese, sodium, phosphorus, sulphur and organic carbon, contained in the phases that can retain Pb in the sediments. The content of Ca, Mg, Fe, K, Mn, Na, P, Pb and S was determined by atomic emission spectrometry with inductively coupled plasma ICP-AES (iCAP6500 Thermo Scientific) in the solutions obtained after digesting the sediments with aqua regia, and the total organic carbon (TOC) was determined by coulometric titration of a solid sample (Coulomat 702 CS/LI, Ströhlein). To assess the quality of the analyses a reference material, WQB-3 lake sediment, was tested.

RESULTS AND DISCUSSION

Lead content in the lake sediments ranged from below 3 to 222 mg kg^{-1}. The average concentration was 37 mg kg^{-1}, the geometric mean 30 mg kg^{-1}, and the median was 33 mg kg^{-1} (Table 1). The geometric mean of the lead content in the sediments of the profundal zone was much higher than an average Pb content in sediments from the littoral zone, which was established as 7 mg kg^{-1} (Lis, Pasieczna 1995). In the majority of the examined samples, the lead concentration was higher than the geochemical background. Only in 5.77% of the samples it was lower than 10 mg kg^{-1}. The lead level below the TEC value (36 mg kg^{-1}) was found in 51.68% of the analyzed samples. Only in 2.88% of cases, the Pb content exceeded 91 mg kg^{-1} (PEL – probable effect level), and the Pb concentration higher than 130 mg kg^{-1} (PEC value) was found only in the sediments from Karczemne Lake. Seven lakes in the analyzed group had a sediment lead level above 100 mg kg^{-1} and these were: Karczemne (222 mg kg^{-1}), Człuchowskie (120 mg kg^{-1}) and Bobięcińskie Wielkie (112 mg kg^{-1}) in the Pomeranian Province, Trześniowskie (119 mg kg^{-1}) and Malcz (103 mg kg^{-1}) in the Lubuskie Province and Nicemino (119 mg kg^{-1}) and Trzesiecko (111 mg kg^{-1}) in the West-Pomeranian Province. Karczemne Lake is located in the vicinity of Kartuzy, Człuchowskie Lake is on the outskirts of Człuchów, Trzesiecko Lake on the outskirts of Szczecinek; there is a village called Łagów on Trześniowskie Lake and Bobięcin on Bobięcińskie Wielkie Lake. Moreover, there is an early medieval settlement from the 9th-12th century on an island on Bobięcińskie Wielkie Lake, and Malcz Lake is

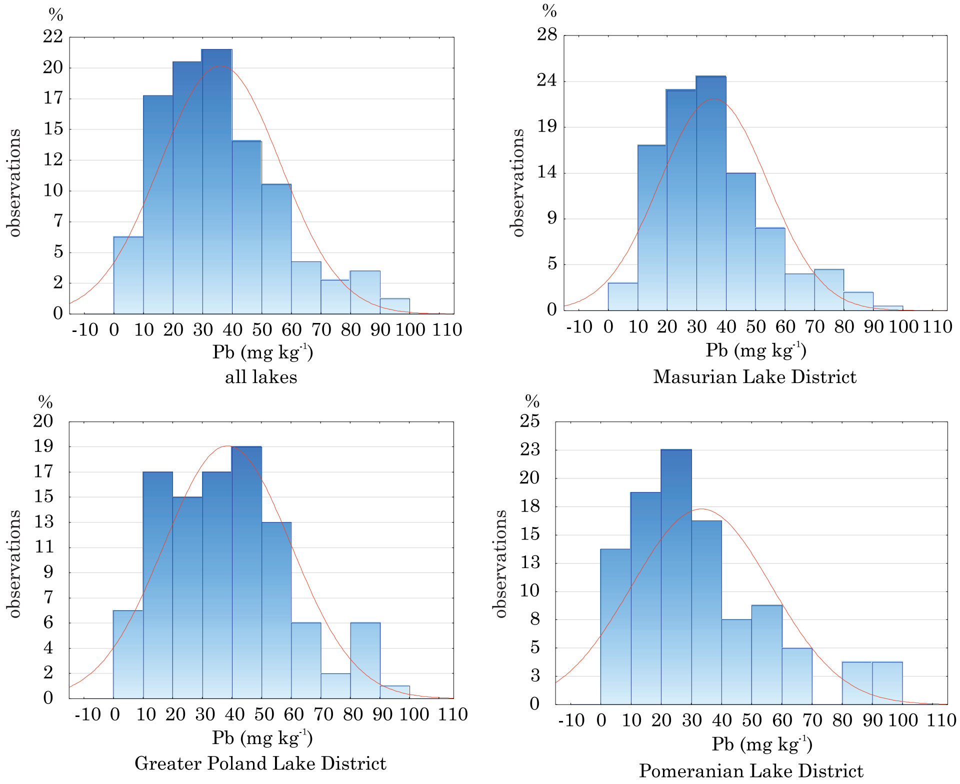
Table 1

Statistical parameters of lead and main elements in lake sediments (n = 409)

Element	Parameter					
	mean	geometric mean	median	minimum	maximum	standard deviation
Al (%)	0.50	0.37	0.39	0.04	2.23	0.40
Ca (%)	13.50	8.91	13.75	0.05	30.63	8.62
Fe (%)	1.55	1.26	1.39	0.10	10.91	1.01
K (%)	0.112	0.081	0.086	0.005	0.510	0.091
Mg (%)	0.32	0.26	0.27	0.01	1.25	0.19
Mn (mg kg^{-1})	901	644	713	32	11770	962
Na (%)	0.026	0.020	0.019	0.006	0.536	0.043
P (%)	0.108	0.087	0.088	0.005	1.925	0.118
Pb (mg kg^{-1})	37	30	33	<3	222	24
S (%)	1.045	0.825	0.933	0.023	4.629	0.687
TOC (%)	7.15	5.93	6.11	0.19	22.90	4.12

located within a military training ground. These lakes were excluded from the analysis of correlations between the content of lead and other selected macroelements in the sediments (scatter graphs, correlation coefficients, factor analysis), and were not considered in the calculation of statistical parameters for sediments from different lakelands.

Lake sediments from the Pomeranian, Greater Poland and Masurian Lake Districts were characterized by a variable lead content. The sediments from the Pomeranian Lake District contained on average 33 mg kg^{-1} Pb (geometric mean 25 mg kg^{-1}, median 27 mg kg^{-1}), from the Greater Poland Lakes 38 mg kg^{-1}, (geometric mean 31 mg kg^{-1}, median 33 mg kg^{-1}), and an average Pb content in the lakes from the Masurian Lakes was 38 mg kg^{-1} (geometric mean 31 mg kg^{-1}, median 38 mg kg^{-1}). This diversity is also visible in the histograms, illustrating the number of samples in different Pb concentration ranges (Figure 1). The largest share of sediments from the Pomeranian Lake District contained 10-40 mg Pb kg^{-1}, in the Masurian Lake District the majority of lake sediments fell into 10-50 mg Pb kg^{-1} range, and in the lakes from Greater Poland the most common range of Pb content was 10 to 60 mg Pb kg^{-1}.



all lakes

Masurian Lake District

Greater Poland Lake District

Pomeranian Lake District

Fig. 1. Histograms presenting lead content in lake sediments

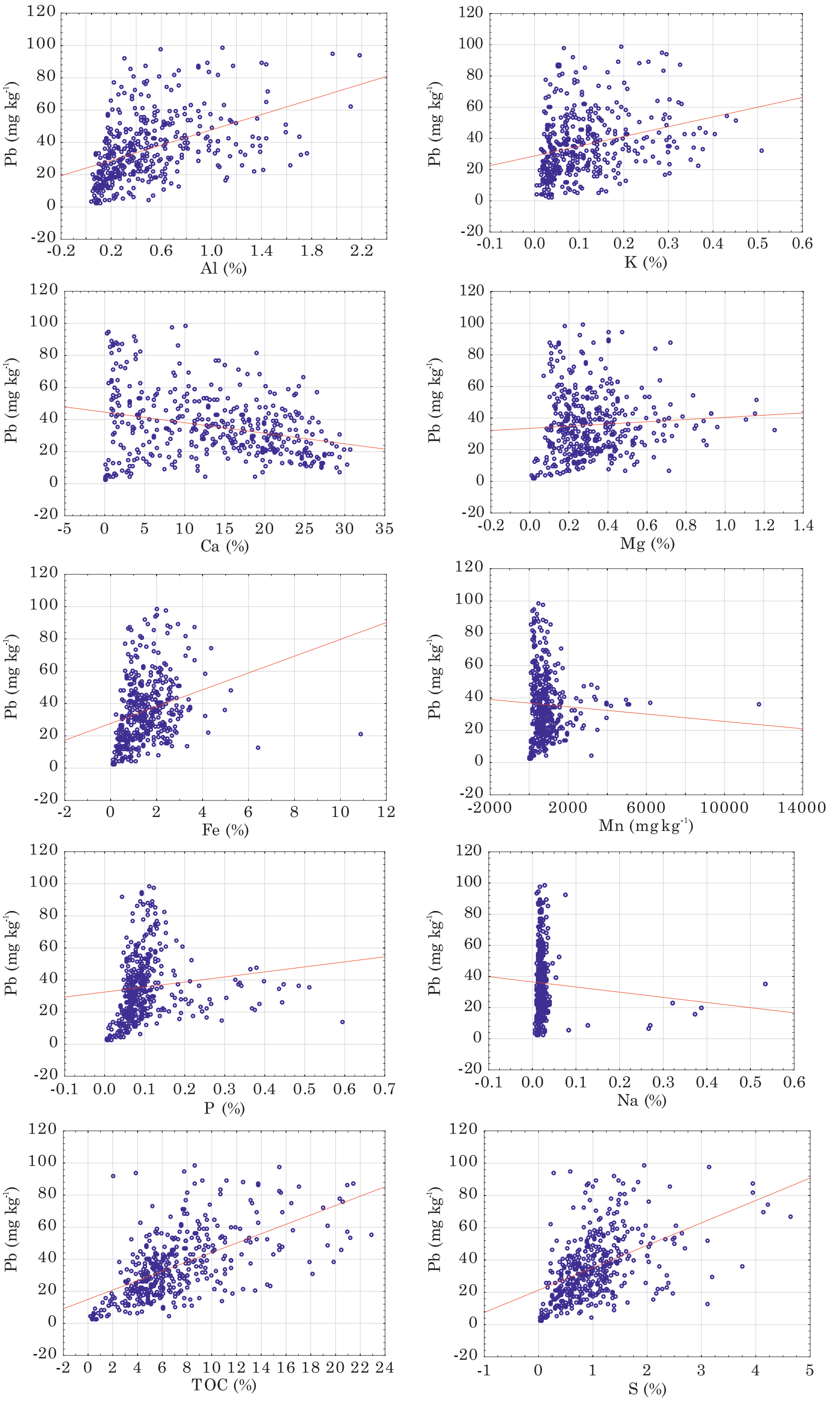


Fig. 2. Scatter graphs displaying the content of Pb and other macroelements

Table 2

Results of factor analysis of the concentration of lead and main elements in lake sediments

Element	Factor 1	Factor 2
Al	0.952	0.145
Ca	-0.629	-0.256
Fe	0.446	0.318
K	0.961	-0.022
Mg	0.780	-0.266
Mn	-0.060	-0.044
Na	0.028	0.191
P	0.128	0.308
Pb	0.343	0.757
S	-0.060	0.722
TOC	0.062	0.734
Baseline	6.966	5.081
Share	0.249	0.181

Lead concentration in the sediments was relatively well correlated with the total organic carbon (r = 0.59), aluminium (r = 0.45) and sulphur (r = 0.47), moderately correlated with iron (r = 0.26) and potassium (r = 0.28), very weakly correlated with phosphorus (r = 0.12), and negatively correlated with calcium concentration (r = -0.28). No correlation was observed for manganese (r = -0.05), magnesium (r = 0.07) and sodium (r = -0.07). Scatter graphs showed a clear relationship between the content of lead and organic carbon, sulphur, aluminium, potassium and iron, a negative correlation with calcium content, a very weak correlation with phosphorus level and lack of any correlation with the presence of manganese, sodium, and magnesium (Figure 2). Strong correlation between the Pb content and the level of organic carbon and sulphur indicates that the lead present in organic matter-rich sediments in deep sits is mostly deposited in the form of sulphides, most likely as PbS or in iron sulphides. The role of iron sulphides in retaining heavy metals in sediments has been described in a number of publications. For example, mercury immobilization in contaminated sediments is facilitated by iron sulphide nanoparticles, and this discovery was the basis for patent applications (JEONG 2007, XIONG et al. 2009). A good correlation between the content of lead and aluminium and potassium suggests that a significant portion of Pb binds to clay minerals, probably those of illite-smectite type, fairly common in the quaternary deposits. Moraine clays contain also montmorillonite and kaolinite (KENIG 2009). The possibility of lead adsorption by clay minerals has been widely discussed (ETCI et al. 2012 , HIZAL, APAK 2012). The correlation between lead and phosphorus was found to be relatively poor. Although numerous works have proved the usefulness of apatites for the immobilization of heavy metals in the contaminated sediments (Arey 2001, Corami et al. 2008), it was reported that the formation of pyromorphite (lead phosphate) was inhibited under reducing conditions (Scheckel et al. 2010).

The factor analysis revealed the presence of two factors that together accounted for nearly 45% of variation (Table 2). The first factor included aluminium, potassium and magnesium, and the second sulphur and organic carbon. The lead share was low in the first factor (0.343), but very high in the second factor (0.757). Based on the results of the factor analysis, it can be assumed that lead deposited in the profundal zone sediments is mainly stored in the form of sulphides.

CONCLUSIONS

1. In most of the analyzed lake sediments, the lead content was greater than the geochemical background in Poland, but only in a few lakes its concentration was high enough to pose a possible threat to aquatic organisms dwelling in the sediments.
2. It was confirmed that the lead content in the sediments was significantly correlated with the content of organic carbon and sulphur, and to a lesser extent with the concentration of aluminium, iron and potassium. A negative correlation was observed for calcium content.
3. Lead concentrations varied among the sediments from various lakelands, being lower in the lakes located within the Pomeranian Lake District than in those from Greater Poland and Masurian Lake Districts.

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SENSITIVITY OF SOIL ENZYMES TO EXCESSIVE ZINC CONCENTRATIONS*

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Abstract

The sensitivity of soil enzymes to soil contamination with zinc was analyzed. A laboratory experiment was performed on sandy loam at pH 7.0, sampled from arable land at a depth of 0 to 20 cm. Soil samples were passed through a sieve with 2 mm mesh size and contaminated with the following zinc doses: 0, 300, 600, 1200 and 2400 mg Zn^{2+} kg^{-1} soil. Zinc was applied in the form of aqueous solution of $ZnCl_2$. Soil was mixed thoroughly with zinc, and its moisture content was brought to 50% capillary water capacity. The samples were incubated at 25°C. Beakers with soil samples were weighed once a week to replenish evaporated water. The activity of soil enzymes: dehydrogenases, urease, acid phosphatase, alkaline phosphatase, catalase, arylsulfatase and β-glucosidase, was determined after 15, 30, 60 and 120 days of the experiment. The results were used to calculate soil resistance (RS), ED_{20} and ED_{50} values.

The results of the study indicate that soil enzymes are characterized by varied sensitivity to excessive zinc concentrations, and that the RS index is a reliable measure of enzymatic responses to zinc pollution. The analyzed enzymes were classified in the following decreasing order in terms of their resistance to zinc: β-glucosidase> acid phosphatase > urease >arylsulfatase = alkaline phosphatase> catalase > dehydrogenases. Zinc continued to exert a negative effect on soil enzymes throughout the experiment (120 days). ED_{20} values for the analyzed enzymes in mg Zn^{2+} kg^{-1} DM soil were determined at: 103 for dehydrogenases, 184 for alkaline phosphatase, 233 for urease, 247 for arylsulfatase, 416 for acid phosphatase, 419 for catalase and 1373 for β-glucosidase.

Key words: soil enzymes, zinc, ED_{20}, ED_{50}, soil resistance (RS) index, soil contamination.

OPORNOŚĆ ENZYMÓW GLEBOWYCH NA NADMIERNE ILOŚCI CYNKU

Abstrakt

Celem badań było określenie wrażliwości enzymów na zanieczyszczenie gleby cynkiem. Doświadczenie przeprowadzono w warunkach laboratoryjnych na glinie piaszczystej o pH 7,0, pobranej z użytku rolnego z warstwy od 0 do 20 cm. Przed rozpoczęciem badań glebę przesiano przez sito o oczkach 2 mm i zanieczyszczono następującymi dawkami cynku: 0, 300, 600, 1200, 2400 mg Zn^{2+} kg^{-1} gleby. Cynk stosowano w postaci

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wodnego roztworu $ZnCl_2$. Następnie po dokładnym wymieszaniu gleby jej wilgotność doprowadzono do 50% kapilarnej pojemności wodnej i poddano inkubacji w cieplarce w temp. 25°C. Jeden raz w tygodniu zlewki przeważano w celu uzupełnienia ewentualnych ubytków wody. Po 15, 30, 60 i 120 dniach inkubacji część zlewek likwidowano i oznaczono aktywność enzymów glebowych: dehydrogenaz, ureazy, fosfatazy kwaśnej, fosfatazy alkalicznej, katalazy, arylosulfatazy i β-glukozydazy. Na podstawie wyników obliczono indeks oporności enzymów (RS) oraz wskaźniki ED_{20} i ED_{50}.

Stwierdzono, że enzymy glebowe mają zróżnicowaną oporność na nadmiar cynku w glebie, a wskaźnik oporności RS jest dobrą miarą ich reakcji na zanieczyszczenie tym metalem. Pod względem zmniejszającej się oporności można je uszeregować następująco: β-glukozydaza> fosfataza kwaśna > ureaza >arylosulfataza = fosfataza kwaśna > katalaza > dehydrogenazy. Negatywne działanie cynku na enzymy glebowe utrzymywało się przez cały okres badań (120 dni).Wartość ED_{20} dla poszczególnych enzymów w mg Zn^{2+} kg^{-1}s.m. gleby wynosiła: dehydrogenazy – 103, fosfataza alkaliczna – 184, ureaza – 233, arylosulfataza – 247, fosfataza kwaśna – 416, katalaza – 419, β-glukozydaza – 1373.

Słowa kluczowe: enzymy glebowe, cynk, ED_{20},ED_{50}, wskaźnik oporności (RS), zanieczyszczenie.

INTRODUCTION

There is a growing body of evidence that intensive farming (Wang et al. 2013) and industrial development (Kucharski et al. 2011) contribute to soil contamination with heavy metals (Kucharski et al. 2000, Wyszkowska et al. 2001, Gillet, Ponge 2002, Kucharski, Wyszkowska 2004, Seifert, Domka 2005, Wyszkowska et al. 2007, Kucharski et al. 2009). In recent years, Roca-Perez et al. (2010) have observed increasing levels of heavy metal contamination in farmland. Trace element deposition in soil can result from anthropogenic factors and from natural accumulation. Polluted wastewater and air are potential sources of heavy metal contamination in the soil environment (Chary et al. 2008, Huang et al. 2009, Sienkiewicz, Czarneckaet al. 2012, Glina, Bogacz 2013, Wang et al. 2013).

Soil enzymes, in particular intracellular enzymes produced by microorganisms (Nielsen, Winding 2002, Kucharski et al. 2011), are robust indicators of changes in soil quality. They participate in the biogeochemical cycling of elements (Nielsen, Winding 2002, Kucharski, Wyszkowska 2004, Wyszkowska et al. 2009, Wyszkowski, Wyszkowska 2009), including organic matter transformation (Wang et al. 2013). Carbon, nitrogen and phosphorus cycling enzymes as well as oxidoreductases are important indicators of soil quality (Bielińska, Żukowska 2002, Rodriguez et al. 2004, Bielińska et al. 2005, Wyszkowska et al. 2010, Qu et al. 2011).

Heavy metals are slowly transformed in the environment (Adriano et al. 2004). High concentrations of hazardous substances and microelements in soil can exert toxic effects on soil microorganisms (Bruins et al. 2000, Jonak et al. 2004) by inducing changes in enzyme activity or the population size of soil-dwelling microbes (Szymańska-Pulikowska 2012, Wyszkowska et al. 2013), which are the main source of soil enzymes. Enzyme activity levels are indicative of the structure and function of microbial communities (Brockett et al. 2012). Wang et al. (2012) observed that in contaminated environments, some bacteria regulate gene expression inside a molecule, which leads to rapid exchange and reduces free zinc concentrations in soil.

Heavy metal absorption by plants and microorganisms is determined by soil's sorption capacity, organic matter content and pH. Huang et al. (2009) and Zhao et al. (2010) observed elevated zinc concentrations in industrial regions as well as in rice fields, which could have very serious implications for consumer health.

The objective of this study was to determine the sensitivity of soil enzymes to soil contamination with zinc.

MATERIALS AND METHODS

Experimental design

A laboratory experiment was performed on samples of sandy loam at pH 7.0, which are characterized in Table 1. Soil was passed through a sieve with 2 mm size mesh before analysis. 100 cm^3 beakers were filled with 100 g of air-dried soil each. Soil samples were contaminated with zinc doses of 0, 300, 600, 1200 and 2400 mg Zn^{2+} kg^{-1} soil, applied in the form of $ZnCl_2$. The soil was mixed thoroughly with zinc, and its moisture content was brought to 50% capillary water capacity. The samples were incubated at 25°C. Beakers were weighed once a week to replenish evaporated water. After 15, 30, 60 and 120 days of the experiment, three beakers from every treatment were emptied to determine the activity of soil enzymes: dehydrogenases, urease, acid phosphatase, alkaline phosphatase, catalase, arylsulfatase and β-glucosidase. The results were used to calculate ED_{20} and ED_{50} values, and the indicators of soil resistance (RS) and soil resilience (RL).

Soil enzymes

Activity of dehydrogenases was determined by the method proposed by Öhlinger (1996). Soil samples were incubated at 37°C for 24 hours using 3% aqueous solution of

Table 1

The physicochemical and chemical properties of soil

Property	Loamy sand
Soil texture (μm)	g kg^{-1}
2000 -50	720
50 - 2	210
2 < 0	70
pH_{KCl}	7.0
	mmol(+) kg^{-1}
Hydrolytic acidity (HAC)	16.05
Sum of exchangeable bases Ca^{++}, Mg^{++}, K^+, and Na^+ (TEB)	75.0
Cation exchange capacity (CEC)	91.05
Base saturation (BS) %	82.30
	g kg^{-1}
$C_{organic}$	7.05
N_{total}	0.67
	mg kg^{-1}
Zn_{total}	16.60

2,3,5-triphenyl tetrazolium chloride (TTC) as the substrate. The absorbance of triphenylformazan (TPF) was measured in a Perkin-Elmber Lambda 25 spectrophotometer at 485 nm. The activity of dehydrogenases was expressed in µmol TPF kg^{-1} d.m. h^{-1}.

The catalase activity was determined according to Alef and Nannpieri (1998) by measuring the volume of potassium permanganate which was used up during titration by the decomposition of hydrogen peroxide to water and oxygen. The results were given in mol O_2 kg^{-1} d.m. soil h^{-1}.

The activity of urease, acid phosphatase, alkaline phosphatase, β-glucosidase and arylsulfatase was determined in accordance with the procedures proposed by Alef and Nannpieri (1998). In the analysis of urease activity, soil was incubated with 10% aqueous solution of urea as the substrate. The quantity of produced N-NH_4 was determined with the use of the Nessler's reagent. The absorbance of ammoniated mercury iodide was measured in a Perkin-Elmber Lambda 25 spectrophotometer at 410 nm. Urease activity was expressed in mmol N-NH_4 kg^{-1} d.m. soil h^{-1}. Soil samples were incubated with 4-Nitrophenyl β-D-glucopyranoside(PNG) to determine β-glucosidase activity, with 4-Nitrophenyl phosphate disodium to measure the activities of acid phosphatase and alkaline phosphatase, and with potassium 4-Nitrophenyl sulfate (PNS) to determine arylsulfatase activity. 4-Nitrophenol (PNP) was the catalysis product for all enzymes, and its absorbance was measured in the Perkin-Elmber Lambda 25 spectrophotometer at 400-420 nm. The results were expressed in mmol PNP kg^{-1} d.m. soil h^{-1}.

Soil resistance (RS)

The soil resistance (RS) index was calculated using the formula proposed by Orwin and Wardle (2004):

$$RS = 1 - \frac{2\left| D_0 \right|}{C_0 + \left| D_0 \right|},$$

$D_0 = C_0 - P_0$,
C_0 – parameter value in control (uncontaminated) soil over time t_0,
P_0 – parameter value in disturbed (contaminated) soil over time t_0.

Statistical analysis

The homogeneity of variance was estimated by the Tukey's test at $p = 0.01$. Pearson's correlation coefficients between the degree of soil contamination with zinc and enzyme activity were calculated. The effect of zinc on the activity of soil enzymes was also evaluated by principal component analysis (PCA). Statistical analyses were performed with the use of Statistica 10.0 software (StatSoft, 2012). Zinc doses which induced a 20% (ED_{20}) and 50% (ED_{50}) decrease in the activity levels of dehydrogenases, catalase, urease, β-glucosidase, acid phosphatase, alkaline phosphatase and arylsulfatase were determined.

RESULTS AND DISCUSSION

According to many authors (Quet al. 2011, Melgar-Ramírez et al. 2012), a heavy metal concentration is the most important indicator of soil quality. The main anthropogenic sources of environmental pollution are mining, transportation, heavy and light industries which contaminate local ecosystems. Qu et al. (2011) observed that heavy metals exert a negative effect on the activity of soil enzymes. In the cited study, the activities of all examined enzymes decreased with an increase in pollution levels. A similar trend was noted in our study (Table 2), where the activities of all enzymes were negatively corre-

Table 2

Enzyme activity in soil contaminated with zinc (kg^{-1} d.m. h^{-1})

Dose Zn^{2+} (mg kg^{-1} of soil d.m.)	Enzyme*						
	Deh (µmol TFF)	Kat (mol O_2)	Glu (mmol PNP)	Ure (mmol $N\text{-}NH_4$)	Pac (mmol PNP)	Pal (mmol PNP)	Aryl (mmol PNP)
15 day							
0	20.291^{b}	0.210^{a}	0.755^{a}	0.725^{b}	0.812^{ab}	1.678^{a}	0.191^{a}
300	11.232^{d}	0.183^{c}	0.716^{a}	0.579^{c}	0.691^{cd}	1.131^{c}	0.153^{b}
600	5.903^{f}	0.157^{e}	0.646^{b}	0.388^{d}	0.632^{de}	0.827^{e}	0.118^{d}
1200	1.790^{h}	0.096^{gh}	0.588^{bc}	0.176^{gh}	0.501^{fg}	0.590^{f}	0.070^{hi}
2400	0.412^{ij}	0.060^{k}	0.505^{d}	0.175^{f}	0.328^{hi}	0.325^{g}	$0.030^{lł}$
r	-0.834	-0.965	-0.973	-0.864	-0.984	-0.895	-0.958
30 day							
0	21.494^{a}	0.195^{b}	0.575^{c}	0.808^{a}	0.893^{a}	1.481^{b}	0.137^{c}
300	12.057^{c}	0.178^{c}	0.536^{cd}	0.577^{c}	0.743^{bcd}	0.956^{d}	0.085^{efg}
600	5.877^{f}	0.155^{e}	0.494^{de}	0.384^{d}	0.476^{fg}	0.827^{e}	0.075^{gh}
1200	1.877^{h}	0.092^{hi}	0.414^{f}	0.114^{h}	0.399^{gh}	0.523^{f}	0.041^{kl}
2400	0.437^{ij}	0.027^{l}	$0.392\mathrm{f}^{gh}$	0.164^{fg}	0.315^{hi}	0.318^{g}	$0.031^{lł}$
r	-0.829	-0.99^{1}	-0.928	-0.827	-0.866	-0.898	-0.868
60 day							
0	20.660^{b}	0.204^{a}	0.490^{de}	0.822^{a}	0.832^{ab}	1.455^{b}	0.120^{d}
300	11.977^{c}	0.168^{d}	0.539^{cd}	0.545^{c}	0.762^{bc}	0.964^{d}	0.091^{ef}
600	5.837^{f}	0.131^{f}	0.440^{ef}	0.332^{e}	0.564^{ef}	0.828^{e}	0.080^{fgh}
1200	1.624^{h}	0.083^{j}	0.410^{fg}	0.109^{h}	0.495^{fg}	0.517^{f}	0.052^{jk}
2400	0.368^{ij}	$0.018^{ł}$	0.352^{gh}	0.153^{fgh}	0.178^{j}	0.315^{g}	0.040^{kl}
r	-0.835	-0.982	-0.897	-0.806	-0.983	-0.906	-0.922
120 day							
0	9.429^{e}	0.135^{f}	0.416^{f}	0.567^{c}	0.757^{bc}	1.441^{b}	0.098^{e}
300	6.049^{f}	0.101^{g}	0.482^{de}	0.294^{e}	0.751^{bcd}	0.817^{e}	0.059^{ij}
600	2.480^{g}	0.086^{hj}	0.406^{fg}	0.299^{e}	0.535^{ef}	0.544^{f}	0.051^{jk}
1200	0.694^{i}	0.055^{k}	0.331^{h}	0.119^{gh}	0.353^{hi}	0.320^{g}	$0.021^{łm}$
2400	0.113^{j}	$0.015^{ł}$	0.265^{i}	0.143^{fgh}	0.24^{ij}	0.32^{g}	0.014^{m}
r	-0.837	-0.975	-0.909	-0.776	-0.937	-0.774	-0.875

* Deh – dehydrogenases, Kat – catalase, Glu– β-glucosidase, Ure – urease, Pac – acid phosphatase, Pal – alkaline phosphatase, Aryl – arylosulphatase;
Enzymes marked with the same letter form a homogeneous group.

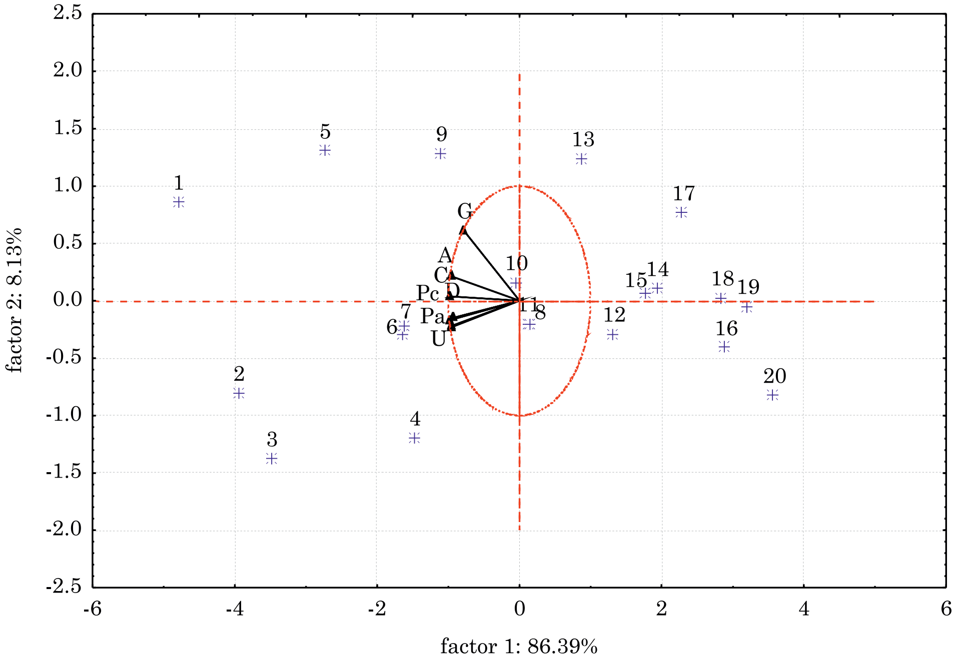

Fig. 1. Enzyme activity in loamy sand contaminated with zinc – the PCA method.

Vectors represent the analyzed variables: D – dehydrogenases, C – catalase, U – urease, Pc – acid phosphatase, Pa – alkaline phosphatase, G – b-glucosidase, a – arylosulphatase

1 - 0 mg Zn^{2+} on 15 day; 2 - 0 mg Zn^{2+} on 30 day; 3 - 0 mg Zn^{2+} on 60 day; 4 - 0 mg Zn^{2+} on 120 day; 5 - 300 mg Zn^{2+} on 15 day; 6 - 300 mg Zn^{2+} on 30 day; 7 - 300 mg Zn^{2+} on 60 day; 8 - 300 mg Zn^{2+} on 120 day; 9 - 600 mg Zn^{2+} on 15 day; 10 - 600 mg Zn^{2+} on 30 day; 11 - 60 mg Zn^{2+} on 60 day; 12 - 600 mg Zn^{2+} on 120 day; 13 - 1200 mg Zn^{2+} on 15 day; 14 - 1200 mg Zn^{2+} on 30 day; 15 - 1200 mg Zn^{2+} on 60 day; 16 - 1200 mg Zn^{2+} on 120 day; 17 - 2400 mg Zn^{2+} on 15 day; 18 - 2400 mg Zn^{2+} on 30 day; 19 - 2400 mg Zn^{2+} on 60 day; 20 - 2400 mg Zn^{2+} on 120 day

lated with the level of zinc contamination. Our observations corroborate the findings of Lee et al. (2009) and Kucharski et al. (2011).

The distribution of vectors around the axis representing the first principal component indicates that the activity of all analyzed enzymes, excluding β-glucosidase, was negatively correlated with this variable (Figure 1). Only the vector representing the first principal component of β-glucosidase was positively correlated with the second principal component. The projection of data onto component space indicates that the activity levels of all enzymes decreased with an increase in zinc concentrations. The highest levels of enzymatic activity were observed on day 15, and the lowest – on day 120, regardless of the degree of soil contamination. The effects of zinc on soil enzymes were also analyzed by Boros et al. (2011) and Wyszkowska et al. (2013), whereas Borowik et al. (2013) evaluated its influence on nitrification.

The examined enzymes were classified in the following decreasing order in terms of their average sensitivity to soil pollution with 2400 mg Zn^{2+} kg^{-1}: dehydrogenases (98% decrease in enzyme activity) > catalase (84%) > alkaline phosphatase = arylsulfatase (79%) > urease (78%) > acid phosphatase (68%) >β-glucosidase (32%).

Soil resistance indicators are effective measures of microbial and enzymatic responses to environmental stress (Borowik et al. 2013, Orwin, Wardle 2004). The data shown in Table 3 demonstrates that the soil resistance (RS) index is highly useful for evaluations of soil quality in zinc-contaminated environments. Low RS values are indicative of long-term toxic effects of zinc. In this study, low RS values were reported on soil incubation days 15 and 120. The resistance of the analyzed enzymes to zinc decreased with an increase in zinc concentrations in soil. Significant negative correlations were observed between RS values and zinc doses. β-glucosidase was most resistant to all concentrations of zinc, whereas dehydrogenases activity was characterized by the lowest resistance to the analyzed pollutant. Based on the data presented in Tables 3 and 4, the analyzed enzymes were arranged in the following decreasing order in terms of their resistance to zinc: β-glucosidase> catalase > acid phosphatase >arylsulfatase> urease >

Table 3

Soil resistance (RS) to zinc on days 15 and 120, determined according to the levels of enzyme activity

Dose Zn^{2+} (mg kg^{-1} of soil d.m.)	Enzyme*						
	Deh	Kat	Ure	Glu	Pac	Pal	Aryl
15 day							
300	0.383^{b}	0.772^{a}	0.665^{a}	0.902^{a}	0.740^{b}	0.508^{a}	0.672^{a}
600	0.170^{c}	0.598^{b}	0.364^{b}	0.748^{bc}	0.638^{c}	0.327^{c}	0.445^{b}
1200	0.046^{e}	0.296^{d}	0.138^{cde}	0.637^{d}	0.446^{e}	0.214^{de}	0.224^{d}
2400	0.010^{g}	0.167^{f}	0.137^{cde}	0.502^{e}	0.253^{f}	0.107^{f}	0.084^{f}
Average	0.152	0.458	0.326	0.697	0.519	0.289	0.356
r	-0.829	-0.929	-0.796	-0.950	-0.983	-0.950	-0.925
120 day							
300	0.472^{a}	0.597^{b}	0.350^{b}	0.726^{bc}	0.982^{a}	0.395^{b}	0.432^{b}
600	0.151^{d}	0.468^{c}	0.358^{b}	0.952^{a}	0.546^{d}	0.233^{d}	0.351^{e}
1200	0.038^{f}	0.254^{e}	0.117^{de}	0.662^{cd}	0.303^{f}	0.125^{f}	0.121^{e}
2400	0.006^{g}	0.058^{g}	0.144^{cd}	0.469^{e}	0.189^{g}	0.125^{f}	0.078^{f}
Average	0.167	0.344	0.242	0.702	0.505	0.220	0.246
*r**	-0.773	-0.975	-0.787	-0.813	-0.852	-0.802	-0.898

* key under Table 2

Table 4

Zinc dose (mg kg^{-1} d.m. soil) responsible for 20% and 50% decrease in enzyme activity, ED_{20} andED_{50}

% decrease in enzyme activity	Enzyme*						
	Deh	Kat	Ure	Glu	Pac	Pal	Aryl
20	103	419	233	1373	416	184	247
50	689	1255	928	3184	1514	991	1082

* key under Table 2

alkaline phosphatase > dehydrogenase. The highest value of ED_{20} was determined for β-glucosidase at 1373 mg Zn^{2+} kg^{-1} d.m. soil, and the lowest one – for dehydrogenases at 103 mg Zn^{2+} kg^{-1} d.m. soil. ED_{50} values were determined at 3184 mg Zn^{2+} for the former and 689 mg Zn^{2+} for the latter enzyme.

Renella et al. (2005) and Lee et al. (2009) attributed the observed decrease in enzyme activity in soils contaminated with heavy metals to their adverse effects on microbial counts. Other authors (Schwartz et al. 2001, Kizilkaya 2004, Gülser, Erdogan 2008, Qu et al. 2011) observed that heavy metals affect enzymes not only by inducing changes in microbial populations, but also by modifying their diversity. The extent to which enzyme activity was inhibited by zinc could have been determined by both of the above factors. Similarly to other elements, excessive concentrations of zinc induce changes in electron transport, cell membrane permeability and contribute to oxidative stress (Wang et al. 2009, Cui, Zhao 2011). Zinc could also lead to enzyme denaturation (Jonak et al. 2004). The noted reduction in enzyme activity could have also resulted from the negative effect of zinc on the physicochemical properties of soil (Lestan et al. 2003, Boros et al. 2011, Cui, Zhao 2011).

CONCLUSIONS

1. Soil enzymes are characterized by varied sensitivity to excessive zinc concentrations in soil. The analyzed enzymes were classified in the following decreasing order in terms of their resistance to zinc: β-glucosidase> acid phosphatase > urease >arylsulfatase = alkaline phosphatase > catalase > dehydrogenases.
2. Zinc continued to exert negative effect on soil enzymes throughout the entire experiment (120 days).
3. The values of ED_{20} for the analyzed enzymes (in mg Zn^{2+} kg^{-1} d.m. soil) were determined at: 103 for dehydrogenases, 184 for alkaline phosphatase, 233 for urease, 247 for arylsulfatase, 416 for acid phosphatase, 419 for catalase and 1373 for β-glucosidase.

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EFFECT OF SLURRY FERTILIZATION ON THE SELENIUM CONTENT AND CATALASE ACTIVITY IN LESSIVE SOIL

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Abstract

The aim of the present research was to determine the total selenium content in soil and plants from a microplot experiment with different nitrogen fertilization regimes, and to identify the relationships of the selenium content in soil and plants versus the soil catalase activity. The experiment was conducted in randomized blocks with three replications. The soil and plant samples were collected from a microplot experiment established at the IUNG in Pulawy. The soil was enriched with mineral nitrogen and with nitrogen supplied in slurry, both applied at doses of 100 kg N ha^{-1}. The total selenium content in soil under each of the crop rotation systems was no more than 0.2 mg kg^{-1}. Data from the references imply that the soil was deficient in selenium. The highest amount of selenium was under winter wheat and spring barley with undersown crop in crop rotation A, and in soil under maize crop rotation B. Slurry fertilization significantly stimulated the activity of catalase in soil, as compared with the control and mineral nitrogen fertilization treatments. The highest catalase activity – nearly double the control – was detected in soil under winter wheat in crop rotation A and under spring barley in crop rotation B; winter wheat, regardless of the type of crop rotation, accumulated on average 0.3 mg Se kg^{-1} d.w. in aerial parts and 0.344 mg Se kg^{-1} d.w. in roots. The highest amounts of selenium in the investigated parts of plants were reported in the control plots and in the plots with slurry fertilization. Mineral fertilization reduced selenium availability to plants. In both crop rotation systems, the highest bioaccumulation of selenium was noted in winter wheat roots from control plots, while the lowest one was detectetd in aerial parts of plants from the plots with slurry fertilization. Despite the fertilization applied, the selenium content in plant roots was higher than its content in aerial parts. The correlation analysis of the results on selenium concentration in soil and plants as well as the catalase activity of soil identified only a significant dependence between the total selenium content and catalase activity in soil from crop rotation B.

Key words: selenium, catalase activity, slurry, plants.

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WPŁYW NAWOŻENIA GNOJOWICĄ NA ZAWARTOŚĆ SELENU W GLEBIE I ROŚLINACH UPRAWIANYCH W ZMIANOWANIU

Abstrakt

Celem pracy było określenie wpływu nawożenia azotem w formie mineralnej oraz w formie gnojowicy od trzody chlewnej na całkowitą zawartość selenu w glebie oraz roślinach występujących w zmianowaniu na tle aktywności katalazy glebowej. Do badań wykorzystano próbki gleby i roślin z doświadczenia mikropoletkowego prowadzonego przez IUNG w Puławach. Zastosowano nawożenie azotem w postaci saletry amonowej w ilości 100 kg N ha^{-1} oraz w formie gnojowicy w ilości 100 kg N ha^{-1}. Doświadczenie wykonano w dwóch zmianowaniach z następującym doborem roślin: A: koniczyna – pszenica ozima – jęczmień jary z wsiewką; B: kukurydza – pszenica ozima – jęczmień jary. Zawartość selenu w glebie i roślinach oznaczono metodą Watkinsona z użyciem spektrofluorymetru F-2000 firmy Hitachi. Całkowita zawartość selenu w glebie, w obu zmianowaniach, nie przekraczała średnio 0,200 mg kg^{-1}. Nawożenie gnojowicą spowodowało istotny wzrost całkowitej zawartości selenu w glebie, w odniesieniu do jego zawartości w obiektach kontrolnych i nawożonych azotem w formie mineralnej. Najwyższą zawartość tego pierwiastka w zmianowaniu A wykazano w glebie pod uprawą jęczmienia jarego z wsiewką koniczyny, natomiast w zmianowaniu B – w glebie pod kukurydzą. Nawożenie azotem w formie gnojowicy istotnie stymulowało aktywność katalazy w badanej glebie w porównaniu z jej aktywnością w obiektach kontrolnych oraz w obiektach nawożonych azotem w formie mineralnej. W glebie ze zmianowania A najwyższą aktywność katalazy – ponad 2-krotnie wyższą – wykazano pod uprawą pszenicy ozimej, natomiast w zmianowaniu B – pod uprawą jęczmienia jarego. Pszenica ozima, niezależnie od rodzaju zmianowania, zgromadziła największą ilość selenu spośród roślin uprawianych w obu zmianowaniach: w częściach nadziemnych średnio 0,300 mg kg^{-1} s.m., natomiast w korzeniach 0,344 mg kg^{-1} s.m. Największą zawartość selenu w badanych roślinach wykazano w obiektach kontrolnych oraz w obiektach, na których stosowano azot w formie gnojowicy. Zastosowanie azotu w formie mineralnej ograniczyło pobieranie tego pierwiastka przez rośliny testowe. Wykazano, że najwyższą zdolność kumulacji selenu miały korzenie pszenicy ozimej pobrane z obiektów kontrolnych, natomiast najmniejszą – części nadziemne jęczmienia jarego z obiektów nawożonych azotem w formie gnojowicy. Niezależnie od zastosowanego nawożenia, zawartość selenu w korzeniach badanych gatunków roślin była większa od jego zawartości w częściach nadziemnych. W warunkach doświadczenia wykazano istotną korelację między aktywnością katalazy a całkowitą zawartością selenu w glebie.

Słowa kluczowe: selen, aktywność katalazy, gnojowica, rośliny.

INTRODUCTION

Selenium (Se) is an essential micronutrient for most plants, animals and people. This element builds selenoproteins, acts against oxidative stress, is involved in the production of thyroid hormones and contributes to the functioning of the immune system (AMOUROUX et al. 2001). The global distribution of selenium in water, air, soils and live organisms is uneven, so that some areas on the Earth are depleted of Se and pose serious health risks to both animals and humans, such as development of cancer and heart diseases. Soil-borne selenium is the primary source of human food Se. Most soils of the temperate humid climate zones and developed from sedimentary rocks contain low selenium levels, insufficient to produce food and animal feed plants with adequate Se content (KABATA-PENDIAS 1998). According to WINKEL et al. (2012), selenium bioavailability is a function of the interaction among the prevailing geochemical parameters, i.e., pH and redox conditions, and soil properties, such as organic carbon, Fe hydroxide, and clay contents, and Se speciation. In soils, Se comes in a broad range of oxidation states: +6 in selenates, +4 in selenites, 0 in elemental Se, and -2 in inorganic and organic selenides. Selenate, which is poorly adsorbed on oxide surfaces thus being the most mobile Se form, can be expected to occur under high oxidative conditions. At low redox potential, it can be reduced to selenite, which has much higher adsorption affinity. It is strongly retained by ligand exchange on oxide surfaces, especially at low pH, which reduces its bioavailability (HARTIKAINEN 2005, PATORCZYK-PYTLIK, KULCZYCKI 2009). The transformation of easily soluble selenates added to acidic or neutral soils into slightly soluble forms is relatively fast. In soil, processes of decomposition and synthesis of mineral and organic matter occur all the time, being monitored and activated by a variety of enzymes. Catalase (EC 1.11.1.6) is an iron porphyrin enzyme which catalyses very rapid decomposition of hydrogen peroxide to water and oxygen (NELSON, COX 2000). The enzyme is widespread in nature, which explains its diverse activities in soil. The activity of catalase, alongside dehydrogenase, is tested to provide information on microbial activities in soil (ACHUBA, PERETIEMO-CLARKE 2008). A study of the Se content in soils and its forms can improve our understanding of Se cycling and balance in geosystems and their impact on health problems.

With the above in mind, the present investigation has been launched to study the total selenium content and catalase activity in soil fertilized with mineral nitrogen and swine slurry, and their impact on the bioavalability of this microelement to crops.

MATERIAL AND METHODS

Soil and plant samples were collected in March (winter wheat) and May (other plants) 2008, from a microplot experiment established by the Department of Plant Nutrition of the Institute of Soil Science and Cultivation in Pulawy. The soil, according to the FAO classification, was Haplic Luvisol with the texture of loamy sand and sandy loam. The experiment was conducted with two crop rotation systems: A (red clover - winter wheat - spring barley + undersown crop) and B (maize – winter wheat – spring barley). Some agrotechnical elements of plants under crop rotations are presented in Table 1. The soil was enriched with mineral nitrogen in the form of ammonium nitrate at the dose of 100 kg N ha^{-1} and in the form of pig slurry at the dose of 100 kg N ha^{-1}. The experiment was designed as split-plot trials with three replications on 1x1 m plots. Soil samples were air-dried and sieved through a 2 mm screen. The plant material was rinsed in deionized water to remove soil particles, separated into aerial biomass and roots, and dried.

Table 1

Some agrotechnical information about the plants in the crop rotations

Plant	Cultivar	Date of sampling	Development stage
Crop rotation A			
Red clover	Hruszowska	25.05.2008	BBCH 33
Winter wheat	Turnia	20.03.2008	BBCH 25
Spring barley with undersown crop	Justina	25.05.2008	BBCH 30
Crop rotation B			
Maize	Ainergy	25.05.2008	BBCH 15
Winter wheat	Turnia	20.03.2008	BBCH 25
Spring barley	Justina	25.05.2008	BBCH 30

The total selenium content in soils and plants was determined applying the Watkinson method (1966) on a Hitachi F-2000 spectrofluorometer. The samples were microwave-digested with concentrated nitric and perchloric acids. The different forms of selenium in the samples were reduced by boiling with 10% HCl. The selenium was complexed with 2,3-diaminonaphtalene (DAN) to yield the fluorescent compound, which was extracted with cyclohexane and read on a spectrofluorometer at the excitation and emission wave lengths of 376 and 519 nm, respectively. The analytical procedures gave satisfactory values for the standard reference material CRM024-050 Resource Technology Corporation (RTC), soil from Western US of a texture of loamy sand; Se 0.558 mg kg^{-1} (certified value 0.540 mg kg^{-1}). The certified reference material was included in each batch of samples for quality control. The bioaccumulation coefficient (BC) was calculated as a ratio of selenium concentration in plant aerial parts or roots to its amount in soil. Catalase activity (CAT) was measured applying the Johnson and Temple method (1964). Soil was incubated with hydrogen peroxide for 20 min at 20^{o}C. The remaining material, H_2O_2, not decomposed by catalase, was treated with *potassium* permanganate in the presence of H_2SO_4. To eliminate probable overestimation of the enzymatic activity due to chemical reduction of added H_2O_2, correction for autoclaved soil (0.1 MPa, 120°C, 30 min) was made. The results were expressed in mmol O_2 kg^{-1} h^{-1}. The soil samples were analysed for organic carbon by wet oxidation with potassium dichromate, total nitrogen following the Kjeldahl method and pH in 1M KCl potentiometrically. All the analyses were performed on triplicate samples. The multi-replication data from the analyses of soil and plant samples underwent statistical procedure consiting of variation analysis for one-factorial experiments carried out in a split-plot design. The data were analysed for treatment effect with the analysis of variance (Anova), after which the Tukey test at $p < 0.05$ was applied. The analysis was carried out using Statistica for Windows software.

RESULTS AND DISCUSSION

General properties of the soil are given in Table 2. The data on total Se concentrations of the soil (Table 3) indicate that slurry application significantly increased the total selenium content in the soil. The results show that the total selenium content did not exceed 0.2 mg kg^{-1}. Such low levels of selenium in soils suggested that plants growing on these soils were deficient in this microelement. According to Kabata-Pendias (1998), the mean total selenium content in soils worldwide is estimated at 0.44 mg kg^{-1}, while its background content in various soil groups ranges from 0.05 to 1.5 mg kg^{-1}, being the lowest in Podzols and the highest in Histosols. Aro and Alfthang (1998) as well as Hartikainen (2005) claim that soils containing less than 0.5 mg Se kg^{-1} are likely to lead to crops and pastures having inadequate selenium concentrations (<0.05 mg kg^{-1} d.w.). In the soil from both crop rotations, the application of slurry significantly increased total selenium compared with the control and soil where mineral fertilization was applied. Sager (2007) stated that average total selenium in slurry ranged from 0.21 to 0.35 mg kg^{-1}. Thus, the increase in Se in slurry-treated soil could have been caused by the amount of this microelement in slurry. The supplementation of

Table 2

Some general properties of the soil

Treatment	Crop rotation A			Crop rotation B		
	red clover	winter wheat	spring barley with undersown crop	maize	winter wheat	spring barley
Organic carbon (g kg^{-1})						
Control	7.55	6.98	6.87	6.95	6.55	6.28
$N_{MINERAL}$	6.98	6.86	6.91	6.55	6.83	6.63
N_{SLURRY}	7.68	7.55	7.86	7.45	7.59	7.56
Total nitrogen (g kg^{-1})						
Control	0.679	0.681	0.678	0.667	0.645	0.643
$N_{MINERAL}$	0.711	0.713	0.723	0.719	0.698	0.715
N_{SLURRY}	0.728	0.731	0.735	0.725	0.718	0.731
pH_{KCl}						
Control	5.5	5.4	5.4	5.3	5.4	5.3
$N_{MINERAL}$	5.3	5.3	5.2	5.2	5.2	5.2
N_{SLURRY}	5.4	5.4	5.3	5.3	5.3	5.5

Table 3

Total selenium content in the soil (mg kg^{-1})

Treatment (I factor)	Crop rotation (factor II)					
	A			B		
	cultivated plant (factor III)					
	red clover	winter wheat	spring barley with undersown crop	maize	winter wheat	spring barley
Control	0.118	0.121	0.133	0.161	0.125	0.122
N_{MINERAL}	0.100	0.131	0.144	0.144	0.125	0.124
N_{SLURRY}	0.124	0.152	0.186	0.177	0.153	0.152
Mean	0.114	0.135	0.154	0.161	0.134	0.133
Mean for treatment (factor I)						
	Control	0.129	N_{MINERAL}	0.126	N_{SLURRY}	0.157
Mean for crop rotation (factor II)						
	A		0.134	B	0.141	
Mean for plants (factor III)						
	Red clover/ maize	0.135	Winter wheat	0.134	Spring barley	0.143
LSD $_{0.05}$	I	0.004	interactions	II/I 0.003 I/II 0.005		
	II	0.002		I/III 0.005 III/I 0.003		
	III	0.002		II/III 0.005 III/II 0.002		

mineral fertilizers decreased the total selenium content in soil, which may be explained in the following way: increased mineral fertilization causes greater root growth and provides plants with a larger volume of soil from which Se can be extracted, an explanation supported by Blagojevic et. al. (1980).

Generally, the organic addittives increased enzyme activities of soil and their application to soil can improve soil structure and stimulate microbiological activity (Jezierska-Tys, Frąc 2009, Jezierska-Tys et al. 2011). Catalase activity of the soil from crop rotation B was significantly higher than in soils treated with mineral nitrogen and from control plots (Table 4). In crop rotation A, CAT activity was significantly stimulated by slurry application only in the soil under winter wheat, as compared to that of control. The correlation analysis of the results on selenium concentration in soil and plants as well as the catalase activity of soil yielded a significant relationship only between the total selenium content and catalase activity in soil from crop rotation B (0.56). Our results coincide with the literature data reviewed by Samuel (2010) and with our earlier studies (Borowska, Koper 2011).

Variation of the Se status in humans largely depends on diet. Plant foods are the major dietary sources of Se in most countries around the world, followed by meat and seafood (Winkel et al. 2012). As shown in Tables 5 and 6, winter wheat accumulated in an average 0.3 mg Se kg^{-1} d.w. in aerial organs and 0.344 mg Se kg^{-1} d.w. in roots, regardless of the crop rotation. The highest amounts of selenium in the investigated parts of plants were

Table 4

Catalase activity (CAT) in the soil (mmol O_2 kg^{-1} h^{-1})

Treatment (I factor)	Crop rotation (II factor)					
	A			B		
	cultivated plant (III factor)					
	red clover	winter wheat	spring barley with undersown crop	maize	winter wheat	spring barley
Control	74.10	163.19	57.34	52.04	75.86	160.54
$N_{MINERAL}$	62.63	157.89	60.86	53.81	70.57	149.96
N_{SLURRY}	71.45	171.13	54.69	67.92	82.92	166.72
Mean	69.39	164.07	57.63	57.92	76.45	159.07
Mean for treatment (factor I)						
	Control	97.16	$N_{MINERAL}$	92.61	N_{SLURRY}	102.46
Mean for crop rotation (factor II)						
		A	97.02	B	97.80	
Mean for plants (factor III)						
	Red clover/ maize	63.64	Winter wheat	120.25	Spring barley	108.34
$LSD_{0.05}$	I	0.066	interactions	II/I 0.034 I/II 0.068		
	II	0.020		I/III 0.069 III/I 0.040		
	III	0.023		II/III 0.033 III/II 0.032		

Table 5

Selenium content in aerial parts of plants (mg kg^{-1} d.w.)

Treatment (I factor)	Crop rotation (II factor)					
	A			B		
	cultivated plant (III factor)					
	red clover	winter wheat	spring barley with undersown crop	maize	winter wheat	spring barley
Control	0.132	0.318	0.145	0.120	0.330	0.140
$N_{MINERAL}$	0.125	0.287	0.132	0.124	0.288	0.129
N_{SLURRY}	0.135	0.265	0.128	0.194	0.324	0.118
Mean	0.131	0.290	0.135	0.146	0.314	0.129
Mean for treatment (factor I)						
	Control	0.196	$N_{MINERAL}$	0.181	N_{SLURRY}	0.195
Mean for crop rotation (factor II)						
		A	0.185	B	0.196	
Mean for plants (factor III)						
	Red clover/ maize	0.139	Winter wheat	0.301	Spring barley	0.132
$LSD_{0.05}$	I	0.013	interactions	II/I 0.003 I/II 0.013		
	II	0.002		I/III 0.013 III/I 0.004		
	III	0.003		II/III 0.004 III/II 0.003		

Table 6

Selenium content in plant roots (mg kg^{-1} d.w.)

Treatment (I factor)	Crop rotation (factor II)					
	A			B		
	cultivated plant (factor III)					
	red clover	winter wheat	spring barley with undersown crop	maize	winter wheat	spring barley
Control	0.129	0.403	0.151	0.125	0.399	0.143
$N_{MINERAL}$	0.126	0.298	0.141	0.132	0.294	0.122
N_{SLURRY}	0.141	0.328	0.132	0.263	0.344	0.123
Mean	0.132	0.343	0.141	0.173	0.346	0.129
Mean for treatment (factor I)						
	Control	0.225	$N_{MINERAL}$	0.185	N_{SLURRY}	0.221
Mean for crop rotation (factor II)						
		A	0.205	B	0.215	
Mean for plants (factor III)						
	Red clover/ maize	0.152	Winter wheat	0.344	Spring barley	0.134
LSD $_{0.05}$	I	0.003	interactions	II/I 0.002 I/II 0.004		
	II	0.001		I/III 0.004 III/I 0.002		
	III	0.001		II/III 0.002 III/II 0.002		

recorded in control plots and in the plots with slurry fertilization. These results confirm that among common cereal crops, wheat is the most efficient accumulator of Se (wheat> rice> maize> barley> oats). Wheat is also the most important Se source for humans. Thus, this cereal is an obvious target crop for agronomic biofortification to increase the dietary Se intake (BROADLEY et al. 2006).

According to TERRY et al. (1992) and MUNIER-LAMY et al. (2007), the value of bioaccumulation coefficient (BC) reflects the plant's capacity for the uptake of nutrients from soil, while telling us about the amount and rate of nutrient translocation from soil solution to aerial organs of plants. The bioaccumulation coefficients (BC) of selenium demonstrated that aerial parts and roots of winter wheat and spring barley from both crop rotation systems absorbed selenium more easily from soil of the control plots or plots where mineral fertilization was applied (Figures 1 and 2). However, the BC factor calculated for maize indicated an opposite tendency, namely the highest amounts were recorded for plants from plots fertilized with slurry (Figure 2).

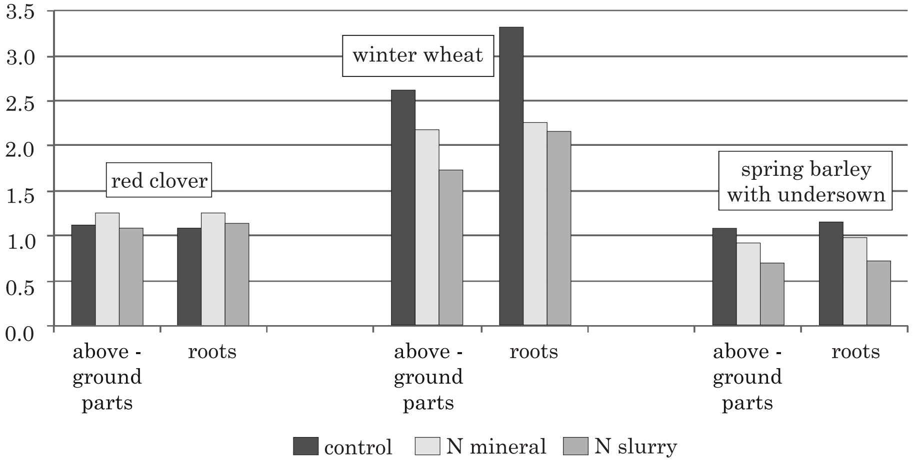

Fig. 1. Bioaccumulation coefficients (BC) for plants in crop rotation A

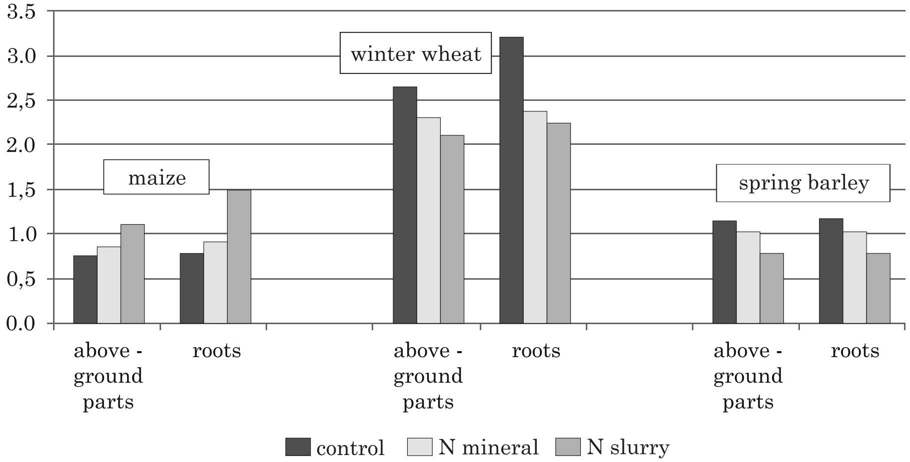

Fig. 2. Bioaccumulation coefficients (BC) for plants in crop rotation B

Winkel et al. (2012) mentioned that a large amount of organic matter can lead to reducing conditions, where both biotic and abiotic mechanisms induce the formation of elemental selenium or metal selenides. Elemental Se is not soluble in water and it is classified as unavailable to biota. The accumulation of selenium by plants may be affected by competition or interactions with sulphur fertilization, as well as by other interactions in soil during the adsorption, transport and transformation processes (Hawkesford, Zhao 2007).

CONCLUSIONS

1. The total selenium content in soil did not exceed 0.2 mg kg$^{-1.}$ In the light of the relevant literature, the analyzed soil is deficient in this microelement.

2. Slurry fertilization significantly stimulated catalase activity in the investigated soil in comparison with the control and soil from plots fertilized with mineral nitrogen.

3. Regaardless of the crop rotation system, winter wheat accumulated in 0.3 mg Se kg^{-1} d.w. in aerial organs and 0.344 mg Se kg^{-1} d.w. in roots, on aveerage. The highest amounts of selenium in the investigated plant parts were obtained from control plots and the plots with slurry fertilization. Mineral fertilization reduced selenium availability to plants. Irrespective of the applied fertilization, the selenium content in plant roots was higher than in aerial organs.

4. The correlation analysis of the results on selenium concentrations in soil and plants as well as the catalase activity in soil showed a significant dependence only between the total selenium content and catalase activity in soil from crop rotation B.

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SUITABILITY OF SOME NITROGEN FERTILIZERS FOR THE CULTIVATION OF EARLY CABBAGE

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Abstract

Early cabbage was grown in a field experiment in two treatments, with and without weed control, to test the following fertilizers: ammonium nitrate and Entec 26-a, a new formula nitrogen fertilizer containing a DMPP nitrification inhibitor, applied in single preplant or split doses, and calcium cyanamide (Perlka), supplied only prior to the planting of cabbage. All the sources of N were used in amounts supplying 150 kg N ha^{-1}. The fertilizer Perlka was applied 2 weeks before planting cabbage transplants, while Entec 26 and ammonium nitrate were introduced to soil 3 days before that. All the fertilizers were incorporated into the soil by harrowing. In the treatments with split N application, top dressing was conducted just after the cabbage plants were uncovered, which took place 3-4 weeks after transplanting. The experiment was established in a one factorial design with four replications.

The results proved high efficiency of nitrogen fertilization, which – when applied at a dose of 150 kg N ha^{-1} - enhanced the yield of early cabbage by about 50% compared to the treatment without N fertilization and weed control. Ammonium nitrate and Entec 26 appeared to be equally valuable sources of this nutrient, both in single and split doses, in each year of the investigation. Calcium cyanamide produced the highest marketable yield of heads with a significantly lower mean content of nitrates in edible parts. Another big advantage of this N source is its high efficiency in reducing weed infestation during the whole vegetation period.

Key words: cabbage, ammonium nitrate, Entec 26, calcium cyanamide, yield, plant composition.

PRZYDATNOŚĆ WYBRANYCH NAWOZÓW AZOTOWYCH W UPRAWIE KAPUSTY WCZESNEJ

Abstrakt

W doświadczeniu wegetacyjnym saletrę amonową i nawóz azotowy Entec 26 zawierający inhibitor nitryfikacji DMPP stosowano w całości przedwegetacyjnie lub w dawkach dzielonych, natomiast cyjanamid wapnia (Perlka) wyłącznie przed sadzeniem rozsady kapusty wczesnej

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w dwóch kombinacjach: z odchwaszczaniem i bez usuwania chwastów. Wszystkie badane nawozy stosowano w łącznej dawce 150 kg N ha^{-1}. Nawóz Perlka wysiewano 2 tygodnie przed sadzeniem rozsady kapusty, zaś saletrę amonową i Entec 26 bezpośrednio przed sadzeniem rozsady kapusty, pod bronę. W obiektach, gdzie stosowano dawki dzielone, nawożenie pogłówne wykonywano 3-4 tygodnie po posadzeniu rozsady, po usunięciu włókniny, którą osłaniano rośliny. Doświadczenie założono w układzie jednoczynnikowym w 4 powtórzeniach. Potwierdzono wysoką skuteczność nawożenia azotem, które w dawce 150 kg N ha^{-1} spowodowało wzrost plonu kapusty uprawianej na zbiór wczesny o ok. 50% w porównaniu z obiektem bez nawożenia i odchwaszczania. Saletra amonowa i nawóz Entec 26 miały zbliżoną wartość jako źródło azotu dla kapusty, niezależnie od metody stosowanej w każdym roku badań. Użycie cyjanamidu wapnia przyczyniło się do uzyskania wyższego plonu handlowego kapusty oraz istotnego zmniejszenia średniej zawartości azotanów w częściach jadalnych, w porównaniu z pozostałymi nawozami. Dużą zaletą tego nawozu jako źródła azotu jest jego wysoka efektywność w redukcji zachwaszczenia przez cały okres uprawy kapusty.

Słowa kluczowe: kapusta, saletra amonowa, Entec 26, cyjanamid wapnia, plon, skład chemiczny.

INTRODUCTION

Nitrogen fertilization is a major factor affecting the yield and nutritional value of vegetable crops. As an elementary constituent of amino acids, proteins, nucleic acids and numerous secondary metabolites, nitrogen plays a crucial role in the metabolism of plants, their growth and development. Suboptimal supply of this element can lead to delayed maturation, inhibited protein synthesis and high reduction of the yield (Rahn et al. 1998). The economic consequences of failing to produce target yield are bad enough to tempt farmers to apply more nitrogen than necessary (Rahn 2002). It is not always easy to ascertain whether nitrogen has been overfertilized because the borderline between optimal and excessive N supplies is rather vague (Peck 1981). An oversupply N fertilization invariably leads to stimulated vegetative growth and may negatively affect the crop's nutritional value by limiting the synthesis of sugars, enhancing the accumulation of nitrates accumulation and contributing to high nitrogen leaching from the soil (Sorensen 1999, Rahn 2000, Neeteson, Carton 2001, Dzida et al. 2013). For white head cabbage, the negative consequences of nitrogen overfertilization are such as inferior storability and worse processing quality due to decreased dry matter and sugar content. Typically, in a temperate climate, no more than 50% of N applied to the soil is effectively used by plants while much nitrogen is leached, causing the contamination of ground and surface water.

Loss of nitrogen can be partly counteracted by split application of N fertilizers, with 50% introduced in a preplant dose, followed by one or two top dressings. However, in the case of cabbage grown for early harvest, the common practice is to apply a single preplant N dose because such cabbage grows for a short time in the field.

Extensive field research has been conducted on sulphur coated urea (Kołota 1982, Wiedenfeld 1986, Brown et al. 1988, Guertal 2000, Hochmuth

2003, Pack, Hutchinson 2003, You Sheng et al. 2005) and polymer coated urea (Drost et al. 2002) as nitrogen sources for vegetables. High production costs and similar or sometimes lower efficiency in comparison to conventional N fertilizers are the reason why these types of fertilizer have not found broad use in practice.

Another way to reduce N losses from soil is to add nitrification inhibitors reducing the conversion of ammonium N form to nitrates, which are most likely to leach. Research on nitrification inhibitors conducted with products such as nitrapyrin (2- chloro 6- trichloromethyl pyridine) and DCD (dicyanamide) did not translate into a widespread commercial use in vegetable crop production (Hendrickson et al. 1978, Kołota, Dobromilska 1985, Amberger 1986, Frye et al. 2002, Hochmuth 2003). Recently, a new formula of a nitrogen fertilizer containing DMPP nitrification inhibitor (3, 4 dimethylpyrazole phosphate) has been developed and made available under the commercial name ENTEC 26 (Hähndel, Zerulla, 2000, 2001, Hähndel, Strohm 2001, Pasda et al. 2001, Paschold et al. 2008, Chohura, Kołota 2011, Kołota, Chohura 2008, Li et al. 2008, Adamczewska-Sowińska, Krygier 2014, Smoleń et al. 2013). Many field studies have demonstrated its higher efficiency than soluble N sources, expressed either by an increased yield level or a lower demand for N application. Another advantage of the use of a nitrification inhibitor together with nitrogen fertilizers is the considerable limitation of the negative impact of NO_3^- on the environment, mainly by reducing its leaching from soil (Li et al. 2008).

Another source of nitrogen highly resistant to leaching is calcium cyanamide, produced by AlzChem Company under the commercial name Perlka, containing 19.8% N. Fertilizer of this type, named Azotniak, was manufactured in Poland and used as an efficient herbicide for the control of dicotyledonous weeds in vegetable and agriculture crops (Hahn 1951). Its production was discontinued in the 1960s because the powder form of the fertilizer was inconvenient for application, causing frequent cases of eye and skin irritation (Brzozowski et al. 1953). Currently, calcium cyanamide is offered in the granulated form, which is safe for people, well adopted to precise application and recommended as preplant fertilizer for a wide range of vegetable crops grown in open field.

The aim of the present study was to evaluate the efficiency of conventional fertilizer compared with one containing nitrification inhibitor and with calcium cyanamide in the cultivation of white head cabbage for early harvest in the spring.

MATERIAL AND METHODS

A field experiment with white head cabbage Reactor F_1 cultivar was conducted in 2009-2011, at the Piastów Horticultural Experimental Station (long.1700 E; lat. 51.05 N) on sandy clay soil with pH of 7.2 and organic matter content of 1.8%. Available forms of phosphorus and potassium expressed in 1 dm^3 of soil were replenished by early spring fertilization up to the standard level for cabbage. i.e. 50 mg P and 150 mg K. The fertilization was composed of triple superphosphate and potassium chloride. The mineral nitrogen content in soil was 24-30 mg N dm^{-3}, depending on the year of study.

The following nitrogen fertilization treatments were included in the study:

1) ammonium nitrate (34% N) applied in a single preplant dose – 150 kg N ha^{-1};
2) ammonium nitrate applied in a split dose (100 kg N ha^{-1} as preplant fertilization and 50 kg N ha^{-1} as top dressing);
3) ENTEC 26 (7.5% N-NO$_3$, 18.5% N-NH$_4$, 13% S+DMPP inhibitor) applied in a single preplant dose 150 kg N ha^{-1};
4) ENTEC 26 applied in a split dose (100 kg N ha^{-1}as preplant fertilization and 50 kg N ha^{-1} as top dressing);
5) Perlka (calcium cyanamide) containing 19.8% N applied in a single preplant dose – 150 kg N ha^{-1};
6) Perlka applied in a single preplant dose – 150 kg N ha^{-1}, to the plots without weed control during the whole growing period;
7) treatment with neither nitrogen application nor weed control during the whole growing period.

As recommended, Perlka was applied 2 weeks, while ENTEC 26 and ammonium nitrate were given 3 days before the transplanting of cabbage and incorporated into the soil by harrowing. In treatments with the split N application, top dressing was conducted just after the removal of covers from plants, i.e. 3-4 weeks after transplanting. The experiment was established in a one factorial design with four replications and a plot area of 8.64 m^2 (2.7x3.2 m).

Seeds of an early cabbage cultivator called Reactor F$_1$, whose growing period in open field is said to last 55-58 days from planting, were sown at the end of February into multicell trays filled with standard peat moss substrate. A single cell capacity was 80 cm^3. The transplants produced in a greenhouse were hardened in an unheated plastic tunnel for the last ten days before planting in a field. Well-developed seedlings at the stage of 5-6 leaves were transplanted in a field in the first decade of April, in a spacing pattern of 45x40 cm. Immediately after planting, the cabbage plants were covered with nonwoven crop cover of 17 g per 1 m^2 thickness, which was kept for the period of 3-4 weeks, depending on the actual weather conditions.

Weed infestation, defined as the number and weight of weeds per unit area, was assessed four weeks after planting. In the later part of the plant growing period, all the plots weeded except treatments 6 and 7, where weeds were left until harvest and evaluated at that time. The final level of weed infestation allowed us to determine the weed control effects caused by Perlka at the end of the growing period.

First harvest of cabbage heads was conducted in mid-June, and the subsequent two were carried out in one-week intervals. Each time, the heads which weighed >1.5 kg and fulfilled the requirements for marketable yield were picked up. The content of dry matter (by drying at 105°C to the constant weight PN-90/A-75101/03), vitamin C (the Tillman's method PN-90/A-7501/11) total and reducing sugars (the Loof-Shoorl method PN-90A/7501/07) and nitrates expressed by the amount of NO_3-N in fresh weighs (Orion ion selective electrode) were determined in samples of edible parts.

The results of the field experiment and chemical analyses were evaluated statistically using analysis of variance for a two-factorial design and the least significant differences were calculated by the Tukey's test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Cabbage is a species with high nutritional demand for nitrogen, which strongly affects the yield and crop quality. According to SADY et al. (1999), production of a ton of marketable cabbage yield is associated with an uptake of approximately 2.0 kg of N. The recommended dose of this nutrient, depending on the duration of a plant growing season, varies from 150 kg N ha^{-1} for cultivars harvested in early spring up to 250 N ha^{-1} for those grown for autumn harvest.

In our study, there was a marked response of early cabbage to this nutrient, which when applied at the dose of 150 N ha^{-1} raised the marketable yield by about 50% on average, irrespective of the form of fertilizer and method of its application, in comparison to the treatment neither N fertilized nor weeded (Table 1). Furthermore, the use of the above N dose caused

Table 1

Effect of different nitrogen fertilization on white head cabbage marketable yield (t ha^{-1})

Type and dose of fertilizer	Year			Mean
	2009	2010	2011	
Ammonium nitrate 150 kg N ha^{-1}	91.9	94.5	85.5	90.6
Ammonium nitrate 100+50 kg N ha^{-1}	87.9	91.7	81.8	87.2
Entec 26 150 kg N ha^{-1}	92.6	93.7	86.2	90.8
Entec 26 100+50 kg N ha^{-1}	90.2	91.6	83.9	88.6
Perlka 150 kg N ha^{-1}	96.3	97.3	89.5	94.4
Perlka 150 kg N ha^{-1} without weeding	94.9	96.3	88.3	93.2
Treatment without N fertilization and weed control	52.8	50.9	35.1	46.2
Mean	86.7	88.0	78.6	84.4

LSD$\alpha_{0.05}$ for: years – 6.2, fertilization – 5.3, interaction – 7.6

a considerable change in the nutritional value of the crop at harvest. Plants grown on plots without N application and with 24-30 mg dm^{-3} of mineral N in the soil at the time of transplanting contained substantially higher amounts of dry matter and vitamin C, while having a lower content of nitrates compared to those supplied with 150 N ha^{-1} (Tables 2, 3). Such a response is in accordance with the findings of SORENSEN (1999) in studies on cabbage, broccoli and leek. The adverse effect of nitrogen fertilization on the content of vitamin C in vegetables has been reported by others (LISIEWSKA, KMIECIK 1996, GAJEWSKI, RDZANOWSKA 2004, KOŁOTA, ADAMCZEWSKA-SOWIŃSKA 2009, CHOHURA, KOŁOTA 2011). Variable effects of nitrogen fertilization were observed in the case of total and reducing sugar content in cabbage heads. The cabbage yield in 2011 was significantly lower than in the other years due to the shortage of rainfall during the stage of head formation in June.

The split application of nitrogen, expected to minimize environmental losses by matching the time of crop nitrogen supply to crop nitrogen demand,

Table 2

Effect of different nitrogen fertilization on the content of nitrate in white head cabbage edible parts (mg NO_3^- kg^{-1} f.w.)

Type and dose of fertilizer	Year			Mean
	2009	2010	2011	
Ammonium nitrate 150 kg N ha^{-1}	600	895	568	688
Ammonium nitrate 100+50 kg N ha^{-1}	542	740	610	631
Entec 26 150 kg N ha^{-1}	475	825	820	707
Entec 26 100+50 kg N ha^{-1}	563	715	930	736
Perlka 150 kg N ha^{-1}	553	555	680	596
Perlka 150 kg N ha^{-1} without weeding	667	620	626	638
Treatment without N fertilization and weed control	450	520	428	466
Mean	550	696	666	637

LSD$\alpha_{0.05}$ for: years – 38, fertilization – 32, interaction – 51

Table 3

Effect of different nitrogen fertilization on dry matter, vitamin C and sugars content in white head cabbage edible parts (mean for years 2009-2011)

Type and dose of fertilizer	Dry matter (%)	Vitamin C (mg 100g^{-1} f.w.)	Sugars (% f.w.)	
			total	reducing
Ammonium nitrate 150 kg N ha^{-1}	6.81	33.9	3.97	3.18
Ammonium nitrate 100+50 kg N ha^{-1}	7.08	34.2	4.12	3.86
Entec 26 150 kg N ha^{-1}	7.18	31.7	4.30	3.96
Entec 26 100+50 kg N ha^{-1}	7.35	34.7	3.74	3.39
Perlka 150 kg N ha^{-1}	7.40	31.5	4.08	3.71
Perlka 150 kg N ha^{-1} without weeding	7.06	34.6	3.81	3.61
Treatment without N fertilization and weed control	7.54	44.9	4.03	3.59

LSD$\alpha_{0.05}$ n.s. 3.7 n.s. n.s.

appeared to be inefficient for cabbage yield. Both fertilizers, ammonium nitrate and Entec 26 used in one dose prior to planting, assured slightly higher marketable yields of cabbage than the treatments in which 1/3 of the total dose was applied as top dressing. Similar effects were observed by SMIT et al. (1996) in a study on Brussels sprouts, which – like white head cabbage – take up most of nitrogen early in the growth period and have roots penetrating deeply into the soil. The above authors indicated that a positive response to split N application could be expected in cultivation of vegetable crops with a shallow and rather poorly developed root system e.g. leek, that is plants which should be given small amounts of nitrogen regularly throughout the growth. A split dose of ammonium nitrate supplied as preplant and top dressing fertilization was associated with a significant decrease of nitrates in cabbage in two out of the three years of the study, while a reverse effect was found in the case Entec 26 use. Dry matter, vitamin C, and sugars in edible parts of cabbage were unaffected by the method of nitrogen application. The highest content of dry matter and vitamin C was observed in the treatment which had been neither nor weeded.

Irrespective of the weather conditions in the particular years and methods of fertilizer application, the efficiency of Entec 26, a new concept nitrogen fertilizer with an added DMPP nitrification inhibitor, was similar to that of ammonium nitrate. Similarly to another study conducted on early leek by KOŁOTA and ADAMCZEWSKA-SOWIŃSKA (2007), the size of marketable yield, level of nitrate accumulation and content of dry matter, vitamin C and sugars in cabbage did not differ significantly under the influence of these N sources. Contrary data are presented by HÄHNDEL, ZERULLA (2001), who found an overall 10% yield increment of different vegetable crops, including a 20% yield rise of lamb lettuce and small radish affected by Entec 26. The above researchers indicate that a fertilization system with DMPP containing fertilizers is less sensitive to unfavorable weather conditions and reduces the risk of surface and ground water pollution.

According to PASDA et al. (2001), positive effects of fertilizers containing a DMPP nitrification inhibitor on crop yield can be especially pronounced at sites with a high precipitation rate or intensive irrigation, and on light sandy soils. Such conditions did not occur in our study, conducted on heavy clay soil with high amounts of water, and for this reason supplemental watering of the plants was unnecessary.

The maximum yield of early cabbage marketable heads in the study was obtained from the treatments supplied with calcium cyanamide, although the difference with the yields from plotss treated with a single preplant dose of ammonium nitrate or Entec 26 were not significant. Practically, it was very important to find that an equally high cabbage yield was harvested from plots without weed control throughout the whole growing period. The results of our field trial confirmed the previous observations by HAHN (1951), indicating that calcium cyanamide was a very efficient compound for weed control. The weed infestation assessment conducted four weeks from the date of planting cabbage transplants on plots supplied with 150 kg N ha^{-1} in the form of calcium cyanamide showed that the number of weeds was reduced by 46.1-49.7% and their fresh weight was 61.8-64.8% lower in comparison to treatments with ammonium nitrate or Entec 26 (Tables 4, 5). This positive effect was even stronger at the end of the growing period, when the number of weeds declined by 64.1% to 68.4% and their aerial biomass decreased by 62.2% to 68.4% of the respective results in the non-weeded control.

The other advantage of calcium cyanamide is the substantial reduction of nitrate accumulation in edible parts of cabbage at harvest. In 3-year average values, the amount of this compound was 688 mg kg^{-1} f.w. in the treatment with 150 kg N ha^{-1} of ammonium nitrate supplied in a single preplant dose, 596 and 638 mg kg^{-1} f.w. in the case of calcium cyanamide use in weed free and non-weeded treatments, respectively. These results coincide that the report by Venter and Fritz (1976), who observed lower accumulation of

Table 4

Effect of different nitrogen fertilization on the number of weeds per 1 m^2

Type and dose of fertilizer	Year			Mean
	2009	2010	2011	
4 week after planting				
Ammonium nitrate 150 kg N ha^{-1}	341	363	324	342
Ammonium nitrate 100+50 kg N ha^{-1}	332	348	315	332
Entec 26 150 kg N ha^{-1}	351	371	345	356
Entec 26 100+50 kg N ha^{-1}	346	359	348	351
Perlka 150 kg N ha^{-1}	192	165	179	179
Mean	312	321	301	312
At harvest time				
Perlka 150 kg N ha^{-1} without weeding	164	142	180	162
Treatment without N fertilization and weed control	284	271	304	286

Table 5

Effect of different nitrogen fertilization on weight of weeds (g m^{-2})

Type and dose of fertilizer	Year			Mean
	2009	2010	2011	
4 week after planting				
Ammonium nitrate 150 kg N ha^{-1}	368	394	385	382
Ammonium nitrate 100+50 kg N ha^{-1}	348	364	355	356
Entec 26 150 kg N ha^{-1}	380	392	376	383
Entec 26 100+50 kg N ha^{-1}	340	354	349	348
Perlka 150 kg N ha^{-1}	145	135	125	135
Mean	298	309	301	303
At harvest time				
Perlka 150 kg N ha^{-1} without weeding	650	509	620	593
Treatment without N fertilization and weed control	1 719	1 620	1 842	1727

nitrates in kohlrabi fertilized with the same source of nitrogen. A relatively high content of nitrates in cabbage fertilized with Entec 26 was probably due to that fact that most nitrogen occurred in the form stabilized by DMPP ammonium and less prone to leaching than from other N sources. For this reason, in early spring season characterized by heavy precipitation, the uptake of this nutrient and the NO_3-N content in plants may be substantial higher. This was especially evident in 2011, when in April and May, i.e. immediately after planting, there were abundant rainfalls. The content of dry matter, vitamin C and sugars was unaffected by calcium cyanamide.

Calcium cyanamide was the first artificial nitrogen fertilizer manufactured industrially, but because of some difficulties with its use in the powder form, its production in Poland was stopped in 1960s. Now, its granular formulation produced mostly by AlzChem, Bavaria, (Germany) gains more interest as a slow-release fertilizer, quite an efficient herbicide for weed control and a beneficial agent improving soil chemical properties by enhancing soil pH level. Its preplant application may eliminate the need of herbicide use for weed control in cabbage as well as the use of fungicides against clubroot (*Plasmodiophora brassicae*), the most dangerous disease of this species. A high success rate of calcium cyanamide in control of clubroot, on a comparable level to fluazinam (Altima 500 SC), was proven by Klasse (1996) and Robak and Gidelska (2009). The application of this fertilizer is associated with a low risk of nitrate leaching and stimulation of the natural diversity and population size of soil microbes (Dixon 1984)

Taking into account the results of our studies and many data from the literature, it can be concluded that calcium cyanamide is a beneficial source of nitrogen for plants, friendly to the environment owing to reduced losses of this nutrient from soil, and a successful weed and soil born disease control substance in cabbage cultivation.

CONCLUSIONS

1. White head cabbage grown for early harvest in the spring showed high response to nitrogen fertilization, which – when applied at the dose of 150 kg N ha^{-1} – assured a high level of marketable yield, irrespectively of the form and method of its application.

2. Split application of ammonium nitrate or Entec 26 appeared to did not improve the yield volume or the nutritional value of cabbage above the level achieved by a single N dose supplied prior to planting.

3. On heavy clay soil with an adequate moisture level throughout the growing season, Entec 26, a nitrogen fertilizer containing a DMPP nitrification inhibitor, was as valuable source of nitrogen as commonly used ammonium nitrate.

4. Calcium cyanamide produced the highest marketable yield of cabbage with a significantly lower content of nitrates. The best advantage of this N source is its high efficiency in reducing weed infestation, which eliminates the need to use chemical or mechanical methods of weed control.

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THE ROLE OF MAGNESIUM IN THE PROTECTION OF ENTOMOPATHOGENIC NEMATODES FROM SOIL POLLUTION WITH OIL DERIVATIVES*

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Abstract

The aim of the paper was to identify the influence of magnesium on the preservation of pathogenic abilities of entomopathogenic nematodes living in soil contaminated with oil derivatives.

Entomopathogenic nematodes living under natural conditions in soil or applied to soil as biopreparations for plant pest control are sensitive to soil contamination with heavy metals and oil derivatives. These nematodes do not respond directly by higher mortality but by a decrease in their pathogenic abilities, which adversely affects the success in searching and eliminating pests.

A Polish commercial preparation called Owinema SC, containing infective juveniles of *Steinernema feltiae* Filipjev (*Rhabditida: Steinernematidae*), was used to test the effect of oil derivatives on pathogenicity and reproduction of entomopathogenic nematodes.

Soil was polluted with petrol, diesel oil and used engine oil; the control was soil unpolluted with oil derivatives. Magnesium sulphate was also added ($MgSO_4 \cdot H_2O$) in the amount of 160 mg per 1 liter of the suspension. The three oil derivatives in concentrations of: 0.3, 0.6 and 1.0 g or in double doses, i.e. 0.6, 1.2 and 2.0 g were poured over weighted soil batches. The control remained intact. Three replications of each treatment were made. Subsequently, *Tenebrio molitor* L. larvae used as bait inesct were placed in containers with the suspension.

The bait insects were kept in the containers for 7 days, and afterwards they were removed from the soil and taken from the traps in order to check their mortality rate.

Furthermore, to test the reproduction ability of entomopathogenic nematodes, dead larvae were moved to “islands” previously prepared according to the Dutky method.

The results were presented as the number of entomopathogenic nematodes per 1 dead larvae of *Tenebrio molitor* L.

Key words: magnesium, entomopathogenic nematodes, contamination with oil derivatives.

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ROLA MAGNEZU W OCHRONIE OWADOBÓJCZYCH NICIENI W PRZYPADKU ZANIECZYSZCZENIA GLEBY SUBSTANCJAMI ROPOPOCHODNYMI

Abstrakt

Celem pracy było rozeznanie wpływu magnezu na ochronę zdolności patogenicznych owadobójczych nicieni przebywających w glebie skażonej substancjami ropopochodnymi.

Owadobójcze nicienie żyjące w warunkach naturalnych w glebie, a także stosowane doglebowo jako biopreparaty do zwalczania szkodników roślin, są wrażliwe na skażenie gleby metalami ciężkimi oraz substancjami ropopochodnymi. Nicienie te nie reagują wówczas śmiertelnością, ale zmniejszeniem zdolności patogenicznych, co wpływa na ich efektywność w wyszukiwaniu i zabijaniu szkodników roślin.

W doświadczeniu laboratoryjnym wykazano, że siarczan magnezu dodany do gleby skażonej substancjami ropopochodnymi zwiększa zdolności patogeniczne nicieni, ale nie reprodukcje wewnątrz owadów żywicielskich. Owinema S.C. – polski preparat komercyjny zawierający larwy inwazyjne *Steinernema feltiae* (*Rhabditis*, *Steinernematidae*) – używano w testach laboratoryjnych nad oddziaływaniem zanieczyszczenia ropopochodnymi na patogeniczność i reprodukcje owadobójczych nicieni.

Glebę zanieczyszczono benzyną, olejem i olejem silnikowym. Dodano siarczan magnezu ($MgSO_4$ H_2O) w dawce 160 mg l^{-1} zawiesiny. Ropopochodne użyto w dawkach 0,3 g, 0,6 g i 1,0 g lub podwójnej, tj. 0,6 g, 1,2 g i 2,0 g. Gleba nie zanieczyszczona stanowiła próbę kontrolną. W pojemniku z glebą umieszczono owady testowe – larwy mączlika młynarka *Tenebrio molitor* L. Owady po 7 dniach wyjmowano i sprawdzono ich śmiertelność związaną z patogenicznością nicieni i zanieczyszczeniem ropopochodnymi.

Zdolności reprodukcji nicieni badano metodą Dutkego na „wyspach" szkiełek w szalkach. Wyniki przedstawiono jako liczebność larw nicieni z 1 martwej larwy owada *T. monitor*.

Słowa kluczowe: magnez, nicienie owadobójcze, zanieczyszczenie ropopochodnymi.

INTRODUCTION

Under natural conditions, entomopathogenic nematodes of the families *Steinernema* and *Heterorhabditis* live in soil and kill insects by introducing into their bodies *Xenorhabdus* sp. *Photorhabdus* sp. bacteria, which act as pathogens. We have learned to culture these nematodes on a big scale and use them as biopreparations against various plant pests (Bedding 1984, Ehlers 2001). We make use of them for the biological control of harmful insects all over the world (Grewal 2005). Plant protection with these organisms is effective and safe to the environment (Webster 1980). At present, integrated agriculture should combine biological methods, such as biopreparations and protection of natural enemies of pests living in the environment, with other non-chemical and chemical methods. Unfortunately, entomopathogenic nematodes are sensitive to soil pollution with heavy metals (Jarmuł, Kamionek 2000) and oil derivatives (Gospodarek, Jaworska 2009, Jaworska, Gospodarek 2009). Although nematodes do not respond directly by elevated mortality, their ability to search and parasitize insects suffers. It was found that magnesium interaction with heavy metal ions positively affects the entomopathogenic activity of nematodes (Jaworska et al. 1999). The aim of the paper was to identify the influence of magnesium on the pathogenic abilities of entomopathogenic nematodes in soil contaminated with oil derivative substances.

MATERIAL AND METHODS

A representative soil sample collected for laboratory experiments was analyzed at the Chemical-Agricultural Station in Krakow. The soil parameters were determined as follows: soil acidity pH in KCl 6.7, i.e. neutral reaction, light soil, liming unnecessary, total nitrogen content 0.161%, and humus content 2.48%. The content of microelements was also assessed. The sample revealed a low concentration of potassium (9.3 mg 100 g^{-1} of soil) and a high content of magnesium and potassium (respectively 5.2 and 17.5 mg 100 g^{-1} of soil).

A Polish commercial preparation called Owinema SC, containing infective juveniles of *Steinernema feltiae* Filipjev (*Rhabditida: Steinernematidae*), was used to test the effect of oil derivatives on the pathogenicity and reproduction of entomopathogenic nematodes. The preparation is manufactured by Owiplant Ltd. Horticultural Enterprise in Poznań. Containers lined with filter paper discs and containing nematodes were prepared, to which the above soil was added and polluted with petrol, diesel oil and used engine oil; the control was soil unpolluted with oil derivatives.

The first test series made use of commercial Owinema. Half a packet (100 g) was divided into two parts, which were then added to 300 ml of distilled water (150 ml each). A 3-ml dose of the suspension was used, corresponding to *ca* 250 000 specimens of infective juveniles per container. In the second series, magnesium sulphate ($MgSO_4 \cdot H_2O$) was added to the other part, in the amount of 16 mg per 1 l of the suspension. Three oil derivatives (mentioned above) in concentrations of: 0.3 g, 0.6 g and 1.0 g were poured over the soil weighted portion. The control remained unchanged. Three replications were made.

Owinema was used again in the second experiment. The remaining half of the packet (100 g) was divided into two parts and each part was thoroughly stirred in 150 ml of distilled water. Neither the number of nematodes nor the amount of added magnesium sulphate changed. The same oil derivatives were applied but in double doses, i.e. 0.6 g, 1.2 g and 2.0 g. The control objects remained unchanged. Three replications were made.

Subsequently, bait insects such as *Tenebrio molitor* L. larvae, purchased in a zoological shop, were placed in the containers with the suspension. In order to prevent their escape from a container, the larvae were first put in “traps” specially prepared from cut drinking straws. Additionally, each straw was specially perforated in order to make easy the host body infestation by nematodes. When insect larvae were put in a trap, its ends were sealed with plasticine. Five traps with larvae were placed in each container. The next stage involved covering the larvae with a 100 g soil weighted portion and then the oil derivatives in three different concentrations were poured over. The average temperature in the laboratory during the experiment was ±31°C.

Bait insects were kept in the containers for 7 days, after which they were removed from the soil and taken from the traps in order to check their mortality. Live specimens were put separately on Petri dishes lined with filter paper and then kept under laboratory conditions. Mortality of *Tenebrio molitor* L. was checked over 7 consecutive days. Mortality was expressed as the percentage of dead specimens in relation to all larvae per replication. *Tenebrio molitor* L. larvae were selected for the tests because they are readily available and easy to culture. The pest is a polyphagous and may be encountered on all plant products. It is a comopolitan species, which prefers closed spaces and does not occur in high concentrations. Another important characteristic of this species is their sensitivity to the infestation with entomopathogenic nematodes, which is why they are often used as traps.

Furthermore, in order to test the reproduction rate of entomopathogenic nematodes, dead larvae were removed to “islands” previously prepared according to the Dutky method, i.e. dead larvae were placed on a watch glass covered with a filter paper disc, and a small amount of distilled water was added to prevent the drying up of larvae.

The suspension with multiplied nematodes was poured from a Petri dish into 100 ml plastic cups, which were next put into a refrigerator and kept at 4°C. This step was repeated several times over two weeks. At the subsequent stage, nematodes were counted under a microscope. The results were presented as the number of entomopathogenic nematodes per 1 dead larvae of *Tenebrio molitor* L.

The significance of differences between the results was tested by means of one-way Anova. The means were differentiated using the Duncan’s test at the significance level $p = 0.05$.

RESULTS

The mean mortality of *Tenebrio molitor* L. larvae (%) is presented in Figure 1.

Significant differences between objects were demonstrated by analyses. The lowest mortality, about 10%, was recorded in the objects where petrol and engine oil were applied at the lowest concentration of 0.3 g. In the control, the mortality reached almost 70% and did not differ significantly from the sample where a dose of 1 g of diesel oil was used. The other objects did not differ markedly between each other, with the mortality fluctuating at around 30%. In the second part of the experiment, when magnesium supplement was applied, the average mortality was higher. The lowest value was

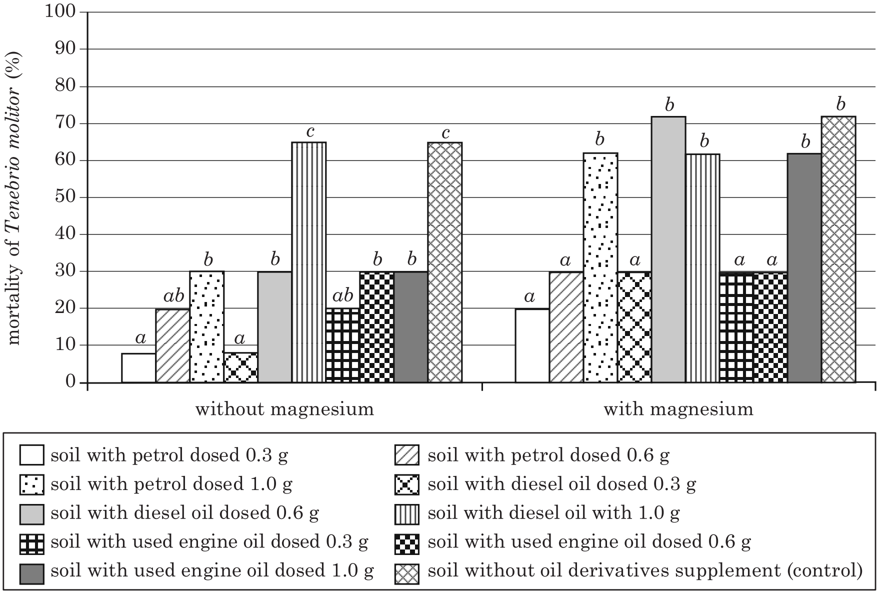

Fig.1. Mean accumulated mortality of *Tenebrio molitor* L. test larvae (%)

noted in the objects where oil derivatives at the lowest concentration (0.3 g) were applied, even though the mortality rate in these treatments increased to over 30%, in which they differed markedly from the other objects. In the control soil with magnesium ions, the mortality of the insects grew to about 80%. Figure 2 shows the results of nematode reproduction in the bodies of dead test larvae.

In the present experiment, the highest reproduction of nematodes in the insect larvae was observed in the control sample, where it reached about 5600 nematodes per 1 insect larva. The lowest value was noted in the object where the highest dose of used engine oil was applied, which differed significantly from the objects where petrol was added in the concentrations of 0.3 and 0.6 g and from the control. The analyses did not show any differentiation in comparison with the other objects. Similarly, after the application of magnesium supplement, the highest number of produced nematodes was observed in the control, where there were *circa* 6040 nematode larvae per insect. The reproduction rate of nematodes observed in the other objects was not significantly higher, despite the applied magnesium.

Figure 3 below shows the mean accumulated mortality in the second experiment in which the commercial preparation Owinema, containing *S. feliate* nematode strain, was used together with a double dose of oil derivatives

In this experiment, considerably increased mortality was observed in comparison with the previous series. A disadvantageous effect of oil derivatives on the test organisms was noticeable. In the variant where no magnesium was added, the lowest mortality was observed in the object with petrol added in the dose of 0.6 g, whereas the highest one was in the treatment

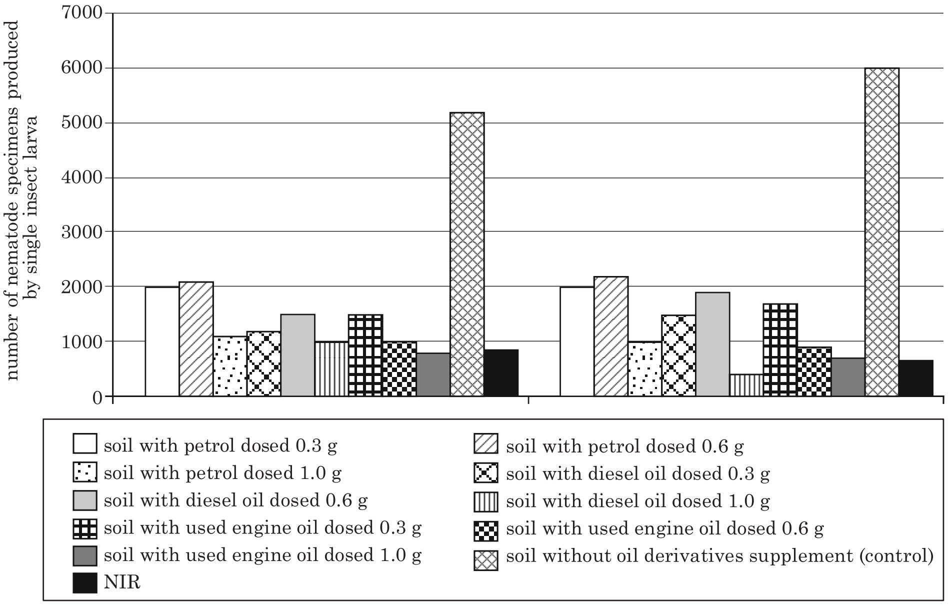

Fig.2. Reproduction of *Steinernema feltiae* (Filipjev) nematodes in the organisms of dead test insects (*T. molitor* L. larvae) kept in the analyzed objects

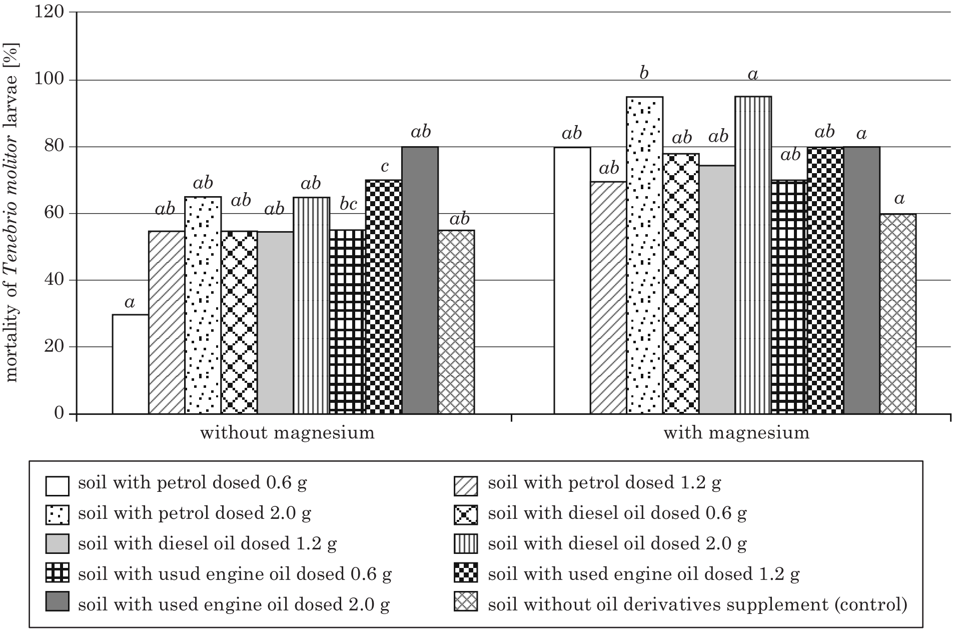

Fig. 3. Mean accumulated mortality of *Tenebrio molitor* L. test larvae (%) under a double level of pollution with oil derivatives

with used engine oil dosed at 2.0 g. In the second part of this experiment, the supplied magnesium additionally raised the mortality of *Tenebrio molitor* L., causing an increase in all average mortality values in all the combinations. The lowest mortality rate was in the control.

Figure 4 presents the results on the nematode reproduction in bodies of dead insect larvae kept in the analyzed objects, in the second series of the experiment, in which *S. feltiae* nematodes from the commercial Owinema preparation were applied. Additionally, the doses of oil derivatives were doubled.

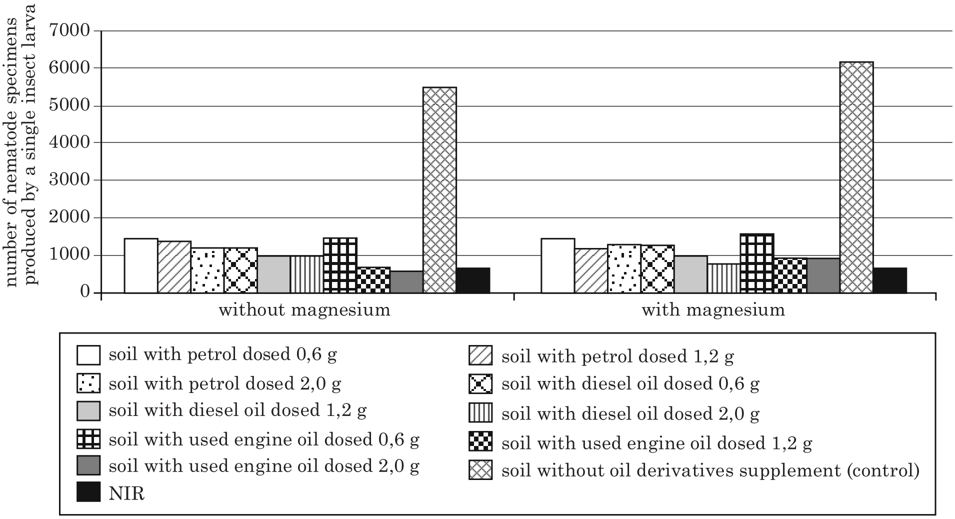

Fig. 4. Reproduction of *Steinernema feltiae* (Filipjev) in the organisms of dead test insects (*T. molitor* L. larvae) kept in the analyzed objects

The only object which significantly differed from the others was the control without any oil derivative (Figure 4). The number of infective juveniles of *S. feltiae* (Filipjev) nematodes isolated from dead *T. molitor* L. larvae bodies at that time in this object without magnesium reached on average 5503 infective juveniles per one dead test insect and 6066 larvae in the sample with magnesium. In the objects polluted with oil derivatives, the highest reproduction (about 2000 infective juveniles) was observed in both cases in the sample with petrol added in doses of 0.3 and 0.6 g. Thus, magnesium did not increase nematode reproduction under oil derivative pollution.

DISCUSSION

Entomopathogenic nematodes, as well as mutualistic bacteria with which they co-exist have been an interesting subject of research for many years. The interest is stimulated by the on-going development of integrated plant protection programmes. Biological methods which take advantage of the phenomenon of parasitism and predation among animal species in the environment play a crucial role in these programmes. Because they are safe in use, biological methods are recommended mainly in organic farming and in integrated production systems (Tomalak 2005).Owing to their ability to seek a host actively, nematodes have gained a considerable advantage over chemical preparations. Such active search for a host organism is the most important characteristic of these organisms, although it looks differently in various strains and species. Factors that affect nematodes may be divided into biotic and abiotic ones. The temperature is an abiotic factor. For the development of nematodes the temperature within the 13-20°C range seems optimal (Ropek 2005). The temperatures during the present experiments were much higher, which might have affected the results. Another possible abiotic factor is the pollution which people creeate in the environment, by consciously or unconsciously. Environmental pollution is one of the crucial issues currently addressed by many scientists. In their investigations, Pezowicz (2002) and Dzięgielewska (2008) demonstrated that environmental pollution poses a serious barrier to the occurrence and spread of nematodes, although these animals may appear even in polluted soils or in urban soils, where they can also be used for biological plant protection. The condition for their durability in the environment is the presence and availability of host insects (Jarmuł, Kamionek 2003). However, the effectiveness of nematode use in programmes of integrated control may be affected by many factors, including soil pollution with oil derivatives. In the research by Jaworska and Gospodarek (2009), soil contamination with oil derivatives caused a significant decline of both pathogenicity and reproductive abilities of entomopathogenic nematodes. Similar results were presented in the paper by Gospodarek and Jaworska (2009). They were also manifested in the present investigations. A negative effect of oil derivatives on the pathogenicity and reproduction of nematodes was observed in the presented research. Pathogenicity of nematodes was defined through the deetrmination of the mortality rate of the test insect larvae, i.e. *Tenebrio molitor* L. Their mortality observed in the experiments might have been affected by the high temperature in the laboratory, which did not favour either the insect larvae or the reproduction of nematodes. Still, the nematodes used for the experiment and the oil derivatives had the strongest influence.

The effect of heavy metals on nematodes depends among others on ionic concentrations and interaction with other ions, but the main effect is produced by the type of ion. Among the ions investigated so far, Cd and Pb ions proved most toxic. In such a case, the mortality of infective juveniles was affected both by the time of contact of nematodes with pollutants (*S.carpocapsae*) and their concentration. The situation looks different for magnesium ions. Instead of being toxic to nematodes, magnesium ison can act synergically, by protecting them against the unfavourable impact of other heavy metals (Jaworska et al. 1999). This dependence was observed also in the present experiment. A supplement of magnesium sulphate positively influenced nematodes' pathogenicity, even under heavy pollution with oil derivatives, although the reproduction rate was not observedd to be higher.

Crude oil and its derivatives negatively affect the environment in various ways (Surygała, Śliwka 1999). Different techniques and technologies of removing contamination with oil derivatives from ground and groundwaters are being developed. Here, at the Department of Agricultural Environment Protection, we have been completed several projects focused on finding out how these pollutants could be removed from the ground by microbiological methods, using entomopathogenic nematodes for checking the efficiency of biocleaning.

CONCLUSION

Magnesium added do the soil contaminated with oil-derivatives increases pathogenic abilities of entomopathogenic nematodes.

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EFFECT OF FOLIAR FERTILIZATION WITH TYTANIT ON THE CONTENT OF SELECTED MACROELEMENTS AND SODIUM IN CELERY

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Abstract

Foliar application of growth regulators or fertilizers containing biostimulators can influence the uptake and accumulation of mineral elements by plants. A pot experiment carried out in two annual series (year of experiment I and II) examined the effect of various concentrations of Tytanit applied once or twice against mineral fertilization (NPK), in comparison with the control object (without fertilization) and the object with mineral fertilization (NPK), on the total content of potassium, calcium, magnesium and sodium in petioles and leaf blades of celery. The experiment involved the application of various concentrations of Tytanit, ranging from 0.001% to 3.6%. The content of selected elements in the plant material was determined after dry mineralization, using the ICP – AES method. The total content of potassium in petioles of celery fertilized with NPK + 3.6% was higher or the same as in the control and in plants fertilized with NPK (I and II series). The results were similar in the case of leaf blades (except for series I). The highest concentration of Tytanit (3.6%) resulted in a reduction of the total calcium content in both of the tested parts of celery and magnesium in petioles, compared with the control. On the other hand, the total content of sodium increased along with the growth of the fertilizer concentration. The highest bioaccumulation of calcium was observed in celery cultivated in the object without fertilization (control). A significantly higher potassium content (mean from both series of research) and over two-fold higher content of calcium were found in celery leaf blades than in petioles, while a higher content of magnesium and sodium appeared in petioles than in leaf blades. The values of (K+Na) : (Ca+Mg) ratios in leaf blades were found to be over two-fold higher than in petioles of celery fertilized with Tytanit.

Key words: petioles and leaf blades of celery, Tytanit concentration, potassium, calcium, magnesium, sodium.

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WPŁYW DOLISTNEGO NAWOŻENIA NAWOZEM TYTANIT NA ZAWARTOŚĆ WYBRANYCH MAKROELEMENTÓW I SODU W SELERZE NACIOWYM

Abstrakt

Stosowanie tytanu w formie oprysku nawozem Tytanit jest jednym z zabiegów agrotechnicznych, których celem jest stymulowanie kwitnienia, zawiązywania owoców i produktywności roślin. W doświadczeniu wazonowym, w dwóch jednorocznych seriach (I i II rok doświadczenia), badano wpływ różnych stężeń Tytanitu stosowanego jedno- i dwukrotnie na tle nawożenia mineralnego (NPK), w porównaniu z obiektem kontrolnym (bez nawożenia) i nawożonym mineralnie (NPK), na ogólną zawartość potasu, wapnia, magnezu i sodu w ogonkach i blaszkach liściowych selera naciowego. W doświadczeniu zastosowano zróżnicowane stężenia Tytanitu – od 0,001 do 3,6%. Zawartość wybranych pierwiastków oznaczono metodą ICP – AES, po mineralizacji materiału roślinnego ,,na sucho". Zawartość potasu w ogonkach liściowych selera nawożonego Tytanitem o stężeniu 3,6% + NPK była większa niż w obiekcie kontrolnym lub nawożonym tylko NPK, lub utrzymywała się na tym samym poziomie (I i II seria). Podobnie było w przypadku blaszek liściowych (z wyjątkiem I serii). Największe stężenie Tytanitu (3,6%) wpłynęło na zmniejszenie zawartości ogólnej wapnia w badanych częściach selera naciowego oraz magnezu w ogonkach liściowych, w porównaniu z obiektem kontrolnym. Zawartość ogólna sodu zwiększała się wraz ze stężeniem nawozu. W blaszkach liściowych selera stwierdzono znacznie większą zawartość potasu (średnio z obydwu serii badań) oraz ponad dwukrotnie większą wapnia niż w ogonkach liściowych. Zawartość magnezu i sodu była większa w ogonkach niż w blaszkach liściowych. Stwierdzono ponad dwukrotnie węższe wartości stosunków (K+Na) : (Ca+Mg) w blaszkach niż w ogonkach liściowych selera naciowego na obiektach nawożonych Tytanitem.

Słowa kluczowe: ogonki i blaszki liściowe selera naciowego, stężenie Tytanitu, potas, wapń, magnez, sód.

INTRODUCTION

Celery (*Apium graveolens* L. *var. dulce* Mill. Pers.) is a valuable vegetable plant owing to its nutritional, flavour and medicinal properties (Dyduch 1987). Cultivated forms developed from wild celery *Apium graveolens var graveolens* grow also in the wild, mainly along the seacoasts of some of the continents. It is a plant of moderately cool climates, yielding well under the climatic conditions of Poland, provided the right choice of cultivars and soil. A high volume and quality of celery yield can be obtained in soils abundant with organic substances and nutrients. To date, the celery production output in Poland has been very low. However, with the current promotion of its nutritive values, constant supply (mainly imported celery) and new cultivation technologies, a rapid increase in the domestic production of this valuable vegetable can be expected.

For some years now, there has been an increased interest in preparations from the group of bioregulators, which have significantly improved the production of plants, particularly vegetables. Foliar application of growth regulators or fertilizers containing biostimulants can affect the uptake and accumulation of mineral components by plants (Bąbelewski, Dębicz 2006, Smoleń, Sady 2009, Smoleń et al. 2010). An active substance of the Tytanit fertilizer is 0.8% titanium (8.5 mg dm^{-3}). This preparation, as indicated in various studies on floriculture, fruit-growing, vegetable-growing, seed production and agriculture, affects growth, quality and development of plants and, consequently, their yield and the quality of sowing material (Hetman, Adamiak 2003, Serrano et al. 2004, Adamiak, Hetman 2007, Marcinek, Hetman 2007, Skupień, Oszmiański 2007, Radkowski, Radkowska 2010).

The aim of the experiment was to examine the effect of the foliar application of Tytanit against mineral fertilization (NPK) on the total content of selected macroelements (potassium, calcium, magnesium) and sodium in petioles and leaf blades of celery and on the weight and molar proportions of those elements in the test plant.

MATERIAL AND METHODS

The experiment was carried out in 2001-2002, in two annual series (year I and II of the experiment). The tested plant was celery of a Dutch cultivar, Tango F_1. Seedlings with 3-4 proper leaves were planted at four plants per pot (of 10 dm^3 capacity) in the first decade of June, in the first and second series of the research. Pots were filled with gardening substrate, containing raised peat with loamy sand (according to PTG), mixed in a 3:1 ratio, and limed with calcium carbonate according to Hh = 1 a month before planting transplants, so that $pH_{H_2O} = 6.60$ was obtained. The total content of selected macroelements in the substrate was (g kg^{-1}): N – 1.89; P – 0.507; K – 0.653; Ca – 11.55; Mg – 1.08; Na – 0.086, and the content of Ti was 32.89 mg kg^{-1}. The content of nitrogen was determined on a CHN auto-analyzer produced by Perkin –Elmer and the content of the other elements was determined using the ICP-AES method after dry mineralization. This greenhouse experiment was set up in a completely random system with three replications. The soil moisture in the pots during the plant growing period was maintained at 60% of the substrate field water capacity. The experiment involved 16 fertilization objects (48 pots), including single and double spraying with Tytanit and its varied concentrations, according to the following design: 1) control object (without fertilization); 2) NPK mineral fertilization in a 1:0.8:1.5 ratio (150 kg N ha^{-1}- ammonium nitrate, P – triple superphosphate, K - potassium sulphate); foliar fertilization with Tytanit against NPK: 3) 0.001%; 4) 0.01%; 5) 0.1%; 6) 1%; 7) 1.2%; 8) 2.4%; 9) 3.6%, which corresponded to 0.043 - 150 mg Ti per pot^{-1} dissolved in 500 cm^3 of water. NPK fertilization was applied one week before planting the seedlings; the first spraying with Tytanit was applied after plant rooting, i.e. in the first decade of July, and the second one was in the first decade of August, in both the first and second series of research. Finally, the celery was harvested at the beginning of October, in both research series.

In the first and the second series, the total content of K, Ca, Mg and Na was determined in the dry matter of petioles and leaf blades of celery. The dry plant material was ground to a particle size of < 0.25 mm, after which a 1g batch was weighed out and transferred to a semi-vitreous chinaware pot, in which the organic substance was oxidized dry in a muffle furnace at 450°C. A dose of 10 ml of diluted HCl (1:1) was poured over crude ash in the pot and the ash was evaporated dry on a sand bath to decompose the carbonates and to separate silica. The content of the pot, after adding 5 ml of 10% HCl, was passed through a solid filter into a 100 ml measuring flask and replenished with distilled water up to the mark. The total content of K, Ca, Mg and Na in the solution was determined on an emission spectrometer with inductively coupled plasma, ICP – AES, produced by Perkin-Elmer. The uptake of the selected macroelements with the yield was calculated on the basis of the dry matter yield from petiole and leaf blades in the first and the second series of research (Malinowska, Kalembasa 2012).

The results were statistically analysed with variation analysis for a two -factor experiment (using FR Analvar 3.2 software for calculations). The significance of differences between means was assessed with the Tukey's test, assuming the significance level of $\alpha = 0.05$ (the control object and the NPK object were not included in the statistical analysis). The values of direct correlation coefficients between the content of K, Ca, Mg and Na and the content of Ti (Malinowska, Kalembasa 2012) in petioles and leaf blades of celery were calculated at $p \leq 0.05$ (Statistica, Version 9.1 StatSoft).

RESULTS AND DISCUSSION

In a two-year experiment on the application of Tytanit in the cultivation of celery, the bioaccumulation of potassium was found to be higher in celery leaf blades than in petioles after a single application of Tytanit, in both research series (Table 1). As a result of the double spraying with Tytanit, the content of the elements in petioles, as compared to leaf blades, increased only in the first series of research. A higher mean content of potassium was found in the examined parts of celery in the second experimental series than in the first one. The Tytanit concentration significantly differentiated the bioaccumulation of potassium in petioles and leaf blades and the number of treatments in the petioles. The application of Tytanit against NPK, in the first and second series of experiment, caused a significant increase in the potassium content in petioles and its reduction in leaf blades, particularly in the first series, as compared to the object fertilized with NPK. The highest concentration of Tytanit (3.6%) resulted in the reduced bioaccumulation of potassium in the examined parts of the test plant, as compared to the concentration 2.4%. Radkowski, Radkowska (2010) found a reduction in the potassium content in meadow sward under the influence of higher concentrations of Tytanit.

Table 1

The total content of potassium in petioles and leaf blades of celery leaves (g kg^{-1} d.m.), in series I and II, in pot experiment

Fertilization object	I series		II series		Mean for petioles	Mean for leaf blades
	petioles	leaf blades	petioles	leaf blades		
Control object	19.06	29.51	11.60	19.20	15.33	19.36
NPK	17.02	28.41	21.84	29.97	19.43	29.19
I spraying of Tytanit						
0.001%+ NPK	17.71	26.09	25.75	31.74	21.73	28.92
0.01% + NPK	18.19	21.99	24.80	29.70	21.50	25.85
0.1% + NPK	20.36	18.25	23.34	30.05	21.85	24.15
1% + NPK	27.19	22.91	25.74	30.66	26.47	26.79
1.2% + NPK	20.13	20.71	24.83	31.70	22.48	26.21
2.4% + NPK	24.23	23.64	23.49	29.64	23.86	26.64
3.6% + NPK	21.62	21.81	22.75	27.14	22.19	24.48
Mean	21.35	22.20	24.39	30.09	22.87	26.15
II spraying of Tytanit						
0.001% + NPK	16.36	19.03	31.40	33.57	23.88	26.30
0.01% + NPK	16.82	22.26	28.28	35.18	22.55	28.72
0.1% + NPK	22.60	22.30	27.78	30.27	25.19	26.29
1% + NPK	29.52	24.73	30.73	27.03	30.13	25.88
1.2% + NPK	31.56	24.08	23.50	29.98	27.53	27.03
2.4% + NPK	29.03	24.81	23.53	27.67	26.28	26.24
3.6% + NPK	21.34	21.18	20.96	26.84	21.15	24.01
Mean	23.89	22.63	26.60	30.08	25.24	26.35
LSD$_{0,05}$ for:						
A – number of treatments	0.24	n.s.	1.01	n.s.	1.56	n.s.
B – concentration of Tytanit	0.69	1.43	2.93	1.09	4.56	1.43
AxB – interaction	0.63	1.30	2.67	1.00	n.s.	0.43
BxA – interaction	0.97	2.02	4.14	1.54	n.s.	0.67

n.s. – non-significant difference

The content of calcium in celery was the highest in the control object (Table 2). In the first series of the experiment, it reached 22.81 g Ca kg^{-1} in petioles, twice as much in leaf blades: 44.11 g Ca kg^{-1}, and in the second series: 22.97 and 56.78 g kg^{-1}, respectively. NPK fertilization resulted in decreasing the content of calcium in petioles by almost 30% and in leaf blades by 20% in comparison to the control object. The foliar application of Tytanit against NPK also caused a reduction of this element in comparison to the control object (without fertilizing). Single spraying resulted in obtaining an average 17.58 g Ca kg^{-1} in petioles, 46.47 g Ca kg^{-1} in leaf blades, while a

Table 2

The total content of calcium in petioles and leaf blades of celery leaves (g kg^{-1} d.m.), in series I and II, in pot experiment

Fertilization object	I series		II series		Mean for petioles	Mean for leaf blades
	petioles	leaf blades	petioles	leaf blades		
Control object	22.81	44.11	22.97	56.78	22.89	50.45
NPK	16.63	37.35	15.72	43.10	16.18	40.23
I spraying of Tytanit						
0.001%+ NPK	18.41	36.75	17.53	48.30	17.97	42.53
0.01% + NPK	16.76	39.81	18.43	48.79	17.60	44.30
0.1% + NPK	14.99	34.57	18.21	57.65	16.60	46.11
1% + NPK	20.03	49.97	20.09	54.07	20.06	52.02
1.2% + NPK	15.64	38.27	19.52	56.03	17.58	47.15
2.4% + NPK	18.36	51.10	19.11	48.55	18.74	49.83
3.6% + NPK	13.26	37.23	15.76	49.44	14.51	43.34
Mean	16.78	41.10	18.38	51.83	17.58	46.47
II spraying of Tytanit						
0.001% + NPK	14.70	34.71	16.41	45.95	15.56	40.33
0.01% + NPK	18.20	44.25	20.25	65.20	19.23	54.73
0.1% + NPK	17.19	35.39	23.63	69.03	20.41	52.21
1% + NPK	20.33	49.78	21.70	56.57	21.02	53.18
1.2% + NPK	17.20	35.40	19.27	47.96	18.24	41.68
2.4% + NPK	15.13	42.06	21.62	55.36	18.38	48.71
3.6% + NPK	13.56	36.94	14.87	42.75	14.22	39.85
Mean	16.62	39.79	19.68	54.69	18.15	47.24
LSD $_{0,05}$ for:						
A – number of treatments	n.s.	0.50	0.71	0.74	0.97	n.s.
B – concentration of Tytanit	0.54	1.45	2.07	2.15	1.57	3.62
AxB – interaction	0.49	1.33	1.89	1.97	1.52	4.13
BxA – interaction	0.76	2.06	2.93	3.05	1.81	6.95

n.s. – non-significant difference

double treatment resulted in 18.15 and 47.24 g Ca kg^{-1}, respectively. A higher mean content of calcium was found in the second series as compared to the first one. In the present experiment, the content of calcium in the test plant biomass was significantly differentiated by the Tytanit concentration and number of treatments, except for petioles in the first series of the study.

The content of magnesium in the examined parts of celery was higher, on average, in the second series than in the first one (Table 3). It was significantly differentiated under the influence of the examined factors. In both series of the experiment, the content of magnesium in petioles from the control object was higher than in petioles from the fertilization objects. In leaf blades, the content of magnesium in the first research series was over twofold higher in fertilization objects than in the control or in the object with NPK, while in the second series, it was slightly higher in the control and NPK objects than in fertilization objects. The application of Tytanit at a concentration of 1% and higher resulted in an increase in the content of magnesium in leaf blades only in the first series of the experiment.

The content of sodium in the examined parts of celery was significantly differentiated by the number of treatments and by the Tytanit fertilization

Table 3

The total content of magnesium in petioles and leaf blades of celery leaves (g kg^{-1} d.m.), in series I and II, in pot experiment

Fertilization object	I series		II series		Mean for petioles	Mean for leaf blades
	petioles	leaf blades	petioles	leaf blades		
Control object	3.30	1.18	5.23	3.55	4.27	2.37
NPK	2.84	1.19	3.96	3.47	3.40	2.33
I spraying of Tytanit						
0.001%+ NPK	2.98	1.81	4.22	3.08	3.60	2.45
0.01% + NPK	3.11	2.41	4.55	3.36	3.83	2.89
0.1% + NPK	2.78	2.67	4.80	3.35	3.79	3.01
1% + NPK	2.99	3.87	4.26	3.24	3.63	3.56
1.2% + NPK	3.17	3.32	3.98	3.06	3.58	3.19
2.4% + NPK	2.86	3.92	3.75	3.08	3.31	3.50
3.6% + NPK	2.43	3.16	3.31	2.79	2.87	2.98
Mean	2.90	3.02	4.13	3.14	3.52	3.08
II spraying of Tytanit						
0.001% + NPK	2.54	2.03	4.28	3.61	3.41	2.82
0.01% + NPK	3.06	2.47	4.42	3.58	3.74	3.03
0.1% + NPK	2.88	2.49	4.71	3.41	3.80	2.95
1% + NPK	2.72	3.87	4.25	3.18	3.49	3.53
1.2% + NPK	2.85	3.82	3.75	3.09	3.30	3.46
2.4% + NPK	2.33	4.11	3.73	3.00	3.03	3.56
3.6% + NPK	2.34	3.42	3.11	2.70	2.73	3.06
Mean	2.68	3.17	4.04	3.23	3.36	3.20
LSD $_{0,05}$ for:						
A – number of treatments	0.03	0.04	0.08	0.04	n.s.	n.s.
B – concentration of Tytanit	0.08	0.11	0.23	0.12	0.48	0.41
AxB – interaction	0.08	0.10	n.s.	0.11	n.s.	n.s.
BxA – interaction	0.12	0.15	n.s.	0.18	n.s.	n.s.

n.s. – non-significant difference

Table 4

The total content of sodium in petioles and leaf blades of celery leaves (g kg^{-1} d.m.), in series I and II, in pot experiment

Fertilization object	I series		II series		Mean for petioles	Mean for leaf blades
	petioles	leaf blades	petioles	leaf blades		
Control object	3.00	2.37	7.86	6.73	5.43	4.55
NPK	2.07	2.37	3.05	2.78	2.56	2.58
I spraying of Tytanit						
0.001%+ NPK	2.05	1.94	3.10	3.17	2.58	2.56
0.01% + NPK	2.00	1.94	3.01	2.74	2.51	2.34
0.1% + NPK	1.99	2.09	3.15	3.12	2.57	2.61
1% + NPK	2.74	2.58	4.46	4.36	3.61	3.47
1.2% + NPK	3.26	2.38	4.47	4.53	3.87	3.46
2.4% + NPK	4.03	4.33	5.98	5.11	5.01	4.72
3.6% + NPK	5.03	3.60	5.65	6.00	5.34	4.80
Mean	3.01	2.67	4.26	4.15	3.64	3.42
II spraying of Tytanit						
0.001% + NPK	1.86	1.69	3.70	3.27	2.78	2.48
0.01% + NPK	2.14	2.78	3.39	3.70	2.77	3.24
0.1% + NPK	2.53	1.70	3.90	3.32	3.22	2.51
1% + NPK	3.69	2.32	6.14	5.02	4.92	3.67
1.2% + NPK	5.24	2.48	6.25	5.09	5.75	3.79
2.4% + NPK	6.51	4.93	9.92	7.55	8.22	6.24
3.6% + NPK	8.43	4.87	8.87	8.87	8.65	6.87
Mean	4.35	2.97	6.03	5.26	5.19	4.11
LSD $_{0,05}$ for:						
A – number of treatments	0.04	0.07	0.14	0.09	0.40	0.33
B – concentration of Tytanit	0.12	0.19	0.40	0.26	1.16	0.95
AxB – interaction	0.11	0.18	0.37	0.25	1.06	0.87
BxA – interaction	0.17	0.27	0.57	0.38	1.64	1.35

applied (Table 4). An increasing concentration of Tytanit resulted in an increase in the content of this element in celery as compared to the control and the object fertilized with NPK in the first series of research, and after its double application in the second series. The content of sodium in petioles of celery fertilized once with Tytanit against NPK (an average from both series of research)was 3.64 g kg^{-1}; after double application: 5.19 g kg^{-1}; in leaf blades: 3.42 g kg^{-1} (single application) and 4.11 g kg^{-1} (double aaplication). The bBioaccumulation of this macroelement was higher in petioles than in leaf blades of celery.

The nutritional value of the plants is determined not only by the absolute content of individual macro- and microelements, but also by their proportions. In the case of macroelements, it is difficult to ensure proper ratios of individual elements or their groups in the plant material, e.g. the (K+Na) : (Ca+Mg) ratio in vegetables. The content of potassium, calcium, magnesium and sodium was determined in the plant materialobtained in the experiment, which enabled the caaclulation of the weight ratio of monovalent to divalent elements. The value of the (K+Na) : (Ca+Mg) ratio in petioles of celery (mean from the series of research) ranged from 0.764 to 1.760, and in leaf blades it ranged from 0.453 to 0.746 (Table 5). Over two-fold higher values of ratios of the analyzed elements were found in celery leaf blades in comparison to celery petioles for the objects fertilized with Tytanit. The narrowest (K+Na) : (Ca+Mg) ratio was calculated for the control objects, i.e. 0.764 in celery petioles and 0.453 in leaf blades. The mean values of weight ratios of the discussed elements in the test plant fertilized twice with Tytanit were slightly broader than after a single application of Tytanit.

Table 5

Weight ratio of (K + Na) : (Ca + Mg) in petioles and leaf blades of celery leaves
(mean for series)

Fertilization object	Petioles	Leaf blades	Mean
	(K + Na) : (Ca + Mg)	(K +Na) : (Ca + Mg)	
Control object	0.762	0.453	0.607
NPK	1.120	0.746	0.934
I spraying of Tytanit			
0.001% + NPK	1.130	0.700	0.915
0.01% + NPK	1.120	0.597	0.859
0.1% + NPK	1.200	0.545	0.873
1% + NPK	1.270	0.544	0.907
1.2% + NPK	1.250	0.589	0.920
2.4% + NPK	1.310	0.588	0.949
3.6% + NPK	1.580	0.632	1.110
Mean	1.270	0.599	0.933
II spraying of Tytanit			
0.001% + NPK	1.410	0.667	1.040
0.01% + NPK	1.100	0.553	0.827
0.1% + NPK	1.170	0.522	0.846
1% + NPK	1.430	0.521	0.976
1.2% + NPK	1.550	0.683	1.120
2.4% + NPK	1.610	0.621	1.121
3.6% + NPK	1.760	0.720	1.240
Mean	1.430	0.612	1.024

Table 6

The ratios of K : Ca, K : Mg, Na : Ca and Na : Mg in petioles and leaf blades of celery leaves (mean for series)

Fertilization object	Petioles				Leaf blades			
	K:Ca	K:Mg	Na:Ca	Na:Mg	K:Ca	K:Mg	Na:Ca	Na:Mg
Control object	1.550	0.502	3.030	0.840	2.550	0.198	7.770	0.487
NPK	0.829	0.281	3.780	1.270	1.320	0.126	8.990	0.828
I spraying of Tytanit								
0.001% + NPK	0.837	0.268	4.260	1.341	1.430	0.134	8.770	0.901
0.01% + NPK	0.813	0.285	4.160	1.450	1.690	0.179	10.300	1.170
0.1% + NPK	0.739	0.275	3.180	1.380	1.860	0.208	10.600	1.110
1% + NPK	0.740	0.192	3.390	0.966	1.930	0.222	7.110	1.071
1.2% + NPK	0.762	0.255	2.630	0.880	1.770	0.207	7.110	0.980
2.4% + NPK	0.767	0.223	2.230	0.628	1.850	0.217	5.450	0.714
3.6% + NPK	0.637	0.204	1.510	0.505	1.720	0.200	4.710	0.500
Mean	0.756	0.244	3.140	1.021	1.750	0.195	7.710	0.921
II spraying of Tytanit								
0.001% + NPK	0.694	0.234	3.540	1.190	1.560	0.172	9.900	1.090
0.01% + NPK	0.879	0.273	4.160	1.202	1.870	0.171	9.610	0.878
0.1% + NPK	0.787	0.239	3.690	1.110	1.890	0.180	11.900	1.182
1% + NPK	0.680	0.186	2.590	0.681	2.000	0.222	9.380	1.090
1.2% + NPK	0.666	0.201	1.830	0.540	1.500	0.211	6.810	1.011
2.4% + NPK	0.702	0.192	1.290	0.347	1.800	0.220	4.560	0.583
3.6% + NPK	0.656	0.208	0.940	0.297	1.630	0.211	3.560	0.477
Mean	0.723	0.219	2.580	0.766	1.750	0.198	7.960	0.902

Table 6 presents molar ratios (mean values from the series of research) between K:Ca, K:Mg, Na:Ca and Na:Mg, assuming K = 1 and Na = 1. The values of calculated ratios between the examined elements in petioles and leaf blades were differentiated under the influence of the fertilization applied. The highest values of molar ratios for the K: Ca and K: Mg of the examined elements in leaf blades of celery were calculated for the control object and after applying lower concentrations of Tytanit. The value of molar ratios between K:Ca and K:Mg in leaf blades of celery was the highest for the control object and objects fertilized with Tytanit against NPK, starting with the concentration of 0.1%, while the lowest value was found for the NPK object. According to MAJKOWSKA-GADOMSKA (2009) and MAJKOWSKA-GADOMSKA, WIERZBICKA (2013), the ratios between the macroelements in eggplant fruits varied between the cultivars and between plants treated and untreated with Asahi SL.

The relationships between the content of K, Ca and Na, and the content of Ti in the dry matter of petioles and leaf blades of celery and between the examined elements were presented in the form of calculated values of direct correlation coefficients (Table 7). The total content of Ti was discussed earlier, in publications by MALINOWSKA, KALEMBASA (2012). A significant positive relation was found in petioles between the content of Ti and the content of Na (r = 0.82) and between the content of Ca and the content of Mg (r = 0.61) in the two-year experiment. A significant positive correlation was also found in leaf blades of the test plant between Ti and Na (r = 0.73), as well as between K and Ca (r = 0.57), Ca and Mg (r = 0.39) and between Ca and Na (r = 0.37).

Table 7

Simple correlation coefficients between the total content of Ti in petioles and leaf blades of celery leaves and the content of K, Ca, Mg and Na, the first and second series of studies

Elements	Petioles					Leaf blades				
	Ti	K	Ca	Mg	Na	Ti	K	Ca	Mg	Na
Ti	1.00					1.00				
K	0.22	1.00				0.02	1.00			
Ca	0.06	0.15	1.00			0.07	0.57*	1.00		
Mg	-0.18	0.13	0.61*	1.00		0.32	0.23	0.39*	1.00	
Na	0.82*	0.11	0.08	0.09	1.00	0.73*	0.20	0.37*	0.24	1.00

$n = 32$, $p < 0.05$, critical value; $r = 0.349$

The uptake of K, Ca, Mg and Na with the yield of celery petioles fertilized with Tytanit was almost twice as high as in the object fertilized with NPK and it was several times higher than in the control object (Table 8). As a result of the double application of the preparation, the mean K, Ca and Na uptake with the petiole yield increased, while the uptake of Mg was reduced. It was observed that the K, Mg and Na uptake was much lower with the yield of leaf blades than with the yield of petioles, except for Ca. The mean Ca uptake with the yield of leaf blades fertilized once with Tytanit was 1.220 g pot^{-1}, after double fertilization: 1.170 g pot^{-1}, and with the yield of petioles: 0.674 and 0.690 g pot^{-1}, respectively. The uptake of the discussed elements depended mainly on the volume of celery yield (MALINOWSKA, KALEMBASA 2012).

KLAMKOWSKI et al. (1999), KLAMKOWSKI, WÓJCIK (2000), ALCARAZ-LOPEZ et al. (2004), SMOLEŃ (2008), SMOLEŃ et al. (2010) confirm the impact of foliar application of growth regulators or fertilizers containing biostimulants on the uptake and accumulation of mineral components by plants. RADKOWSKI, RADKOWSKA (2010) report that foliar application of Tytanit at appropriate concentrations can raise the content of potassium, calcium and magnesium in meadow sward by almost 80%, and the content of sodium by 60% in relation to the control object. According to other authors (MARCINEK, HETMAN 2008), the application of Tytanit resulted in an increase in the nitrogen content in

Table 8

Uptake of selected macroelements and sodium (mg pot^{-1}) in the yield of petioles and leaf blades of celery (mean from the results of two series)

Fertilization object	Petioles				Leaf blades			
	K	Ca	Mg	Na	K	Ca	Mg	Na
Control object	0.097	0.145	0.027	0.034	0.160	0.420	0.019	0.038
NPK	0.449	0.374	0.079	0.059	0.750	1.030	0.060	0.066
I spraying of Tytanit								
0.001% + NPK	0.679	0.568	0.114	0.082	0.639	0.940	0.054	0.057
0.01% + NPK	0.882	0.722	0.157	0.105	0.708	1.210	0.079	0.064
0.1% + NPK	0.832	0.632	0.144	0.098	0.618	1.180	0.077	0.067
1% + NPK	1.120	0.851	0.154	0.153	0.750	1.460	0.099	0.097
1.2% + NPK	0.845	0.661	0.135	0.146	0.616	1.110	0.075	0.081
2.4% + NPK	0.857	0.673	0.119	0.180	0.679	1.270	0.089	0.120
3.6% + NPK	0.934	0.611	0.121	0.225	0.759	1.340	0.092	0.149
Mean	0.878	0.674	0.135	0.141	0.681	1.220	0.081	0.091
II spraying of Tytanit								
0.001% + NPK	1.060	0.689	0.151	0.123	0.576	0.880	0.062	0.054
0.01% + NPK	0.886	0.756	0.147	0.109	0.695	1.320	0.073	0.078
0.1% + NPK	0.995	0.806	0.150	0.127	0.660	1.310	0.074	0.063
1% + NPK	0.934	0.652	0.108	0.153	0.631	1.300	0.086	0.090
1.2% + NPK	1.070	0.710	0.118	0.224	0.633	0.980	0.081	0.089
2.4% + NPK	0.920	0.643	0.106	0.288	0.651	1.210	0.088	0.155
3.6% + NPK	0.852	0.573	0.110	0.349	0.718	1.190	0.091	0.205
Mean	0.960	0.690	0.127	0.196	0.652	1.170	0.079	0.105

Sparaxis tricolor bulbs, and in a reduction of other macroelements. Our research and investigations carried out by other authors imply that the effect of the foliar application of Tytanit on the chemical composition of plants is closely determined by the concentration of the preparation, plant species and atmospheric conditions.

CONCLUSIONS

1. It was found experimentally that the foliar application of Tytanit against NPK fertilization differentiated bioaccumulation of the examined macroelements (K, Ca, Mg) and Na in petioles and leaf blades of celery. The highest concentration of Tytanit (3.6%) resulted in a reduction of the total content of potassium, calcium and magnesium in the examined parts of the

celery, as compared to the concentration 2.4%. On the other hand, the total content of sodium increased along with the growth of fertilizer concentration. The highest bioaccumulation of calcium was observed in celery cultivated in the object without fertilization (the control).

2. A significantly higher potassium content (mean from both series of research) and an over two-fold higher content of calcium were found in leaf blades of celery as compared to petioles, while the content of magnesium and sodium was higher in petioles than in leaf blades.

3. Foliar application of Tytanit against NPK can ensure an optimal – with resepct to the nutritional value – (K+Na) : (Ca+Mg) ratio in petioles and leaf blades of celery.

4. The uptake of the elements depended mainly on the volume of celery yield. In petioles of celery fertilized with Tytanit, it was almost twice as high as in the object fertilized with NPK, and a few times higher than in the control object. Double application of Tytanit increased the mean uptake of K, Ca and Na with the yield of petioles and reduced the uptake of Mg.

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DISTRIBUTION OF NICKEL IN FRACTIONS EXTRACTED WITH THE BCR PROCEDURE FROM NICKEL-CONTAMINATED SOIL

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Abstract

The objective of the study was to evaluate the impact of liming and the application of waste organic material, i.e. sewage sludge, on the content and distribution of nickel in the fractions extracted with the BCR procedure from soil contaminated with this metal. The study was carried out on soil after a 3-year pot experiment, which included the following factors: I – nickel used in the incremental amounts 0, 50, 100 mg Ni kg^{-1} soil; II – liming (0 Ca and Ca according to 1 Hh of soil); and III – the addition of sewage sludge (with and without the addition of sewage sludge at the introducing dose of 2 g C kg^{-1} soil).

The test plant was cocksfoot harvested four times (four swaths) in each plant growing season. The total content of nickel was determined with ICP-AES and its fractions with the three-stage BCR procedure.

The introduction of nickel into the soil resulted in an increase in its total content and in all fractions as well as in its percentage in the exchangeable fraction. Liming reduced the mobility of nickel and decreased its content in the reducible fraction, whil inccreasing it in the residual fraction. The application of sewage sludge contributed to an increase in the total content of nickel in soil and its proportion in the oxidizable fraction. Liming and the application of sewage sludge reduced the mobility of nickel.

Lime and waste organic material (i.e. sewage sludge) were found to be suitable materials for reduction of the mobility of nickel in soil contaminated with this metal.

Key words: soil, liming, sewage sludge, nickel fractions.

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ROZMIESZCZENIE NIKLU WE FRAKCJACH WYDZIELONYCH WG PROCEDURY BCR W GLEBIE ZANIECZYSZCZONEJ TYM METALEM

Abstrakt

Celem pracy była ocena wpływu wapnowania i wprowadzenia do gleby odpadowego materiału organicznego – osadu ściekowego na zawartość i rozmieszczenie niklu we frakcjach wydzielonych wg procedury BCR w glebie zanieczyszczonej tym metalem. Badaniami objęto glebę po trzyletnim doświadczeniu wazonowym, w którym uwzględniono następujące czynniki: I – nikiel stosowany we wzrastających dawkach (0, 50, 100 mg Ni kg^{-1} gleby); II – wapnowanie (0 Ca i Ca wg 1 Hh gleby); III – dodatek osadu ściekowego (bez dodatku i z dodatkiem osadu ściekowego w dawce wprowadzającej 2 g C kg^{-1} gleby).

Rośliną testową była trawa kupkówka pospolita (*Dactylisglomerata* L.), zbierana każdego roku w czterech pokosach (odrostach). Ogólną zawartość niklu oznaczono metodą ICP-AES, a jego frakcje trzystopniową metodą BCR.

Wprowadzenie niklu do gleby spowodowało nie tylko wzrost jego zawartości ogólnej i we wszystkich frakcjach, a jednocześnie zwiększenie procentowego udziału we frakcji wymiennej. Wapnowanie gleby ograniczyło mobilność niklu, zmniejszył się jego udział we frakcji redukowalnej, zwiększył we frakcji rezydualnej. Zastosowany osad ściekowy spowodował zwiększenie ogólnej ilości niklu w glebie i jego udziału we frakcji utlenialnej. Wapnowanie i osad ściekowy ograniczyły mobilność niklu.

Wapnowanie oraz zastosowany w doświadczeniu odpadowy materiał organiczny – osad ściekowy okazały się dobrymi materiałami zmniejszającymi mobilność niklu na glebach zanieczyszczonych tym metalem.

Słowa kluczowe: gleba, wapnowanie, osad ściekowy, frakcje niklu.

INTRODUCTION

Excessive accumulation of toxic substances in soil, including heavy metals, is one of the side effects of the development of civilization (WYSZKOWSKA et al. 2009) Heavy metals are a special type of contaminants, as they are elements which do not break down to basic compounds (VONDRACKOVA et al. 2013). They are found in all types of soil, even in those which are regarded as non-polluted. Their content is low in most soil types, not exceeding several mg kg^{-1} of soil and sometimes even tenths or hundredths of that (KUCHARSKI et al. 2009). The accumulation of heavy metals in soil induces negative changes in its physical, chemical and biological properties, which may have an impact on the availability of many macro- and microelements and, subsequently, on the soil yielding capacity and chemical composition (WYSZKOWSKA et al. 2009). The group of heavy metals includes nickel, which - due to its toxicity and persistence in the environment as well as a high bioaccumulation factor – is classified as a List II substance in the European Commission Dangerous Substances Directive. The World Health Organization (WHO) classified nickel compounds into group I, i.e. carcinogenic substances, while metallic nickel was included in group IIB, i.e. potentially carcinogenic to humans (NAKONIECZNY 2007). Its presence in soil is associated with parent rocks and with industrial (paper, plating, chemical industry), urban and agricultural pollution, including wrong fertilization with organic waste materials (sewage sludge) (Filipek-Mazur et al. 1999, Cempel, Nikel 2006, Kalembasa, Kuziemska 2010).

Nickel is a heavy metal which is essential for the growth and development of living organisms, but in excess it can be toxic (Kuziemska, Kalembasa 2009). It is relatively easily absorbed by plants proportionally to its content in soil, until it reaches a threshold concentration in plant tissues. The toxicity of this metal to plants has been reported in regions with higher nickel concentrations in soil. The visible symptoms of nickel toxicity include chlorosis and necrosis of leaves, shorter root system, other morphological pathologies in the root system (Marschner 1995, Spiak et al. 2003). Diagnosis of risks to the natural environment through measuring the total concentration of metals, including nickel, in soil and in natural and organic fertilizers, omits many important factors that determine the mobility and bioavailability of elements (pH, content of organic matter, content of clay minerals). Reliable evaluation requires determination of individual forms and fractions which constitute a total content of a given metal (Świetlik, Trojanowska 2008, Pakuła, Kalembasa 2009). Among the numerous methods of eco-toxicological examination of soil, speciation of heavy metals is particularly important because it provides for making a reference to their bioavailability (Qvevauviller 2003, Kalembasa et al. 2009, Reid et al. 2011, Jaske, Gworek 2011, Jaremko, Kalembasa 2011).

The objective of the study was to determine the impact of liming and the application of waste organic material, i.e. sewage sludge, on the content and distribution of nickel in the fractions extracted with the BCR procedure from soil contaminated with this metal.

MATERIAL AND METHODS

The study was carried out on soil after a 3-year pot experiment conducted in a research station of the Siedlce University of Natural Sciences and Humanities. The pot trials were run in 2006-2008, in a randomized model with three replications.

The study included the following factors:

1) contamination of soil with nickel – 0, 50 i 100 mg Ni kg^{-1} soil;
2) liming – 0 Ca (without liming) and Ca (liming) at a dose calculated according to the soil hydrolytic acidity 1;
3) waste organic material – without sewage sludge and with sewage sludge, obtained from a sewage treatment plant in Siedlce, applied at a starter dose of 2 g C kg^{-1} soil.

Nickel in the form of $NiCl_2$ $6H_2O$ water solution, liming with $CaCO_3$ and sewage sludge were applied to the soil from April to May 2006.

The study material (Table 1) was collected from the 0-20 cm horizon of lessive soil, whose texture was typical of loamy sand (Kuziemska, Kalembasa 2008). The chemical composition of the sewage sludge is presented in Table 2.

The following determinations were made in the soil: hydrolytic acidity according to PN-R-04027:1997, pH by potentiometric method according to PN-ISO 10390:1997, the content of organic carbon by the oxidation-titration method according to PN-ISO 14235:2003, total nitrogen content by elemental analysis on a Perkin-Elmer Series II 2400 CHN analyzer with a thermal conductivity detector (TCD), the content of available phosphorus and potassium by the Egner-Riehm method according to PN-R-040022:1996, and the total nickel content by atomic emission spectrometry with inductively coupled plasma (ICP-AES) on a Perkin-Elmer Optima 3200 RLspectrometer, after digestion in a mixture of perchloric acid and nitric acid (1:2) (Kopec, Gondek 2002).

Table 1

Some properties of soil used in the pot experiment

Hh cmol(+) kg^{-1}	pH 1 M KCl	$C_{org.}$	$N_{tot.}$	Available		Total
		g kg^{-1} of soil		P	K	Ni
				mg kg^{-1} of soil		mg kg^{-1} of soil
2.22	5.6	7.900	0.980	69.00	75.00	5.670

Table 2

Chemical composition of sewage sludge from Siedlce

Component	(g kg^{-1} d.m.)	Component	(m gkg^{-1}d.m.)
N	60.50	Cd	1.990
P	31.20	Pb	50.50
K	4.280	Ni	20.60
Ca	39.60	Fe	10850
Mg	8.420	Cu	137.7
$C_{org.}$	371.0	Zn	1276.8
Organic matter	640.0	Cr	30.14
Dry matter (g k g^{-1})		180.0	

The sludge used in the experiment was analyzed by the same methodology as in the analysis of soil. Additionally, the dry matter content was determined by the drying-weight method according to PN-EN 12880:2004, while the content of phosphorus, potassium, calcium, magnesium and heavy metals was aassessed by atomic emission spectrometry after dry digestion in a muffle furnace and dissolution of the ash in 10% HCl solution.

The test plant was cocksfoot (*Dactylisglomerata* L.) harvested four times (four swaths) in each plant growing season (every 30 days).

Table 3

A diagram of the BCR metal sequential extraction method

Fraction	Name of fractions	Extraction reagents	pH
F_1	exchangeable and acid soluble	0.1 M CH_3COOH	3.0
F_2	reducible	0.5 M NH_2OHHCl	1.5
F_3	oxidizable	8.8 M H_2O_2 + 1M CH_3COONH_4	2.0
F_4	residual	calculated as difference between total content and sum of three previously separated fractions	-

The soil was analyzed each year of the experiment, after the last grass swath. The total content of nickel was determined with the ICP-AES method and its fractions with 3-step sequential fractionating proposed by the Community Bureau of Reference – BCR (Rauret et al. 1999). The scheme of the procedure is given in Table 3.

RESULTS AND DISCUSSION

Evaluation of the status of nature by measuring the total content of metals in certain compartments of the natural environment (soil, natural and organic fertilizers) very often omits factors that determine the mobility and bioavailability of elements. A reliable assessment of risks to the environment requires determination of individual forms and fractions that constitute the total content of a given metal (Węglarzyk 2001, Wójcikowska-Kapusta, Niemczuk 2009).

Our studies demonstrated that the total content of selected metals in waste organic material used in the experiment (Table 2) did not exceed the permissible limits (The Regulation of the Minister of the Environment). The applied sewage sludge contained large amounts of organic matter and basic plant nutrients, which is why it should be used as fertilizer.

The total content of nickel, its distribution in the individual fractions and their percentages in soil sampled each year after cultivation of the test plant are presented in Tables 4-9. In all experimental years, the lowest total mean content of nickel was detected in the soil of control objects (without application of nickel) and the highest concentration was measured in the soil supplemented with 100 mg Ni·kg^{-1} soil.

The use of nickel in the mineral form contributed to an increase in its total content as well as in all determined fractions. This correlation was confirmed in the subsequent years of the experiment, when - in the objects treated with nickel – the nickel content in the exchangeable fraction (F_1) decreased while increasing in the residual fraction (F_4), which indicated its immobilization by different soil-associated factors. In the first year of the experiment,

Table 4

The content (mg kg^{-1} of soil) of nickel in fractions determined by the BCR method in the analyzed soil after the first year of cultivation

Fertilization	Fractions	Liming					
		0 Ca			Ca to 1 Hh		
		dose of nickel (mg kg^{-1} of soil)			dose of nickel (mg kg^{-1} of soil)		
		0	50	100	0	50	100
Without organic fertilization	F_1 F_2 F_3 F_4	0.530 0.760 1.120 3.210	39.20 8.290 3.980 3.830	73.90 13.60 10.80 7.400	0.400 0.610 1.010 3.610	31.00 6.120 4.220 14.06	57.20 10.20 9.840 28.46
Sum of fractions	Σ	5.620	55.30	105.3	5.630	55.40	105.7
Sludge from Siedlce	F_1 F_2 F_3 F_4	0.380 0.920 2.000 2.440	30.30 6.700 11.20 7.600	54.60 13.12 20.42 11.86	0.300 0.840 1.820 2.790	25.30 6.050 9.020 15.53	46.80 10.12 14.92 34.06
Sum of fractions	Σ	5.740	55.80	105.9	5.750	55.90	105.9
			F_1	F_2	F_3	F_4	Σ
$LSD_{(0.05)}$ for:							
doses of nickel			12.28	2.010	9.947	13.92	0.310
sludge from Siedlce			n.s.	1.545	n.s.	10.06	n.s.
liming			n.s.	n.s.	3.648	n.s.	0.228

n.s.–not significant

Table 5

The percentage share of the nickel fraction in the analyzedsoil after the first year of cultivation

Fertilization	Fractions	Liming					
		0 Ca			Ca to 1 Hh		
		dose of nickel (mg kg^{-1} of soil)			dose of nickel (mg kg^{-1} of soil)		
		0	50	100	0	50	100
Without organic fertilization	F_1 F_2 F_3 F_4	9.430 13.52 19.93 57.12	70.89 14.99 7.200 6.920	69.92 12.87 10.22 6.990	7.100 10.83 17.94 64.13	55.96 11.05 7.620 25.37	54.11 9.650 9.310 26.93
Sum of fractions	Σ	100.0	100.0	100.0	100.0	100.0	100.0
Sludge from Siedlce	F_1 F_2 F_3 F_4	6.620 16.03 34.84 42.51	54.30 12.01 20.07 13.62	51.55 12.39 19.28 16.78	5.230 14.61 31.65 48.51	45.26 10.82 16.14 27.78	44.19 9.560 14.09 32.16
Sum of fractions	Σ	100.0	100.0	100.0	100.0	100.0	100.0

Table 6

The content (mg kg^{-1} of soil) of nickel in fractions determined by the BCR method in the analyzed soil after the second year of cultivation

Fertilization	Fractions	Liming					
		0 Ca			Ca to 1 Hh		
		dose of nickel (mg kg^{-1} of soil)			dose of nickel (mg kg^{-1} of soil)		
		0	50	100	0	50	100
Without organic fertilization	F_1	0.620	36.20	66.80	0.430	25.12	48.10
	F_2	0.830	9.020	11.21	0.900	4.980	10.28
	F_3	1.180	4.420	9.890	1.000	5.520	11.08
	F_4	2.850	5.260	17.30	3.170	19.38	35.44
Sum of fractions	Σ	5.480	54.90	105.2	5.500	55.00	104.9
Sludge from Siedlce	F_1	0.400	26.12	45.12	0.330	22.06	43.08
	F_2	0.990	8.950	14.00	0.560	7.280	10.12
	F_3	2.370	16.32	28.12	1.140	12.75	21.08
	F_4	1.880	3.910	18.34	3.630	13.31	31.12
Sum of fractions	Σ	5.640	55.30	105.6	5.660	55.40	105.4
			F_1	F_2	F_3	F_4	Σ
LSD$_{(0.05)}$ for:							
doses of nickel			11.80	2.628	8.985	9.916	0.302
sludge from Siedlce			n.s.	n.s.	n.s.	7.436	n.s.
liming			n.s.	n.s.	7.162	n.s.	0.177

n.s.–not significant

Tabela 7

The percentage share of the nickel fractions in the analyzed soil after the second year of cultivation

Fertilization	Fractions	Liming					
		0 Ca			Ca to 1 Hh		
		dose of nickel (mg kg^{-1} of soil)			dose of nickel (mg kg^{-1} of soil)		
		0	50	100	0	50	100
Without organic fertilization	F_1	11.31	65.94	63.50	7.820	45.67	45.83
	F_2	15.14	16.43	10.66	16.36	9.050	9.800
	F_3	21.53	8.060	9.100	18.18	10.04	10.56
	F_4	52.02	9.570	16.44	57.64	35.24	33.81
Sum of fractions	Σ	100.0	100.0	100.0	100.0	100.0	100.0
Sludge from Siedlce	F_1	7.090	47.23	42.72	5.830	39.81	40.87
	F_2	17.55	16.18	13.26	9.890	13.14	9.60.
	F_3	42.02	29.51	26.64	20.14	23.01	20.00
	F_4	33.34	7.080	17.38	64.14	24.04	29.53
Sum of fractions	Σ	100.0	100.0	100.0	100.0	100.0	100.0

Table 8

The content (mg kg^{-1} of soil) of nickel in fractions determined by the BCR method in the analyzed soil after the third year of cultivation

Fertilization	Fractions	Liming					
		0 Ca			Ca to 1 Hh		
		dose of nickel (mg kg^{-1} of soil)			dose of nickel (mg kg^{-1} of soil)		
		0	50	100	0	50	100
Without organic fertilization	F_1	0.440	31.12	60.12	0.350	22.16	43.60
	F_2	0.980	10.15	12.56	1.020	5.380	10.08
	F_3	1.080	4.460	8.040	1.000	6.210	9.460
	F_4	2.900	8.790	23.88	3.050	20.85	41.66
Sum of fractions	Σ	5.400	54.60	104.9	5.420	54.60	104.8
Sludge from Siedlce	F_1	0.320	23.68	42.80	0.300	20.09	36.68
	F_2	1.060	10.21	12.84	0.640	6.080	9.860
	F_3	2.510	14.12	23.10	1.390	10.86	18.96
	F_4	1.670	6.990	26.36	3.250	17.97	39.50
Sum of fractions	Σ	5.560	55.00	105.1	5.580	55.00	105.0
LSD$_{(0.05)}$ for:			F_1	F_2	F_3	F_4	Σ
doses of nickel			10.87	2.136	7.315	17.43	0.180
sludge from Siedlce			n.s.	2.035	n.s.	n.s.	n.s.
liming			n.s.	n.s.	5.903	n.s.	0.121

n.s.–not significant

Table 9

The percentage share of the nickel fractions in the analyzed soil after the third year of cultivation

Fertilization	Fractions	Liming					
		0 Ca			Ca to 1 Hh		
		dose of nickel (mg kg^{-1} of soil)			dose of nickel (mg kg^{-1} of soil)		
		0	50	100	0	50	100
Without organic fertilization	F_1	8.150	57.00	57.31	6.460	40.59	41.60
	F_2	18.15	18.59	11.97	18.82	9.860	9.620
	F_3	20.00	8.170	7.660	18.45	11.37	9.030
	F_4	53.70	16.24	23.06	56.27	38.18	39.75
Sum of fractions	Σ	100.0	100.0	100.0	100.0	100.0	100.0
Sludge from Siedlce	F_1	5.750	43.05	40.72	5.370	36.53	34.98
	F_2	19.06	18.56	12.22	11.47	11.05	9.390
	F_3	45.14	25.67	21.98	24.91	19.75	18.06
	F_4	30.05	12.72	25.08	58.25	32.67	37.62
Sum of fractions	Σ	100.0	100.0	100.0	100.0	100.0	100.0

in the objects where nickel was applied at 50 mg $Ni·kg^{-1}$ of soil, the average percentage of the exchangeable fraction (F_1) in its total content was 56.6%, in the second year it was 49.66%, whereas in the third year it was only 44.29%. In the objects treated with 100 mg $Ni·kg^{-1}$ of soil, the values were 54.94%, 48.23% and 43.64% in the first, second and third year, respectively.The average percentage of nickel in its total content in the extracted fractions of soil into which this metal had not been introduced, in the subsequent years of the experiment, was arranged in the following order of decreasing values: $F_4>F_3>F_2>F_1$.

Throughout the study, the total content of nickel depended not only on its amount introduced into soil as $NiCl_2$ $6H_2O$, but also on the organic matter in added waste material. The application of sewage sludge resulted in a minor, yet statistically significant, increase in the total content of nickel in soil despite the fact that its concentration in the sewage sludge was only 20.6 mg Ni kg^{-1} d.m. (Table 2). Similar results related to the impact of sewage sludge on the concentration of nickel and other metals in soil were reported by Filipek-Mazur et al. (1999) and Iżewska et al. (2009).

Regardless of the time of sampling, the sewage sludge used in our study contributed to an increase in the total content of nickel and in its proportion in the oxidizable fraction (F_3), which confirmed the positive impact of organic matter on limiting its mobility and was consistent with the studies conducted by Gondek, Filipek-Mazur (2003) and Latosińska, Gawdzik (2011). Organic matter contained in the sludge created organometallic compounds with mobile forms of nickel. These compounds are difficult to mineralize and therefore not available for plants and do not cause pollution of soil (Jakubus 2012).

The study did not reveal any significant impact of liming on the total content of this metal, but in the first and third year of the experiment it caused some “immobilization of nickel”, which was confirmed by a decrease in its content in the reducible fraction (F_2) and an increase in its concentration in the residual fraction (F_4) in the first and second year of the experiment. The study did not show any significant impact of liming on the amount of nickel in the exchangeable fraction (F_1), but there was a tendency towards a decrease in the percentage of this fraction under the influence of this factor. This correlation was observed in each year of the experiment.

The values of the soil pH in three consecutive years are presented in Table 10. The limed soil had a significantly higher pH than the non-limed soil. The other two experimental factors did not have any significant impact on the discussed parameter. The analysis of correlation did not reveal any significant relationship between pH and distribution of nickel in the extracted fractions, regardless of the time of sampling. This relationship is not consistent with the work of other authors and previously conducted studies, which demonstrated a significant effect of soil acidification on the content of mobile forms of nickel (Chen, Wong 2006, Siebielec et al. 2007, Kucharski et al. 2009). This issue requires further study.

Table 10

pH of soil in 1 M KCl

Fertilization	Years	Liming					
		0 Ca			Ca to 1 Hh		
		dose of nickel (mg kg^{-1} of soil)			dose of nickel (mg kg^{-1} of soil)		
		0	50	100	0	50	100
Without organic fertilization	I	6.640	6.580	6.120	7.460	7.400	7.380
	II	6.520	6.490	6.010	7.400	7.330	7.350
	III	5.970	5.840	5.800	6.640	6.590	6.800
Sludge from Siedlce	I	6.780	6.690	6.680	7.480	7.460	7.420
	II	6.710	6.520	6.530	7.450	7.260	7.260
	III	5.990	5.800	5.800	6.600	6.710	6.720
		Years	I	II	III		
$LSD_{(0.05)}$ for:							
doses of nickel			n.s.	n.s.	n.s.		
sludge from Siedlce			0.184	0.352	0.165		
liming			n.s.	n.s.	n.s.		

n.s.–not significant

Summarizing the results of our study, it is concluded that the addition of nickel into the soil, liming and application of sewage sludge modified the total content of nickel in the soil and its proportion in the fractions extracted with the BCR procedure. The introduction of nickel into the soil resulted in an increase in its total content and, more importantly, in its percentage in the exchangeable fraction (F_1) directly absorbed by plants, which poses a risk to the growth and development of cultivated plants (Spiak et al. 2003, Kuziemska, Kalembasa 2008). It should be taken into consideration that the amount of nickel in fraction F_1 decreased in the consecutive years of the experiment, especially in soils enriched with nickel in doses of 50 and 100 mg kg^{-1}, which confirms its immobilization.Liming reduced the mobility of nickel by decreasing its content in the reducible fraction (F_2) and increasing its proportion in the residual fraction (F_4), which further confirms the data reported in numerous publications indicating that liming is one of the factors limiting the mobility of metals (Kalembasa, Kuziemska 2010).

The application of sewage sludge resulted in an increase in the total content of nickel in the soil and its proportion in the fraction bound to organic matter (F_3), which limited its availability to plants.

Similar correlations were reported by Gondek, Filipek-Mazur (2003) who found that nickel and other metals were bound by humus in soil polluted with urban contamination.

Lime and waste organic material (i.e. sewage sludge) were found to be effective in reducing the mobility of nickel in soil contaminated with this metal.

CONCLUSIONS

1. The total content of heavy metals in the sewage sludge used in the experiment did not exceed the permissible limits.

2. The application of $NiCl_26H_2O$ into the soil resulted in an increase in its total content and in the individual fractions as well as an increase in the percentage of the exchangeable fraction in its total content.

3. Liming contributed to an increase in the nickel content in the residual fraction (F_4) and reduced its proportion in the exchangeable fraction (F_1)

4. The sewage sludge limited the mobility of nickel by increasing its content in the oxidizable fraction (F_3).

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THE EFFECT OF POLIMAG S FERTILIZER ON THE MACRONUTRIENT CONTENT OF THE EDIBLE PARTS OF THREE WELSH ONION (*ALLIUM FISTULOSUM* L.) CULTIVARS

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Abstract

Welsh onions are characterized by a relatively high storage potential, high nutritional value and mild flavor, which contribute to their growing popularity among consumers. The aim of this study was to determine the effect of Polimag S fertilizer applied at two doses on the macronutrient content of edible parts of three Welsh onion cultivars.

A two-factorial field experiment was performed in a randomized block design with three replications. It was set up in the Experimental Garden of the University of Warmia and Mazury in Olsztyn, and conduccted in 2010-2011. The experimental factors were: (1) Welsh onion cultivars Long White Ishikura, Parade and Performer, grown from seedlings for bunch harvest, and (2) the application of mixed fertilizer, Polimag S, at two doses of 0.072 kg m^{-2} and 0.144 kg m^{-2}. The seedlings were grown in a greenhouse, where each year seeds were sown in boxes (50 cm x 30 cm), between 14 and 20 March, to grow seedlings. At the two- or three-leaf stage, they were planted out in a field between 17 and 26 April. Forty-two seedlings were planted on a plot, at the 20 cm x 30 cm spacing. Polimag S, applied as a supplemental fertilizer at two different doses, was mixed with soil immediately before the transplanting of seedlings. A single harvest was carried out manually. Marketable yield consisted of healthy Welsh onion plants, with thickened leaf-bases of more than 1 cm in diameter, free from diseases, pests and mechanical damage. The chemical composition of Welsh onion plants was evaluated immediately after harvest. The concentrations of macronutrients (total N, P, K, Mg, Ca, S) were determined in edible parts of Welsh onion plants.

Statistical analysis revealed no significant effect of a cultivar on the macronutrient content of whole Welsh onion plants, scapes and leaves. Leaves were characterized by the highest macronutrient concentrations. Polimag S at a 50% dose contributed to a significant increase in the total nitrogen and phosphorus content of whole plants. Wide Ca:Mg, Ca:P, K:Mg and K:(Mg+Ca) ratios were noted in whole Welsh onion plants.

Key words: Welsh onion, Polimag S, scapes, leaves, macronutrients.

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WPŁYW NAWOŻENIA POLIMAGIEM S NA ZAWARTOŚĆ MAKROELEMENTÓW W CZĘŚCIACH JADALNYCH TRZECH ODMIAN SIEDMIOLATKI (*ALLIUM FISTULOSUM* L.)

Abstrakt

Siedmiolatka charakteryzuje się dość dobrą trwałością pozbiorczą, dużą wartością dietetyczną oraz łagodnym smakiem, który wzbudza coraz większe zainteresowanie konsumentów warzyw. Celem badań była ocena zawartości makroelementów w częściach jadalnych 3 odmian siedmiolatki nawożonej zróżnicowanymi dawkami Polimagu S.

W latach 2010-2011 – w Ogrodzie Zakładu Dydaktyczno-Doświadczalnego Uniwersytetu Warmińsko-Mazurskiego w Olsztynie – przeprowadzono 2-czynnikowe doświadczenie polowe w układzie losowanych bloków, w 3 powtórzeniach. Badano wpływ następujących czynników: odmiany siedmiolatki Long White Iskihura, Parade, Performer przeznaczonej na zbiór pęczkowy oraz nawożenie nawozem wieloskładnikowym Polimag S w dwóch dawkach – 0,072 kg m^{-2} i 0,144 kg m^{-2}. Siedmiolatkę uprawiano z rozsady. Każdego roku, między 14. a 20, marca nasiona wysiewano w szklarni-mnożarce do skrzynek wysiewnych o wym. 50x30 cm. W kolejnych latach rośliny w fazie 2-3 liści sadzono pojedynczo na polu między 17. a 26. kwietnia. Na poletku sadzono 42 rośliny w rozstawie 20x30 cm. Uzupełniające nawożenie Polimagiem S w dwóch zróżnicowanych dawkach zastosowano mieszając nawóz z glebą bezpośrednio przed sadzeniem rozsady. Zbiór roślin wykonywano jednorazowo, ręcznie. Do plonu handlowego zaliczano rośliny o średnicy cebuli powyżej 1 cm, zdrowe, nieporażone przez choroby i szkodniki, bez uszkodzeń mechanicznych. Ocenę składu chemicznego siedmiolatki wykonano bezpośrednio po zbiorze. W częściach jadalnych określono zawartość makroelementów (N ogólny, P, K, Mg, Ca, S).

Analiza statystyczna nie wykazała istotnego wpływu odmiany na zawartość badanych składników w całej roślinie, łodydze rzekomej oraz liściach asymilacyjnych. Analizując zawartość makroelementów w poszczególnych częściach jadalnych siedmiolatki wykazano, że najbardziej zasobne w te pierwiastki były liście asymilacyjne. Zastosowanie 50% nawożenia uzupełniającego Polimagiem S wpłynęło istotnie na zwiększenie zawartości N ogólnego i P w całych roślinach. W całych roślinach siedmiolatki stwierdzono zwiększone proporcje między Ca:Mg, Ca:P oraz K:Mg i K:(Mg+Ca).

Słowa kluczowe: siedmiolatka, Polimag S, łodyga rzekoma, szczypior, makroelementy.

INTRODUCTION

Welsh onion (*Allium fistulosum* L.) in Poland is grown on a small scale. In contrast, it is popular and widely used in the Far East. Owing to their mild, sweet flavor and delicate consistency, whole Welsh onion plants are used as ingredients in various dishes. Recent years have witnessed a growing popularity of Welsh onions in the Polish cuisine, particularly in the spring (RABINOWITCH, BREWSTER 1990, TENDAJ, MYSIAK 2006, ŻURAWIK et al. 2013).

The yield and quality of Welsh onions are depend on a cultivar and an adequate supply of nutrients. Mineral fertilizers are widely used in horticultural farming. Advanced fertilization programs rely on compound fertilizers which meet the specific nutrient requirements of different crop species. Bulbous vegetables should be fertilized in the spring with Polimag S, the next generation mixed fertilizer that contains sulphur trioxide, because this chemical compound is recommended to plants sensitive to chloride excess. Polimag S can be mixed with urea, ammonium nitrate and nitrochalk immediately before sowing, and with potassium sulphate at any time before application (Filipek-Mazur, Gondek 2005).

The aim of this study was to determine the effect of cultivar and Polimag S fertilizer applied at different rates on the macronutrient content of whole Welsh onion plants, scapes and leaves.

MATERIAL AND METHODS

In 2010-20122, a two-factorial field experiment was performed in a randomized block design with three replications in the Experimental Garden of the University of Warmia and Mazury in Olsztyn. The effects of the following experimental factors were determined:

- Welsh onion cultivars Long White Ishikura, Parade and Performer grown from seedlings for bunch harvest;
- application of mixed fertilizer Polimag S, at two doses of 0.072 kg m^{-2} and 0.144 kg m^{-2}, referred to as 50% and 100% doses. Polimag S contained 10% N, 8% P, 15% K and 35% S.

Seedlings were grown in a greenhouse, where onion seed had been sown (2 g m^{-2}) in boxes (50 cm x 30 cm), each year between 14 and 20 March. The seedlings were prepared in line with the bulbous vegetable planting guidelines (*Rozsada warzyw*... BN-88/9125-08). The substrate used for seedling production was highmoor peat saturated with minerals and nutrients: N-NO$_3$ – 100, P – 80, K – 215, Ca – 1240, Mg – 121 g dm^{-3}, pH in H_2O – 5.9, salt concentration – 1.5 g dm^{-3}.

In order to develop a fertilization program, chemical analyses of soil samples were performed at the Chemical and Agricultural Station in Olsztyn, under Accreditation Certificate no. AB 277 issued by the Polish Center for Accreditation in Warsaw. The soil in which Welsh onion seedlings were planted was found to be abundant in phosphorus and potassium, and no supplemental fertilization with those elements was needed throughout the experiment. Due to the low nitrate nitrogen content of soil, nitrogen was applied at a single dose of 30 kg ha^{-1} in the form of ammonium nitrate, before transplanting the seedlings.

Seedlings at the two- or three-leaf stage were individually planted out in the field between 17 and 26 April. Forty-two seedlings were planted at the 20 cm x 30 cm spacing. The surface area of the plot was 2.52 m^2. Polimag S, applied as a supplemental fertilizer at two different doses, was mixed with the soil immediately before transplanting the seedlings.

A single harvest was carried out by hand. Marketable yield consisted of healthy Welsh onion plants, with thickened leaf-bases of more than 1 cm in diameter, free from diseases, pests and mechanical damage.

The chemical composition of Welsh onion plants was evaluated immediately after harvest. Plant material was collected from the marketable yield in each replication, to obtain an average sample per treatment. The concentrations of macronutrients (total N, P, K, Mg, Ca, S) were determined in edible parts of Welsh onion plants. The results were highly similar in both years of the study, hence the data are given as means from 2010-2011 for whole plants, scapes and leaves. Samples of plant material were prepared for analysis as follows: whole Welsh onion plants were cleaned, divided into scapes and leaves, which were dried at 45°C and 35°C respectively, and ground in an electric mill. The samples were forwarded to the laboratory at the Chemical and Agricultural Station in Olsztyn, where they were mineralized in concentrated sulphuric acid to determine the content of total nitrogen – by potentiometry, phosphorus – by the vanadium-molybdate method, potassium and sodium – by flame photometry, magnesium – by atomic absorption spectrometry (AAS), calcium – by flame photometry.

The significance of differences between means was estimated by constructing the Tukey's confidence intervals at a 5% significance level. All calculations were done in the Statistica 10 application.

RESULTS AND DISCUSSION

Welsh onions are characterized by a relatively high storage potential, high nutritional value and mild flavor, which contribute to their growing popularity among consumers. (Franczuk 2000, Tendaj, Mysiak 2006, Tendaj, Mysiak 2007, Żurawik et al. 2013). Minerals aid in maintaining the body's acid-base balance and contribute to the health and strength of the skeletal system (Filipek-Mazur, Gondek 2005). The levels of the analyzed macronutrients in Welsh onion plants were similar to those reported for the leek by Biesiada et al. (2007) and for the onion by Błażewicz-Woźniak et al. (2008). In the present study, a cultivar had no significant effect on the macronutrient concentrations in edible parts of Welsh onion plants, whereas the supplemental fertilization with Polimag S affected the levels of total nitrogen and phosphorus. Jurgiel-Małecka and Suchorska-Orłowska (2008) noted similar macroelement concentrations in edible parts of common onions, tree onions and shallots at different levels of nitrogen fertilizer.

The total nitrogen content in whole Welsh onion plants ranged from 11.90 g kg^{-1} d.m. to 25.00 g kg^{-1} d.m., and was significantly affected by the dose of Polimag S and the fertilizer x cultivar interaction (Table 1). The above values are similar to those reported for Welsh onions by Kołota et al. (2012), and lower than those noted in a study on tree onions (Orłowski, Jadczak 2003). An increase in the total nitrogen content was observed in whole Welsh onion plants harvested from plots fertilized with Polimag S at 50% dosage. Welsh onions cv. Long White Ishikura from the treatment with

Table 1

Total nitrogen content in edible parts of Welsh onion plants, subject to cultivar and Polimag S dose (means of 2010-2011), g kg^{-1} d.m.

Fertilization*	Cultivar			
	Long White Ishikura	Parade	Performer	mean
Whole plants				
Control	12.80	14.60	11.90	13.10
50%	25.00	19.90	14.40	19.80
100%	12.70	12.70	18.00	14.50
Mean	16.80	15.70	14.80	15.80
LSD$_{0.05}$ for: cultivar fertilizer interaction	n.s. 0.300 0.400			
Scapes				
Control	11.00	12.00	8.000	10.00
50%	21.00	24.00	8.000	18.00
100%	9.000	10.00	13.00	11.00
Mean	14.00	15.00	9.000	13.00
LSD$_{0.05}$ for: cultivar fertilizer interaction	n.s. 0.500 0.600			
Leaves				
Control	14.40	17.10	15.60	15.70
50%	28.80	15.60	20.60	21.60
100%	16.50	15.30	22.40	18.00
Mean	19.90	16.00	19.50	18.40
LSD$_{0.05}$ for: cultivar fertilizer interaction	n.s. n.s. n.s.			

* Key: control – treatment that received no fertilizer, 50% – treatment that received 0.072 kg m^{-2} Polimag S, 100% – treatment that received 0.144 kg m^{-2} Polimag S

Polimag S at 50% had the highest total nitrogen content, and control plants had the lowest nitrogen concentration (a significant difference). An analysis of the fertilizer x cultivar interaction revealed that plants of cv. Performer from the control treatment had the lowest total nitrogen content.

Fertilization and the interaction between the experimental factors had a significant effect on the total nitrogen content of Welsh onion scapes, which ranged from 8.00 g kg^{-1} d.m. to 24.00 g kg^{-1} d.m. The scapes of cv. Parade from the treatment with Polimag S at a 50% dose had the highest total nitrogen content, whereas the lowest nitrogen concentrations were noted in the scapes of cv. Performer from the control treatment and from the treatment with Polimag S at a 50% dose (significant differences). A similar nitrogen content was observed in the scapes of cv. Long White Ishikura fertilized with Polimag S at a 100% dose.

Welsh onion leaves contained more total nitrogen than scapes (18.40 g kg^{-1} d.m. vs. 13.00 g kg^{-1} d.m. on average, means of 2010-2011). The total nitrogen content of leaves ranged from 14.40 g kg^{-1} d.m. to 28.80 g kg^{-1} d.m., and it was similar to that reported by ORŁOWSKI and JADCZAK (2003) in tree onion leaves. The leaves of cv. Long White Ishikura from the treatment with Polimag S at a 50% dose and from the control treatment had the highest and the lowest total nitrogen content, respectively (a non-significant difference).

The cultivar had no effect on the phosphorus content of whole Welsh onion plants (Table 2), which was significantly affected by the doses of Polimag S and the fertilizer x cultivar interaction. Polimag S contributed to an increase in phosphorus concentrations in Welsh onions, which werethe highest in plants harvested from plots fertilized with Polimag S at a 50% dose. The experimental factors and their interaction had no significant effect on the phosphorus accumulation in Welsh onion scapes and leaves, which reached 2.000 g kg^{-1} d.m. and 3.000 g kg^{-1} d.m. on average, respectively (means of 2010-2011). Similar results were reported by KOŁOTA et al. (2012).

The potassium content of whole Welsh onion plants was not significantly affected by the experimental factors. The fertilizer x cultivar interaction had a significant effect on potassium concentrations, which ranged from 13.40 g kg^{-1} d.m. in control plants of cv. Performer to 18.80 g kg^{-1} d.m. in control plants of cv. Parade (Table 3). The observed values are lower than those reported by KOŁOTA et al. (2012) for Welsh onions grown in the Wrocław region. The potassium content in edible parts of Welsh onions was not significantly influenced by a cultivar, fertilizer or their interaction. Welsh onion leaves contained more potassium than scapes (19.00 g kg^{-1} d.m. vs. 13.00 g kg^{-1} d.m. on average, means of 2010-2011).

Differences in the levels of magnesium, calcium and sulphur in Welsh onions were statistically non-significant (Tables 4, 5 and 6). Average magnesium concentrations were as follows: whole plants – 1.000 g kg^{-1} d.m., scapes – 0.900 g kg^{-1} d.m., leaves – 1.100 g kg^{-1} d.m.. The respective average calcium levels were 11.40 g kg^{-1} d.m., 10.30 g kg^{-1} d.m. and 12.30 g kg^{-1} d.m.

Sulphur is a component of volatile compounds such as aliphatic triesters, polysulfides and sulfoxides. Allium vegetables, including onions and garlic, are high in beneficial *sulphur* compounds that give them their distinctive *flavor* and aroma. Sulphur prevents excess accumulation of nitrates (V) and reducing sugars, thus increasing the quality and processing suitability of those vegetables (EL-FAYOUMY, EL-GAMAL 1998). The average sulphur content

Table 2

Phosphorus content in edible parts of Welsh onion plants, subject to cultivar and Polimag S dose (means of 2010-2011), g kg^{-1} d.m.

Fertilization*	Cultivar			
	Long White Iskihura	Parade	Performer	mean
Whole plants				
Control	2.800	2.600	2.700	2.700
50%	3.600	3.800	3.700	3.700
100%	2.500	2.500	2.500	2.500
Mean	3.000	3.000	3.000	3.000
LSD$_{0.05}$ for: cultivar fertilizer interaction	 n.s. 0.1 0.1			
Scapes				
Control	2.000	3.000	2.000	2.000
50%	3.000	4.000	2.000	3.000
100%	2.000	2.000	3.000	2.000
Mean	2.000	3.000	2.000	2.000
LSD$_{0.05}$ for: cultivar fertilizer interaction	 n.s. n.s. n.s.			
Leaves				
Control	3.000	3.400	2.800	3.000
50%	4.000	2.800	3.000	3.200
100%	2.800	2.600	3.000	2.800
Mean	3.200	2.900	2.900	3.000
LSD$_{0.05}$ for: cultivar fertilizer interaction	 n.s. n.s. n.s.			

* Key: see Table 1

of whole Welsh onion plants, scapes and leaves reached 2.300 g kg^{-1} d.m., 1.700 g kg^{-1} d.m. and 2.700 g kg^{-1} d.m., respectively.

According to KOTOWSKA and WYBIERALSKI (1999), the quality of edible plant parts is largely determined by the K:Mg, Ca:Mg and K:(Mg+Ca) ratios. WRÓBEL and MARSKA (1998) and MAJKOWSKA-GADOMSKA and WIERZBICKA (2008) demonstrated that the optimal Ca:Mg ratio should approximate 3, and the Ca:P ratio should be within the 1.200-2.200 range because higher ratios are indicative of nutritional magnesium or phosphorus deficiency. In the present

Table 3

Potassium content in edible parts of Welsh onion plants, subject to cultivar and Polimag S dose (means of 2010-2011), g kg^{-1} d.m.

Fertilization*	Cultivar			
	Long White Iskihura	Parade	Performer	mean
Whole plants				
Control	15.80	18.80	13.40	16.00
50%	18.10	17.70	16.10	17.30
100%	15.70	14.70	16.60	15.70
Mean	16.50	17.10	15.40	16.30
LSD$_{0.05}$ for: cultivar fertilizer interaction	 n.s. n.s. 0.02			
Scapes				
Control	13.00	16.00	10.00	13.00
50%	18.00	19.00	11.00	16.00
100%	11.00	11.00	13.00	12.00
Mean	14.00	15.00	11.00	13.00
LSD$_{0.05}$ for: cultivar fertilizer interaction	 n.s. n.s. n.s.			
Leaves				
Control	18.30	21.90	16.80	19.00
50%	18.30	16.80	20.90	18.60
100%	19.90	18.30	20.60	19.60
Mean	18.80	19.00	19.40	19.00
LSD$_{0.05}$ for: cultivar fertilizer interaction	 n.s. n.s. n.s.			

* Key: see Table 1

Table 4

Magnesium content in edible parts of Welsh onion plants, subject to cultivar and Polimag S dose (means of 2010-2011), g kg^{-1} d.m.

Fertilization*	Cultivar			
	Long White Iskihura	Parade	Performer	mean
Whole plants				
Control	1.000	1.000	0.900	1.000
50%	1.300	1.200	1.000	1.200
100%	0.700	0.800	1.200	0.900
Mean	1.000	1.000	1.000	1.000
LSD$_{0.05}$ for: cultivar fertilizer interaction	 n.s. n.s. n.s.			
Scapes				
Control	1.000	0.900	0.700	0.800
50%	1.300	1.300	0.700	1.100
100%	0.700	0.700	1.100	0.800
Mean	1.000	1.000	0.800	0.900
LSD$_{0.05}$ for: cultivar fertilizer interaction	 n.s. n.s. n.s.			
Leaves				
Control	1.100	1.200	1.100	1.100
50%	1.400	1.100	1.300	1.200
100%	0.800	1.000	1.300	1.000
Mean	1.100	1.100	1.200	1.100
LSD$_{0.05}$ for: cultivar fertilizer interaction	 n.s. n.s. n.s.			

*Key: see Table 1

Table 5

Calcium content in edible parts of Welsh onion plants, subject to cultivar and Polimag S dose (means of 2010-2011), g kg^{-1} d.m.

Fertilization*	Cultivar			
	Long White Iskihura	Parade	Performer	mean
Whole plants				
Control	12.50	10.70	11.30	11.50
50%	12.00	11.70	14.10	12.60
100%	9.400	10.30	10.70	10.10
Mean	11.30	10.90	12.00	11.40
LSD$_{0.05}$ for: cultivar fertilizer interaction	n.s. n.s. n.s.			
Scapes				
Control	15.00	10.00	8.200	11.00
50%	15.80	9.000	12.80	12.50
100%	8.400	8.700	5.600	7.500
Mean	13.00	9.200	8.800	10.30
LSD$_{0.05}$ for: cultivar fertilizer interaction	n.s. n.s. n.s.			
Leaves				
Control	10.00	11.40	14.30	11.90
50%	8.100	14.30	15.30	12.50
100%	10.40	11.80	15.70	12.60
Mean	9.500	12.50	15.10	12.30
LSD$_{0.05}$ for: cultivar fertilizer interaction	n.s. n.s. n.s.			

* Key: see Table 1

Table 6

Sulfur content in edible parts of Welsh onion plants, subject to cultivar and Polimag S dose (means of 2010-2011), g kg^{-1} d.m.

Fertilization*	Cultiver			
	Long White Iskihura	Parade	Performer	mean
Whole plants				
Control	1.900	2.400	2.200	2.200
50%	2.400	2.800	2.400	2.500
100%	2.000	2.000	2.400	2.100
Mean	2.100	2.400	2.300	2.300
LSD$_{0.05}$ for: cultivar fertilizer interaction	n.s. n.s. n.s.			
Scapes				
Control	1.700	1.900	1.700	1.800
50%	1.700	1.800	1.700	1.700
100%	1.600	1.900	1.700	1.700
Mean	1.700	1.900	1.700	1.700
LSD$_{0.05}$ for: cultivar fertilizer interaction	n.s. n.s. n.s.			
Leaves				
Control	2.000	2.800	2.600	2.500
50%	3.100	3.700	3.000	3.300
100%	2.300	2.100	3.100	2.500
Mean	2.500	2.800	2.900	2.700
LSD$_{0.05}$ for: cultivar fertilizer interaction	n.s. n.s. n.s.			

*Key: see Table 1

Table 7

Ca:Mg, Ca:P, K:Mg and K: (Mg+Ca) ratios in Welsh onion plants, subject to cultivar and Polimag S dose (means of 2010-2011)

Fertilization*	Cultivar			
	Long White Iskihura	Parade	Performer	mean
Ca:Mg				
Control	12.50	10.70	12.60	11.50
50%	9.200	9.800	14.10	10.50
100%	13.40	12.90	8.900	11.20
Mean	11.30	10.90	11.80	11.40
Ca:P				
Control	4.500	4.100	4.200	4.300
50%	3.300	3.100	3.800	3.400
100%	3.800	4.100	4.300	4.000
Mean	3.800	3.600	4.000	3.800
K:Mg				
Control	15.80	18.80	14.90	16.00
50%	13.90	14.80	16.10	14.40
100%	22.40	18.40	13.80	17.40
Mean	16.50	17.10	15.40	16.30
K:(Mg+Ca)				
Control	1.200	1.600	1.100	1.300
50%	1.400	1.400	1.100	1.300
100%	1.600	1.300	1.400	1.400
Mean	1.300	1.400	1.200	1.300

*Key: see Table 1

study, the Ca:Mg and Ca:P ratios were inadequate, and ranged from 8.90 to 14.10 and from 3.100 to 4.500, respectively (Table 7).

Wide K:Mg and K:(Mg+Ca) ratios were noted in edible parts of Welsh onion plants. According to Radkowski et al. (1999), optimal K:Mg and K:(Mg+Ca) ratios are 6:1 and 1.600-2.200, respectively. The highest K:Mg ratio was noted in the whole plants of cv. Long White Ishikura, and the lowest was in the leaves of cv. Performer, both fertilized with Polimag S at a 100% dose. The K:(Mg+Ca) ratio was in the 1.100-1.600 range. Polimag S at a 100% dose increased the K:Mg and K(Mg+Ca) ratios (Table 7). Our results are consistent with the findings of Wilk and Rabikowska (1985), and Kulczycki (2006).

CONCLUSIONS

1. Our statistical analysis revealed no significant effect of a cultivar on the macronutrient content of whole Welsh onion plants, scapes and leaves.

2. The analysis of macronutrient levels in whole Welsh onion plants, scapes and leaves showed that leaves were characterized by the highest macronutrient concentrations.

3. Polimag S at a 50% dose contributed to a significant increase in the total nitrogen and phosphorus content of whole plants.

4. Wide Ca:Mg, Ca:P, K:Mg and K:(Mg+Ca) ratios were noted in whole Welsh onion plants.

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NITROGEN FRACTIONS AND AMINO ACID CONTENT IN ALFALFA AND RED CLOVER IMMEDIATELY AFTER CUTTING AND AFTER WILTING IN THE FIELD*

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Abstract

The aim of this study was to determine the composition of nitrogen fractions in alfalfa and red clover, which differ in proteolytic activity, and to evaluate the effect of wilting on changes in nitrogen fractions in alfalfa and red clover herbage. Total nitrogen was divided into protein and non-protein nitrogen, and the amino acid profile of protein was analyzed. Buffer-soluble nitrogen (BSN), including buffer-soluble protein nitrogen (BSPN) and non-protein buffer-soluble nitrogen (NPBSN), was determined. The NPBSN fraction was further subdivided into peptide nitrogen, amino acid nitrogen, neutral detergent-insoluble nitrogen (NDIN) and acid detergent-insoluble nitrogen (ADIN). Wilting in the field to 40% dry matter content (the swath was tedded once) did not reduce the total nitrogen content of alfalfa and red clover herbage, although it affected the concentration of the BSNfraction, in particular NPBSN, and free amino acid nitrogen. During alfalfa wilting, the soluble protein content decreased and the concentrations of non-protein nitrogen compounds increased, mostly due to an increase in free amino acid nitrogen. A reverse trend was observed during red clover wilting – the concentrations of non-protein nitrogen compounds decreased and soluble protein content increased. A decrease was also noted in peptide nitrogen, NDIN and ADIN. Wilting of alfalfa and red clover had no adverse effect on the amino acid profile of protein. The concentrations of essential amino acids that limit milk protein synthesis, i.e. Lys, Met, His and Arg, did not decrease. Legume wilting in the field contributes to an increase in the concentrations of soluble nitrogen in the plant material intended for ensiling.

Key words: alfalfa, red clover, nitrogen fractions, amino acids, wilting.

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ZAWARTOŚĆ FRAKCJI AZOTOWYCH ORAZ AMINOKWASÓW W ZIELONKACH Z LUCERNY I KONICZYNY CZERWONEJ BEZPOŚREDNIO PO SKOSZENIU ORAZ PO PODSUSZENIU NA POKOSACH

Abstrakt

Celem pracy była charakterystyka składu frakcji azotowych w zielonkach z lucerny i koniczyny czerwonej, surowcach roślinnych różniących się potencjałem proteolitycznym, oraz ocena wpływu podsuszania na skład frakcji azotowych w tych zielonkach. Rozdzielono azot ogólny na azot białkowy i azot niebiałkowy oraz określono skład aminokwasowy białka. Ponadto określono związki azotowe rozpuszczalne w buforze (BSN), rozdzielono je na białkowe związki azotowe rozpuszczalne w buforze (BSPN) i niebiałkowe związki azotowe rozpuszczalne w buforze (NPBSN). We frakcji NPBSN wyodrębniono azot peptydowy oraz azot aminokwasowy, wydzielono także związki azotowe o niskiej rozpuszczalności (azot nierozpuszczalny w neutralnym detergencie -NDIN) i całkowicie nierozpuszczalne (azot nierozpuszczalny w kwaśnym detergencie-ADIN). Podsuszenie na pokosach do 40% suchej masy z zastosowaniem jednokrotnego zabiegu przetrząsania nie spowodowało ubytku azotu ogólnego w zielonkach z lucerny i koniczyny czerwonej, natomiast wpłynęło na koncentrację całej frakcji rozpuszczalnej w buforze, szczególnie jej części niebiałkowej, oraz azotu w formie wolnych aminokwasów. W czasie podsuszania zielonki z lucerny malał udział białek rozpuszczalnych, natomiast wzrastał udział związków niebiałkowych, przede wszystkim za sprawą wzrostu azotu wolnych aminokwasów. W koniczynie czerwonej w trakcie podsuszania – odwrotnie niż w lucernie – malała frakcja związków niebiałkowych, natomiast wzrastała frakcja łatwo rozpuszczalnych białek, zaobserwowano wyraźny ubytek azotu w formie peptydowej oraz frakcji trudno rozpuszczalnych i nierozpuszczalnych. Podsuszenie zielonek z lucerny i koniczyny czerwonej nie pogorszyło profilu aminokwasowego białka. Nie stwierdzono ubytku aminokwasów egzogennych limitujących syntezę białka mleka, tj: Lys, Met, His, Arg. Zabieg podsuszania zielonek z motylkowatych na pokosach wpływa na zwiększenie udziału form rozpuszczalnych azotu w surowcu przed zakiszaniem.

Słowa kluczowe: lucerna, koniczyna czerwona, frakcje azotowe, aminokwasy, podsuszanie.

INTRODUCTION

Due to high prices of protein supplements, in particular soybean meal, recent years have witnessed a revival of interest in high-protein roughages such as legume herbage and silage. Alfalfa and red clover are most widely used fodder legumes in the moderate climate zone (ŻUK-GOŁASZEWSKA et al. 2010). Legumes have a high total protein content, a low water-soluble carbohydrate (WSC) content and high buffering capacity, which makes them difficult to ensile (PURWIN et al. 2011). Alfalfa and red clover need to be wilted before ensiling to improve silage quality. Water loss during wilting contributes to an increase in the osmotic pressure in plant cells. As a result, water contained in cells is unavailable to clostridia, thus reducing the risk of their growth, whereas a higher sorption capacity supports the proliferation of lactic acid bacteria. Wilting encourages lactic acid fermentation, minimizes proteolysis and inhibits the growth of butyric acid bacteria responsible for secondary fermentation and silage quality deterioration (DAWSON et al. 1999). Proteolysis begins in plant cells after cutting. The percentage of protein that is hydrolyzed during ensiling is determined by the proteolytic potential, i.e.

protease activity and substrate availability. Alfalfa has the highest proteolytic potential. Red clover contains high levels of polyphenol oxidase (PPO), which protects protein against proteolysis. Studies on the technology of bale silage production, conducted to date, have focused on the effect of herbage wilting and chemical additives on the rate of fermentation, aerobic stability and microbiological quality of silage. The effect of wilting on nitrogen fractions and amino acids in forages has been analyzed under laboratory conditions (PAPADOPOULOS, MCKERSIE 1983, MEESMAN et al. 1994, GUO et al. 2008). CAVALLARIN et al. (2005) investigated the effect of wilting in the field on the composition of nitrogen fractions and amino acid profile of sainfoin. ARRIGO (2006) studied the effect of wilting on the amino acid profile of mixtures of different grasses and grass and clover. However, little attention has been paid to the effect of wilting on changes in the composition of nitrogen fractions and amino acid in legumes.

The aim of this study was to determine the composition of nitrogen fractions in alfalfa and red clover, which differ in proteolytic activity, and to evaluate the effect of wilting on changes in nitrogen fractions in alfalfa and red clover herbage. Total nitrogen was divided into protein nitrogen and non-protein nitrogen. McDougall's buffer (simulating ruminant saliva) was used to determine buffer-soluble nitrogen (BSN), including buffer-soluble protein nitrogen (BSPN) and non-protein buffer-soluble nitrogen (NPBSN). The NPBSN fraction was further subdivided into peptide nitrogen, amino acid nitrogen, neutral detergent-insoluble nitrogen (NDIN) and acid detergent-insoluble nitrogen (ADIN).

MATERIAL AND METHODS

The study was conducted in 2009 at the Agricultural Experiment Station of the University of Warmia and Mazury in Olsztyn, located in Łężany. The experimental plant materials comprised the first cut herbage of alfalfa (cv. Alba) and red clover (cv. Nike) in the second year of growing. Nitrogen top-dressing was not applied, and phosphorus and potassium were applied at 80 kg P_2O_5 and 100 kg K_2O per ha. Alfalfa and red clover were harvested at the beginning of flowering. Primary herbage samples were collected at four locations within each field, from the complete depth of the swath, along the length of 0.3 m, and were combined to obtain bulk/consolidated samples. Average samples were taken from each location (4 n). Samples were collected immediately after cutting and after 36-hour wilting (the swath was tedded once), each time from the same part of the field. In the field, samples were placed in liquid nitrogen containers and were stored at -25°C. PPO activity was determined in selected samples immediately after thawing. The remaining samples were dried at 40°C using Binder FED 115 drying ovens with forced convection, and were ground in a Retsch SK 100 cross beater mill to the particle size of 1 mm. Changes in the following nitrogen fractions during ensiling were determined: protein nitrogen, buffer-soluble nitrogen (BSN), including buffer-soluble protein nitrogen (BSPN), peptide nitrogen, amino acid nitrogen,ammonium nitrogen, neutral detergent-insoluble nitrogen (NDIN) and acid detergent-insoluble nitrogen (ADIN).

Immediately after cutting and after wilting, samples were assayed for: proximate chemical composition – by standard methods (AOAC, 2005), WSC – by the anthrone method (Thomas 1977), buffering capacity – as described by McDonald and Henderson (1962), NDF, ADF and ADL – by the method proposed by Van Soest et al. (1991), PPO activity – according to Halasińska et al. (2001), protein nitrogen – with the use of trichloroacetic acid (TCA), as described by Guo et al. (2008), BSN – using McDougall's buffer, according to the procedure of Hedqvist and Uden (2006). Fodder samples (1.6 g) were mixed with 40 ml of McDougall's buffer (adjusted to pH 6.8), and were incubated for one hour at 39°C in a water bath shaker. Samples were centrifuged at 3000 g^{-1} for 10 minutes. The protein nitrogen content of the supernatant was determined by the Kjeldahl method. The BSN fraction was subdivided into BSPN, peptide nitrogen, amino acid nitrogen and ammonia nitrogen. To determine BSPN, 20 ml BSN extract was mixed with 2 ml of TCA, and the mixture was incubated for one hour in ice bath at 2-3°C. Samples were centrifuged at 5000 g^{-1} for 25 minutes at 4°C in a fixed-angle rotor centrifuge. The supernatant was analyzed using the same procedure as described above for BSN. BSPN was calculated as the difference between BSN and TCA-soluble nitrogen. Peptide nitrogen was calculated as the difference between alpha-amino nitrogen in hydrolyzed TCA-soluble nitrogen and free amino acid nitrogen (Hedqvist, Uden 2006). Amino acid nitrogen was measured as total free amino acids determined in samples deproteinized with TCA, using an amino acid analyzer equipped with a lithium column (Winters et al. 2002). The amino acid content of hydrolyzates was determined with the use of the AAA 400 INGOS amino acid analyzer with a sodium column. The tryptophan content was determined according to Standard PN-EN ISO 13904: 2005.

The results were processed statistically by two-way ANOVA. A model describing chemical composition, buffer capacity, PPO activity, composition of nitrogen fractionsof herbageand changes in amino acid concentrations accounted for the effects of legume species, wilting and the species x wilting interaction. The significance of differences between means (species and wilting groups) was estimated by the Duncan's test.

RESULTS AND DISCUSSION

The effect of forage species and wilting on the chemical composition of alfalfa and red clover herbage is presented in Table 1. Significant ($p < 0.01$) differences were found between alfalfa and red clover in the content of dry

Table 1

Chemical composition (g·kg^{-1} d.m.), buffer capacity (ml kg^{-1} d.m.), PPO activity (J kg^{-1} d.m.) of herbage

Item	Alfalfa		Red clover		SEM	Treatment		SxW
	fresh	wilted	fresh	wilted		S	W	
Dry matter (g kg^{-1})	188	429	204	423	7.13	**	**	ns
Crude ash	70.8	78.6	65.6	86.1	2.70	**	**	ns
NDF	443	462	438	456	9.01	**	*	ns
ADF	376	383	350	378	4.17	**	*	ns
ADL	82.4	86.8	62.0	69.2	5.78	**	**	ns
WSC	70.3	58.9	120	102	2.86	**	**	ns
Buffer capacity	12.8	10,3	8.26	7.46	0.59	**	**	ns
PPO activity	82	11	185	56	15.8	**	**	**

S – species; W– wilting; SEM – standard error of the mean; ns – not significant; ** significant at $P < 0.01$; * significant at $P < 0.05$

matter, crude ash, structural and water-soluble carbohydrates, buffering capacity and PPO activity. Our findings confirm the results of previous research (Purwin et al. 2010) showing that alfalfa is characterized by lower concentrations of water-soluble carbohydrates, higher buffering capacity and a faster rate of lignification, compared with red clover. In both legume species, wilting contributed to a decrease in WSC levels and buffering capacity ($p < 0.01$), and an increase in the content of crude ash, NDF, ADF ($p < 0.05$), ADL ($p < 0.01$) and PPO activity ($p < 0.01$). The increase in the content of crude ash and structural carbohydrates, observed during wilting, is relative and results from WSC loss during respiration processes (Muck 1988). The buffering capacity decreased due to reduced intracellular activity, in particular reduced concentrations of short-chain fatty acids in the cytoplasm (Krebs cycle); the buffering capacity of short-chain fatty acids is substantially higher than that of protein (McDonald et al. 1991). The decrease in PPO activity was related to changes in osmolarity in the cells of alfalfa and red clover plants (Givens, Rulquin 2004).

The total nitrogen content of herbage dry matter was higher ($p < 0.01$) in alfalfa than in red clover (Table 2). Wilting had no influence on the total nitrogen content, which points to a minimal mechanical loss during tedding and raking (Slottner, Bertilsson 2006). Protein nitrogen had a higher ($p < 0.05$) share of total nitrogen in fresh and wilted red clover (765 and 695 g kg^{-1} total nitrogen) than in alfalfa (702 and 642 g kg^{-1} total nitrogen). Irrespective of forage species, wilting decreased (by *ca* 9%) the protein/total nitrogen ratio in comparison with green herbage ($p < 0.01$). In a study by Papadopoulos and McKersie (1983), wilting of the first cut herbage of alfalfa and red clover for 6 and 24 hours led to a decrease in the protein nitrogen

Table 2

Composition of nitrogen fractions (g kg^{-1} total nitrogen) of herbage

Item	Alfalfa		Red clover		SEM	Treatment		SxW
	fresh	wilted	fresh	wilted		S	W	
Total nitrogen (g kg^{-1} d.m.)	29.6	28.0	26.5	26.5	0.37	**	ns	ns
Protein nitrogen	702	642	765	695	20.1	*	**	ns
BSN	272	255	180	178	3.19	**	**	**
NPBSN	109	143	123	114	3.59	**	**	**
BSPN	163	112	56.7	64.2	4.18	**	ns	**
Peptide N	84.1	83.0	87.4	67.7	4.47	**	ns	**
Amino acid N	24.9	60.1	35.6	46.6	1.63	**	**	**
NDIN	96.1	97.4	107	90.4	2.62	**	ns	**
ADIN	68.6	69.6	76.8	64.6	1.87	**	ns	**

BSN – buffer soluble nitrogen; NPBSN – non protein buffer soluble nitrogen; BSPN – buffer soluble protein nitrogen; NDIN – neutral detergent insoluble nitrogen; ADIN – acid detergent insoluble nitrogen; S – species; W – wilting; SEM – standard error of the mean; ns – not significant; ** significant at $P < 0.01$; * significant at $P < 0.05$

content, from 915 to 836 and 748 g kg^{-1} of total nitrogen in alfalfa (a decrease by 8.8% and 18.3%, respectively), and from 959 to 924 and 889 g kg^{-1} total nitrogen in red clover (a decrease by 3.7% and 7.3%, respectively). The cited authors found that forage species and wilting had a significant effect on the protein/total nitrogen ratio. Guo et al. (2008) reported that the wilting of alfalfa forage for 10 hours, from the dry mater content of 230 to 330 g kg^{-1}, decreased the protein nitrogen content by 2.47% (from 850 to 829 g kg^{-1} of total nitrogen). Considerable differences in protein nitrogen levels in fresh and wilted herbage, noted in the above study, could have resulted from different rates of water loss during wilting, determined by environmental conditions (temperature, humidity, air flow) and by the properties of plant material (percentage shares of leaves and stems, which differ among species, and the growth stage at which the plants were harvested). Cavallarin et al. (2005) demonstrated that mechanical conditions may limit proteolysis and catabolism of essential amino acids by reducing field wilting time. The content of BSN and its fractions and the concentrations of NDIN and ADIN varied between the analyzed legume species ($p < 0.01$).

The wilting of alfalfa and red clover herbage affected the concentration of the BSN fraction, and in particular NPBSN, as well as free amino acid nitrogen.The wilting process had no effect on the content of soluble proteins, NDIN and ADIN. The legume species x wilting interaction ($p < 0.01$) was noted with respect to all soluble and insoluble nitrogen fractions (except for protein nitrogen), which indicates that the nitrogen compounds contained in the analyzed forage species differed in their response to wilting. During alfalfa wilting, the content of all soluble nitrogen fractions (BSN) decreased, including soluble proteins (by 32%), whereas the concentrations of non-protein nitrogen compounds increased (by 30%), mostly due to an increase in free amino acid nitrogen (by 140%), and peptide nitrogen levels remained unchanged. The content of ADIN and NDIN in total nitrogen did not change during alfalfa wilting. During red clover wilting, the BSN content did not change significantly and, unlike in alfalfa, the concentrations of non-protein nitrogen compounds decreased (by 7%) and soluble protein content increased (by 13%). A significant increase in free amino acid nitrogen (by 31%) noted in alfalfa was not reported in red clover, in which a substantial peptide nitrogen loss (by 23%) was observed. The NDIN and ADIN concentrations decreased in wilted red clover by 15% and 16%, respectively.

In a study by Guo et al. (2008), the wilting of alfalfa herbage for 10 hours, from the dry matter content of 230 to 330 g kg^{-1}, decreased the protein nitrogen content by 2.47%, and increased the non-protein nitrogen content: N-NH$_3$ by 22.5% (from 2.8 to 3.43 g kg^{-1}total nitrogen), N-AA by 2.7% (from 66.5 to 68.3 g kg^{-1} total nitrogen), peptide nitrogen by 23.8% (from 80 to 99 g kg^{-1} total nitrogen). The cited authors noted a 3% decrease in buffer-soluble protein (from 570 to 554 g kg^{-1} of total nitrogen), a 7% increase in protein fraction B$_2$ (from 135 to 145 g kg^{-1} of total nitrogen), and a decrease in insoluble nitrogen (from 125 to 103 g kg^{-1} of total nitrogen); the ADIN content increased, whereas the NDIN content decreased.

Wilting and forage species had no influence on total amino acids per 100g protein (Table 3). Legume species affected the levels of Thr, Ser, Pro, Cys, Met, Lys ($p < 0.01$), Ala, Val, Trp and His ($p < 0.05$). Alfalfa protein contained higher concentrations of Pro, Cys, Met, Trp and Lys, whereas red clover protein had a higher content of Thr, Ser, Ala, Val, Tyr and Hys. Wilting in the field increased the levels of Pro, Trp ($p < 0.01$) and His ($p < 0.05$), but decreased the Glu content ($p < 0.05$) in both forage species. The concentrations of Thr and Ser increased ($p < 0.01$) in wilted alfalfa and decreased in wilted red clover. The Cys content decreased ($p < 0.01$) in alfalfa protein and increased in red clover protein. The varied effects of wilting on amino acids in red clover and alfalfa protein were confirmed by significant ($p < 0.01$) interactions with regard to Thr, Ser, Pro, Val, Cys, Met, Trp ($p < 0.01$), Leu and Phe ($p < 0.05$).

In an experiment carried out by Winters et al. (2000), percentages of total amino acid nitrogen and free amino acid nitrogen in total nitrogen in the green herbage of hybrid ryegrass (perennial ryegrass x Italian ryegrass) were 78.6% and 4.34%, respectively, and after two days of ensiling under natural moisture conditions the respective values reached 74.6% and 8.28%. In the present study, the increase in free amino acid nitrogen noted during wilting over similar periods of time (from 2.49% to 6.01% total nitrogen in alfalfa, and from 3.56% to 4.66% total nitrogen in red clover) indicates a hi-

Table 3

Composition of alfalfa and red clover protein (g 100 g^{-1} crude protein)

Amino acid	Alfalfa		Red clover		SEM	Treatment		
	fresh	wilted	fresh	wilted		S	W	SxW
Asp	14.6	12.88	15.08	14.17	0.385	ns	ns	ns
Thr	1.71	3.73	3.61	3.56	0.259	**	**	**
Ser	1.59	3.88	4.23	3.93	0.328	**	**	**
Glu	10.16	6.83	11.03	6.97	0.194	ns	*	ns
Pro	5.71	7.77	5.70	6.53	0.264	**	**	**
Gly	3.62	4.21	4.17	4.31	0.102	ns	*	ns
Ala	4.19	4.49	4.62	4.75	0.089	*	ns	ns
Val	4.10	5.02	5.35	4.83	0.158	*	ns	**
Cys	1.82	1.76	0.86	1.29	0.119	**	**	**
Met	1.93	1.52	0.89	1.43	0.115	**	ns	**
Ile	4.83	3.82	4.60	3.99	0.082	ns	ns	ns
Leu	6.86	6.72	5.71	7.19	0.220	ns	ns	*
Tyr	3.50	3.57	4.05	5.57	0.343	*	ns	ns
Phe	3.88	3.71	3.34	4.75	0.201	ns	ns	*
Trp	1.33	1.37	0.94	1.58	0.071	*	**	**
Lys	4.75	4.93	3.71	3.94	0.189	**	ns	ns
His	3.06	3.17	3.22	3.58	0.069	*	*	ns
Arg	4.40	3.65	3.74	3.52	0.152	ns	ns	ns
Total	82.04	83.03	84.85	85.89	15.6	ns	ns	ns

S – species; W – wilting; SEM – standard error of the mean; ns – not significant; ** significant at $P < 0.01$; * significant at $P<0.05$

gher proteolytic potential of alfalfa and more effective proteolysis-inhibiting mechanism in red clover. ARRIGO (2006) compared different methods of grass and clover mixture conservation, reporting the highest deviation from the initial total amino acid content in the protein of field-dried forage. According to the cited author, a decrease in amino acid levels was due to leaf loss during the mechanical treatment (cutting and tedding repeated four times). Amino acid loss in the dry matter of field-dried forage, compared with fresh forage, reached 14% and 7% in forage harvested at the early and late stage of maturity, respectively (p = 0.99).

In our study, changes in amino acid concentrations did not result from changes in the protein content of leaves and stems caused by mechanical loss, as confirmed by the absence of differences in the total nitrogen content. The changes were most likely due to protein catabolism that occurs in plant cells until the dry matter content reaches 40% (JARRIGE et al., 1995).

Guo et al. (2008) reported that the concentrations of essential amino acids were higher ($p < 0.05$) in wilted alfalfa than in fresh alfalfa herbage. In a study of Cavallarin et al. (2005), the total amino acid content of sainfoin in herbage was not significantly affected by wilting and mechanical conditioning. Significant differences were observed in the levels of individual amino acids – wilting caused an increase in the concentrations of Asp and Pro and a decrease in the content of Leu, Lys and Arg. Arrigo (2006) demonstrated that Lys was most susceptible to degradation, in particular in forages harvested at early stages of maturity; three methods of drying, i.e. control drying, drying in the barn and drying in the field, caused Lys degradation in 19%, 28% and 32%, respectively. Met, Phe and Leu were most resistant to changes during the drying. In our study, the Pro content increased, whereas the levels of Asp, Lys and Arg remained unchanged. The observed increase in Pro and decrease in Glu could have resulted from the fact that Pro is synthesized by transamination of ornithine or reduction of glutamic acid in the presence of alpha-Ketoglutaric acid. An increase in Pro and Leu as well as a decrease in Arg were also reported by Ashbell et al. (1986) and Seyfarth et al. (1989), as cited in Cavallarinet al. (2005). According to the above authors, amino acid (in particular Lys) losses are largely determined by the wilting time and temperature. Arrigo (2006) noted the highest decrease in Lys during the drying. In our experiment, the Lys content did not decrease during the wilting, most likely because the swath was tedded only once, at a relatively high moisture content of the herbage. This suggests that the Lys loss results mostly from the leaf protein loss. In addition, in our study, the sward was cut at the beginning of flowering. According to Arrigo (2006), forages harvested at late stages of maturity are characterized by smaller amounts of leaves and a slightly increased initial amino acid content in dry matter; they also dry at a faster rate and, consequently, are less prone to proteolysis.

CONCLUSION

Wilting in the field to the 40% dry matter content (the swath was tedded once) did not reduce the total nitrogen content of alfalfa and red clover herbage, but significant changes in the composition of nitrogen fractions were observed between cutting and ensiling. Wilting affected the concentration of the BSN fraction, in particular NPBSN, and free amino acid nitrogen. The nitrogen fractions of the analyzed forage species differed in their response to wilting. During alfalfa wilting, the soluble protein content decreased and the concentrations of non-protein nitrogen compounds increased, mostly due to an increase in free amino acid nitrogen. A reverse trend was observed during red clover wilting, in which the concentrations of non-protein nitrogen compounds decreased and soluble protein content increased. A significant increase in free amino acid nitrogen was not reported, while a peptide nitrogen loss was observed. NDIN and ADIN concentrations decreased only in red clover.The wilting of alfalfa and red clover increased the ratio of peptide-bound to free amino acids, but had no adverse effect on the amino acid profile of protein. The concentrations of essential amino acids that limit milk protein synthesis, i.e. Lys, Met, His and Arg, did not decrease.

Legume wilting in the field contributes to an increase in the concentrations of soluble nitrogen in the plant material intended for ensiling. However, due to an increase in the osmolarity within plant cells and a decrease in buffering capacity, it could restrain changes in nitrogen fractions during the ensiling process.

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CHANGES IN SULPHUR DIOXIDE CONCENTRATIONS IN THE ATMOSPHERIC AIR ASSESSED DURING SHORT-TERM MEASUREMENTS IN THE VICINITY OF OLSZTYN, POLAND

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Abstract

Sulphur dioxide is one of the principal gases responsible for the quality of atmospheric air. Air pollution, even relatively low one, is not indifferent to human health. In 2007-2009, an investigation was carried out to follow changes in the concentration of sulphur dioxide in atmospheric air, in different time intervals, depending on the air temperature and humidity. This paper draws on results of continuous measurements taken at the Station of Monitoring the Immission of Air Pollutants in Olsztyn-Kortowo. The measured concentrations of SO_2 were presented graphically as means for different time intervals. It has been demonstrated that changes in SO_2 concentrations were mainly induced by drops in temperatures, emissions and other industrial actitivities, as well as the course of the growth and development of plants. The highest concentrations appeared at noon and the lowest ones were at night. Analysis of similarities verified hourly variations in the SO_2 concentrations in air. Four distinc groups were distinguished: nocturnal (1:00-7:00 a.m.), mid-day (10:00 a.m.-3:00 p.m.), a group with two time intervals (8:00-9:00 a.m.; 4:00-7:00 p.m.) and late evening (8:00 p.m. - midnight). Considering the aerial concentration levels of this gas in monthly intervals, high similarity was observed between the early spring versus the autumn and winter seasons. Dependences between SO_2 concentrations and temperature were confirmed by analysis of the correlation coefficients and linear regression equations. Likewise, relationships between SO_2 concentrations and air humidity were analyzed in different time intervals and presented both graphically and statistically, by computing correlations and regression equations. Irrespective of the source of emission and meteorological con-

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ditions, the SO_2 concentration remained on a low level and did not exceed the permissible threshold either in the vicinity of Olsztyn or in the whole region of Warmia and Mazury.

Key words: SO_2, hourly, daily, monthly and annual cycles, temperature, humidity.

ZMIANY STĘŻENIA DWUTLENKU SIARKI W POWIETRZU ATMOSFERYCZNYM W POMIARACH KRÓTKOOKRESOWYCH W REJONIE OLSZTYNA

Abstrakt

Dwutlenek siarki jest jednym z ważniejszych gazów odpowiedzialnych, za jakość powietrza atmosferycznego. Zanieczyszczenia powietrza nawet na stosunkowo niskim poziomie nie są obojętne dla zdrowia człowieka. W latach 2007-2009 badano zmiany stężenia dwutlenku siarki w powietrzu atmosferycznym – w różnych przedziałach czasowych – w zależności od temperatury i wilgotności powietrza. W pracy wykorzystano wyniki z pomiarów ciągłych Stacji Monitoringu Imisji Zanieczyszczeń Powietrza w Olsztynie-Kortowie. Określone stężenia SO_2 przedstawiono graficznie jako średnią z różnych okresów pomiarowych. Wykazano, że na zmiany stężenia wpływały głównie spadki temperatury, działania emisyjne i gospodarcze oraz wynikające z przebiegu wegetacji roślin. Najwyższe wartości stężeń stwierdzono w godzinach południowych, najniższe w nocnych. Analiza podobieństw potwierdziła zróżnicowanie godzinowe stężenia SO_2 w powietrzu. Wydzielono wyraźnie 4 grupy: nocną (1:00-7:00), okołopołudniową (10:00-15:00), grupę w 2 przedziałach czasowych (8:00-9:00; 16:00-19:00) oraz wieczorną (20:00-24:00). Rozpatrując stężenie tego gazu w powietrzu w zależności od stężenia miesięcznego, znaczne podobieństwo wykazano w okresie wiosenno-letnim oraz jesienno-zimowym. Zależności między stężeniem SO_2 a temperaturą potwierdzono, dyskusyjnie, współczynnikami korelacji i równaniami regresji liniowej. Podobnie, zależności między stężeniem SO_2 a wilgotnością powietrza analizowano w różnym przedziale czasowym w ujęciach graficznym, a także statystycznym, wykazując związki korelacyjne i równania regresji. Niezależnie od źródeł emisji i warunków meteorologicznych, stężenie SO_2 utrzymywało się na niskim poziomie i nie przekraczało dopuszczalnych norm w rejonie Olsztyna i na całym obszarze Warmii i Mazur.

Słowa kluczowe: SO_2, cykle godzinowe, dobowe, miesięczne, roczne, temperatura, wilgotność.

INTRODUCTION

Sulphur dioxide is a widespread atmospheric air contaminant, emitted from a variety of sources, mainly from industrial plants (KOPER, SIWIK-ZIOMEK 2005). Sulphur compounds, according to KOMARNISKY et al. (2003), are among the most ubiquitous atmospheric pollutants, playing a crucial role in the chemistry of the Earth's atmosphere. For example, they strongly affect the climate and weather (CHARLSON et al. 1992, ROBOCK 2000). Large amounts of contaminants reach the troposphere from natural and anthropogenic sources (SPEIDEL et al. 2007). The volume and range of variation of actual concentrations of pollutants are strongly dependent on the current meteorological conditions, including ambient temperature (ROGALSKI, LENART 2011), and on changes occurring in the atmopshere (CZARNECKA et al. 2007, YOUNG-MIN et al. 2002). Larger quantities of sulphur dioxide in wintertime together with its weaker transformation into other sulphur compounds are responsible for a higher fluctuation in the concentrations of SO_2.

Sulphur dioxide in the air is responsible for interfering the proper functions of a healthy human (Atkinson et al. 2001, Tunnicliffe 2001). The noxious character of this compound intensifies as the SO_2 concentration in the air goes up (Ibald-Mulli et al. 2001, Sarnat 2005, Peden, Reed 2010). The atmopsheric concentration of sulphur dioxide also differentiates phytotoxic effects of the compound on plants and in soil, in which both excess and deficit of sulphur ought to be avoided (Rogalski 2006, Skwierawska et al. 2014). The mechanism through which SO_2 spreads in the air is very complex. Emission from point sources depends on such factors as climatic conditions, land relief, height and construction of chimneys, land development, etc. Thus, research and observations of SO_2 distribution must be carried out continuously and meticuously (Young-Min et al. 2002, Rogalski, Lenart 2004).

Considering the versatility of deletrious effects produced by sulphur dioxide in the environment, this study has have been undertaken to demonstrate changes in concentrations of this compound measured in several time intervals, whose momentary fluctuations resulted in specific effects.

MATERIAL AND METHODS

In 2007-2009, observations were conducted on the variation in sulphur dioxide concentrations in the air, which were based on results of measurements taken at the Station of Monitoring the Immission of Air Pollutants, managed by the Chair of Air Protection and Environment Toxicology, at the University of Warmia and Mazury in Olsztyn. The station is situated in the southern outskirts of Olsztyn, on Kortowskie Lake, at a site located at the following geographical coordinates φ = 53°27′N and λ = 20°27′E, 104 m above sea level. The station lies about 600 m from the largest housing estates of Olsztyn, a town with the population of 174,941 (www.polskainfo.pl). There are some industrial facilities in the near vicinity of the station, about 1000 m away, including a petrol station and a municipal thermal power plant. At a distance of 400 m to 600 m from the station there is a dual carriageway and some town buildings. With this localization it was possible to measure concentrations of the air contamination with sulphur dioxide in the context of spatial impact. The localization of the station and the collection methods comply with the requirements set in the Regulation of the Minister of the Environment (2002). The air sucked in at the height of 4 m above the ground level is directed into an air-conditioned contains an immission analyzers.

The concentration of SO_2 was measured by an MLU 100E fluorescence analyzer. The data were subjected to microprocessing, which generated the results as means from the measurements. The information collected at the station was transmitted at one-hour intervals to the main computer, where it was stored and processed in a CS 5 system. The results of measurements underwent statistical elaboration with Statistica v. 10, while the classifica-

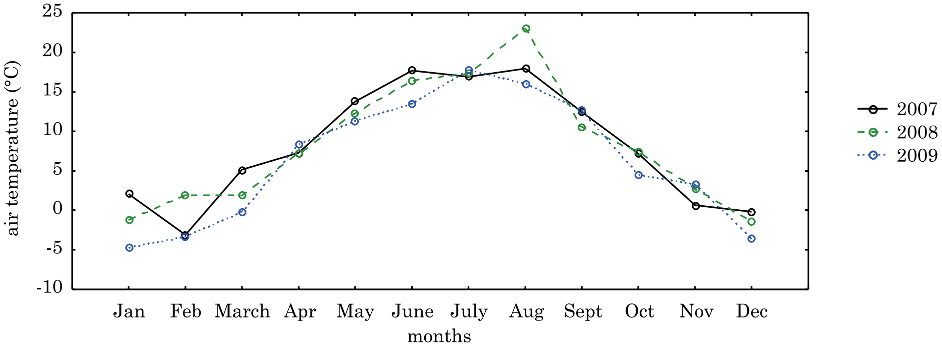


Fig. 1. Mean monthly air temperatures at the monitoring station in Olsztyn-Kortowo in 2007-2009

tion and principal component analyses were performed with the MVPS v.3.1 programme. Relationships between the air temperature and relative humidity versus concentrations of sulphur dioxide in the air were uidentified by the Spearman's rank correlations (rs) and Pearson's linear correlation at $p = 0.01$ (Gołaszewski et al. 2003). Parallel to the analysis of sulphur dioxide concentrations, the air temperature was measured using a MICROS STEP air temperature sensor, set in the range of -50°C to +80°C and at sensitivity of 0.01. The air humidity was determined with a MICROS SRH humidity sensor, composed of a plate with a variable absorbtion coefficient.

The average air temperature for the whole period of observations was 6.92°C, although it was higher in 2007 and in 2008, when it reached 8.5°C and 7.28°C, respectively, with an exceptionally warm winter in 2008 (Figure 1). In that year, the air temperature dropped below 0°C only in January and December, when it was still four-fold higher than in the analogous time in the previous year. An indicator suggesting high variability of the weather condtions in the analyzed winter seasons was the number of days with sub-zero temperatures. In 2007 and 2008, the number of such days was similar: 59 and 53, respectively. The year 2009 was the coldest, with the average temperature of 6.31°C, and freezing temperatures persisting until April. January 2009 was an extremely cold month, with the monthly mean temperature of -4.76°C and the lowest temperature of -20.7°C. There were 92 days with freezing temperatures throughout the winter season.

During the research period, the air humidity remained on a high level (above 85%), peaking to 93.7% in 2008, being slightly lower in 2007 (87.9%) and declining to 80% in 2009 (Figure 2). It should be added that the measured relative air humidity was characterized by a considerably large number of extreme results.

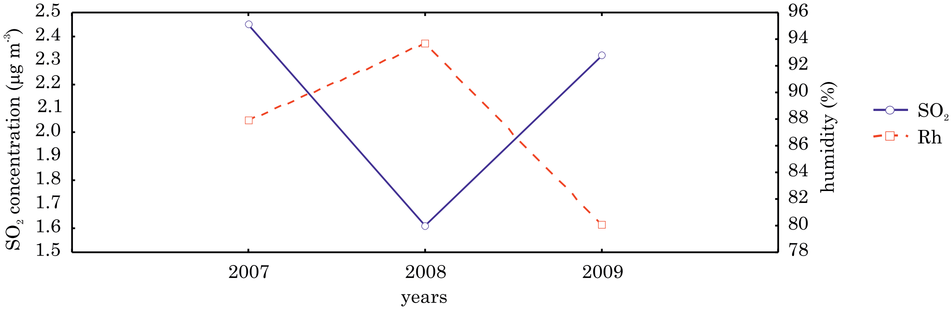


Fig. 2. Relationships between SO$_2$ concentration and relative humidity in 2007-2009

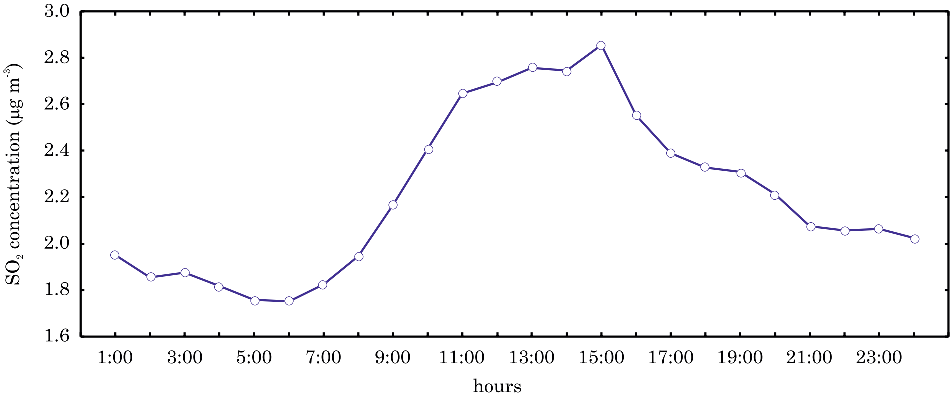


Fig. 3. Daily courses of mean one-hour concentrations of sulphur dioxide in the air in Olsztyn-Kortowo in 2007-2009

RESULTS AND DISCUSSION

Different emission sources and volumes of emitted pollutants, mostly affected by anthropogenic factors, constantly modify concentrations of sulphur dioxide in the atmospheric air. Conditions like these were also responsible for the aerial levels of SO$_2$ in and around Olsztyn-Kortowo (Figure 3). There were distinct differences during the day, e.g. lower and more stable concentrations at night hours, higher concentrations in the afternoon hours, with the peak around 3.00 p.m. followed by decreasing levels in the late afternoon until late at night. The meteorological conditions played an important part. Other significant factors included anthropogenic conditions such as increasing economic activity, traffic flow, heating of houses, etc. A similar course of daily fluctuations was reported by ANDRZEJEWSKA, OLSZEWSKI (2008) and PEREZ (2001). Considering daily mean values measured in individual months and years, the achieved variation of SO$_2$ concentrations was closer to the actual state owing to a smaller number of averaged values. The mean daily concentration of sulphur dioxide in the air was low (2.34 μg m^{-3}), but the maximum value reached 9.02 μg m^3. However, daily values in individual

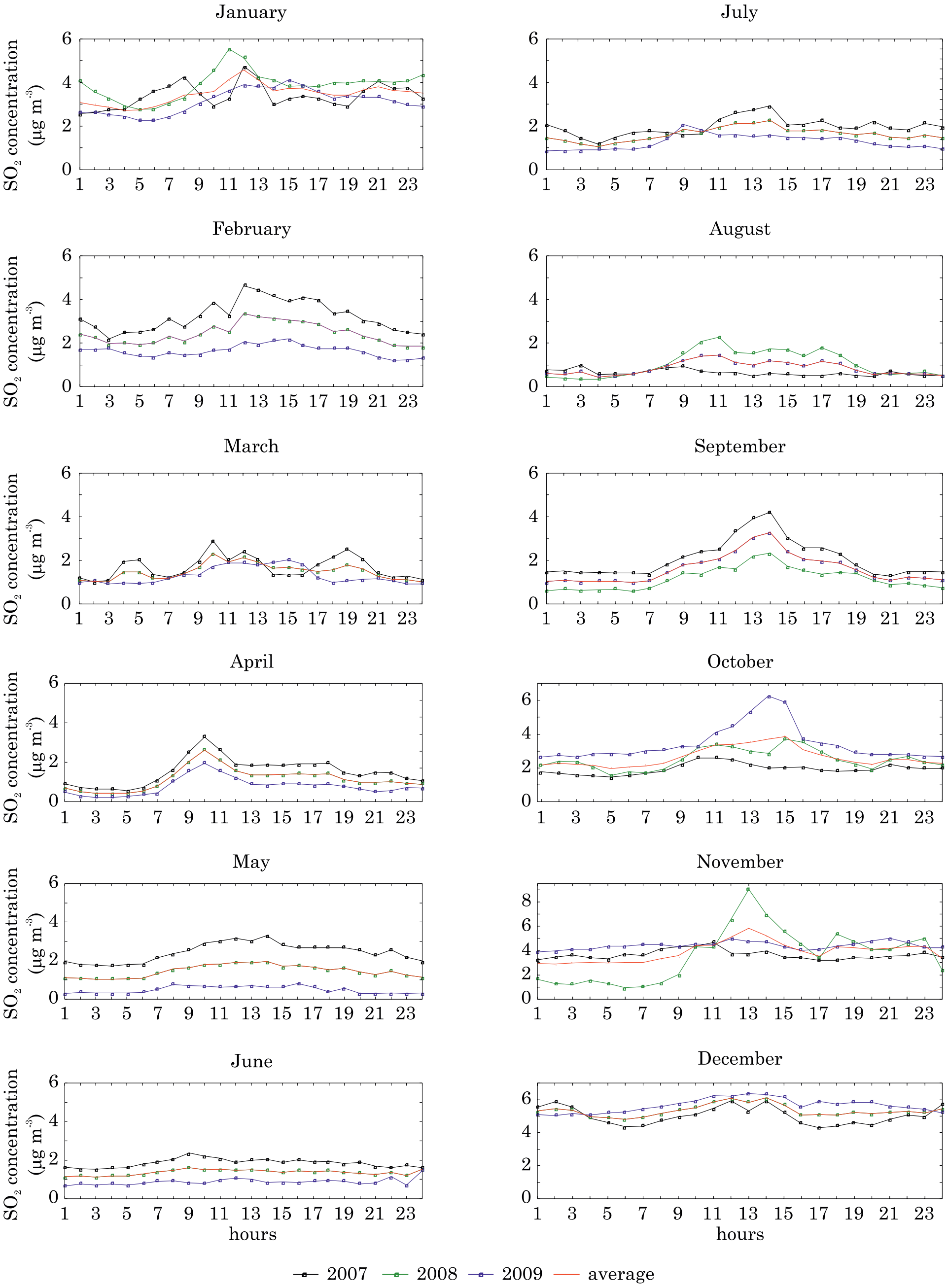


Fig. 4. Daily courses of mean one-hour SO$_2$ concentrations in the air in Olsztyn-Kortowo in individual months during the years 2007-2009

months were varied (Figure 4). The graphic presentations of changes in the SO_2 air concentrations, shown hour by hour, imply considerable shifts in daily sets. In 2007, larger dispersion of concentrations appeared in winter months, especially in January, February and March, with the peak concentrations between 9 a.m. and 1 p.m., and in December during night and afternoon hours. Similar variations occurred in 2008, with the maximum values determined in November and December. In the coldest year, 2009, the highest concentrations of SO_2 in air began to be noted as early as October and continued until December, mostly in afternoons. The course of daily fluctuations in SO_2 concentrations, presented by total values gathered during the research, support our previous observations that SO_2 concentrations fluctuated more in 2007 than in 2008-2009. The summarized data give us a complete picture of greater variability of sulphur dioxide concentrations in winter than in summer.

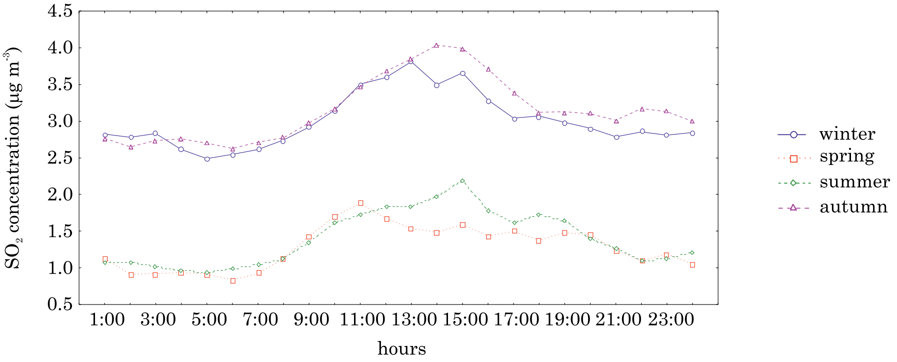


Fig. 5. Averaged courses of mean one-hour concentrations of sulphur dioxide in seasons of the year during the years in Olsztyn-Kortowo

When analyzing SO_2 concentrations in the seasons of the year (Figure 5), it was demonstrated that daily fluctuations in winter and autumn were characterized by an approximately same course of curves, and that those curves assumed a shape similar to the ones achieved in summer and in spring. Presumably, an increase in the sulphur dioxide concentrations was mostly due to emission from the thermal heating of houses in residential estates near the monitoring station (suburbs like Dajtki, Słoneczny Stok). Similar results were reported by Kolbarczyk, Kolbarczyk (2007), who conducted observations in several localities in north-western Poland. There, the highest SO_2 concentrations were recorded between 11.00 a.m. and 4.00 p.m., with an evident peak noticed at 3.00 p.m. Thus, daily fluctuation cycles in SO_2 concentrations were largely shaped by a set of meteorological conditions. Road transport is thought to be another important source of atmospheric pollutants (Liping, Yaping 2005). The highest SO_2 concentrations in the summer between 3 and 4.00 p.m. is attributed to a more intensive traffic flow, because most of the through roads near Olsztyn cross the city *(www.gospodarka.olsztyn.eu)*. The concentration of SO_2 measured at weekends was lower than on weekdays, and showed a stronger relationship with the vehicle traffic. Similar results were obtained by Vandaele et al. (2002), Mieszalski (1981) showed that under daylight the diffusion of sulphur into plant leaves is more rapid than in the dark. Plants involved in phytoremediation contributed heavily to the daily changes in SO_2 concentrations in the atmosphere. Decreased sulphur emmission into the air may lead to the depletion of this element from soil, thus depressing crop yields and bioavailability of other nutrients, which invariably deteriorates the quality of yields (Jankowski et al. 2008, 2014). In turn, the form of sulphate or elemental sulphur applied as fertilizer modifies the content of total carbon in soil (Majcherek et al. 2013).

It is worth mentioning that the decomposition of organic matter under anaerobic conditions leads to the generation of hydrogen sulphide. The released hydrogen sulphide, which is an unstable compound, is quickly broken down to the final product, i.e. SO_2 (Syed et al. 2006, Tamminen, Andersen 2007). Another factor which could have induced differences in daily concentrations of sulphur dioxide in the air is the woodland surrounding the monitoring station (an urban park and a forest). Deciduous trees dominant in those tree communities are able to absorb more SO_2 than conifers.

A study by Rogalski and Lenart (2011) proved that the power of dependence between the concentration of SO_2 and the air temperature (T) was expressed by the highest values of correlation coefficients (rs), which were statistically significant only in the months:

February: $SO_2 = 1.6811 - 0.1980 \times T$ $rs = -0.4606$

April: $SO_2 = 0.3633 + 0.2672 \times T$ $rs = +0.4274$

June: $SO_2 = 0.2091 + 0.0726 \times T$ $rs = +0.3869$

In February, growing temperature caused a decrease in the concentration of SO_2 in the air. In April and June, the effect was opposite, most probably due to interactions with other meteorological elements or an influx of contaminated air.

The analysis of similarities in SO_2 concentrations in the atmospheric air in Olsztyn-Kortowo, in 2007-2009, shown in hourly intervals, revealed four distinct groups of concentrations related to different times of the day (Figure 6). The night and early morning hours (from 1.00 to 7.00 a.m.) reached a 97% similarity level. Another branch consisted of morning hours (8.00-9.00 a.m.) and afternoon hours (4.00-7.00 p.m.). In this group, the following afternoon hours: 4.00-5.00 p.m. and 6.00-7.00 p.m., were most similar (97%). The third cluster consisted of late evening and night hours (8.00 p.m. to midnight), where close similarity in terms of sulphur dioxide concentrations was noticed between 10.00 and 11.00 p.m. (98%). The fourth group was made up of the midday hours (10.00 a.m. to 3.00 p.m.). Ranges of SO_2concentrations in the atmospheric air in those hours overlapped each other to a large extent, thus verifying their high similarity.

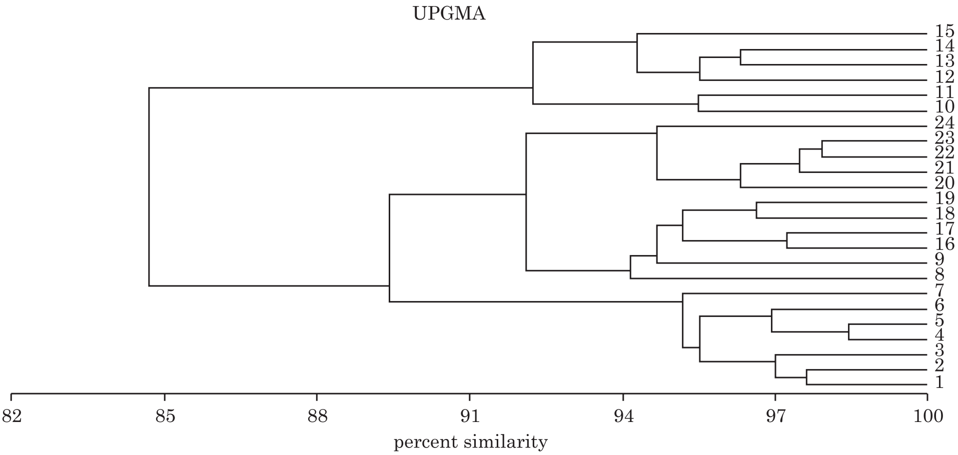


Fig. 6. An hourly dendogram of the similarity of SO_2 concentrations in the air in Olsztyn-Kortowo in 2007-2009

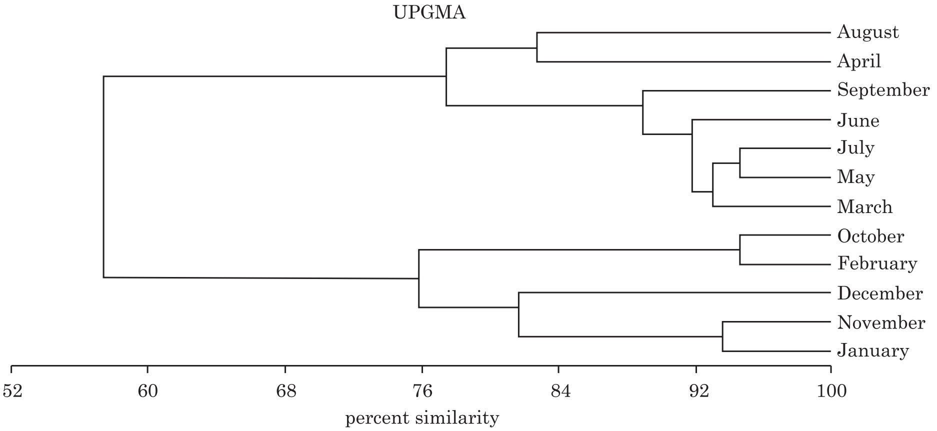


Fig. 7. Dendogram of monthly similarities in SO_2 concentrations in the atmsopheric air in Olsztyn-Kortowo in 2007-2009

The analysis of clusters based on the per cent similarity of SO_2 concentrations in a monthly cycle (Figure 7) showed a considerably distinct character of these values in the atmopsheric air in Olsztyn-Kortowo, in 2007-2009. The autumn and winter months were distincly different from the spring and summer ones. In the former group of months, the highest similarity (95%) with respect to the atmospheric SO_2 concentrations in Olsztyn-Kortowo was detected for October and February. The SO_2 concentrations similar to the ones in that group were demonstrated in December and in November and January, which additionally formed a sub-group of their own, with a similarity level of 94%. Another group (95%) consisted of the SO_2 concentrations recorded in July and May, to which the concentrations of atmospheric sul-

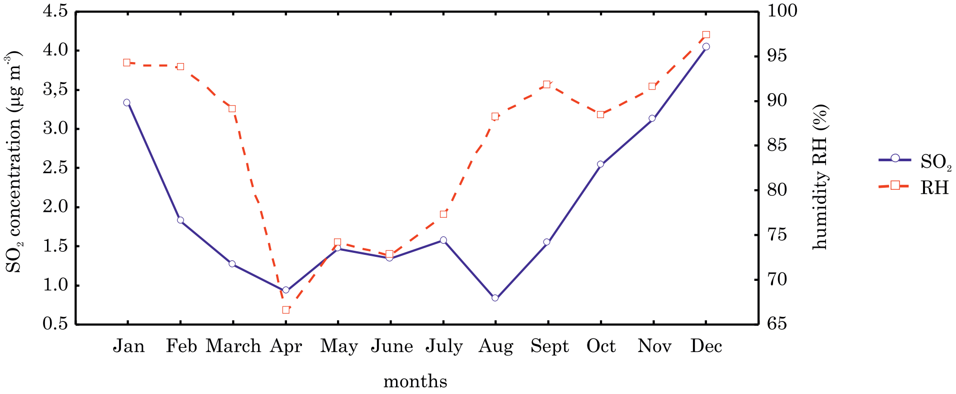


Fig. 8. Relationships between SO_2 concentration and humidity in individual months measured in Olsztyn-Kortowo, in 2007-2009

phur dioxide were most similar in March (93%) and June (92%). The least similar level of SO_2 in the atmospheric air was characteristic for the group composed of August and April (77%), versus the group made up of March, May, July, June and September.

The annual course of air humidity, in monthly intervals, against the backdrop of SO_2 concentrations, is illustrated in Figure 8. From April to July, the air humidity was not shown to have any impact on modifications in the concentration of SO_2 in air. This might have been caused by higher temperatures prevailing in that season or else the occurrence of some other ele-

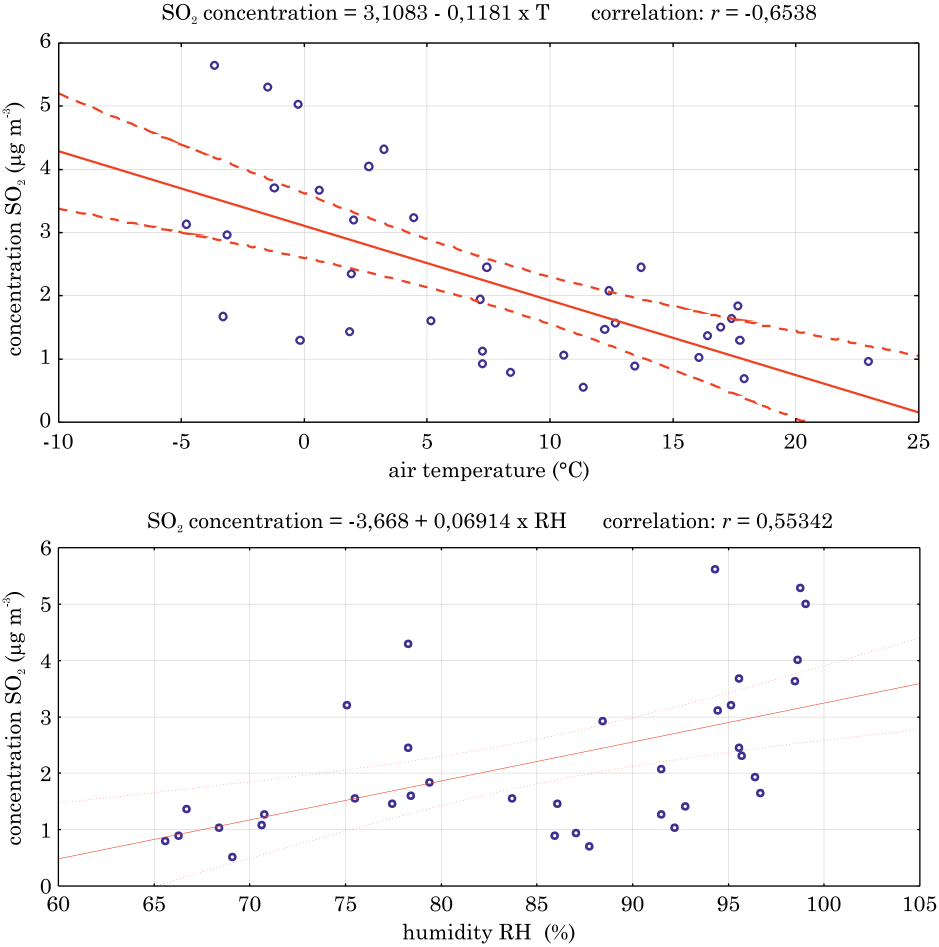
Table 1

Relationships between SO_2 concentrations and humidity from January 2007 to December 2009 in Olsztyn-Kortowo

Test period	Spearman's rank-order correlation		Regression equation
	r_S	p	
January	-0.228313	0.050405	SO_2= 10.6493– 0.0782 x RH
February	-0.227116	0.066670	SO_2= 6.7037– 0.0498 x RH
March	-0.072198	0.583557	SO_2= 0.58 + 0.0088 x RH
April	-0.462549	0.000257	SO_2= 2.8621– 0.0289 x RH
May	0.241314	0.022718	SO_2= 0.3887 + 0.0151 x RH
June	0.075987	0.476571	SO_2= 1.1159 + 0.0034 x RH
July	0.096585	0.403365	SO_2= 1.3966 + 0.0028 x RH
August	-0.111528	0.380278	SO_2= 84.4197 + 0.0007 x RH
September	-0.072223	0.498738	SO_2= 2.1251 - 0.0066 x RH
October	-0.298917	0.006714	SO_2= 6.3823 - 0.0429 x RH
November	-0.346826	0.001412	SO_2= 5.2664 - 0.0215 x RH
December	-0.045240	0.684658	SO_2= 10.4166 - 0.0626 x RH

RH – relative humidity of the air (%)

ments stabilizing the characteristics of air. In order to describe dependences between SO_2 concentrations and air humidity, the results of measurements were submitted to analysis of correlation rs and regression equations were developed (Table 1). Our analysis of the issuing data implies that the highest values of the correlation coefficient, which were statistically significant at the same time, occurred in the following months: April, October and November. The correlation and regression variations may have been due to less efficient SO_2 oxygenation and, consequently, a higher level of this gas in the air. A similar observation was reported by Kleniewska (2004). Besides, the aggregated effect of meteorological elements could have led to some synergistic interactions between the air temperature and humidity. Regardless of the above, the air flowing to Olsztyn might have demostrated elevated sulphur dioxide contamination. Based on the regression equations, it can be estimated that


SO_2 concentration = 3,1083 - 0,1181 x T correlation: r = -0,6538

SO_2 concentration = -3,668 + 0,06914 x RH correlation: r = 0,55342

Rys. 9. Relationships between SO_2 concentration versus the air temperature and humidity in individual months measured in Olsztyn-Kortowo, in 2007-2009 the amount of SO_2 in the atmospheric air depends on the known meteorological elements. The graphic depiction of values (Figure 9), including the dispersion of data, defined the character of relationships between the variables. According to IGRASHI et al. (2006), to assess the influence of humidity on the SO_2 concentration in air one would have to further analyze relationships of other gases and ions present in the atmosphere with air humidity.

CONCLUSIONS

1. The variation in the concentrations of sulphur dioxide in the air is the function of aggregated dependences between the abiotic variables, emission processes caused by anthropogenic activity and – to a large extent – the growth and development of plants. Regardless of the above relationships, the effect of air temperature and humidity on SO_2 concentrations in the air has been proven.
2. Daily concentrations of sulphur dioxide in the air showed some coincidence with periodic drops in ambient temperature and with rush hours causing more intensive traffic in Olsztyn.
3. The highest values of sulphur dioxide concentrations occured at noon, lower in the morning and afternoon hours and the lowest ones were at night.
4. In autumn, relative humidity revealed a stronger influence on the concentration of sulphur dioxide in the air. In the other seasons, the correlation of these variables was weak or very weak.
5. The nature of the dependence, identified by the regression equation, between the concentration of sulphur dioxide and temperature was that of a reversely proportional relationship, while that between the SO_2 concentration and air humidity was a proportional one.
6. Irrespective of the source of emission and influx of air masses, the concentration of sulphur dioxide in and around Olsztyn-Kortowo remained on a low level, never exceeding the thresholds, not even duirng extremely high fluctuations.

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IMPACT OF LIGNITE MINE WATERS FROM DEEP SEATED DRAINAGE ON WATER QUALITY OF THE NOTEĆ RIVER

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Abstract

Open-pit lignite mines affect many compartments of the environment. Surface mines cause changes in the catchment basin, re-shaping the land relief, modifying soil properties and depressing lake water levels as well as the groundwater table. Although the environmental concerns raised by this type of mines have been widely surveyed, we lack sufficient information provided by research reports on regarding the influence of lignite mines there on surface water bodies. In general, there are two types of mine waters from brown coal mining: runoff from the surface and water percolating from deep seated drainage. This paper discusses the impact of lignite mine waters from a deep seated drainage system in the Lubstów Mine on the quality of water in a lowland river. Lignite had been excavated in Lubstów until 2009, and untreated mine waters had been discharged to the Noteć River. The aim of the study was to assess possible changes of the river water quality after the long-term contamination with mine waters. For the assessment, three sites were selected (one above and two below the mine water inflow) for water sampling in order to perform chemical analyses according to standard methods (spectrophotometry, atomic absorption spectroscopy). Properties of mine waters, such as pH, conductivity, phosphorus, nitrates, sulphates, alkalinity and heavy metals, were analysed in samples taken directly from the canal which carried discharged mine waters to the Noteć River.

The results showed that lignite mine waters from deep seated drainage generally caused minor changes in river water quality, except alkalinity, in which the water quality below the discharge point (site B) was significantly worse than at the upper site (A). Chemically, site C was similar to site A.

Key words: Noteć River, mine waters, lignite, Kujawskie Lakeland, freshwater quality.

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WPŁYW WÓD KOPALNIANYCH Z ODWODNIENIA GŁĘBINOWEGO ODKRYWKI WĘGLA BRUNATNEGO NA JAKOŚĆ WÓD NOTECI

Abstrakt

Odkrywki węgla brunatnego oddziałują na wiele aspektów środowiska. Kopalnie odkrywkowe wpływają na zmiany zachodzące w zlewni, w tym na strukturę krajobrazu, zmiany w otaczających odkrywkę glebach, obniżanie lustra wód jeziornych oraz gruntowych. Zagadnienia te były wielokrotnie badane, jednak w przypadku oceny wpływu wód kopalnianych na jakość wód rzecznych zakres tych prac nie był szeroki i trudno znaleźć większą liczbę znaczących pozycji literaturowych. Zasadniczo wyróżniane są 2 typy wód kopalnianych z odkrywek węgla brunatnego: wody z odwodnienia powierzchniowego oraz wgłębnego. W pracy zaprezentowano wyniki badań nad wpływem wód kopalnianych z odwodnienia wgłębnego odkrywki węgla brunatnego Lubstów na wartości wskaźników jakości wód rzeki Noteci. Pozyskiwanie węgla z odkrywki Lubstów trwało do 2009 r., a badane wody kopalniane zgodnie z wymogami środowiskowymi odprowadzano do rzeki bez oczyszczania. Celem badań było określenie możliwych zmian jakości wody po kilku latach zrzutu. Wskaźniki jakości wody analizowano na 3 stanowiskach (1 powyżej zrzutu – stanowisko A, 2 poniżej zrzutu – B i C), stosując standardowe metody (spektrofotmetrię, absorpcyjną spektrometrię atomową). Wody kopalniane oceniano na podstawie prób pobranych bezpośrednio z kanału zrzutowego. Określono większość istotnych wskaźników jakości wody, takich jak odczyn pH, przewodność elektrolityczna, stężenia fosforu, azotu azotanowego, siarczanów, metali ciężkich, alkaliczność itp.

Wykazano nieznaczny wpływ wód kopalnianych z odwodnienia wgłębnego na jakość wód Noteci. Jedynie w przypadku alkaliczności zaobserwowano istotną zmianę bezpośrednio poniżej zrzutu z odkrywki (stanowisko B). W punkcie oddalonym o 1 km od zrzutu wód kopalnianych (stanowisko C) jakość wód była zbliżona do jakości wody w punkcie badawczym powyżej zrzutu (stanowisko A).

Słowa kluczowe: Noteć, wody kopalniane, węgiel brunatny, Pojezierze Kujawskie, jakość wód powierzchniowych.

INTRODUCTION

Open-pit lignite mines affect all compartments of the environment. Surface mines cause changes in the catchment basin, reshaping the land relief, modifying soil characteristics, and depressing lake water levels as well as groundwater tables (ILNICKI 1996). The environmental concerns raised by surface mine exploitation have been widely studied, although there is a shortage of research reports concerning the impact lignite mines on surface water quality (YOUNGER, WOLKERSDORFER 2004). At the same time, other environmental issues such as the impact on groundwater and mine wastewater treatment have been described in detail (JANIAK 1992, HILDMANN, WONSCHE 1996, GRÜNEWALD 2001, DOMSKA, RACZKOWSKI 2008, KAVOURIDIS 2008, HANCOCK, WOLKENSDROFER 2012, and others).

The removal of mine waters from a deep seated drainage system at the Lubstów Surface Lignite Mine to the Noteć River (Kujawskie Lakeland, central Poland) began in 2003, affecting the river water discharge and in some cases the water quality parameters. Studies were undertaken to evaluate the impact of the mine waters on the Noteć, a small lowland river, and this paper contains results from the surveys carried out in 2008 and 2009. The total volume of mine water discharged at the surveyed site (MW) was 6,939,433 m^3 in 2008 (0.13 m^3 s^{-1}), while the average river water discharge (1961-2005) at the nearest river monitoring site in Łysek was about 0.89 m^3 s^{-1}, meaning that the contribution of mine waters to the total river discharge was about 17% (Wachowiak 2010). This is a considerably large volume of water, considering a relatively low annual precipitation in this region (about 538 mm), which results in low water levels in lakes and watercourses (Staniszewski, Szoszkiewicz 2010).

The sites selected for the evaluation of Noteć River water quality were situated below Brdowskie Lake and above Gopło Lake, a secondary recipient of mine waters. The lakes Brdowskie, Modzerowskie and Przedeckie affect the quality of water in the Noteć River above the surveyed sites, and are similar in terms of water quality and aquatic plant structure (Staniszewski, Szoszkiewicz 2002, 2004, 2005, 2010). The Noteć River had been analysed previously in its upper sections, where both eutrophic and mesotrophic conditions were identified (Staniszewski 2001).

MATERIAL AND METHODS

Water from the Noteć was sampled at three sites in 2008 – 2009. In 2008 and 2009, it was submitted to analyses of the content of dissolved forms of metals, while in 2009 other water quality parameters were assessed. The selected sites were: Dziadoch (A) – above the mine waters discharge site, Nykiel (B) and Ignacewo (C) – both below the mine waters discharge site. The Ignacewo site is situated in the Goplańsko-Kujawski Area of Protected Landscape. There were no other significant sources of potential contamination of water around the selected experimental sites and this part of the catchment was dominated by a rural landscape with patches of woodland or grassland and a few scattered houses. Mine waters were sampled from the canal which carries mine waters to the Noteć (Dziadoch MW – Nykiel). Additional chemical data were obtained from the Konin Coal Mine (personal communication, M. Modelska-Babiak, KWB Konin). All samples were collected to 0.5 l polyethylene bottles and the following analyses of water were performed:

- pH reaction and conductivity – electrometrically;
- soluble reactive phosphates – samples passed through 0.45 µm pore size membrane filters, ascorbic acid method;
- nitrates – samples passed through 0.45 µm pore size filters, cadmium reaction method;
- sulphates – samples passed through 0.45 µm pore size filters, spectrophotometry;

- total phosphorus – the acid persulfate digestion method;
- total concentrations of dissolved metals (Fe, Cd, Zn, Mg, Ca, K, Na) – atomic absorption spectroscopy (AAS) in averaged samples analyzed according to standard methods after passing through 0.45 µm pore size filters.

The bottom sediment in the canal five years after it had been opened (May 2009) underwent chemical analyses, including pH reaction, sediment conductivity, concentrations of total nitrogen (the Kjeldahl method), phosphorus and potassium (the Egner method).

The location of each site was selected so as to obtain a reliable picture of the potential impact of mine waters on the river, assuming that river and mine waters mixed completely. Site A (Dziadoch) lies 50 metres above the mine water discharge and presents the following characteristics: mineral and organic bottom sediments, a very small river slope and tree (mostly black alder) canopy shading on both river banks. Mine waters (site MW) were collected directly from a 40-meter canal, partly made of concrete, and near the canal, from the river, where the bed was composed of stones, pebbles and gravel. Mine waters were discharged through this canal from April 2003 to May 2009. According to the data from the Konin Coal Mina Company (MODELSKA-BABIAK 2011 – personal communication), as much as 58% of surface mine waters flew to the river through that outflow site in 2003, decreasing to about 40% in the subsequent years. Sites Nykiel (site B, 100 m below discharge) and Ignacewo (C, about 1 km below discharge) were similar in terms of their landscape relief and land use (mostly rough pastures and scattered houses).

RESULTS AND DISCUSSION

The levels of phosphorus, nitrates and other parameters in the mine waters were acceptable from the view of sustaining the functions of a river ecosystem. In fact, they did not cause changes to the river water quality (Table 1, Figure 1). Mine waters should meet standards set for wastewater (the Journal of Law no 137, item 984), and the measured values were much below these thresholds, i.e. 30 mg N_{NO3} dm^{-3}, 500 mg SO_4 dm^{-3}, etc. In comparison to the river water quality standards, some parameters such as total phosphorus and alkalinity were above the thresholds in class II, meaning that in the long term they could affect the river ecosystem.

The results show that the concentrations of dissolved metals at the sites below the discharge canal were either lower (zinc) or the same (cadmium, iron) as in the site above. The mine water concentrations of zinc were lower than in the Noteć River and lower than the average values reported by GRABIŃSKA et al. (2006) for Polish rivers flowing through agricultural landscapes (the Narew, Biebrza, Rozoga and the Pisa), ranging from 0.034 to 0.044 mg Zn dm^{-3}.

Table 1

Average and maximum values of main water quality parameters in the Noteć River (sites A, B, C) and in mine waters (site MW) in 2009

Parameters	Site A		Site MW *		Site B		Site C	
	average	max	average	max	average	max	average	max
pH reaction **	7.83	7.91	7.58	7.63	7.75	7.89	7.73	7.86
Conductivity (mS cm^{-1})	0.662	0.720	0.720	0.758	0.685	0.725	0.568	0.634
Total phosphorus (mg P l^{-1})	0.15	0.17	**0.82*****	**2.86**	0.20	0.38	0.16	0.22
Soluble reactive phosphates (mg PO_4 l^{-1})	0.25	0.31	0.22	0.27	0.29	0.39	0.40	0.66
Nitrates (mg N-NO_3 l^{-1})	0.73	1.40	0.06	0.10	0.63	1.30	0.45	1.30
Alkalinity (mg $CaCO_3$ l^{-1})	202.5	230	**257.5**	**260**	216.3	240	245.0	**260**

* results for mine waters were compared to river standards to facilitate comparisons with the Noteć River water quality; ** median for pH reaction; *** bolded numbers – above water quality class II (Journal of Law No 257, item 1545).

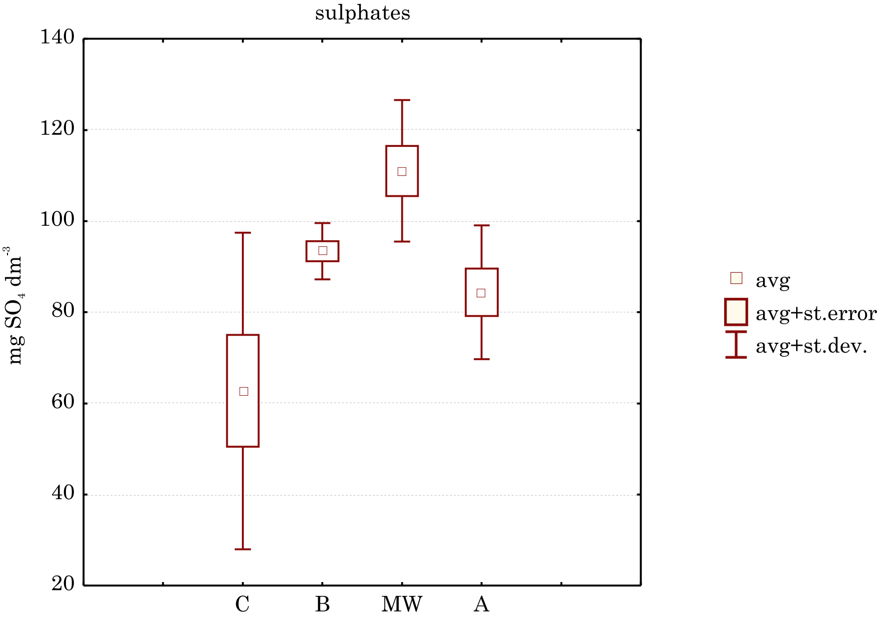

Fig. 1. Comparison of average concentrations of sulphates in the Noteć River and in mine waters in 2009: A – site above mine water discharge, B, C – sites below mine water discharge, MW – mine waters

The analyses carried out in 2008-2009 demonstrated the content of dissolved Fe in the river water was below detectability limits (Table 2). In samples collected in 2004 (M. Modelska-Babiak – personal communication), an average total iron concentration was 1.145 mg Fe dm^{-3} (the threshold for waste waters is 10 mg Fe dm^{-3}), which could explain the presence of iron in sediments from the mine waters canal (Table 3).

Concentrations of magnesium in the Noteć River were similar to those in other rivers in the Polish Lowlands but higher than in lowland water bodies situated in rural areas (Koc et al. 2008). A range of potassium in Polish rivers is wide: from 1.4 to 10.3 (Koc et al. 2004) with an average of about 4.67 mg K dm^{-3}, although the Weser River, for example, it reached 42 mg K dm^{-3} (Grzebisz et al. 2004). The low level of K in mine waters (3.9-5.9 mg K dm^{-3}) did not worsen the quality of the river water (Table. 4).

Changes in the water chemistry below the mine water discharge point were not significant; however, even small changes in water quality can influ-

Table 2

Average and maximum total concentrations of dissolved heavy metals at sampling sites based on analyses carried out in 2008-2009

Parameters (mg dm^{-3})	Site A		Site MW		Site B		Site C	
	average	max	average	max	average	max	average	max
Zn	0.03	0.08	0.01	0.04	0.02	0.05	0.02	0.04
Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.008
Fe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3

Chemical characteristics of sediments in the mine water canal from the Lubstów Surface Mine versus selected Polish data for river sediments

Parameter	Unit	Sediments from mine waters canal	River sediments (Odra R., Wilga R., Kwisa R. Wigierski National Park rivers*)
pH	-	7.49	4.5 - 8.8
EC	mS cm^{-1}	0.50	
C_{org}	%	28.1	
$N_{tot.}$		0.44	
P (as P_2O_5)	mg kg^{-1}	0.17	
K (as K_2O)		3.86	
Fe	mg kg^{-1}	218 542.6	2900 - 45 908
Mn		5353.9	272.1 - 2101
Pb		47.1	5 - 153
Zn		28.2	28 - 1226

* data from publications: Helios-Rybicka (2005), Korabiewski (2005) and others

Table 4

Average total concentrations of dissolved light metals at sampling sites based on analyses carried out in 2008-2009

Parameters (mg dm^{-3})	A	MW	B	C
Mg	14.9	18.2	15.1	13.8
Ca	90.9	88.8	89.4	84.3
K	5.9	3.0	5.7	3.9
Na	19.6	11.0	19.0	14.4

ence the species structure of aquatic organisms such as benthic algae or invertebrates. For instance, a small increase of the Cd contamination can affect the river biota (Iwasaki, Ormerod 2012). Macro-invertebrates are very susceptible to environmental stressors, and therefore are included in several biological indices broadly used in environmental surveys (Birk, Hering 2006, Buffagni et al. 2006).

The results of the Wilcoxon test showed that differences found between the river sites were significant only for alkalinity, conductivity and pH (Table 5). There were significant differences in the chemistry of mine waters and the Noteć River water with respect to such quality parameters as sulphates, alkalinity, pH, conductivity and zinc concentration. The levels of phosphorus, nitrates, dissolved metals and the other parameters in the mine waters were acceptable from the point of view of sustaining the river ecosystem.

If concentration of metals in mine waters were higher, Gopło Lake, lying below the sampling sites, and especially its southern end receiving water from the Noteć, would be endangered. The morphological characteristics of lakes make them similar to settling tanks, which is why they collect most of mineral and organic material transported by watercourses.

Another objective of the study was to analyse parameters of the sediment in the canal. Studies on heavy metal concentrations in sediments have been conducted by many authors in recent years, as this is an important

Table 5

Significant differences between pairs of sites using the Wilcoxon test at different *p*

Pairs of sites	Zn	Conductivity	Sulphates	Alkalinity	pH
	p				
MW - A	0.027	0.017	0.011	0.011	0.011
MW - B			0.017	0.017	0.011
MW - C			0.049		
A - B		0.017		0.025	
A - C				0.011	0.049
B - C				0.029	

issue for the environmental quality, research and analytical problems (Boszke et al. 2002, Soylak et al. 2002, Helios-Rybicka 2005, Korabiewski 2005, Szyczewski et al. 2009, and others).

The deposit formed after 5 years of discharging mine waters built up a 2-cm thick layer, consisting mostly of iron (218 542.6 mg kg^{-1}; i.e., 21.9%) and manganese (5353.9 mg kg^{-1}; i.e., 0.54%), which gave it the red colour (Table 3). The determined Fe and Mg levels are significantly different than those reported by Helios-Rybicka (2005) and Korabiewski (2005). Besides, alkaline pH (7.49) and the high content of organic substance (C_{org} = 28.1%) are the chemical features antagonistic to the high levels of Fe, particularly under anoxic conditions.

The acidification capacity, which could be produced by Fe compounds, is generally created by hydratation processes, yielding considerable concentrations of protons and in some cases hydroxyl compounds, as illustrated by these formulas:

$Fe^{2+} + 6H_2O \leftrightarrow Fe(OH)_3 + 3HO_3^+$,

$Fe^{2+} + H_2S + 2\ H_2O \rightarrow FeS + 2H_3O^+$; but

$Fe_2O_3 + 3\ H_2O \leftrightarrow 2Fe(OH)_3$

The third reaction may be considered as intrinsically responsible for alkaline pH, which could be harmful to the environment. In the investigated ecosystem, the direct manifestation is the brownish colour of the wastewater due to high concentrations of dissolved organic matter (mostly fulvic). The geochemical background values of metals in lake or river sediments or deposits, defined by the Polish Geological Institute, are Pb = 15 and Zn = 73 mg kg^{-1} (Bojakowska, Sokołowska 1998). On the other hand, the threshold values for class I (uncontaminated sediments) are 30 mg kg^{-1} Pb and 125 mg kg^{-1} Zn (Bojakowska 2001). The data contained in Table 5 show that the Zn content need not give rise to concern, since its content is below the geochemical background and the class I threshold. This might not be true about lead. It appears that the investigated sediment is slightly contaminated by Pb, moderately by Mn and polluted by the Fe compounds.

CONCLUSIONS

The concentrations of sulphates, phosphates, nitrates and the conductivity in the Noteć River below the mine water discharge point were not high, did not affect the water quality and should not pose a threat to aquatic plants.

The concentrations of dissolved metals in mine and river waters were low, but the total levels of metals were higher due to the elements contained in the layer of sediments in the canal carrying mine waters to the river. Levels of heavy metals should be strictly controlled and technical measures might have to be taken to reduce their level in mine waters because of possible harmful pressure on the river biota.

The analyses of the sediment showed a high concentration of iron, which resulted from the chemical composition of mine waters discharged in 2004-2008.

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A MINERAL PROFILE OF WINTER OILSEED RAPE IN CRITICAL STAGES OF GROWTH – NITROGEN

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Abstract

Winter oilseed rape is the key oil crop in temperate climate regions of the world. Yield of seeds depends the most on nitrogen management throughout the season. The evaluation of N status in the oil-seed rape canopy was studied in 2008, 2009, and 2010 seasons. The one factorial experiment to verify the formulated hypothesis, consisting of six treatments, was as follows: absolute control (AC), NP, NPK, NPK + MgS - $^1/_3$ rate of total planned rate applied in Spring (NPKMgS1), NPKMgS - 1.0 rate in Autumn (NPKMgMgS2), NPK+MgS - $^2/_3$ in Autumn + $^1/_3$ in Spring (NPKMgS3). Plant samples were taken at three stages: i) full rosette (BBCH 30), ii) the onset of flowering (BBCH 61), iii) maturity (BBCH 89). The total plant sample was partitioning in accordance with the growth stage among main plant organs such as leaves, stems, straw, and seeds. Yield of biomass, nitrogen concentration and content were determined in each part of the plant. The study showed an existence of two strategies of dry matter and nitrogen accumulation by oil-seed rape throughout the season. In 2008, and partly in 2010 revealed the strategy of yield formation relying on relatively slow, but at the same time a permanent increase in nitrogen and biomass accumulation during the season. This strategy resulted in a high seed density in the main branch. The size of this yield component significantly depended on nitrogen content in leaves at the onset of flowering. In 2009 revealed the second strategy of yield formation by oilseed rape. Its attribute was a very high rate of nitrogen accumulation during the vegetative growth, resulting in a huge biomass of leaves at the onset of flowering. This growth pattern, as corroborated by yield of seeds, was not as effective as the first one. The main reason of its lower productivity was the reduced seed density, especially in pods of the secondary branch, resulting from an excessive nitrogen content in leaves at the onset of flowering. Irrespectively on the strategy of nitrogen management by the oilseed rape canopy, the best predictor of the final yield was nitrogen content in seeds. It can be concluded that any growth factor leading to the nitrogen sink decrease, such as reduced plant density and/or disturbed N management throughout the season, can be considered as a factor negatively impacting yield of seeds.

Key words: nitrogen, plant parts, N partitioning, yield structural components, yield of seeds.

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PROFIL MINERALNY RZEPAKU OZIMEGO W KRYTYCZNYCH FAZACH ROZWOJU – AZOT

Abstrakt

Rzepak ozimy jest kluczową rośliną oleistą w umiarkowanych pod względem klimatu regionach świata. Plon nasion w największym stopniu zależy od gospodarki azotem w okresie wegetacji. Ocenę gospodarki azotem w łanie rzepaku przeprowadzono w latach 2008-2010. Hipotezę badawczą weryfikowano w 1-czynnikowym doświadczeniu, obejmującym 6 kombinacji: kontrola absolutna (AC), NP, NPK, NPK – $^1/_3$ całkowitej planowej dawki MgS wiosną (NPKMgS1), NPK + 1,0 dawka MgS jesienią (NPKMgS2), NPK + MgS – $^2/_3$ dawki jesienią i $^1/_3$ – wiosną (NPKMgS3). Próbki roślin pobierano w 3 stadiach: i) rozety (BBCH 30), ii) początku kwitnienia (BBCH 61), iii) dojrzałości fizjologicznej (BBCH 89). Pobrane próbki roślin dzielono, zależnie od fazy rozwoju rzepaku, na główne części, jak liście, łodygi, słoma + plewy, nasiona. Plon biomasy, koncentrację i akumulację azotu oznaczano w każdej części rośliny. Wykazano funkcjonowanie dwóch strategii akumulacji biomasy i azotu przez łan rzepaku. W 2008 r., a w mniejszym stopniu w 2010 r., ujawniła się strategia formowania plonu, polegająca na względnie powolnej, lecz ciągłej akumulacji azotu i biomasy w okresie wegetacji. Ta strategia formowania plonu przez rzepak przejawiła się dużą liczbą nasion w łuszczynach pędu głównego. Ten element struktury plony wykazał istotną i dodatnią reakcję na ilość zakumulowanego azotu w liściach na początku kwitnienia rzepaku. W 2009 r. ujawnił się drugi model formowania plonu przez rzepak. Plonotwórcza istota tej strategii przejawiała się bardzo intensywną akumulacją azotu, a tym samym biomasy przez łan rzepaku w okresie przed kwitnieniem. Strategia ta, jak wynika z plonu nasion, nie była tak efektywna jak pierwsza, dominująca w 2008 roku. Główną przyczyną mniejszej efektywności tej strategii była zredukowana liczba nasion w łuszczynach, zwłaszcza pędów bocznych, wynikająca z nadmiaru azotu w liściach na początku kwitnienia. Niezależnie od strategii gospodarki azotem w łanie rzepaku, najważniejszym wskaźnikiem finalnego plonu nasion był potencjał nasion do akumulacji tego składnika. Stwierdzono, że jakikolwiek czynnik wzrostu prowadzący do spadku potencjału ujścia fizjologicznego azotu, jak zbyt mała liczba roślin lub też zakłócona gospodarka azotem w łanie w okresie wegetacji, może być traktowany jako ujemnie kształtujący plon.

Słowa kluczowe: azot, części rośliny, rozdział azotu , elementy struktury plonu, plon nasion.

INTRODUCTION

Winter oilseed rape is the most important oil crop in temperate regions across the world. As documented by SUPIT et al. (2010), the yield potential of this crop in the Central European countries like the Czech Republic, Germany and Poland as evaluated during the period 1996-2005 was at the level of 3.6, 4.1, and 3.7 t ha^{-1}. Whereas, the actual yields in this period were lower, presenting the level of 2.5, 2.7 and 2.2 t ha^{-1}, respectively. In the period, 2006-2012 actual yields in these three countries raised up, achieving the level of 2.940 ± 0.153, 3.466 ± 0.430, 2.618 ± 0.272 t ha^{-1} (FAOSTAT 2013). There are numerous factors responsible in the yield gap development. In the present farming practice, the domination of nitrogen fertilizer over potassium and phosphorus fertilizers is an obvious matter. The superfluous role of nitrogen in plant growth is well documented, taking into account its physiological functions (RUBIO et al. 1993). However, an efficient exploitation of fertilizer nitrogen requires its balancing using a broad set of nutrients.

In the primary step, it refers to phosphorus and potassium (Grzebisz et al. 2010*a*; Grzebisz et al. 2013). In the period 1989-2005, as documented by Grzebisz et al. (2012), the ratio of N:P_2O_5:K_2O consumption in Central European countries underwent a significant extension, resulting in a high sensitivity of broad-leafed crops, like oilseed rape to the course of weather. Therefore, the key reason of the existing yield gap in oil-seed rape production is the imbalanced fertilization of crops due to N excess, leading during Spring's vegetation to an increased sensitivity of winter oilseed rape to water shortages (Abbasian, Rad 2011, Albert et al. 2012). The second step in an efficient usage of externally applied nitrogen requires is its balancing by secondary nutrients, like sulfur and magnesium (Spychaj-Fabisiak et al. 2011, Szczepaniak et al. 2013).

The yield of winter oilseed rape is a result of development processes, which efficiency is decisive for development of yield component. Two of four key yield components, such as the number of pods per plant, numbers of seeds per pod are highly sensitive to nitrogen supply during spring growth of oilseed rape (Diepenbrock 2000). However, the impact of in-season management of nitrogen taken up by the crop during both a vegetative and reproductive period is weakly recognized. The objective of the conducted study was to evaluate an impact of nitrogen accumulation and its partitioning among the main plant organs of winter oilseed rape in two critical stages such as the rosette and the beginning of flowering on development of structural yield components, and consequently, on final yield of seeds.

MATERIALS AND METHODS

Studies on nitrogen status of winter oil-seed in critical stages of yield formation were carried out during three consecutive growing seasons 2007/08, 2008/09, and 2009/10 at Donatowo in the private farm. The field experiment was established on a soil originated from a loamy sand underlined by a sandy loam, classified as Albic Luvisol. Soil fertility level as indicated by the main agrochemical characteristics was satisfactory for producing high yield of seeds (Table 1). The one factorial trial, replicated four times, consisted of six treatments. The detailed description, including the rate and form of fertilizer are given in Table 2.

Each year of study, winter wheat preceded oilseed rape. The size of the individual plot was 100 m^2. The variety *Chagall* was sown in the last decade of August. Phosphorus and potassium were applied prior to sowing in doses adjusted to the soil test rating and treatment. Phosphorus was applied in the form of di-ammonium phosphate and potassium in accordance with the treatment schedule. Magnesium and sulfur were applied to the crop in the form of magnesium sulfate during the season as presented in Table 2. Nitrogen (ammonium saltpeter) was applied at the rate of 27 kg N ha^{-1} before

Table 1

Agrochemical characteristics of the soil

Year	pH (1M KCl)	Content of nutrients (mg kg^{-1} soil)						N_{min}
		P	rating	K	rating	Mg	rating	kg ha^{-1}
2008	6.36	96.3	very high	151.1	high	45	medium	65.6
2009	6.55	89.1	very high	164.3	high	52	high	74.8
2010	5.96	68.4	high	103.8	medium	75	very high	68.0

Table 2

Arrangement of the experiment: composition, rates and timing

Code of the treatment	Treatments	N		P	K		Mg		S	
		time and rate of applied fertilizer (kg ha^{-1})								
		A**	S***	A	A	S	A	S	A	S
AC	control	0	0	0	0	0	0	0	0	0
NP	N + P*	27	187	30.1	0	0	0	0	0	0
NPK	NPK	27	187	30.1	149.4	0	0	0	0	0
NPKMgS1	NPK + MgS1	27	187	30.1	99.6	49.8	0	5.4	0	6.0
NPKMgS2	NPK + MgS2	27	187	30.1	149.4	0	16.3	0	18.0	0
NPKMgS3	NPK + MgS3	27	187	30.1	99.6	49.8	10.8	5.4	12.0	6.0

* di-ammonium phosphate, ** Autumn, *** Spring

sowing, 102 kg N ha^{-1} before Spring's regrowth and at the remaining part at the end of the rosette stage. Fungicide and insecticide applications were sprayed when required. At maturity, plants were harvested from the area of 15 m^2 using a plot combine harvester. Total seed yield was adjusted to 8% moisture content.

Plant material for assessment of dry mater and nitrogen dynamics were sampled from an area of one m^2 in three consecutive stages of wheat growth according to the BBCH scale: 30, 61 and 89. At each measurement date, the harvested plant sample was partitioning, in accordance with the development stage, into subsamples of leaves, stems, seeds, straw and then dried (65°C). Finally, at each stage, total and sub-sample, dry matter per one m^2 was recorded. At BBCH 89, the whole sub-sample was divided into the main raceme – the principal branch and secondary branches. Number of pods and seeds per pod were counted separately for each sub-group. Nitrogen concentration in plant samples was determined by a standard macro-Kjeldahl procedure, with accuracy of 0.1 mg N (PN-ENISO20483:2007p). Nitrogen content in a particular plant part was calculated by multiplication of its concentration and respective biomass.

The experimentally obtained data were subjected to the conventional analysis of variance using computer programs Statistica 7. The differences between treatments were evaluated with the Tukey's test. In tables and figures, results of the F test (***, **, * indicate significance at the $P < 0.1\%$, 1%, and 5%, respectively) are given. Path analysis was conducted based on correlation coefficients taking yield of seed and/or its structural components as effects and nitrogen accumulation in plant parts as independent variables. The path diagram, showing direct and indirect path coefficients have been elaborated based on the highest value of the correlation coefficient for a particular set of variables. In the second step of diagnostic procedure, the stepwise regression was applied to define the optimal set of variables for a given crop characteristic. In the computing procedure, a consecutive variable was added to the multiple linear regressions in the step-by-step manner. The best regression model was chosen based on the highest *F*-value for the entire model and significance of all independent variables (Konys, Wiśniewski 1984).

Acronyms applied in the paper:

MBY - yield of the main branch (inflorescence),
SBY - yield of secondary branches,
TSY - total yield of seeds,
PD - plant density,
SBP - number of secondary branches per plant,
PMB - number of pods per the main branch,
PSB - number of pods per the secondary branch,
SMB - number of seeds per a pod of the main branch,
SSB - number of seeds per a pod of the secondary branch,
WSM - weight of 1000 seeds of the main branch,
WSB - weight of 1000 seeds of the secondary branch,
STY - yield of straw,
N_{RT} - nitrogen content in oilseed rape at the rosette stage,
N_{LE} - nitrogen content in leaves of oilseed rape at the beginning of anthesis,
N_{SH} - nitrogen content in shoots of oilseed rape at the beginning of anthesis,
N_{ST} - nitrogen content in straw of oilseed rape at maturity,
N_{SE} - nitrogen content in seeds of oilseed rape at maturity.

RESULTS AND DISCUSSION

Yield of seeds and yield forming components

Yield of oilseed rape responded significantly to the increased input of nutrients, balancing the N rate. The optimal composition of applied nutrients was modified by the course of weather, in consecutive years of study (Tables 3, 4). The detailed analysis of the total yield of seeds showed that the main branch constituted around $^1/_3$ of the whole yield. The impact of the principal inflorescence yield, based on the R^2, on the yield of seeds was much lower as found for the yield produced by secondary branches:

Table 3

Distribution of precipitation during oilseed rape vegetation, the synoptic station at Brody

Period	Consecutive months during crop vegetation (mm)											
	VIII	IX	X	XI	XII	I	II	III	IV	V	VI	VII
1957-2010	69.3	41.4	33.0	34.3	40.0	31.0	31.3	35.3	29.9	46.7	59.5	75.4
2007/2008	82.3	27.5	18.4	25.0	27.8	74.4	13.2	45.2	60.4	23.5	15.0	47.9
2008/2009	94.4	14.5	50.7	18.4	36.6	17.5	32.4	37.6	10.0	82.2	99.0	66.0
2009/2010	31.9	58.2	62.8	40.3	49.1	19.1	15.3	38.5	33.4	83.6	21.2	121.6

1) $\mathrm{TSY} = 2.399\mathrm{MBY} + 0.95$ for $n = 18$, $R^2 = 0.66$ and $p < 0.001$,

2) $\mathrm{TSY} = 1.282\mathrm{SBY} + 0.508$ for $n = 18$, $R^2 = 0.93$ and $p < 0.001$.

Yields of the main branch and secondary branches responded in the different manner to external conditions, i.e., to the weather and applied nutrients. In the first case, weather impact was constant, affecting yield of the principal inflorescence irrespectively on fertilization treatments. In the second case, yield of seeds was a result of interaction of both factors. In 2008, the highest yield of seeds was harvested in the NPKMgS2 treatment, where magnesium and sulfur applied were applied in the full rate in Spring. The same trend was noted in 2010, but yields were much lower. In 2009, the highest yield was achieved in the NPK treatment. The observed year-to-year variability of yield can be partly explained by a high variability of the course of weather. The first year of study was characterized by a mild drought, which prevailed throughout the whole 2007/08 season, except April, which was moist. In contrast, in the 2008/2009 season, distribution of precipitation was normal, except May and June, which were wet, leading to delayed flowering. In the third, 2009/10 season, Autumn was moist and cold, leading to a sharp decrease in plant density (Table 4). Plants of oilseed rape responded to applied magnesium and sulfur the most in 2008, when a mild water stress took place just before the onset of stem elongation. The observed crop response implicitly corroborates the hypothesis by Grzebisz (2013), concerning the inductive impact of magnesium and sulfur on N management, under a gentle water stress.

Table 4

Statistical evaluation of seeds yield and yield components

Factor	Factor level	MBY SBY TSY			PD	SBP	PMB	PSB	SMB	SSB	WMB	WSB
		yield (t ha^{-1})			no m^{-2}	no plant^{-1}			no pod^{-1}		(g)	
Fertilization treatments (FT)	AC	1.035^{a}	1.846^{a}	2.881^{a}	35.2	6.21^{a}	45.1	125.0^{a}	12.1^{a}	10.0	5.63	5.23
	NP	1.260^{ab}	2.554^{b}	3.814^{b}	36.4	7.49^{b}	46.8	127.0^{a}	13.5^{ab}	12.7	5.97	5.50
	NPK	1.240^{ab}	3.119^{bc}	4.359^{bc}	33.9	8.00^{b}	48.2	180.8^{b}	13.7^{abc}	10.9	5.92	5.44
	NPKMgS1	1.405^{b}	2.692^{bc}	4.097^{bc}	35.4	7.11^{ab}	44.3	169.2^{ab}	16.9^{c}	9.5	5.88	5.35
	NPKMgS2	1.394^{b}	3.156^{c}	4.550^{c}	37.3	7.41^{ab}	45.0	177.3^{b}	16.2^{bc}	10.1	5.71	5.38
	NPKMgS3	1.339^{b}	3.052^{b}	4.391^{bc}	38.1	7.67^{b}	48.7	168.1^{ab}	13.9^{abc}	9.3	6.02	5.42
Years (Y)	2008	1.454^{b}	3.335^{c}	4.750^{c}	35.7	6.91^{a}	53.2	142.9^{a}	14.4^{b}	14.7^{c}	5.30^{a}	4.73^{a}
	2009	1.535^{b}	2.717^{b}	4.252^{b}	51.2	7.00^{a}	48.5	149.7^{a}	11.5^{a}	6.0^{a}	5.47^{a}	5.11^{b}
	2010	0.887^{a}	2.158^{a}	3.047^{a}	21.3	8.04^{b}	37.4	181.0^{b}	17.3^{c}	10.5^{b}	6.81^{a}	6.31^{c}
Fertilizing treatments (FT)		***	***	***	ns	***	ns	**	***	ns	ns	ns
Years (Y)		***	***	***	***	**	***	**	***	***	***	***
FT x Y		ns	*	*	ns	**	ns	ns	**	ns	ns	ns

F – probability values: *,**, *** of 0.05, 0.01, 0.001, ns – no significantly different; ^{a} the same letters means a lack of significant differences

An analysis of yield forming components is a useful tool for explaining yield of seed variability (DIEPENBROCK 2000). The course of weather, in consecutive seasons was the principal factor affecting development of yield components. The primary one, number of plants per m^2, showed the highest year-to-year variability, changing from about 20 in the wet 2010 to 50 in the normal 2009. Any effect of fertilizing treatments was observed. The same type of response was observed for the weight of thousand seeds. The compensation effect of low plant density was significant, as documented by much higher TSW in 2010, compared to other years. The same dominating trend has been observed for the number of seeds in pods of the secondary branches. The impact of weather conditions on development of this yield component was highly specific. Plants of oilseed rape produced the highest number of seeds in the semi-dry 2008, followed by 2010, and the lowest in the wet 2009. The next canopy component, the number of secondary branches per plant (SPB) is an important yield forming element, highly responsive to plant density (DIEPENBROCK 2000). This oilseed rape characteristic significantly depended both on fertilizing treatments, but was modified by the course of weather in consecutive years (Table 4). A detailed analysis of SPB showed that in 2008, it was around 7, independently on the treatment. In the wet, 2010, plants fertilized with NPK produced eight branches per plant, but others around seven. Plants grown in the control plot produced about 5, but those fertilized with NP, NPK and NPKMgS3 on average 9. The top response od SPB was an attribute of the NP treatment. The effect of potassium in the NPK treatment was positive, resulting in the highest, on average, value of this plant characteristic. These results implicitly corroborate an importance of the fresh applied phosphorus as a growth compensation factor under low plant density. Number of pods per plant is an important factor, affecting yield of seeds (DIEPENBROCK 2000). In the case of the main branch, this component was only affected by the course of weather, decreasing in the order: 2008 > 2009 > 2010. Number of pods developed by secondary branches was on average several times higher, responding to fertilization treatments and years, but not to interaction of both factors. Potassium was the key nutrient impacting positively this plant characteristic, as noted in the NPK treatment. Addition of magnesium and sulfur, averaged over years, did not results in the pod number increase, mainly due to its sharp decrease in 2009. This yield component showed an extremely high flexibility, as affected by plant density, as documented in 2010. Number of seeds per pod showed a quite different response to tested factors, taking into account the principal inflorescence and secondary branches. Number of seeds developed in pods of the main branch responded to applied nutrients, but at the same time was modified by the weather course in consecutive seasons. In the first two years, the highest number of seeds, independently on its real values, was an attribute of the NPKMgS1. In the third year, the effect of MgS was even more pronounced, as documented by 25% increase in the number of seeds as compared to the NPK treatment. This trend indicates on magnesium and sulfur as an important factor for seed set in the pod, especially in low density oilseed crop. In contrast, number of seeds per pod of the secondary branch was much lower, responding only to weather conditions during vegetation. The compensation effect was not observed, because the highest seed pod density was an attribute of the semi-dry 2008 year.

In order to define the key yield forming component, the path and stepwise analyses were used, but evaluated separately for each part of the total yield. The yield produced by the MBY was, as indicated by both, the correlation and path coefficients, significantly affected by the number of plants per unit area (PD, Figure 1a). The straight effects of other variables were small (< 0.1) for SB, PMB, PSB, and WSB, but large and positive (> 0.5) for SMB, SSB, and WMS. These three variables impacted, however, negatively the PD direct coefficient. The stepwise regression showed that yield produced by the principal branch was the best explained by plant density, as a single yield predictor. The improvement of the MBY prognosis accuracy requires an implementation of two other yield forming components, such as the PMB and especially the SMB:

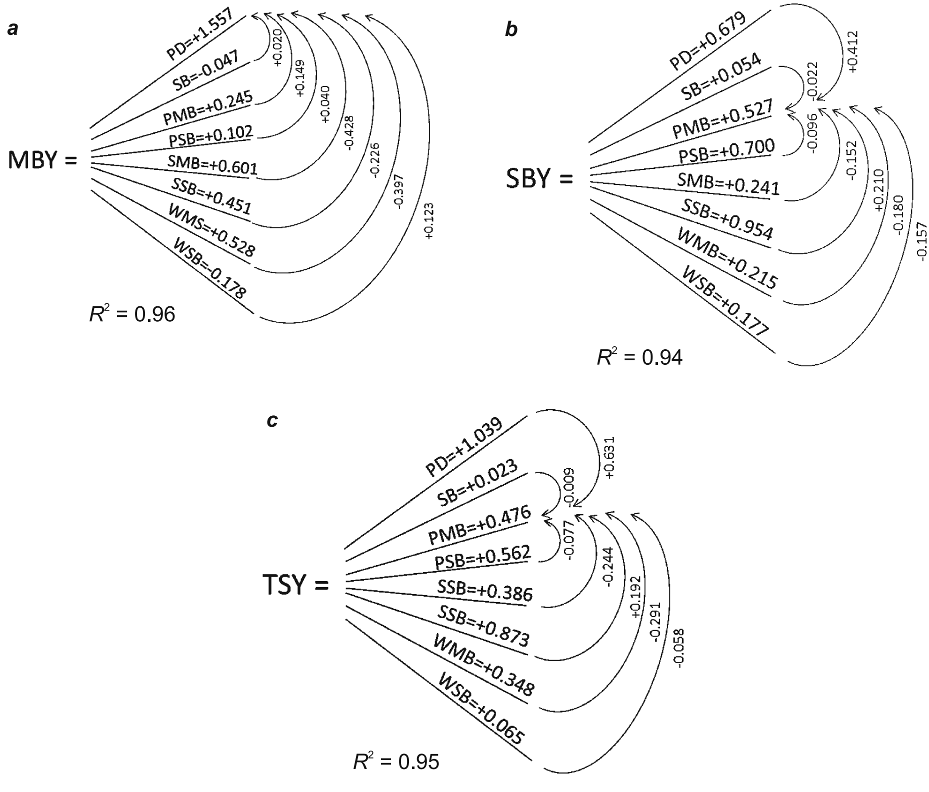

Fig. 1. Path diagrams: The arrangement of yield forming components impacting both directly and indirectly yield of oilseed rape produced by: *a* – the main inflorescence, *b* – secondary branches, *c* – whole plant

1) MBY = 0.507 + 0.021 PD for $n = 18$, $R^2 = 0.70$ and $p < 0.00002$,

2) MBY = -0.491 + 0.03PD + 0.047SMB for $n = 18$, $R^2 = 0.82$ and $p < 0.0000$,

3) MBY = -1.514 + 0.027PD + 0.02PMB + 0.064SMB for $n = 18$, $R^2 = 0.93$ and $p < 0.00000$.

These three regression models implicitly indicate on a balance between plant density and development of the main inflorescence structural components. Too dense plant canopy results in the lower number of seeds per pod of the principal inflorescence and thousand seed weight (Table 4).

The yield of secondary branches, decisive for the total yield of seeds, was determined by the quite different set of yield forming components. The number of pods per the main branch, as presented in the path diagram, was the single component, discriminating the most yield produced by subordinate branches (Figure 1*b*). Its direct effect was, however, much weaker, compared with several other yield components, which impact decreased in the order: SSB > PSB > PD. The value of the correlation coefficient for PMB was increased mainly due to the indirect but large impact of plant density (PD). The stepwise regression models implicitly showed that the best set of yield forming components is composed by the number of pods and seeds per pod of the main and secondary branch (PMB, PSB, SMB, SSB):

1) SBY = -0.554 + 0.071PMB for $n = 18$, $R^2 = 0.54$ and $p < 0.00054$,

2) SBY = -3.774 + 0.104PMB + 0.116SMB for $n = 18$, $R^2 = 0.72$ and $p < 0.00008$,

3) SBY = -4.789 + 0.1PMB + 0.007PSB + 0.SMB for $n = 18$, $R^2 = 0.82$ and $p < 0.00002$,

4) SBY = -3.976 + 0.06PMB + 0.015PSB + 0.036SMB + 0.099SSB for $n = 18$, $R^2 = 0.89$ and $p < 0.00000$.

The fourth regression model in the spite of the lowest p value was biased by insignificant effect of seed number per the main branch.

The total yield of seeds is a result of productivity of the main and secondary branches. The dominating yield forming effect of the number of pods per the principal stem has been fully corroborated by the path analysis (Figure 1*c*). However, the largest direct impact was an attribute of other components such as PD > SSB > PSB. The first variable was a decisive component for explanation of the final yield of seeds, having a large effect as results from the value of the indirect path coefficient. The best set of prognostic variables is composed of three structural components, such as the number of pods per the principal and subordinate branch and the number of seeds per pod of the main branch:

1) TSY = -0.59 + 0.1PMB for $n = 18$, $R^2 = 0.60$ and $p < 0.00016$,

2) TSY = -4.113 + 0.136PMB + 0.126SMB for $n = 18$, $R^2 = 0.72$ and $p < 0.00007$,

3) TSY = -5.5 + 0.13PMB + 0.01PSB + 0.131SMB for $n = 18$, $R^2 = 0.83$ for $p < 0.00001$.

This model is in close agreement with the regression model developed for oilseed rape by Grzebisz et al. (2010*b*). The key exception between both models is plant density as shown above, instead of the number of secondary branches. The last two sets of regression models implicitly show that both yield produced by subordinate branches, and the total yield can be explained at the moderate level of prognosis (ca 70%) by analyzing the response of the main branch components such as the number of pods and number of seed in the pod of the first inflorescence to interaction of seasons and applied nutrients. The best model, allowing to explain above 90% of oil-seed rape variability yield requires a different set of components (Figure 1c), including the number of plants per unit area, number of pods and seeds per pod of the secondary branch. This set of yield forming components is principally affected by year-to-year variability.

Dry matter partitioning

Dry matter yield and its partitioning among main oilseed rape organs was, except the rosette stage, governed by interaction of seasons, as the dominant factor, and fertilization treatments, as the minor one (Table 5). At the rosette stage, plant biomass was only significantly affected by applied fertilizers, showing on average, a considerably higher production in all treat-

Table 5

Statistical evaluation of oilseed rape biomass partitioning among plant parts during growth (g m^{-2})

Factor	Factor level	BBCH 31	BBCH 61		BBCH 89		
		LE[*]	LE	SH	ST	SE	TO
Fertilization treatments (FT)	AC	221.4^{a}	112.8^{a}	384.4^{a}	1007.5^{a}	288.1^{a}	1295.6^{a}
	NP	310.8^{b}	202.9^{b}	534.9^{b}	1269.0^{b}	381.4^{b}	1650.5^{b}
	NPK	298.0^{b}	199.7^{b}	507.0^{b}	1498.7^{b}	435.9^{bc}	1934.6^{b}
	NPKMgS1	304.0^{b}	192.7^{b}	557.9^{b}	1515.1^{b}	409.7^{bc}	1724.8^{b}
	NPKMgS2	296.8^{b}	195.4^{b}	531.2^{b}	1538.5^{b}	455.0^{c}	1933.6^{b}
	NPKMgS3	263.6^{ab}	195.7^{b}	540.6^{b}	1431.6^{b}	439.1^{bc}	1870.6^{b}
Years (Y)	2008	217.3	142.4^{a}	432.7^{a}	1603.2^{b}	475.0^{c}	2078.0^{b}
	2009	290.6	268.0^{b}	659.5^{b}	1205.0^{a}	425.2^{b}	1630.2^{a}
	2010	285.4	139.2^{a}	436.2^{a}	1222.1^{a}	304.5^{a}	1526.5^{a}
Fertilization treatments (FT)		**	**	**	***	***	***
Years (Y)		ns	***	***	***	***	***
FT x Y		ns	*	**	**	*	*

[*] LE, SH, ST, SE, TO – leaves, shoots, straw, seeds, total, respectively;
F – probability values: *,**, *** of 0.05, 0.01, 0.001, ns – no significantly different;
[a] the same letters means a lack of significant differences

ments fertilizing with nitrogen compared to the absolute control. Therefore, it collaborates the hypothesis that, plant density cannot be considered as the decisive yield forming factor (Hűhn 2001). At all subsequent stages of oilseed rape growth, its biomass was significantly affected by interaction of both factors. Its impact was, however, quite different before and after flowering, as resulted from analysis of biomass yield and its partitioning among main plant organs. In 2009, it has been noted during the period extending from the rosette to the onset of flowering an accelerated increase of the whole biomass of oilseed rape. The observed differences in the rate of biomass increase were much stronger for leaves compared to stem. The biomass of leaves doubled in 2009 as compared to other years, whereas it was only by $^{1}/_{3}$ higher for shoots. Quite distinct pattern of dry matter yield and distribution has been documented in the period extending from the onset of flowering to maturity. At this stage, the total plant biomass decreased in the descending order: 2008 (100%) > 2009 (78.4%) = 2010 (73.5%). However, its partitioning differentiated significantly for main organs. With respect to seeds, it decreased in the following order: 2010 (100%) > 2009 (89.5%) > 2010 (64%). At the same time, the yield of straw was by $^{1}/_{3}$ higher in 2008 compared to other years. The first model followed the strategy of oilseed crop growth termed by Barłóg and Grzebisz (2005*a*) as “fast biomass accumulation” and the second as “slow but permanent biomass accumulation.” The first strategy, dominating in 2009, resulted in a high biomass increase at the onset of flowering, leading subsequently to dramatic reduction in seed density. The second one, enabled the growth of pods, as underlined by the higher number of seeds per plant, as observed in 2008, up to maturity, resulted in the significant yield increase. This strategy reveals under conditions of the extended period of N uptake, but without a sharp elevation during the pre-anthesis period of growth, as hypothesized by Barłóg and Grzebisz (2005*b*). The higher yield of straw (STY) can be, therefore, considered as the key factor corroborating this hypothesis. The vegetative part of oilseed plants, including both stems and threshed pods, was the most important single factor explaining variability of the final yield of seeds, especially in 2008, characterized by the highest yield of seeds:

1) all years: $\mathrm{TSY} = 0.622 + 0.0025\mathrm{STY}$ for $n = 18$, $R^2 = 0.63$, and $p < 0.001$,
2) 2008: $\mathrm{TSY} = 1.526 + 0.002\mathrm{STY}$ for $n = 6$, $R^2 = 0.87$, and $p < 0.001$.

These two equations implicitly corroborate the hypothesis by Grzebisz (2013), who documented a positive impact of simultaneously application of magnesium and sulfur on yield components and yield of cereals under conditions of the mild water stress. Such conditions prevailed in 2008, in which the highest yield was harvested in the treatment with full rate of Mg and S applied in Autumn. The mode of this two nutrients action has been explained by a significant increase in vegetative biomass of oilseed plants following the beginning of flowering. As shown in Table 4, plants with an elevated dry matter yield at the beginning of lowering, as observed in 2009, impacted negatively the seed density of the secondary branch. This crop yield characteristic was significantly affected by oil-seed rape vegetative biomass production following the beginning of flowering ($DM_{89\text{-}61}$):

$$SSB = 5.639 + 0.732DM_{89\text{-}61} \qquad \text{for } n = 18,\ R^2 = 0.45, \text{ and } p < 0.01.$$

This equation corroborates the hypothesis that development of structural components of oilseed yield depends the most on the course of weather just before and after flowering. The observed increase in the seed density, as observed in 2008, resulted from a prolonged increase in the dry matter yield of vegetative parts. This is a result of pod growth, as a prerequisite of seed density. This is in agreement with Ludlow and Muchow (1990), who documented for cereals an increased growth of vegetative organs during the post-flowering period in response to mild drought. This positive response took place in treatments with MgS rate, applied in Autumn. The action of these nutrients resulted in additional uptake of nitrogen. The same type of crop response was reported by Grzebisz (2013) for cereals, maize and sugar beets. The contrastive growth conditions, resulting in the excess of biomass production during the pre-anthesis period of growth, which took place in 2009, leading finally to reduction in the seed number. This phenomenon indirectly proves the finding by Habekotte (1993), who showed a positive impact of plant biomass production by oilseed plants since the onset of flowering on seed density.

Nitrogen concentration and accumulation

Nitrogen concentration in the main part of oilseed rape plants was variable in consecutive stages of growth (Table 6). At the rosette stage, the

Table 6

Statistical evaluation of nitrogen concentration in parts of oilseed plant in critical stages of growth, (g kg^{-1} d.m.)

Factor	Factor level	BBCH 31	BBCH 61		BBCH 89	
		LE*	LE	SH	ST	SE
Fertilization treatments (FT)	AC	36.2^{a}	31.2^{a}	16.3^{a}	5.2^{a}	30.0^{a}
	NP	50.2^{b}	43.0^{b}	23.3^{b}	8.3^{b}	34.9^{b}
	NPK	52.4^{b}	40.0^{b}	25.1^{b}	8.0^{b}	35.9^{b}
	NPKMgS1	51.0^{b}	42.7^{b}	24.3^{b}	7.7^{b}	35.6^{b}
	NPKMgS2	49.9^{b}	42.2^{b}	24.3^{b}	0.73^{ab}	35.2^{b}
	NPKMgS3	51.6^{b}	41.9^{b}	23.8^{b}	8.1^{a}	34.9^{b}
Years (Y)	2008	44.2^{a}	36.8^{a}	19.7^{a}	5.6^{a}	30.0^{a}
	2009	5.11^{b}	47.7^{b}	23.3^{b}	11.0^{b}	35.9^{b}
	2010	50.4^{b}	36.7^{a}	25.6^{b}	5.7^{a}	37.3^{b}
Fertilization treatments (FT)		***	***	***	***	***
Years (Y)		***	***	***	***	***
FT x Y		*	ns	ns	ns	ns

* see Table 5; F – probability values: *,**, *** of 0.05, 0.01, 0.001, respectively, ns – no significantly different; ^{a} the same letters means a lack of significant differences

applied nitrogen fertilizer was the key factor impacting nitrogen concentration in leaves, but modified by the course of weather. Plants fertilized with nitrogen showed its concentration at the level of 5% d.m. This value is considered as an optimal for high-yielding plantation (WOJNOWSKA et al. 1995). It is necessary to focus attention on a much lower N concentration in 2008 compared to other two years. At flowering, this trend underwent a change for leaves, but not for shoots. In leaves, a significantly higher concentration was noted in 2009. At maturity, the same trend as for leaves at anthesis was observed for straw. The almost double concentration of N in straw in plants harvested in 2009 as compared to other years, indirectly indicates on a much lower N remobilization during the post-flowering period. The excess of nitrogen in vegetative plant parts was a result of an insufficient development of the sink capacity as indicated by the reduced seed density per plant.

The content of nitrogen in the oilseed rape canopy, as a result of its concentration and plant biomass, progressed throughout plant development stages. Both experimental factors impacted this crop characteristic only at maturity (Table 7). At the rosette stage the amount of nitrogen in the canopy responded to applied fertilizer N, but the key differences were due to variable weather in consecutive seasons. The most significant changes in nitrogen content took place in the period extending from the rosette up to the onset of flowering. As a rule, the amount of accumulated N raises up, but

Table 7

Statistical evaluation of nitrogen accumulation and partitioning in oilseed plant parts in critical stages of growth (g m^{-2})

Factor	Factor level	BBCH 31	BBCH 61		BBCH 89		NHI
		LE[*]	LE	SH	ST	SE	%
Fertilization treatments (FT)	AC	8.00^{a}	3.60^{a}	6.57^{a}	4.95^{a}	8.43^{a}	63.0
	NP	15.60^{b}	9.48^{b}	12.43^{b}	10.49^{b}	13.16^{b}	55.6
	NPK	15.75^{b}	8.50^{b}	12.90^{b}	11.96^{b}	15.44^{b}	56.4
	NPKMgS1	15.62^{b}	8.68^{b}	13.49^{b}	9.69^{b}	14.45^{b}	59.9
	NPKMgS2	14.81^{b}	8.52^{b}	13.05^{b}	10.86^{b}	15.87^{b}	56.7
	NPKMgS3	13.59^{b}	8.45^{b}	12.90^{b}	11.36^{b}	15.17^{b}	57.2
Years (Y)	2008	12.12^{a}	5.37^{a}	8.64^{a}	9.16^{b}	14.44^{b}	60.4^{b}
	2009	15.10^{b}	13.16^{b}	15.80^{b}	13.48^{c}	15.42^{b}	53.3^{a}
	2010	14.47^{b}	5.08^{a}	11.23^{a}	7.02^{a}	11.39^{a}	$61,8^{b}$
Fertilization treatments (FT)		***	***	***	***	***	ns
Years (Y)		***	***	***	***	***	***
FT x Y		ns	ns	ns	*	*	ns

[*] see Table 5; F – probability values: *,**, *** of 0.05, 0.01, 0.001, respectively, ns – no significantly different; ^{a} the same letters means a lack of significant differences

the observed degree of the increase was the year dependent. It was significant in 2008 and 2010, but almost doubled in 2009. Nitrogen partitioning among plant organs also showed year-to-year variability. In 2008, N content in leaves constituted 38%, whereas in 2009 – 45%, and in 2010 – 31% of its total amount in the canopy. At maturity, the pattern of nitrogen accumulation was considerable different. Effect of applied fertilizers was modified by the course of weather. The nitrogen harvest index achieved 60% in 2008 and 2010, but only 53% in 2009, in spite of the highest total N accumulation. These figures indirectly indicate on insufficient size of the sink capacity, as referred to the seed density.

In order to explain the impact of nitrogen management on yield, both paths and stepwise analyses were applied. Yield of seed produced by the main branch was, as indicated by almost equal values of both correlation and direct path coefficients, depended on the amount of N accumulated in seeds (Figure 2*a*). The path coefficient was a result of indirect and large effect of N content in leaves (negative) and in shoots (positive) at flowering. This observation has been corroborated by the stepwise analysis, as presented by the set of equations, taking into account all three sets of variables, including also N accumulated in the rosette:

1) $MBY = 0.257 + 0.074N_{SE}$ for $n = 18$, $R^2 = 0.57$ for $p < 0.001$,

2) $MBY = 0.448 - 0.036N_{RT} + 0.096N_{SE}$ for $n = 18$, $R^2 = 0.65$ for $p < 0.00034$,

3) $MBY = 0.645 - 0.54N_{LE} + 0.036N_{SH} + 0.075N_{SE}$ for $n = 18$, $R^2 = 0.78$ for $p < 0.0007$.

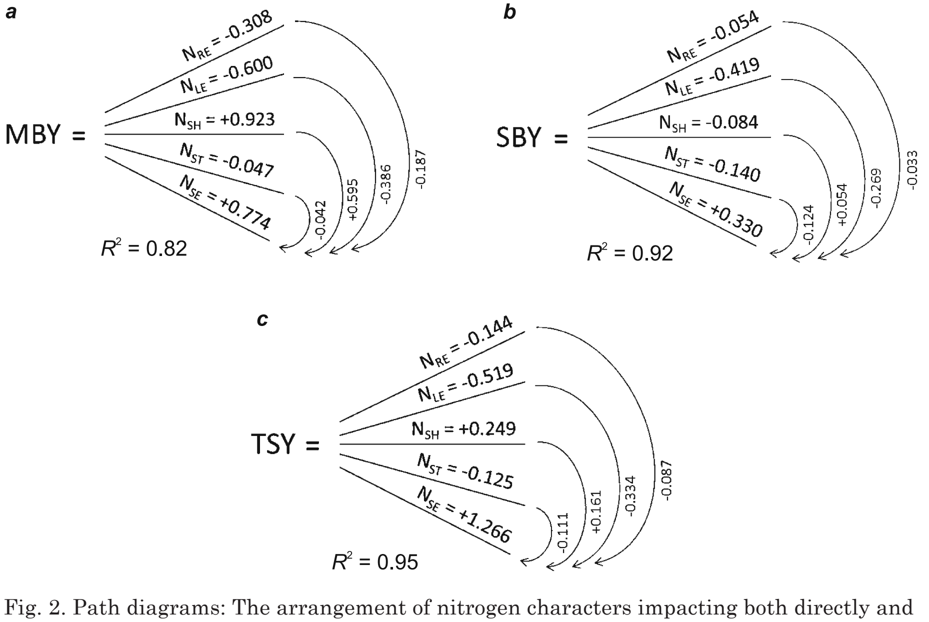

Fig. 2. Path diagrams: The arrangement of nitrogen characters impacting both directly and indirectly yield of oilseed rape produced by: *a* – the main inflorescence, *b* – secondary branches, *c* – whole plant

Yield of seeds produced by secondary branches was the most affected by nitrogen content in seeds (Figure 2*b*). However, its direct impact was significantly, but negatively corrected by all other variables, the most by nitrogen content in leaves. These observations have been fully corroborated by the developed stepwise regression models:

1) $SBY = 0.179 + 0.186N_{SE}$ for $n = 18$, $R^2 = 0.72$ for $P < 0.00001$,

2) $SBY = 0.263 - 0.1N_{LE} + 0.267N_{SE}$ for $n = 18$, $R^2 = 0.92$ for $p < 0.00000$.

The total yield of seeds, as referred earlier, was significantly depended on the yield produced by secondary branches. As results from analysis of both correlation and path coefficients, yield of seeds was governed the most by N content in seeds (Figure 2*c*). It has been, however, corrected by the amount of N accumulated in leaves at anthesis. This entire model has been fully corroborated by the developed stepwise regression models:

1) $TSY = 0.437 + 0.26N_{SE}$ for $n = 18$, $R^2 = 0.80$ and $p < 0.00000$,

2) $TSY = 0.527 - 0.107N_{LE} + 0.346N_{SE}$ for $n = 18$, $R^2 = 0.92$ and $p < 0.00000$.

The presented above three sets of equations clearly show that excess of nitrogen in leaves leads to yield decrease. In order to explain this phenomenon, it has been investigated the impact of nitrogen content in parts of oilseed rape during the growth season on the yield component performance. It has been documented that the number of pods (PMB) and number of seeds per pod (SMB), developed by the first inflorescence, are the key yield components for the moderate level of yield prognosis. The PMB dependency on nitrogen content in plant parts can be explained by the amount of N accumulated in seeds. This conclusion is supported by both correlation and path coefficients (Figure 3*a*). However, the path coefficient has been significantly corrected by N content in leaves and shoots at the beginning of flowering. This dependency has been corroborated by the stepwise regression model as presented below:

$PMB = 40.84 - 2.78N_{LE} + 1.87N_{SH} + 1.74N_{SE}$ for $n = 18$, $R^2 = 0.5$ and $p < 0.003$.

The second variable, SMB has been the most determined by the content of nitrogen in shoots. Its direct impact was negative in contrast to positive and large values of coefficients for N_{LE} and N_{SE} (Figure 3*b*). The N_{SH} coefficient has been positively corrected by nitrogen content in leaves (large indirect impact) and seeds (moderate) but at the same time negatively by its content in straw at maturity (large). The interactional effect of these two major variables has been fully corroborated by the developed regression model:

$SMB = 10.67 + 1.35N_{LE} - 1.25N_{SH}$ for $n = 18$, $R^2 = 0.57$, and $p < 0.0018$.

These two sets of equations implicitly corroborate the hypothesis that the performance of pods and seeds in the main branch was governed by the balance of N content in leaves and shoots at the beginning of anthesis. The excess of nitrogen in shoots at the beginning of flowering and in straw at maturity was the main reason for seed number reduction per pod, leading to decrease a seed capacity for N utilization.

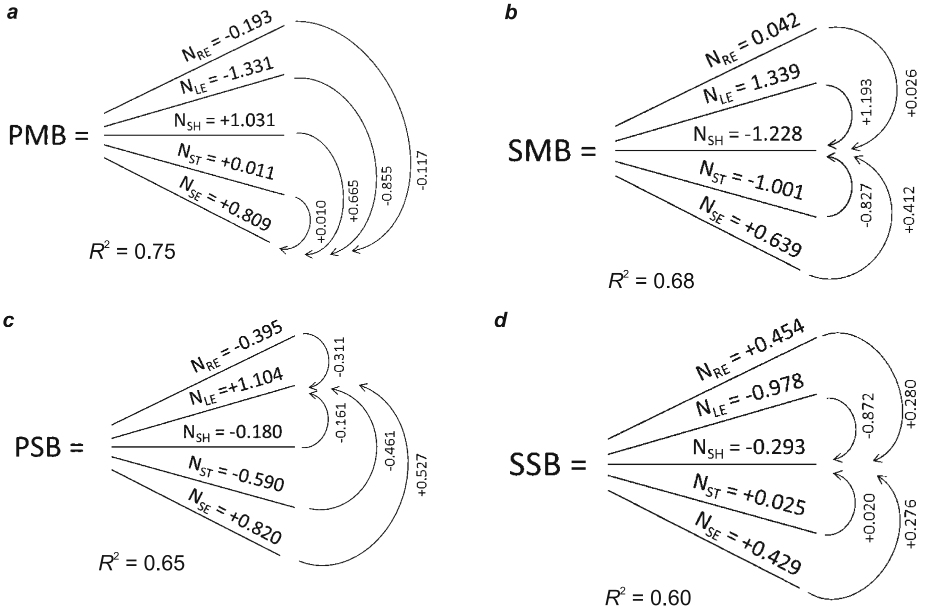

Fig. 3. Path diagrams: The arrangement of nitrogen characters impacting on yield structural components: *a* – number of pods per the main inflorescence, *b* – number of seeds per pod of the main inflorescence, *c* – number of pods per the secondary branch, *d* – number of seeds per the secondary branch

The higher level of yield prognosis requires the different set of data, composed mainly of PSB and SSB. The studied N accumulation variables had almost the same impact on PSB as results from analysis of correlation but not from path coefficients (Figure 3c). The highest values of both coefficients were the attribute of N_{LE}, which impact was significantly corrected by all, except N_{SH}, N variables. However, the significant prognosis can only be conducted using nitrogen content in leaves as the single predictor:

$PSB = 91.15 + 5.62N_{LE}$ for $n = 18$, $R^2 = 0.49$, and $p < 0.0013$.

The content of nitrogen in shoots and leaves at the beginning of anthesis showed, as results from values of correlation coefficients, the highest but negative impact on the seed density of secondary branches, SSB. This yield the structural components have been controlled the most by N content in leaves (Figure 3*d*). However, based on the developed stepwise regression model the highest accuracy of prognosis can be achieved, introducing only N content in shoots as the single significant variable:

$SSB = 14.54 - 0.525N_{SH}$ for $n = 18$, $R^2 = 0.31$, and $P < 0.01$.

Both equations implicitly indicate on the contrastive effect of N content in leaves at the beginning of flowering on the number of pods and seeds per pod of the secondary inflorescences. Plants following the strategy “slow by permanent biomass accumulation” showed the tendency to develop more pods of high seed density. This trend revealed a significant impact of applied magnesium and sulfur on components of yield performance in the first branch (Table 4). This pattern of N economy was observed in 2008. The post -anthesis increase in dry matter production, mainly pods, was the prerequisite of high yield of seeds. This fact corroborates the hypothesis developed by Diepenbrock (2000), who indicated the length of pods as the key attribute of the high-yielding oil-seed plantation. Plants followed the strategy "fast biomass accumulation" could develop a reasonable number of pods, but in expense of seed density, especially for pods of secondary branches. It seems probably that the prolonged pre-anthesis growth due to oversupply of nitrogen resulted in overproduction of vegetative biomass, as occurred in 2009. This phenomenon can be explained by a shortage of carbohydrate production for developing pods since the onset flowering, finally leading to seed density reduction (Habekotte 1993).

CONCLUSION

The conducted study implicitly documented that yield of oilseed rape significantly depends on the dominant strategy of plant growth during the pre- and post-anthesis periods. The study showed that the yield of seeds depends on the seed capacity to accumulate nitrogen. Plants following the strategy "slow by permanent biomass accumulation," which revealed in 2008 could continue biomass production from the onset of flowering. The highest yield of seeds was related to the increasing seed density in the first inflorescence as affected by magnesium and sulfur supply. This strategy revealed also a positive impact of N content in leaves at flowering stage of growth on seed density in the principal inflorescence. The second pattern of canopy performance by oilseed rape, which took place in 2009, termed as "fast biomass accumulation" was not as productive as the first one. It resulted in the seed density reduction, as a prerequisite of the decrease of the N physiological sink size, leading to the seed yield decrease. There was found a negative impact of N content in leaves on seed density in secondary branches.

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CONTENTS OF Cu, Zn, Cd, Pb AND Fe IN RAINWATER EFFLUENTS DISCHARGED TO SURFACE WATERS IN THE CITY OF POZNAŃ

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Abstract

Progressing urbanization results in the potential increase of pollution sources such as wastes, industrial or municipal sewage, which may contain hazardous inorganic pollutants such as e.g. metal ions and their compounds. Urbanization has a negative effect also on soil, as a consequence of an increasing share of impermeable surfaces such as pavements, parking lots, housing developments and public buildings. The area of a hardened (impermeable) surface is exposed to intensive surface runoff during rain and as a result pollutants are transported through the storm drainage system directly to surface waters.

The aim of this study was to determine the effect of metals contained in rainwater discharged from subcatchments of various land use types on the quality of surface waters (the Cybina River and the Antoninek reservoir) in the city of Poznań, as well as investigate whether metal contents in rainwater effluents depend on the relief and character of the immediate catchment.

Investigations were conducted in 2009 along the lower section of the Cybina River. Water samples from the river and reservoir were collected each time when collecting rain water samples. Water and sewage samples were collected 15 times within a year. Analyses were conducted on 5 selected catchments (with different land uses) drained by storm water drainage system discharged to the Cybina River and the Antoninek reservoir. Water was collected from 13 sampling points (5 of which were sewer outfalls and 8 were situated on the river or the reservoir above and below the sewage discharge).

Higher concentrations of all tested elements were recorded in rainwater in comparison to the water samples coming from the river or the reservoir. Canonical Variate Analysis (CVA) model presenting differences between water concentrations of Cu, Zn, Cd, Pb and Fe and environmental variables (rainfall intensity measured in a four-point scale, location as divided into industrial areas multi- and single-family housing as well as location of sewage discharge to the river on the reservoir) showed elevated concentrations of these elements especially in water

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collected directly from the storm water sewer. Elevated metal concentrations in storm system effluents did not have a significant effect on the content of these pollutants in the river or the reservoir. It could have been caused by the intensive immobilization of elemental ions in bottom deposits followed by the metal uptake by aquatic organisms, mainly plants.

Key words: rainwater, pollution, surface waters, copper, zinc, cadmium, lead, iron.

ZAWARTOŚĆ Cu, Zn, Cd, Pb I Fe W ŚCIEKACH DESZCZOWYCH ODPROWADZANYCH DO WÓD POWIERZCHNIOWYCH MIASTA POZNANIA

Abstrakt

Postępujący proces urbanizacji powoduje potencjalne zwiększenie źródeł zanieczyszczeń, jak odpady, ścieki przemysłowe czy komunalne, które mogą zawierać groźne zanieczyszczenia nieorganiczne, m.in. jony metali oraz ich związki. Proces ten oddziałuje niekorzystnie także na glebę, powodując zwiększenie udziału powierzchni nieprzepuszczalnych, jak chodniki, parkingi, budynki mieszkalne i użytkowe. Teren o powierzchni utwardzonej (nieprzepuszczalnej) jest narażony na intensywny spływ powierzchniowy podczas deszczu i transport substancji zanieczyszczających kanalizacją deszczową bezpośrednio do wód powierzchniowych.

Celem badań było określenie wpływu metali zawartych w wodzie deszczowej odpływającej ze zlewni cząstkowych o różnym użytkowaniu gruntów na jakość wód powierzchniowych (rzeki Cybiny i zbiornika Antoninek) na terenie Poznania, a także zbadanie, czy zawartość metali w ściekach deszczowych zależy od ukształtowania i rodzaju zlewni bezpośredniej.

Badania prowadzono w 2009 r. wzdłuż dolnego odcinka rzeki Cybiny. Próbki wody pobierano z rzeki i ze zbiornika Antoninek podczas opadów deszczu, 15 razy w ciągu roku. Do badań wybrano 5 zlewni (o odmiennym sposobie użytkowania) odwadnianych kanalizacją deszczową uchodzącą do rzeki Cybiny i zbiornika Antoninek. Wodę pobierano z 13 stanowisk (5 to wyloty z kanałów, 8 usytuowano na rzece lub zbiorniku powyżej i poniżej ujścia ścieków).

W wodzie deszczowej stwierdzono większe stężenie wszystkich badanych pierwiastków w porównaniu z wodą pochodzącą z rzeki lub zbiornika. Analiza CVA (Canonical Variate Analysis), która umożliwia określenie zależności między stężeniami Cu, Zn, Cd, Pb i Fe oraz zmiennymi środowiskowymi (intensywność opadów atmosferycznych mierzona w 4-stopniowej skali, lokalizacja uwzględniająca tereny przemysłowe, zabudowa wielo- i jednorodzinna, lokalizacja punktów poboru wody deszczowej) jednoznacznie wskazała na wyższe stężenie badanych pierwiastków w wodzie pobranej bezpośrednio z kolektorów deszczowych. Podwyższone stężenie metali w ściekach deszczowych nie wpłynęło znacząco na zawartość tych zanieczyszczeń w rzece i zbiorniku. Mogło to być spowodowane intensywną immobilizacją jonów pierwiastków w osady denne, a następnie pobraniem metali przez organizmy wodne, głównie rośliny.

Słowa kluczowe: ścieki deszczowe, zanieczyszczenie, wody powierzchniowe, miedź, cynk, kadm, żelazo.

INTRODUCTION

As a consequence of spatial management and urbanization the urban space is being gradually developed by roads, pavements, housing and public buildings (Wardas et al. 2010). This leads to an increased percentage share of impermeable surfaces such as concrete or asphalt in the urban space at the simultaneous reduction of permeable and semi-permeable surfaces such as natural subsoils, tree and shrub plantings. This phenomenon results in the accelerated surface runoff and promotes migration of pollutants to surface waters (Göbel et al. 2007).

Numerous pollutants may be found in rainwater, e.g. compounds of metals such as arsenic, mercury, chromium, lead, cadmium, nickel, barium, zinc, vanadium, tin, silver, strontium, aluminium, copper or iron. Quality of rainwater depends to a considerable degree on the type of catchment, season of the year, type of pavements, intensity of vehicle traffic, but also dry and wet deposition within a given catchment (Shirasuna et al. 2006).

Metals in rainwater in urbanized areas originate mainly from dusts from combustion plants, iron and steel industry, non-ferrous metallurgy, waste incineration plants, cement or glass-making industries (Göbel et al. 2007).

Significant sources of certain metals such as Cu and Zn include impermeable roof surfaces, which are most frequently produced from materials containing these metals, additionally roofing materials, roof gutters or downpipes may be covered by special coatings containing Al or Pb.

Pavements of roads and parking lots with intensive vehicle traffic are important potential sources of metals. Direct pollutant sources include products formed as a result of abrasion of pavements, tires, brake shoes as well as leaks of gear oil, lubricant, brake fluid or corrosion products. Tire wear leads to the release of oxides of such metals as Zn, Pb, Cu, Cr and Ni, while wear of brake shoes produces mainly oxides of Ni Cr, Cu and Pb. In turn, Fe may originate from worn brake drums.

Rainwater quality to a considerable degree depends on the form of pollutants. The stronger a metal is bound with solid particulates, the greater the effect of intensive, but short-term precipitation on total load (Dougherty et al. 2006, Budai, Clement 2007).

In Poland for many years rainwater flowing to the receiving water tank was considered clean. As a result, precipitation water discharged to the sewerage system was analyzed extremely rarely and those analyses concerned only several indexes such as pH, BOD_5, COD, chlorides, sulfates or electrolytic conductivity. Only recently rainwater has been included in the sewage category, resulting in the necessity of its treatment prior to its discharge to surface waters (*Ordinance of the Minister of the Environment* 2006).

The aim of the presented study was to determine the effect of metals contained in rainwater sewage flowing in from subcatchments differing in land use types (single- and multi-family housing development, industrial areas) on quality of surface waters (the Cybina River and the Antoninek reservoir) in the city of Poznań.

MATERIALS AND METHODS

Site description

Investigations were conducted in 2009 along the lower section of the Cybina River, being a right-bank tributary to the Warta River. The analyzed section of the Cybina is situated in the city of Poznań, between Lakes Swarzędzkie and Maltańskie, i.e. between 4.3 and 8.7 km of the river course, counting from its confluence to the Warta. Mean multiannual flow rate in the river at this section is 0.67 m^3 s^{-1}. In the river course at this section four small dam reservoirs were constructed or reconstructed in the 1980's, which main objective was to intensify the process of water self-purification.

Analyses concerning the effect of rainwater sewage on surface waters were conducted on five selected catchments drained by storm water drainage systems discharged to the Cybina and the small Antoninek reservoir located in its area. Catchment no. 1 draining the single-family housing district is 42.7 ha in area, of which paved areas occupy 28.9% (Figure 1*a*). A settlement tank and a separator for sewage pre-treatment are located in front of the outfall sewer outlet to the Cybina. Catchment no. 2 covers the area of a vehicle manufacturing plant (Volkswagen), including parking lots for new cars and employee parking plots as well as a section of an expressway of approx. 800 m. The drained catchment is 56.8 ha in area, of which 51.6% are paved surfaces (Figure 1a). Before being discharged to the Cybina sewage is pretreated in an Imhoff tank, adapted for this purpose after a former domestic sewage and process wastewater treatment plant. Plot no. 3 covers mainly the Antoninek Glassworks. It is 4.78 ha in area, of which 88% are paved (Figure 1*b*). Rainwater sewage is discharged to the Antoninek reservoir. Plot no. 4 occupies the area of workshops, a car showroom and parking lots of Polcar,

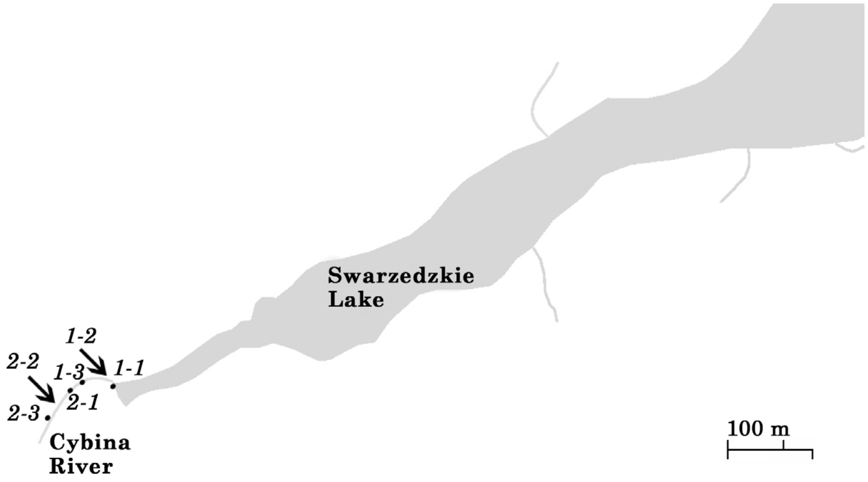


Fig. 1*a*. The location map of water sampling points below Lake Swarzędzkie

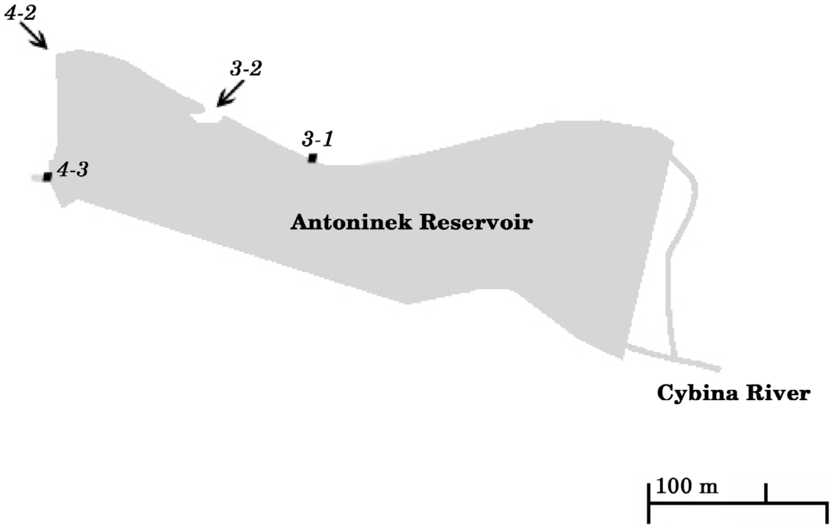


Fig. 1*b*. The location map of water sampling points in the Antoninek reservoir

a car dealer also offering automobile repair services. The catchment area is 4.33 ha, of which 76% are paved. Rainwater is also discharged to the Antoninek reservoir (Figure 1*b*). Plot no. 5 covers the area of a small multi-family housing district. This catchment is 7.37 ha in area, with only 26.7% being paved (Figure 1*c*). The limited paved area and a slight inclination of the area results in the amount of discharged waters being lower than those of the other catchments.

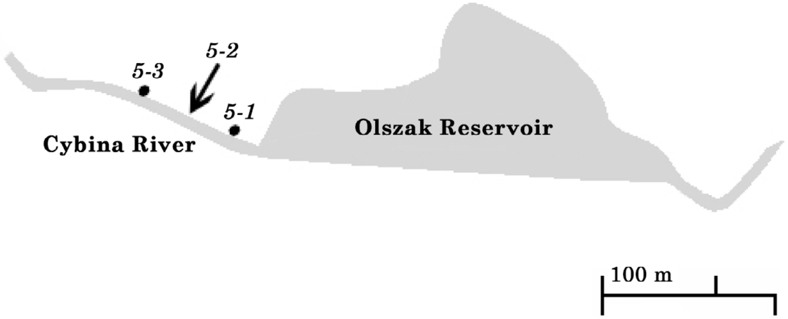


Fig. 1*c*. The location map of water sampling points below the Olszak reservoir

Sampling

Water samples from the river and reservoir were collected each time when collecting rain water samples. Water and sewage samples were collected 15 times within a year. A total of 13 sampling points were established in the river in the vicinity of rainwater sewage outfalls from the identified subcatchments and directly on these outfalls. Five of them were storm water sewage outfalls (denoted with black arrows), 8 were situated on the river and the water reservoir constituting storm water receiving waters. The sampling points on the river were always situated above and below sewage outfalls (denoted with black points); however, in the case of the reservoir due to the location of sewage outfalls in close vicinity only one station was established above and below both outfalls (Figure 1*b*).

Analytical methods

Water samples from the river and reservoir were collected from the current from the subsurface water layer. Storm water sewage was collected directly from the outflow from the sewer. Samples for chemical analyses were collected at each sampling point in three replications and they were analyzed separately.

Five metals (Cu, Zn, Cd, Pb and Fe) were detected in water and sewage samples. Quantitative analyses of elements were performed by flame atomic absorption spectrometry FAAS (ISO 9964-1-1993) as well as inductively coupled plasma mass spectrometry ICP-MS (ISO 17294-2-2003). Specification and parameters for individual methods were presented below (Tables 1, 2).

Statistical analysis was performed using the analysis of variance (Anova). In analysis parametric test Matched Anova for normal data was used. This test is used to compare two or more sets of matched data, and it tests the null hypothesis that the mean difference between the sets is zero.

Table 1

Instrumental characteristics and setting for ICP-MS

Spectrometer	**ELAN DRC II (Perkin Elmer Sciex, Canada)**
Nebulizer	Meinhard
Spray chamber	Cyclonic
Interface	Pt cones
Mass analyzer	Quadrupole
RF power (W)	1200
Ar gas flow rates (L min^{-1}): plasma auxiliary nebulizer	 15 1.2 0.94
Internal standard	Sc^{45}, Y^{89}, Tb^{159}

Table 2

Operating parameters for F-AAS

Ion	Wavelength (nm)	Lamp intensity (mA)	Split (nm)
Fe	248.3	10.0	0.2
Zn	213.9	10.0	1.0

If $p < 5\%$ then the null hypothesis is rejected and at least one of the sets is significantly different.

In order to indicate which explanatory variables are the best predictors of sampling points, taking into account also types of catchment area as well as rainfall intensity discriminatory analysis was used, which made it possible to construct the CVA [canonical variation analysis – a linear variant of Fisher's discriminatory analysis (LDA)]. Calculations using the Canoco 4.5 for Windows program were presented graphically in the CanoDraw for Windows program. Thanks to this statistical method it may be stated which environmental variables [rainfall intensity measured in a four-point scale, location as divided into industrial areas, multi- and single-family housing development as well as the position of sewage discharge to the river or the reservoir (above the storm sewer, directly from the sewer and below the storm sewer)] determine the distribution of concentrations for analyzed elements in storm water sewage and in surface waters. Explanatory variables used in discriminatory analysis in Canoco as environmental variables. Then a canonical correspondence analysis (CCA) was selected using Hill's scaling.

In order to determine the boundary level of significance the Monte Carlo permutation test was performed. All variables were analyzed and next these variables were included in the model, which contributed the most to the discrimination of groups based on the values of p and F for the analyzed variable. This process was repeated to the time when the value of p dropped below 0.05 for the tested variable.

Differencies between the catchments were tested using an analysis of variance on repeated measures using that general linear models approach and Tukey' s test. Statistical analyses were done Statistica version 6.0 software.

RESULTS

Analysis of distribution for concentrations of selected elements (Cu, Zn, Cd, Pb and Fe) in the Anova test

The analysis given below was conducted for elements, which values within a given group were statistically significantly correlated ($p < 0.05$) – Table 3. The Anova test was applied in order to compare three and four paired groups of variables with a distribution consistent with a normal distribution.

The figures given below (1 - 5) illustrate the distribution of concentrations for Cu, Zn, Cd, Pb and Fe. Definitely the highest concentrations of all the elements were recorded in water collected from rainwater sewers. In case of copper the highest value (amounting to over 120 $\mu g\ l^{-1}$) was observed in the water from sewer no. 1 (Figure 2*a*). A greater effect on the concentrations of copper in river water was observed for water from sewer no. 2, which is connected with a greater amount of rainwater influents. Concentrations in river water below that sewer were so high that they exceeded acute

Table 3

Mean and calculated values of *p* in the Anova test for Cu, Zn, Cd, Pb and Fe (μg l^{-1})

Catchment		Single-family housing district			Area of a vehicle manufacturing plant			Antoninek Glassworks		Area of workshops		Area of a multi-family housing district		
		1-1.	**1-2.**	**1-3.**	**2-1.**	**2-2.**	**2-3.**	**3-1.**	**3-2.**	**4-2.**	**4-3.**	**5-1.**	**5-2.**	**5-3.**
Cu	average	2.75	32.56	3.26	2.59	15.88	8.00	2.37	10.23	19.87	2.35	1.60	8.65	2.94
	***p*- value**	0.011*			0.001*			0.049*				0.007*		
Zn	average	16.25	52.97	16.32	25.84	246.82	90.90	20.14	870.70	156.91	11.55	7.73	87.20	15.93
	***p*- value**	0.046*			0.001*			0.019*				0.020*		
Cd	average	0.01	0.10	0.02	0.02	0.21	0.07	0.16	0.44	0.31	0.03	0.01	0.21	0.04
	***p*- value**	0.049*			0.035*			0.011*				0.007*		
Pb	average	0.39	5.18	0.71	0.43	4.63	2.45	10.43	15.68	18.58	7.06	1.35	17.55	3.15
	***p*- value**	0.016*			0.004*			0.164				0.044*		
Fe	average	143.53	441.28	150.86	244.18	460.84	292.68	278.17	649.11	652.04	315,87	432.18	2049.21	317.50
	***p*-value**	0.045*			0.014*			0.050				0.046*		

values for some aquatic animals, e.g. cladocerans *Daphnia pulicaria* and *Ceriodaphnia dubia*. Phytoplankton species, especially cyanobacteria, are very sensitive to copper. That is a reason of the frequent use of copper as an algicide (Padovesi-Fonseca, Philomeno 2004). These high concentrations of copper are probably one of the reasons of the observed cyanobacteria decline in the Antoninek Reservoir (Gołdyn et al. 2010). More evident differences in the concentration levels were shown for zinc. In sewer no. 3 maximum

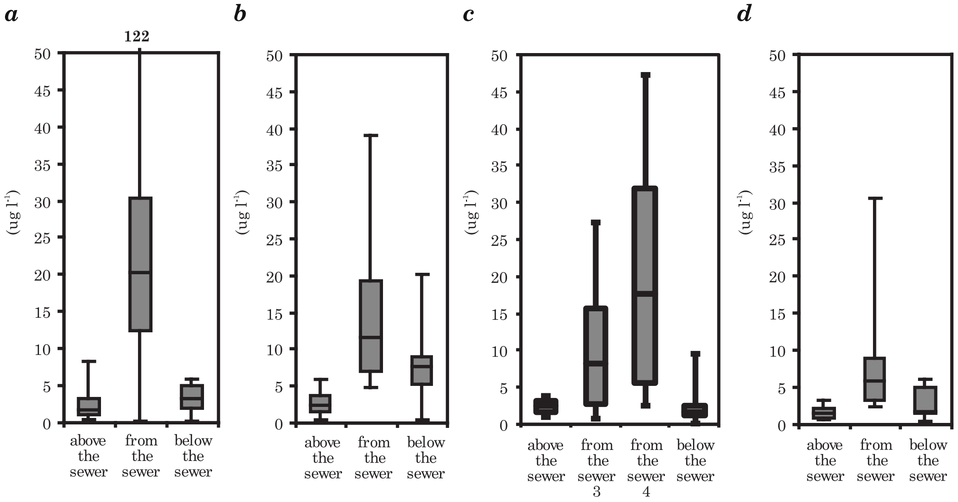


Fig. 2. The distribution of Cu concentrations in the annual cycle: *a* – catchment of the single-family housing district, *b* – catchment of the area of a vehicle manufacturing plant, *c* – catchments of the Antoninek Glassworks and the area of workshops, *d* – catchment of the area of a multi-family housing district

values exceeded 7 mg l^{-1} (Figure 3*c*). It is the value exceeding the criteria of organoleptic effects for pollutants, listed by the US EPA (2009). Also for cadmium the highest concentration was observed in waters from sewers nos. 3 and 4 (Figure 4*c*) located in industrial areas. Discharge of these waters to the reservoir in Antoninek resulted in a rapid precipitation of cadmium to bottom deposits, since at testing station 4.3 in the vicinity of the outflow of water from the reservoir the concentration of this element was much lower, never exceeding the chronic value of 0.25 μg l^{-1} (EPA 2009).

When analyzing the distribution of lead concentrations it may be stated that the greatest differences in concentrations were found at sewer no.

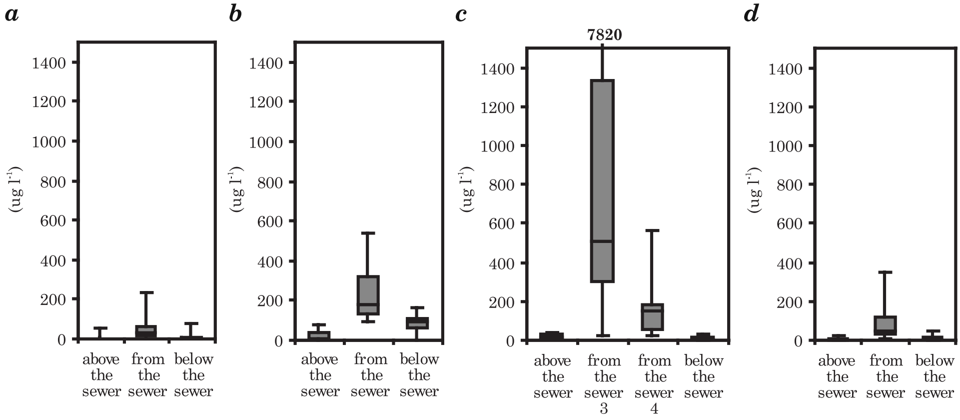


Fig. 3. The distribution of Zn concentrations in the concentrations in the annual cycle: *a* – catchment of the single-family housing district, *b* – catchment of the area of a vehicle manufacturing plant, *c* – catchments of the Antoninek Glassworks and the area of workshops, *d* – catchment of the area of a multi-family housing district

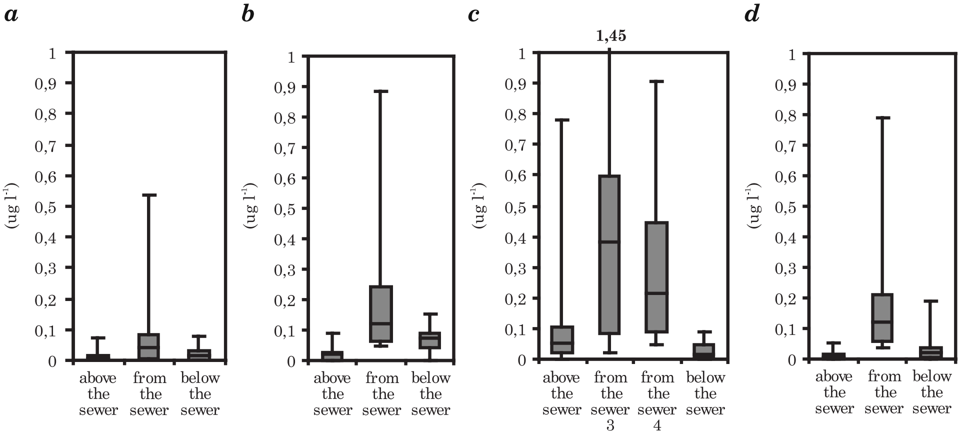


Fig. 4. The distribution of Cd concentrations in the annual cycle: *a* – catchment of the single-family housing district, *b* – catchment of the area of a vehicle manufacturing plant, *c* – catchments of the Antoninek Glassworks and the area of workshops, *d* – catchment of the area of a multi-family housing district

5 in comparison to the concentrations of this element in samples of water coming from the river above and below the sewer (Figure 5*d*). An analogous situation occurred in case of iron, when the highest concentrations were also recorded at sewer no. 5 (Figure 6*d*). In most cases high concentrations of these parameters in sewers had an effect on elevated levels of concentrations in water sampled below the sewer Only in case of the Antoninek reservoir usually instead of an increase in the concentrations their levels were found

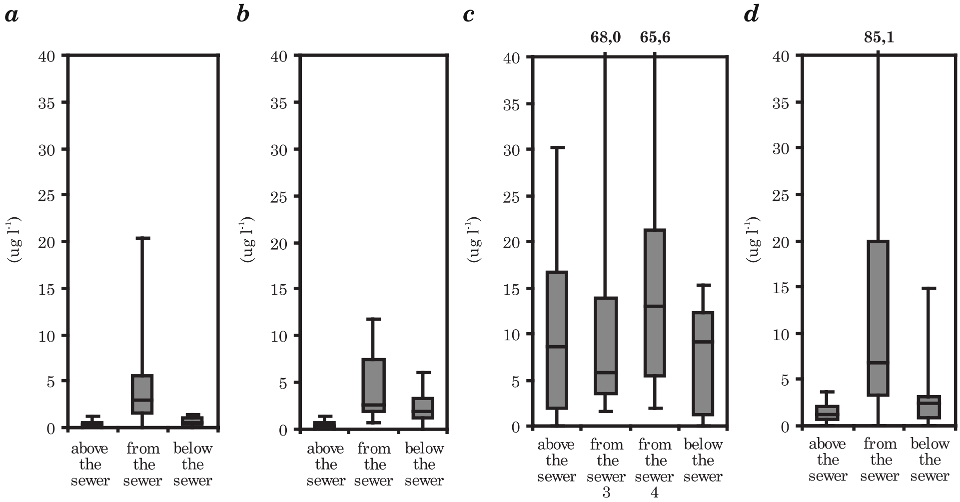


Fig. 5. The distribution of Pb concentrations in the annual cycle: *a* – catchment of the single-family housing district, *b* – catchment of the area of a vehicle manufacturing plant, *c* – catchments of the Antoninek Glassworks and the area of workshops, *d* – catchment of the area of a multi-family housing district

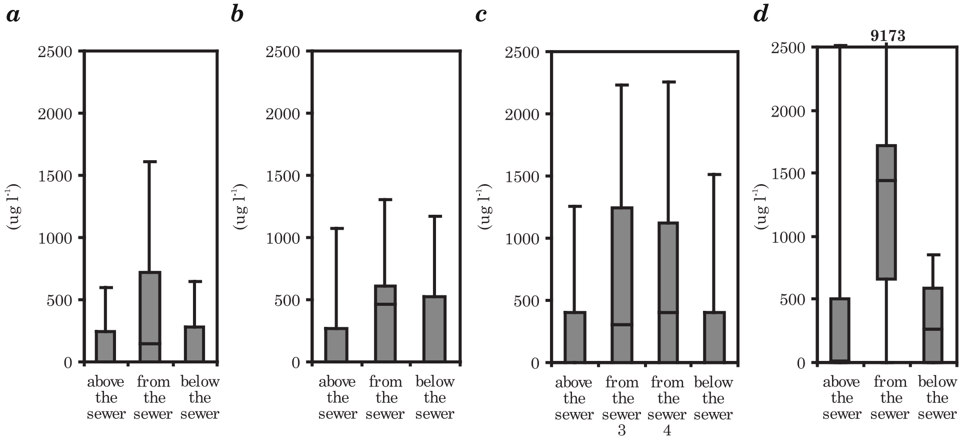


Fig. 6. The distribution of Fe concentrations in the annual cycle: *a* – catchment of the single-family housing district, *b* – catchment of the area of a vehicle manufacturing plant, *c* – catchments of the Antoninek Glassworks and the area of workshops, *d* – catchment of the area of a multi-family housing district

to decrease in its waters, despite the high values in discharged stormwaters. This indicates that in the reservoir there occurs a rapid sedimentation of pollutants supplied both with stormwater and river waters, connected with the lothic conditions being replaced by lenthic conditions.

Canonical Variate Analysis (CVA) model – a canonical variant of Fisher's linear discriminatory analysis (LDA)

This model was constructed based on the discriminatory analysis. The aim of the conducted analyses was to verify which environmental variables may influence the distribution of concentrations of tested elements in waters of the Cybina River as well as the Antoninek reservoir. Canonical Variate Analysis (CVA), a canonical variant of Fisher's linear discriminatory analysis (LDA) was used in the construction of the model.

The discriminatory analysis compared the effect of different variables on the distribution of concentrations of tested elements in water. The following parameters were included in the analyses: rainfall intensity measured in a four-point scale, location as divided into industrial areas, multi- and single -family housing as well as the location of sewage discharge to the river or the reservoir (above the sewer, directly at the sewer and below the storm water sewer).

Forward stepwise analysis was performed in order to verify which variables to the highest degree determine the distribution of concentrations of the tested elements.

The model given below (Figure 7) presents dependencies between tested parameters (Cu, Zn, Cd, Pb and Fe) and variables included in the analysis. All analyzed elements were characterized by elevated concentrations in water collected directly from the storm water sewer, with higher concentrations of investigated pollutants in storm water sewage. For lead and cadmium

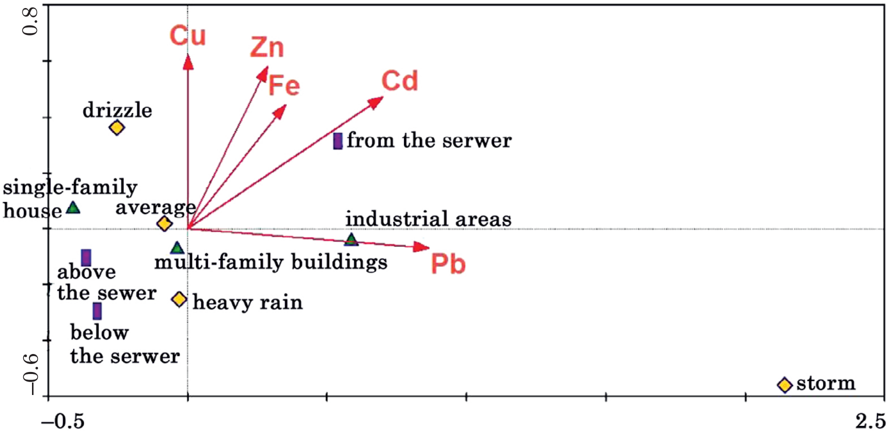
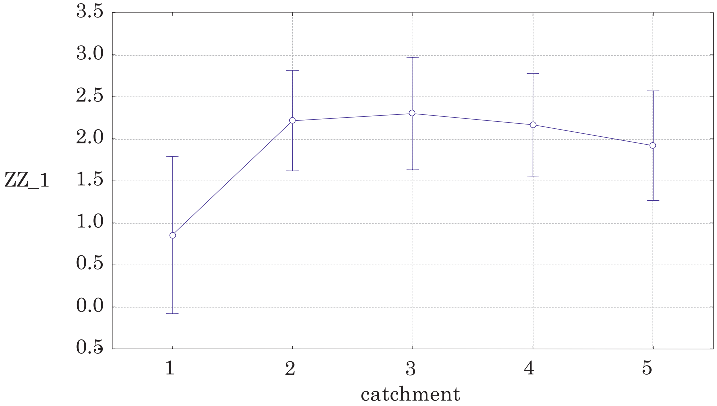


Fig. 7. The CVA model (n = 137) – dependencies between analyzed elements and variables (rainfall intensity, location as divided into industrial areas, multi- and single-family housing development as well as the location of sewage inlet to the river or the reservoir (above the sewer, directly at the sewer and below the storm water sewer, $p < 0.05$

the highest concentrations were recorded during the most intensive rainfall in industrial areas. Higher copper concentrations were detected in water coming from the catchments with predominant single-family housing development during the least intensive rainfall.

The analysis of variance on repeated measures using that general linear models approach was done (Figure 8). The model tests two factors (catchment, concentration of examined metals) and interaction between analysed factors. In the analysis Tukey's test was applied which made it posssible to find the difference between the catchments with respect to the level of examined metals. It was shown that catchment no. 1 draining the single-family housing district differ significantly from the other studied catchments.



ZZ_1 – expected boundary values

Fig. 8. The differencies between the catchments no 1-5 with respect to the level of examined metals [F(4, 160)=5.0811, $p = 0.0007$]

DISCUSSION

When analyzing recorded data it may be stated that rainwater contained a considerable load of metals such as Cu, Zn, Cd, Pb or Fe in comparison to water collected from the Cybina River or the Antoninek reservoir. The highest values were found for Fe, while the lowest for Cd. The highest levels for Cu were recorded in rainwater collected from catchment no. 1 (mean 32.56 μg l^{-1}), which could probably have been caused by the frequent use of copper roof finish elements in single-family housing. In countries of Central Europe the mean copper emission from roof surfaces was 1.1 g m^{-2} $year^{-1}$, while for zinc it was 3.0 g m^{-2} $year^{-1}$. Water coming from catchment no. 3 had the highest Zn and Cd values, which is typical of industrial areas, i.e. the Antoninek Glassworks in this case. Such high values were obviously a consequence of the high share of paved area (impermeable) in the catchment, amounting to as much as 88%. The highest Pb concentration was recorded in rainwater coming from catchment no. 4 (workshops, showrooms, parking lots), with the mean lead level in rainwater at the storm water sewer amounting to 18.58 μg l^{-1}. Catchment no. 5 with multi-family housing development was characterized by rainwater with an elevated Fe concentration. This area had a slight percentage (26.7%) of paved areas, which may indicate that this element is only partially immobilized with the soil sorption complex.

When referring the recorded results to literature data similar trends may be found in the distribution of concentrations for the tested elements. Studies conducted by GÖBEL et al. 2007 concerned rainwater collected from an unpaved catchment (gardens, green areas), roofs and roads with varying degrees of vehicle traffic. It was shown that water flowing directly from roofs had higher concentrations of such elements as Zn or Cu, while water collected from traffic arteries had higher Pb concentrations in relation to the other tested locations. In the case of Pb and Zn, the above mentioned findings were confirmed in a study by GNECCO et al. (2005). A study by GÖBEL et al. (2007) showed a considerable effect of the type of pavement, from which rainwater flows to the receiving waters on its pollution with metals.

Studies conducted by KANG et al. in the years 2005 - 2007 were connected with the evaluation of water quality in the Yeongsan River in the vicinity of the city of Gwangju (Korea) depending on the weather. Results showed a marked effect of precipitation intensity on pollution of tested waters. This was the case particularly with such metals as Cd, Cu or Zn.

Metal pollution of rainwater is also influenced by the manner of catchment use. Analyses conducted in Queensland State, Australia, showed that water rich in Zn abd Pb flows from areas with housing land use. In contrast, rainwater collected from industrial areas had higher Pb and Fe concentrations in comparison to water collected from the other sampling points (HERNGREN et al. 2005).

In recent years much attention has been focused on disposal methods for metals polluting waters (SHRESTHA et al. 2003). One of the methods to remove metals from water may be connected with the establishment of a series of flow-through ponds with a total length adapted to the river water flow rate. Effectiveness of this method was specified for Cu at 70%, while for Zn it was 94 - 95% (WALKER, HURL 2002). In turn, in the experiment conducted by JANG et al. (2005) 3 types of mulch were evaluated as potential sorbents of heavy metals from water flowing from urbanized areas. It was shown that mulch from bark of deciduous trees is the best sorbent of heavy metals in urban areas. It seems that establish the biological barriers can increase the inflow of heavy metals because some plants and algae, called hyperaccumulators, such as *Thlaspi caerulenscens* J. Presl & C. Presl*, Salix vilminalis, Brassica juncea* (L.) Czern., *Ceratophyllum demersum* L., *Potamogeton crispus* L. and *Oenanthe aquatica* (L.) demonstrate a particularly high ability to accumulate metals in unusually high concentrations (SALT et al. 1998, Obarska-Pempkowiak, Kołecka 2005, Senze et al. 2009, Świerk, Szpakowska 2011, RAJFUR 2013). Also common reed (*Phragmites australis* (Cav.) Trin. ex Steud.), which is a widely distributed species worldwide, proves useful in cleaning eutrophic lakes and waste waters (Kozłowska et al. 2009).

CONCLUSIONS

1. Elevated concentrations of all tested metals were found in water collected from storm water sewers. The highest concentrations in rainwater were recorded for Fe (max. 7820 μg l^{-1} – catchment no. 5) and for Zn (max. 7820 μg l^{-1} – catchment no. 3), while the lowest concentration in rainwater was detected for Cd.
2. Rainwater coming from catchments of industrial and commercial land use (catchments nos. 3 and 4) had higher concentrations of such elements as Zn, Cd and Pb, while rainwater coming from catchments with predominant single-family housing developments had higher Cu concentrations in comparison to water collected from the other catchments.
3. Values of measured concentrations were most probably influenced by the share of paved surfaces in the total area of the immediate catchment and by the land use of the catchment.
4. No significant negative effect of rainwater was found on surface waters in the city of Poznań. This may indicate intensive immobilization of these elements into bottom deposits and tissues of aquatic vegetation, mainly hydromacrophytes.
5. The CVA model presenting dependencies between water concentrations of Cu, Zn, Cd, Pb and Fe and environmental variables showed that all analyzed elements were characterized by elevated concentrations in water collected directly from the storm water sewer.

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INFLUENCE OF CADMIUM DOSE AND FORM ON THE YIELD OF OAT (*AVENA SATIVA* L.) AND THE METAL DISTRIBUTION IN THE PLANT*

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Abstract

The influence of two levels of artificial Cd soil contamination (2 and 20 mg Cd kg^{-1} of soil) on the weight of oat plants, chlorophyll content in leaves, rate of photosynthesis, stomatal conductivity and transpiration rate was researched in a pot experiments with *Avena sativa* L. Another objective was to detect the effect of cadmium contamination of soil on the content of cadmium in the dry mass of oat panicles, stems, upper green and bottom yellow leaves and roots. The soil contamination was applied in the forms of nitrate $Cd(NO_3)_2$, chloride $CdCl_2$ and sulphate $CdSO_4^{2-}$.

High correlation was found between the measured levels of photosynthesis rate, stomatal conductivity and transpiration rates, but no correlation occurred between these levels and the cadmium content in leaves. In the variants with Cd contamination, insignificantly higher levels of photosynthesis rates were observed in the measurements than in the zero variant. A 10-fold higher Cd application dose significantly manifested itself by a higher content of Cd in all the analyzed parts of plants, including generative organs. A several-fold higher Cd level was found in the roots than in other parts of the plant, whereas the lowest Cd content was observed in panicles.

However, the results obtained by measuring the cadmium content in stems and green leaves were not significant. In most treatments, a notably higher Cd content was determined in bottom yellow leaves than in upper green leaves. This indicates Cd accumulation in senescent tissues and its difficult reutilization. The highest variance was discovered in treatments with the accompanying SO_4^{2-} anion. While estimating the effect of accompanying anions on the Cd content, significant differences were observed only under the higher level of Cd contamination.

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The increase in the Cd content in bottom yellow leaves after $CdSO_4$ application was significant when compared with the treatment in which $Cd(NO_3)_2$ was applied and insignificant versus the variant with $CdCl_2$. On the other hand, a higher and more significant content of Cd in phtosynthetically active green leaves was measured in the treatment with $CdCl_2$ than with $Cd(NO_3)_2$.

Key words: cadmium, photosynthesis rate, translocation, interaction, oat.

WPŁYW DAWKI I FORMY KADMU NA PLON I DYSTRYBUCJĘ METALU W OWSIE (*AVENA SATIVA* L.)

Abstrakt

W eksperymentach z *Avena sativa* L. zbadano wpływ 2 poziomów sztucznego zanieczyszczenia gleby Cd (2 i 20 mg Cd kg^{-1} gleby) w postaci $Cd(NO_3)_2$, $CdCl_2$ i $CdSO_4^{2-}$ na masę roślin owsa, zawartość chlorofilu w liściach, tempo fotosyntezy, przewodność szparkową i szybkość parowania, a także na zawartość kadmu w suchej masie wiechy, łodyg, górnych i dolnych zielonych i żółtych liści oraz korzeni. Wysoki poziom korelacji stwierdzono między mierzonymi poziomami intensywności fotosyntezy, przewodności szparkowej i szybkości transpiracji, nie wykazano jednak korelacji między tymi poziomami i zawartością kadmu w liściach. W wariantach z zanieczyszczeniem Cd zaobserwowano nieznacznie wyższy poziom fotosyntezy w pomiarach w porównaniu z wariantem zerowym. W przypadku 10-krotnie większej intensywności stosowania kadmu wykazano istotnie większą zawartość Cd we wszystkich monitorowanych częściach roślin, w tym w organach generatywnych. Kilkakrotnie wyższy poziom Cd niż w innych częściach rośliny stwierdzono w korzeniach, a najmniejszy w wiechach, jednak dane uzyskane podczas pomiaru zawartości kadmu w łodygach i zielonych liściach nie były znaczące. W porównaniu z górnymi liśćmi, zauważalnie większą zawartość Cd w większości poddanych eksperymentowi roślin stwierdzono w dolnych żółtych liściach. Wskazuje to na akumulację Cd w starzejących się tkankach i trudności z jego przetworzeniem. Największą różnicę wykazano w próbkach z anionem towarzyszącym SO_4^{2-}. Podczas ewaluacji wpływu anionów towarzyszących na zawartość Cd istotne różnice wystąpiły jedynie w przypadku wyższego poziomu zanieczyszczenia Cd. Znaczący wzrost zawartości Cd po zastosowaniu $CdSO_4$ zaobserwowano w dolnych żółtych liściach, w porównaniu z próbkami, w których zastosowano $Cd(NO_3)_2$ natomiast nieznaczny – w porównaniu z próbkami, w których użyto $CdCl_2$. Zdecydowanie największą zawartość Cd w zielonych liściach o aktywnej fotosyntezie stwierdzono w roślinach poddanych działaniu $CdCl_2$ w porównaniu z tymi, w przypadku których użyto $Cd(NO_3)_2$.

Słowa kluczowe: kadm, tempo fotosyntezy, translokacja, interakcja, owies.

INTRODUCTION

Plants absorb cadmium from soil mainly in the form of Cd^{2+} cations. The bioavailability of soil-borne Cd can be affected by its total content, the form of chemical bond and other factors, such as the type of soil, CEC (cation exchange capacity), pH, temperature, organic material content, soil redox potential and effects of other ions (ADRIANO 2001, CLEMENS et al. 2002, HASAN et al. 2009, KACALKOVA et al. 2009). During the cadmium uptake by plant roots, competition occurs between heavy metals and certain mineral nutrients that have similar chemical characteristics (TLUSTOS et al. 2006).

The kinetics of Cd^{2+} uptake indicates the presence of two transport mechanisms – lower and higher Cd^{2+} activity in the solution (Costa, Morel 1993). Absorption may occur in complex inorganic forms of $CdCl^{+}$, $CdCl_2$ and $CdSO_4$, or in the form of an organic phytometalophore complex (McLaughlin et al. 1996).

The Cd root uptake in plants mainly proceeds through Ca^{2+}, Fe^{2+} and Zn^{2+} transporters (Clemens 2006). The nonspecific ZIP IRT transporter, which is involved in the ferrum uptake, has been mentioned. By contrast, higher specificity to Fe was expressed in other transporters (Plaza et al. 2007).

The transport of cadmium through plasma membranes of root cells depends on the Cd concentration in a nutrient medium. The saturated character of Cd absorption indicates that the uptake is achieved through a carrier-mediated system (Hart et al. 1998). Duarte et al. (2007) used *Halimione portulacoides* as a citric acid chelator in their experiments. At a dose of 25 μM, both the uptake of cadmium and its translocation increased; at 50 μM, a further increase of Cd uptake by roots was apparent.

In as much as Cd can make use of other transporters, its uptake can be blocked on one side of the cell wall. On the other hand, Cd can block the uptake of other cations. Cadmium uptake from soil increases at a higher Cd content in soil and a low pH value. Such ions as Ca^{2+} and, in some cases, Zn^{2+} can occupy sorption spaces better than Cd^{2+} and displace them in the soil solution. Interactions between Cd and Zn have often been described in the literature (McKenna et al. 1993, Welch 1995).

At high concentration levels, the results of determinations of Cd in a nutrient medium demonstrated a marked reduction in the concentrations of Mg, P, K, Mn, Cu and Zn in plant tissues (Abu-Muriefah 2008, Kikuchi et al. 2009). After experimental Cd contamination of soil, less potassium was determined in grains of oat and in aerial organs and roots of yellow lupine and radish. A contrary effect was also noted, with higher K concentrations in oat straw and roots, as well as in roots of maize (Ciecko et al. 2004). Lower K, Ca and Mg content in tissues with a high Cd concentration in a nutrient medium was noted in cucumbers and tomato plants (Burzynski 1988) and in maize (Walker et al. 1977).

The uptake of Cd may also be influenced by anions. Phosphates may affect Cd uptake by the formation of stable complexes, particularly at higher pH (Bolan et al. 2003). Regarding mobile anions, these are mainly Cl^{-} and SO_4^{2-}. Results by Zhao et al. (2004) confirmed an increased uptake after the application of KCl, along with K_2SO_4. Chlorides and sulphates created complexes with Cd^{2+} and, in this way, increased the Cd^{2+} uptake by plants. Nitrogen fertilization increased the Cd content in oats, especially at high nitrogen doses (160 kg N ha^{-1}) (Eurola et al. 2003).

Cadmium penetrates through the epidermis into the cortex of roots. It can move within the cortex through the apoplast or symplast. At the endoderm level, cadmium enters the symplast and conductive tissues, where it flows through elements of the xylem. Here, for example, it creates complexes with ligands, organic acids and phytochelatins. But most Cd (60-80%) remains in roots (MENCH et al. 1989). Therefore, the transport of Cd from the roots to aerial parts of a plant is limited (CATALDO et al. 1983). GUO, MARSCHNER (1995) present results indicating that phytochelatins may also be involved in the translocation of Cd to aerial plant organs. This suggests a relationship between higher concentrations of cadmium and Cd-bound phytochelatins in the roots of maize and other plant species.

As mentioned above, roots are the main organ for the accumulation of Cd. Cadmium can accumulate in the vacuoles of root cells and, through the tonoplast, it is transported by several mechanisms, for example the Cd^{2+}/H^{+} antiport or *via* a phytochelatin-Cd transporter (HART et al. 1998), which may be Mg-ATP dependent (SALT, RAUSER 1995). Phytochelatins are obviously also involved in the detoxication of heavy metals through chelation into vacuoles. Zinc phytates were found in cells of the root extension zones, where small vacuoles are located, but Cd bound with phytine acid was not indicated (VANSTEVENINCK et al. 1992). Cadmium ions can create complexes with soluble compounds, such as organic acids. Phytochelatines are degraded in vacuoles by hydrolases, or they are transported back into cytosol (LEOPOLD et al. 1999).

The most common symptoms of the toxic effect of Cd include the browning of root hairs, root tips and necrotic spots on leaves. Thus, Cd influences the structure of leaves and brings about many physiological changes. This is related to the negative effect of Cd on the production of chlorophyll, carotenoids and the activity and reduction of the regeneration of enzymes which participate in the fixation of CO_2 (DE FILIPPIS, ZIEGLER 1993, GOUIA et al. 2003, EKMEKCI et al. 2008). Consequently, a decrease in the gas exchange parameters also occurs, the degree of stomata opening is reduced and the transpiration and photosynthetic rates are decreased (DI TOPPI, GABBRIELLI 1999). At the same time, with these diminishing life parameters, the synthesis of defensive and reparation proteins is increased. This is true, for example, about glutathione, which is connected with the onset of the plant's adaptation to cadmium (HALL 2002).

The average content of cadmium equal 0.26 mg kg^{-1} of soil (median 0.18 mg kg^{-1}) was established, based on the results of monitoring agricultural soils in the Czech Republic. Minimum and maximum values ranged between 0.18 - 4.56 mg kg^{-1} of soil (all in a total solution of aqua regia) (POLAKOVA et al. 2010). According to the Regulation of the Ministry of Environment of the Czech Republic, number 13/1994 Coll., the maximum permissible amount of cadmium in soils is 1 mg kg^{-1}.

The goal of this study was to study the translocation of cadmium to the aerial parts of oat plants and its impact on selected physiological parameters.

Cadmium was applied in the nitrate, chloride and sulphate forms, in dosages of 2 and 20 mg kg^{-1} of soil. The lower dose of cadmium is double the maximum permissible content in soil. The higher dose was established based on a preliminary experiment, during which notable visual symptoms of phytotoxicity in oat plants were absent. Cadmium content was also evaluated in individual parts of oat plants: roots, stem, lower yellowing leaves, upper photosynthetically active leaves and panicles. Differences in the cadmium content of yellowing leaves and the green, photosynthetically active leaves indicates a possibility of the reutilization of Cd.

Another objective was to investigate the phytotoxicity of Cd, particularly its impact on yield, chlorophyll content and the physiological characteristics: photosynthetic rates (P_N), transpiration rates (E) and gas conductivity (g_s).

MATERIAL AND METHODS

The research was carried out as a pot experiment in an outdoor environment. The experiment took place in Černožice (a region of Hradec Kralove, the Czech Republic), situated at the altitude of 255 m above sea level. The experimental crop was the common oat (*Avena sativa* L.), Atego variety. Oat was grown in alluvial soil, which was clay loamy in texture. The agricultural and chemical characteristics of the soil medium were as follows: pH/KCl – 6.20; content of available P 30.0 mg kg^{-1}, K 382.2 mg kg^{-1}, Mg 166.2 mg kg^{-1}, Ca 6700.0 mg kg^{-1} (in the Mehlich III. leach), CEC 211.5 mmol kg^{-1}. The total heavy metal content in *aqua regia* was Cd <0.5; Ni 16.1; As 9.9; Cr 30.6; Pb 42.8; Hg 0.179; Zn 182.0 mg kg^{-1}. Thus, the soil was characterized by a high content of potassium, at the K/Mg ratio 2.29, and a low phosphorus content. The high potassium content may influence the absorption of Cd and other cations. On the other hand, the effect of accompanying anions on Cd absorption may be more obvious. The soil placed in pots for the trials was air-dried, homogenized and sieved through a 10-mm stainless-steel sieve.

The experimental pots were 10-L plastic pots with perforated bottoms (pot dimensions: height 0.28 m; top diameter 0.25 m; bottom diameter 0.21 m). Each pot was placed in a polyethylene bowl, with a diameter of 26 mm m and a depth of 40 mm. A 10-kg batch of air-dried and sieved (≤10 mm) soil was put into each 10-L plastic pot. Seven variants of cadmium treatment were started in the experiment, with three replications. The scheme of the experiment and doses of chemicals per experimental pot are given in Table 1. Except for the control variant, the nitrogen dose in all variants was levelled by means of NH_4NO_3. In order to provide a nutrient supply, a weighed amount of chemicals was dissolved in 300 ml of distilled water (1 day) and the solution was evenly applied to the soil in the experimental pots. Phosphorus or potassium nutrition was not performed.

Subsequently, thirty grains of oat (*Avena sativa* L.) were sown into each pot in May. Normally developed plants were singled out in June, i.e., twenty

Table 1

Scheme of the experiment

Variant No.	Concentration Cd mg kg^{-1}/ accompanying anion	Chemicals (g^{-1} / pot)			
		$Cd(NO_3)_2.4H_2O$	$CdCl_2.2.5H_2O$	$CdSO_4.8H_2O$	NH_4NO_3
I.	control	-	-	-	-
II.	Cd 2 / NO_3^-	0.055	-	-	1.130
III.	Cd 20 / NO_3^-	0.549	-	-	1.001
IV.	Cd 2 / Cl^-	-	0.041	-	1.144
V.	Cd 20 / Cl^-	-	0.405	-	1.144
VI.	Cd 2 / SO_4^{2-}	-	-	0.046	1.144
VII.	Cd 20 / SO_4^{2-}	-	-	0.456	1.144

-five best-developed plants remained in each pot. The pots were placed outdoors, on an experimental plot of land. Individual pots were spaced at 0.15 m from each other and their position within the group at the site was regularly changed. Plants were grown under standard light and temperature conditions. Soil moisture was kept at ~60% of WHC by watering with distilled water. The average daily temperature levels for individual ten-day periods and months at the Hradec Kralove site, located approximately 4 km away, are given in Table 2.

Plants were harvested on 5 of August 2009, at the phase of milky ripeness (75 DC). Twenty whole plants were harvested from each experimental pot and their panicles, stems, upper green leaves and lower yellowing leaves were collected separately. The individual samples were placed into paper bags and immediately dried in a drying apparatus (Venticell 707; Ilabo, Borsovska, Kyjov, CZ) at a temperature of 65°C until they reached constant mass (*ca* 48 hours). The experimental pots were turned upside down and the roots of the harvested plants were also taken. The roots were washed three times in distilled water and also dried at 65°C. The dried parts of the plants were then ground in a laboratory mill into fine powder.

Table 2

Average daily temperatures in 2009 at the Hradec Kralove (the Czech Republic) site in °C

Month	1st decade (mean)	2nd decade (mean)	3rd decade (mean)
May	-	19.0	13.8
June	14.4	19.4	15.2
July	18.6	17.6	21.0
August	16.6	-	-

Source: Czech Hydrometeorological Institute

The measurement of physiological characteristics, i.e. photosynthesis rate (P_N), transpiration rate (E) and gas conductance (g_s), took place at the stage of 71 DC, by means of an infra-red gas analyser LCpro+ (ADC Bio-Scientific Ltd., UK). The chlorophyll content was measured by a chlorophyll -meter CCM 200 (OptiScience, USA).

Mineralization of the dried and ground samples was carried out by the dry combustion method in a combustion muffle furnace (CALOR SN 305; MIWY, Lipnik n. Becvou, CZ). A portion of each sample in a quartz cup was 0.2-1 g. The maximum incineration temperature was 450°C. While it was still warm after combustion, the ash was dissolved in a 5% solution of nitric acid on a heating plate, qualitatively transferred into a calibrated test-tube and replenished with de-mineralized water up to the mark line. The actual determination of the cadmium content was carried out by atomic absorption spectrometry (Atomic Absorption Spectrometer SOLAAR M5; Thermo Electron Spectroscopy Ltd., Solaar House, Cambridge, UK). The identifiable limit of atom absorption of cadmium was 0.050 mg l^{-1}. A mixed-model procedure, with a repeated statement for variants, was used to analyse the content of cadmium in the oat plants. Data from each plant part was tested separately. Analysis of variance (the Wilks' lambda, $P < 0.05$) was used to determine significant differences. All statistical tests presented in this study were performed using a Statistica 9.1 (StatSoft Inc., Tulsa, OK, U.S.A.) software package.

RESULTS AND DISCUSSION

During the experiment, the growth, development and health of the plants were recorded. No incidence of diseases or pests was identified and no symptoms of deficiency or damage resulting from higher doses of cadmium were observed in any of the groups. In groups II - VII, no statistically significant differences in the weight of dry matter of aerial parts of the plants were observed (Table 3). Any larger decrease in the plant weight in the variants with Cd contamination may have also been partly prevented by the dose of nitrogen. No significant decrease of the chlorophyll content was observed and no correlation between the Cd content and chlorophyll content in leaves was identified. In the experiments carried out by Lagriffoul et al. (1998) and Cheng et al. (2002), a decrease in the chlorophyll content was

Table 3

Weight of the dry matter (g)/pot of the aerial parts of oat plants

Variants	I	II	III	IV	V	VI	VII
Mean	175.39	163.44	172.95	160.96	167.1233	157.3567	155.21
SD (*n* = 3)	5.99	5.99	5.991	5.99	5.99	5.99	5.99

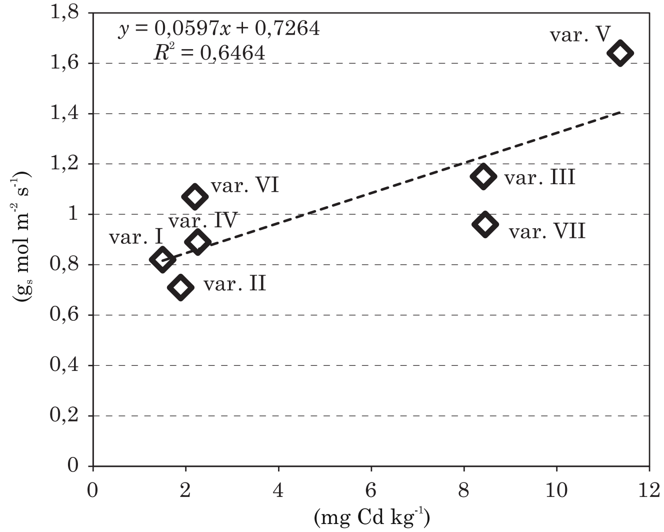

Fig. 1. Relationship between Cd content in green leaves (axis x) and gas conductance (g_s, axis y)

observed only at the highest levels of Cd contamination. Figure 1 shows the relation between the Cd content in green leaves and gas conductance (g_s).

In the variants with the Cd nutrition, insignificantly higher levels of photosynthesis rates were identified in the measurements as compared with the control variant. The initial response of plants to stress can be the intensification of metabolic processes. Our results imply that oat is fairly tolerant and adaptable to stresses induced by higher Cd^{2+} ion content. For comparison, VASSILEV et al. (2004) reported the following findings for barley: the maximal shoot Cd concentration of 41 ± 8 mg Cd kg^{-1} DW without any visual toxicity symptoms on the shoots occurred at 28 mg Cd kg^{-1} of sand. However, at the highest contamination level (42 mg Cd kg^{-1} of sand), reduced leaf gas exchange, photosynthetic pigments content and electron transport activity, but not altered lipid peroxidation status of thylakoids were found.

Table 4 gives data on Cd content in particular treatments of the experiment. Statistically significant differences in the content of Cd, at $p \geq 0.05$, are evident in the individual parts of the oat plant, i.e. panicles, stems, upper green leaves, lower yellow leaves and roots. Significantly, as is seen in Table 4, the highest content of cadmium was observed in roots. A higher content of Cd in roots than in the other parts of plant was indicated in all the treatments where Cd was applied (II-VII.).

These results are confirmed by numerous sources in the literature, which document high content of Cd in roots relative to the comparatively simple uptake from the soil environment and difficult translocation of Cd from roots to aerial parts of a plant. OBATA, UMEBAYASHI (1993) mention that over 50% of cadmium intake remains in roots. The same results (60-80 % in roots) were

Table 4

Changes in the cadmium content (mg kg^{-1}) in all the analyzed parts of oat plants relative to different variants of cadmium nutrition and accompanying anions nutrition

Specification	Var. I		Var. II		Var. III		Var. IV		Var. V		Var. VI		Var. VII	
	av.	SD	av.	SD	av.	SD	av.	SD	av.	SD	av.	SD	av.	SD
Panicles	0.12^{a}	0.01	$1,59^{a}$	0.28	5.88^{a}	0.50	2.31^{a}	0.46	5.98^{a}	0.20	2.47^{a}	0.65	5.85^{a}	0.68
Stalks	1.56^{b}	0.20	3.68^{a}	0.43	18.17^{a}	1.53	3.72^{a}	1.17	19.37^{ab}	1.76	3.98^{ab}	0.54	17.37^{a}	0.81
Green leaves	1.50^{b}	0.25	1.89^{a}	0.14	8.42^{a}	1.11	2.26^{a}	0.58	11.37^{ab}	1.60	2.19^{a}	0.43	8.47^{a}	1.04
Yellow leaves	1.37^{b}	0.30	8.34^{a}	1.65	44.63^{b}	5.25	9.93^{b}	0.12	55.97^{b}	5.64	11.63^{b}	1.18	62.70^{b}	10.62
Roots	1.82^{b}	0.26	31.4^{b}	3.95	194.67^{c}	20.0	40.90^{c}	3.79	235.33^{c}	23.38	42.20^{c}	4.05	219.33^{c}	18.41

NB: Statistically significant difference between parts of plant a, b, c, d - $P < 0.05$; av. = average; SD = standard deviation further documented by Cataldo et al. (1983) or Mench et al. (1989). By contrast, the lowest content of Cd was indicated in panicles, which did not significantly vary from the content of Cd in stems and green leaves. It is generally understood that the concentration of Cd in plants decreases in the sequence of root> leaves> fruit> seeds (Sharma et al. 2006).

A significantly higher content of Cd in lower yellowed leaves than in green leaves was determined in most of the treatments, except treatments I, II and V. That suggests the accumulation of Cd in senescent tissues and its difficult reutilization. The biggest difference was detected in the treatment with accompanying SO_4^{2-} (treatment VII). Higher doses of sulphate could be predicted to cause higher accumulation Cd in yellow leaves and lower reutilization in other parts of the plant. Our earlier experiments, in which the translocation of zinc (Tuma et al. 2008) and nickel (Tuma et al. 2010) were examined, provided similar results.

These findings may confirm the important role of sulphur in the synthesis of phytochelatins, with which cadmium creates complexes. These complexes, of low and medium molecular weight, are bound to unstable acid sulphides (S^{2-}), which in turn are stabilized by complexes. These complexes obviously play an important role in the detoxication of cadmium (Nocito et al. 2007, Khan et al. 2008), after which they are transported into vacuoles, where they are dissociated and Cd creates complexes with acids (Di Toppi, Gabbrielli 1999).

Statistical significances between individual treatment variants are shown in Figure 2, in contrast to Table 4. Evidently, a 10-fold higher supply of cadmium (III, V and VII) was significantly linked to higher Cd content in all parts of plants, including generative organs, in which (see above) the lowest content of Cd was detected. This is related to the simple uptake of Cd through the root system. Thus, the amount of Cd absorbed by plants depends on its concentration in the nutrient medium (Holm et al. 1995). Most of the Cd which remains accumulated in roots is transported into all aerial parts of the plant when the degree of soil contamination increases.

Page, Feller (2005) suggested that, in this case, Cd was released more slowly from the roots to the leaves and subsequently redistributed only at trace levels in the phloem to the youngest leaves. Hart et al. (1998) mention that results indicate that excess Cd accumulation in durum wheat grain is not correlated with the seedling-root influx rates or root-to-shoot translocation, but may be related to phloem-mediated Cd transport to the grain.

The influence of the accompanying mobile anions (NO_3^-, Cl^-, SO_4^{2-}) on the content of Cd in roots was not significant. A significant increase in the content of Cd appeared in leaves, but only at lager cadmium loading.

A significant increase in the content of Cd in bottom yellow leaves was indicated in treatment VII, after the application of $CdSO_4$, relative to treatment III ($Cd(NO_3)_2$); the said increase was insignificant compared with treatment VI ($CdCl_2$). On the other hand, the highest cadmium content in photo-

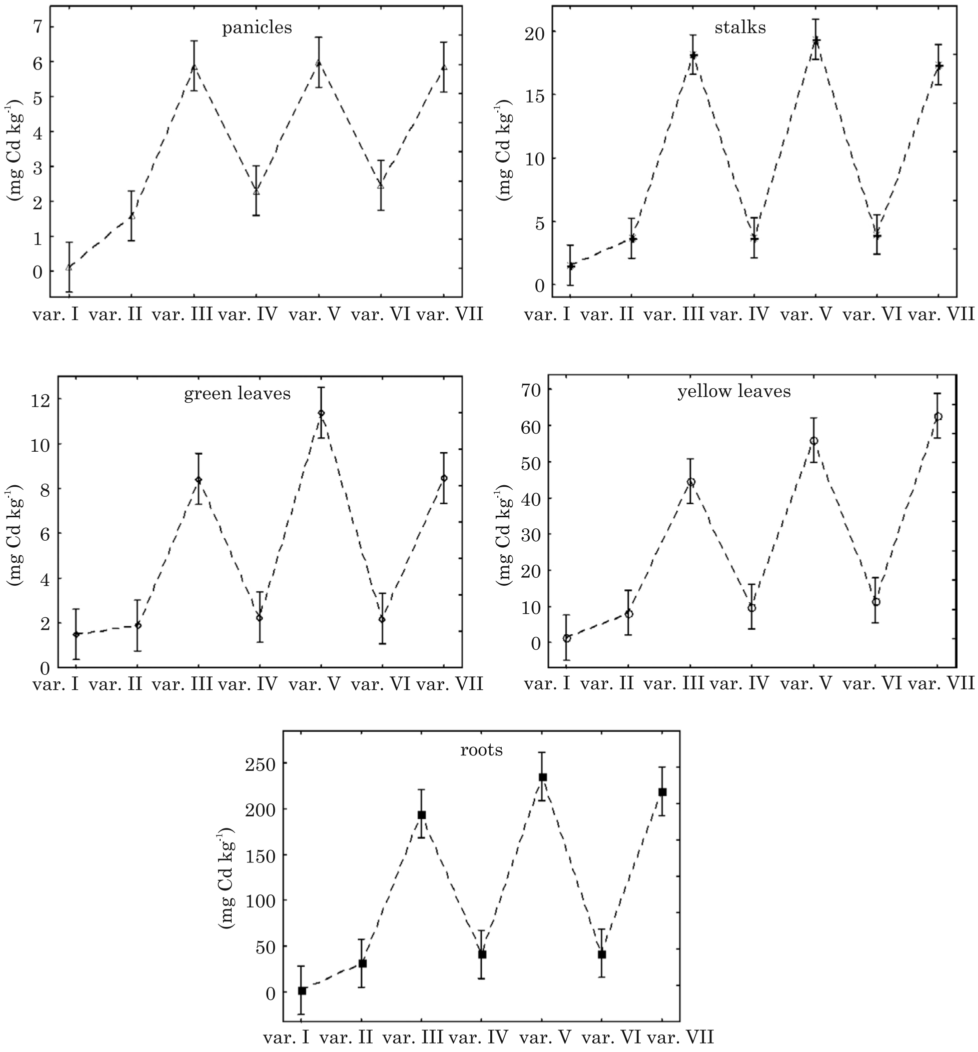

Fig. 2. Changes in the cadmium content according to the treatment variants. Error bars represent means ± SD ($n = 3$)

synthetically active green leaves appeared in treatment VI ($CdCl_2$). Herren, Feller (1997) state that the long distance translocation of cadmium may depend on the availability of other elements. McLaughlin et al. (1996) mention that sulphate had just a limited effect on the availability Cd for chard, especially when compared with Cl^-.

These results have been contrasted with some earlier work on the effect of Cl^- salinity on Cd availability in Swiss chard. Possible mechanisms explaining a weaker effect of SO_4^{2-} compared to Cl^- on Cd^{2+} availability have been proposed. Experiments by Zhao et al. (2004) showed that Cl^- and SO_4^{2-} anions increased Cd uptake by plants, which can be interpreted as Cl^- and SO_4^{2-} readily complexing with Cd^{2+}, thereby increasing the bioavailability of Cd^{2+} in soils. The effect of potassium itself on the plant uptake of Cd was also observed.

In our experiments, cadmium behaved somewhat differently than nickel (Tuma et al. 2010) and zinc (Tuma et al. 2008). Nickel was much more easily transported through the plant. In the treatments in which $NiSO_4$ was applied in higher doses, a significant increase in Ni content was observed in all the analyzed parts of plants, including panicles. Roots were the only exception. Zinc is less mobile in plants than nickel. Nevertheless, an increase in Zn content was found in the treatments with $ZnSO_{4,}$ compared to treatments with the application of Zn $(NO_3)_2$. The effect was significant in stems at both lower and higher nutrition, and in upper green and bottom yellow leaves of oat plants at higher nutrition. Obviously, the mobility of Cd through the plant is substantially lower than that of Ni and lower than the mobility of Zn, so the influence of the accompanying mobile anions on the translocation of Cd was less significant (Zeller, Feller 1999, Zhu et al. 2003, Yuruk, Bozkurt 2006, Gondek 2010).

CONCLUSIONS

In all the variants with Cd contamination, the weight of aerial parts of oat plants decreased only insignificantly. No significant decrease in the chlorophyll content in leaves was observed. Based on the measured photosynthesis rate, stomatal conductivity and transpiration rate, no correlation between these characteristics and the cadmium content in leaves was proven. Oat developed fairly good tolerance to stress induced by a higher cocentration of Cd^{2+} ions.

A 10-fold higher Cd application dose manifested itself significantly by a higher content of Cd in all the analyzed parts of plants, including generative organs. A several-fold higher Cd content was determined in roots than in the other parts of the plant, whereas the lowest content of Cd was observed in panicles. However, the results obtained by measuring the content of cadmium in stems and green leaves were not significant. A notably higher content of Cd in most treatments was determined in bottom yellow leaves than in upper green leaves. This indicates the accumulation of Cd in senescent tissues and difficulties in its reutilization. The biggest difference was discovered in the treatments with the accompanying anion SO_4^{2-}. A higher sulphate application level, along with Cd, could lead to higher accumulation and deposition of Cd in yellow leaves, and to lower reutilization of the metal in other parts of the plant. The assessment of the effects of accompanying anions on Cd content revealed significant differences only at higher levels of

Cd contamination. An increase in the Cd content after $CdSO_4$ application was determined in bottom yellow leaves. It was significant versus the treatment in which $Cd(NO_3)_2$ was applied and insignificant compared with variants in which $CdCl_2$ was applied.

On the other hand, significantly the highest content of Cd in green leaves with active photosynthesis was measured in the treatment with $CdCl_{2,}$ compared to the treatment with $Cd(NO_3)_2$. The above results were certainly connected with the lower mobility of Cd through the plant, as compared with Ni and Zn.

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CONTENT OF TOTAL ZINC AND ITS FRACTIONS IN SELECTED SOILS IN THE PROVINCE OF PODLASIE

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Abstract

The aim of this research was to determine the total content of zinc and its fractions in arable soils of the Province of Podlasie (*województwo podlaskie*) and to assess the correlation between the zinc content, its fractions and physicochemical properties of soils.

The research material consisted of samples taken from arable soils in 81 points of the province, with one point chosen in the majority of the districts. Each point was located on mineral soil used as arable land, without any external source of contamination like roads or industrial plants. Samples were taken after harvest, from the arable layer, determined to lie within the depth of 0-30cm. The samples were submitted to determinations of the basic physicochemical properties of soil. The content of total zinc was determined after mineralization in *aqua regia*, by the flame atomic absorption spectrometry method. The content of zinc fractions was determined by the BCR method. There were four fractions determined: exchangeable, bound with oxides and hydroxides, bound with organic matter and residual zinc.

The physicochemical properties of soils were highly differentiated except the grain-size composition. The content of total zinc was typical of arable uncontaminated soils and fluctuated in a wide range. The content and shares of zinc fractions in total zinc were as follows: for very light and light soil: organic>bound with oxides and hydroxides>exchangeable>residual; for medium-heavy soil: organic>exchangeable>bound with oxides and hydroxides>residual. Distribution of zinc in particular fractions was dependent to some degree on pH, content of organic matter and grain-size composition.

Key words: soils, zinc, properties, fraction.

ZAWARTOŚĆ CYNKU OGÓŁEM I JEGO FRAKCJI W WYBRANYCH GLEBACH WOJEWÓDZTWA PODLASKIEGO

Abstrakt

Celem pracy było określenie całkowitej zawartości cynku oraz jego frakcji w glebach uprawnych Podlasia użytkowanych jako grunty orne oraz określenie zależności między zawartością cynku, jego frakcji a własnościami fizykochemicznymi gleb.

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Materiał badawczy stanowiły próbki pobrane z gleb uprawnych w 81 punktach woj. podlaskiego. Wybrano po jednym punkcie w większości gmin tego województwa. Zlokalizowane były one na glebach mineralnych użytkowanych jako grunty orne, pozbawione zewnętrznych źródeł zanieczyszczeń, jak drogi czy zakłady przemysłowe. Próbki pobrano z warstwy uprawnej, określonej na głębokość 0-30 cm, po zbiorze roślin uprawnych. W próbkach oznaczono podstawowe właściwości fizykochemiczne gleb.

Oznaczono zawartość cynku ogólnego, po mineralizacji w wodzie królewskiej, metodą atomowej spektrometrii absorpcyjnej płomieniowej. W glebach oznaczono zawartość frakcji cynku metodą BCR. Oznaczono 4 frakcje, tj. dostępną, związaną z tlenkami i wodorotlenkami, związaną z substancją organiczną i rezydualną.

Stwierdzono, że właściwości fizykochemiczne badanych gleb były bardzo zróżnicowane, z wyjątkiem składu granulometrycznego. Zawartość cynku ogółem była typowa dla niezanieczyszczonych gleb uprawnych i mieściła się w szerokich granicach. Zawartość i udział frakcji cynku w ogólnej jego zawartości układały się w następujący szereg w przypadku gleb bardzo lekkich i lekkich: organiczna > związana z tlenkami i wodorotlenkami > wymienna> rezydualna, a w przypadku gleb średnich: organiczna> wymienna> związana z tlenkami i wodorotlenkami> rezydualna. Rozmieszczenie cynku w poszczególnych frakcjach zależało w pewnym stopniu od wartości pH, zawartości węgla organicznego oraz składu granulometrycznego gleb.

Słowa kluczowe: gleby, cynk, właściwości, frakcje.

INTRODUCTION

Zinc is an essential microelement in the nutrition of crops, especially maize, which covers increasingly larger acreage of farmland in Poland (Kabata-Pendias 2002, Spychaj-Fabisiak, Długosz 2006). The main source of zinc for plants is soil. The content of this microelement depends on the type of parent rock and soil texture. However, the content of the available form of zinc depends mainly on soil reaction, content of organic carbon, sorptive capacity, share of exchangeable cations in the sorptive complex, as well as organic and mineral fertilisation which supplies this microelement and factors which modify physicochemical properties of soils and thus the zinc availability (Dąbkowska-Naskręt, Różański 2009, Czekała, Jakubus 2000). The share of phytoavailable forms in the total zinc content is highly differentiated and depends on many factors. With the same total content, there could be different amounts of available forms of this metal, from low to very high, up to a toxic level (Czekała, Jakubus 2000).

In Poland, the total content of metals in soil, including zinc, serves to estimate soil contamination degrees (Kabata-Pendias 2002). Apart from the total content, the quantity of available form extracted in 1 mol dm^{-3} HCl is determined. Most often, the content of metals, including zinc - which is said to be available - is higher than that of the easily available fractions. There are several methods for determination of these fractions, but is seems that the BCR method can meet the expectations (Sutherland, Tack 2003).

The aim of this research was to determine the total content of zinc and its fractions in arable soils in the Province of Podlasie (*województwo podlaskie*), which underlie arable land, and to assess the correlation between zinc content, its fractions and physicochemical properties of soils.

MATERIAL AND METHODS

The research material consisted of samples taken from arable soil in 81 points in the whole prrovince, with one point selected in almosst every district. Each point was located on mineral soil used as arable land, without any external source of contamination like roads or industrial plants. Samples were taken after harvest, from the arable layer, determined to lie within the depth of 0-30 cm. The following crops were grown: maize (14 points), grain (53 points), rape (2 points), buckwheat (2 points) and grass (the other points). The samples were submitted to the determinations of basic physicochemical properties: grain-size distribution by the Cassagrande's method with Pruszynski's modification, organic carbon content by the Tiurin's method, pH in 1 mol dm^{-3} KCl by potentiometry. The soils were assinged agronomical categories, and divided into three groups: very light, light and medium-heavy soil. Heavy soils were excluded because they does not occur in the Province of Podlasie. The soils were grouped acording to pH, agronomical category and content of organic carbon. This arrangement served as the background for the presetation of average percentages of zinc fractions in its total zinc content.

The content of total zinc was determined after mineralization in *aqua regia*, by the flame atomic absorption spectrometry method. Afterwards, the metal was determined in the fractions: exchangeable, bound with Fe and Mn oxides and with organic matter by the modified BCR method (Community Bureau of Reference, currently Standards, Measurement and Testing Programme). The extraction of exchangeable, water and light acids soluble part of zinc (1 stage, fraction I), bound with iron and manganese hydroxides (2 stage, fraction II) and bound with organic matter (3 stage, fraction III) was also performed. In the first stage, acetic acid was used (concentration 0.11 mol dm^{-3}); the second one used hydroxylamine hydrochloride (concentration 0.5 mol dm^{-3}), whereas at the third one involved hydrogen peroxide and ammonium acetate (concentration 1 mol dm^{-3}, pH=2). Zinc was determined by the flame technique on an atomic absorption spectrophotometer. The percentages of particular fractions in the total content of zinc were calculated. The correlation coefficients between physicochemical properties of soils and total zincc content and its fractions were calculated with the use of Statistica programme.

RESULTS AND DISCUSSION

Among very light and light soils, loamy sand and poorly loamy sand are most common, whilee sandy clays prevail among medium-heavy soils. The content of fine fraction in very light soils ranged from 4 to 10%, in light soils – from 11 to 20%, and in medium-heavy soils – there was approximately 22% of fine fraction on average, in the range of 20-28% (Table 1).

Table 1

Physicochemical properties of soils

Soils		Characteristics of soils			
		pH	C_{org} (g kg^{-1})	content of fine fraction (%)	total Zn (mg kg^{-1})
Very light *n* = 10	min-max	4.0-5.8	8-21	4-10	13.5-38.6
	$\bar{x}$	4.6	17	9	29.6
Light *n* = 25	min-max	4.2-6.9	10-22	11-20	19.4-52.5
	$\bar{x}$	5.2	18	16	33.5
Medium-heavy *n* = 46	min-max	4.1-7.8	7-42	21-28	14.5-58.8
	$\bar{x}$	6.1	24	22	35.1

The pH of very light soils was highly varied, from 4.0 to 5.8. The pH of light soils ranged from 4.2 to 6.9, but was higher in medium-heavy soils than in very light and light soils. The analyzed soils are characteristic for Podlasie, where most soils are light and acidic. The content of organic carbon in the soils was differentiated and ranged from 8.0 to 42.0 g kg^{-1}.

The content of total zinc in the first group soils was in a wide range, from 13.5 to 38.6 mg kg^{-1}. There was more of zinc in the second group of (light) soils: from 19.4 to 52.5 mg kg^{-1}. Medium-heavy soils contained from 14.5 to 58.8 mg kg^{-1} of total zinc (Table 1). This diversity corresponds to the relationship between total zinc and grain-size distribution in soils. The correlation coefficient between total zinc content and fine grain fraction was 0.29 (Table 2). Higher correlation coefficients between the total content of zinc and colloidal parts of soils are reported by DĄBKOWSKA-NASKRĘT and RÓŻAŃSKI (2009). The total content of zinc in the analyzed soils is the same as

Table 2

Correlation coefficients between total zinc and its fractions in soils and properties of soils

Variable	Total Zn	Fraction I	Fraction II	Fraction III	Fraction IV
pH	0.12	0.01	0.18	0.02	0.29*
C org	0.1	-0.14	0.05	0.22*	0.12
Content of fine fraction	0.25*	-0.04	0.2	15	0.24*

* confidence level $p < 0.05$

the natural content of this element in soils of Poland (Kabata-Pendias 2002). The results of total zinc in the soils are similar to those obtained by Skorbiłowicz et al. (2002), who demonstrated that total zinc fluctuated from 13.2 to 54.8 mg kg^{-1} in acid soils in Podlasie.

In the exained soils grouped by acidification, the percentage of particular zinc fractions as compared to total Zn was only slightly differentiated (Figure 1). The content of available zinc (fraction I) was the highest in strongly acidic soils, reaching about 30% of total Zn. The zinc content in this fraction was lower in weakly acidic and acidic soils, increasing in soils with higher pH. Many authors (Ge et al. 2000, Kucharzewski et al. 2004, Kalembasa, Pakuła 2006) emphasize increased zinc solubility in more acidic soils. The current results show that the release of available zinc forms is not only pH dependent, especially in soils with pH higher than 6.6, where an increase in the content of the most soluble zinc form extracted with weak acetic acid was noted. A decrease in the content of exchangeable zinc, as argued by Lorenz et al. (1997), is connected with an increase in the share of zinc bound with organic matter due to the formation of complex organic-mineral anions, which are able to sustain high mobility of zinc in soils with elevated pH.

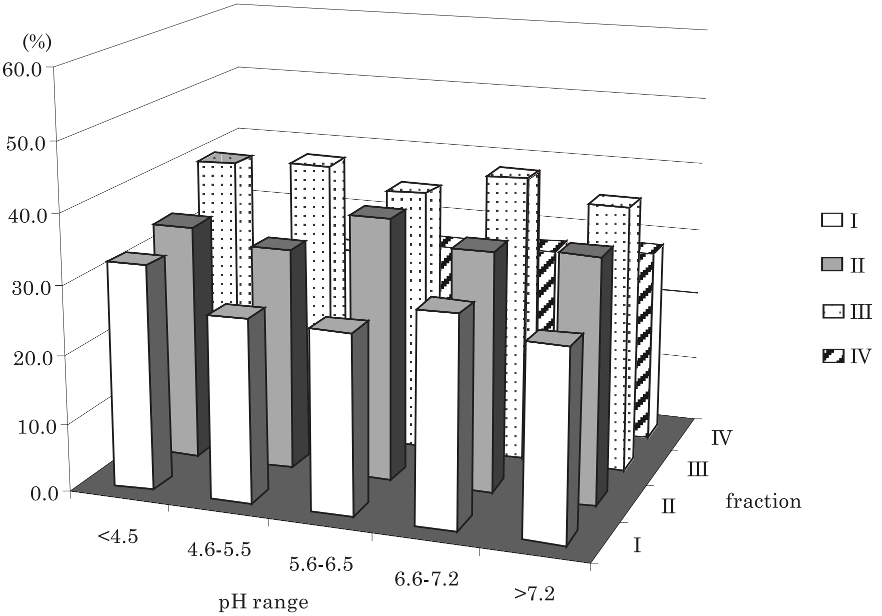

Fig. 1. Percentage of zinc fractions in total content in soils depending on pH

The share of zinc bound with organic matter (fraction III) in the analyzed soils was higher than in fraction I. An increase in the content of fraction III in soils with pH 4.6-5.5 and 5.6-6.5 was related to a decrese in the content of fraction I. In soils with different pH ranges, the relationships between fractions changed. The share of mobile fractions was decreasing, while the share of more stabile fractions was increasing, which was especially evident in the case of acidic and light acidic soils. In soils with pH higher than 7.2, the share of the residual fraction higher by a few per cent than in soils with lower reaction. The supports the claim by Lorenz et al. (1997) that zinc is transferred in acidic soils from the residual fraction into soluble and potentially soluble fractions, while the reverse transfer occurs in soils with higher pH. This is implied by values of the correlation coefficient between pH and the residual fraction (stabile) – Table 2.

No significant influence of the grain composition of soils on zinc content in particular fractions was noted (Figure 2). Some tendency can be seen towards decreasing the content of exchangeable fraction while increasing the content of fine fraction. A higher agronomical category of soils was associated with a decreasing zinc mobility. The share of exchangeable fraction in light and medium-heavy soils was 10% lower than in very light soils. In very light soils, there was an evident transfer of most stabile fraction (residual) into fraction III with organic matter. In light and medium soils, the relationship between fractions was similar and can be described as follows: the highest share of zinc was in fraction with organic matter, the second mosst abundant was zinc bound with oxides and hydroxides of manganese and iron, followed by residual fraction and exchangeable fraction. Similar shares of zinc fractions were reported by Kabała and Singh (2006). In the current study, soils formed from sand had most zinc (40% on average) in the available fraction, follwoed by zinc in the fraction with organic matter (25%), and the leastzinc was found in the residual soil fraction.

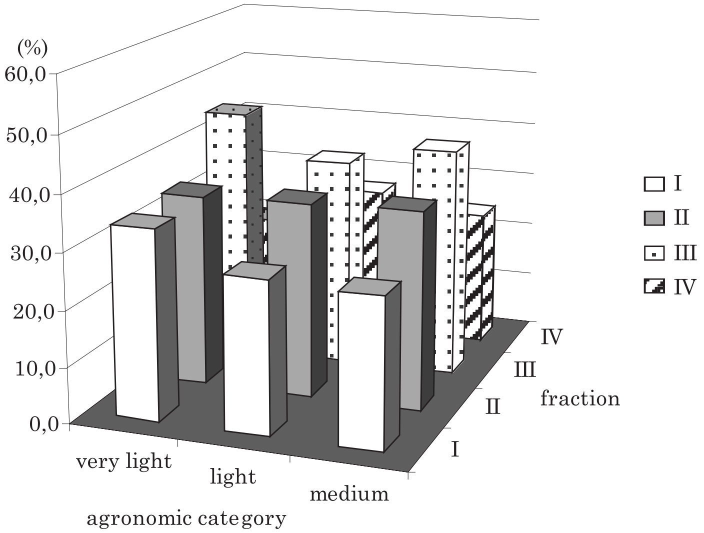

Fig. 2. Percentage of zinc fractions in total content in soils depending on the agronomical category

The correlation coefficients for the first three zinc fractions and fine fraction were not significant. The significant relationship was observed only for the residual fraction and total content of zinc (Table 2).

The next factor affecting the solubility of zinc is the content of organic matter in soils (Lorenz 1997, Korzeniowska, Stanisławska-Glubiak 2004, Kucharzewski et al. 2004).

The share of exchangeable zinc in soils containing up to 30 g kg^{-1} of carbon was similar. The increase of carbon content above 30 g kg^{-1} caused the decreased zinc mobility in the analyzed soils (Figure 3). Oxides and hydroxides of manganese and iron (fraction II) had a higher share of bound zinc. The samples were characterized by a significant content of zinc bound with organic matter (fraction III). Most of zinc in this fraction, at the expense of the exchangeable fraction, was noted in soils with the content of carbon above 40 g kg^{-1}. The soils contained the least zinc in the residual fraction. Different results were obtained by Dąbkowska-Naskręt and Bartkowiak (2002), who studied soils with a similar grain-size composition and found most zinc in the residual fraction. Those soils were different from the ones analyzed in our research by the reaction (neutral and alkaline), which was probably the cause of differences in the share of zinc fractions.

Organic matter forms quite strong bonds with zinc, whicch resulted in a slight influence of the organic carbon content on exchangeable zinc (low correlation coefficients) – Table 2. Similar results were obtained by Mercik et al. (2004). According to these authors, the content of soluble zinc in sandy soils is increasing with a higher content of organic carbon, which can be seen

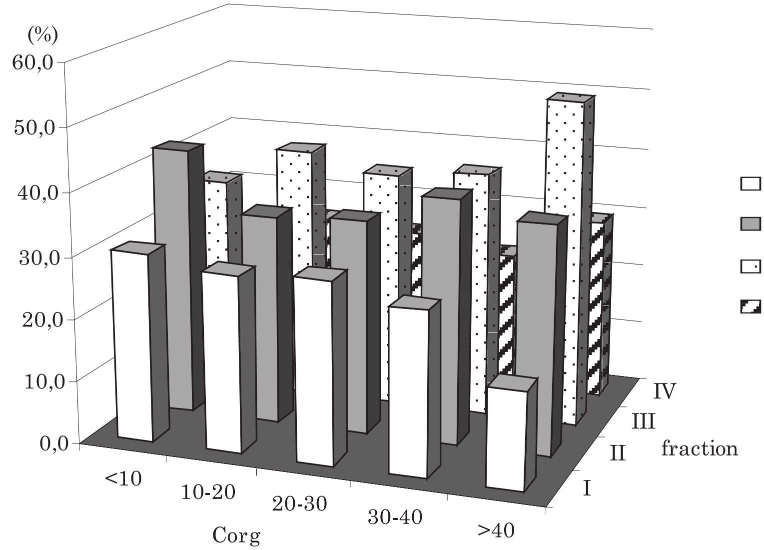

Fig. 3. Percentage of zinc fractions in total content in soils depending on the content of organic carbon (g kg^{-1})

in soils with the carbon content up to 40 g kg^{-1}. A strong relationship (correlation coefficient above 0,7) between zinc and organic matter was proven by Korzeniowska and Stanisławska-Glubiak (2004).

Some of the studied samples were characterized by a significant content of organic carbon, despite the lower share of fine fraction, which affected the binding of zinc in fraction III, and which indicates the correlation coefficient between C_{org} and fraction III as well. Ge et al. (2000) proved that zinc which is soluble in soils with neutral and alkaline reaction occurs mainly as organic complexe, whereas in acid soils is appears as exchangeable ions.

CONCLUSIONS

1. The content of total zinc was typical of arable uncontaminated soils and fluctuated in a wide range.
2. The content and shares of zinc fractions in total zinc were as follows: for very light and light soils: organic>bound with oxides and hydroxides>exchangeable>residual; for medium-heavy soil: organic>available>bound with oxides and >residual.
3. Distribution of zinc in particular fractions to some degree depended on pH and the content of organic matter; to a lesser degree, it was dependent on the grain-size composition of soil.

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INFLUENCE OF LIMING AND MINERAL FERTILIZATION ON THE COPPER CONTENT IN GRAIN OF SPRING BARLEY (*HORDEUM VULGARE* L.) AND WINTER WHEAT (*TRITICUM AESTIVUM* L.) CULTIVATED ON LOESSIAL SOIL

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Abstract

The paper presents research on the copper content in grain of winter wheat and spring barley cultivated in 1986-2001, on grey-brown podzolic soils developed from loess (static fertilization experiment) underlying a field located in the Rzeszów Foothills (SE Poland). The experiment was set up by the random sub-block method, on a field under a static fertilization trial composed of a four-year crop rotation system and the NPK Mg or NPK Mg Ca fertilization system. The first variable was liming (A) and the second one consisted of different mineral fertilization variants (B). The basic level of fertilization ($N_1P_1K_1$) was 80 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 120 kg K_2O ha^{-1} under spring barley and 90 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, 100 kg K_2O ha^{-1} under winter wheat. The crop rotation was the following: in 1986-1989 – potatoes, spring barley, fodder cabbage, winter wheat; in 1990-1993, 1994-1997, 1997-2001 – potatoes, spring barley, fodder sunflower, winter wheat. Mineral fertilization included NPK fertilization with constant Mg fertilization, and differentiated NPK fertilization with constant Mg and Ca (liming) fertilization. Liming was performed in 1985, 1989, 1993 and in 1997 (4 t ha^{-1} CaO). The experiment included 14 fertilization variants with 4 replications. The copper content in plants was determined by FAAS after mineralization of plant samples in a mixture of HNO_3:$HClO_4$:H_2SO_4 in the 20:5:1 ratio. Liming decreased the copper content in spring barley grain but had no statistically significant effect on the copper content in winter wheat grain. Mineral fertilization did influence the copper content winter wheat grain, but decreased it grain obtained from the plot with lower P fertilization together with NK fertilizer. However the interaction (liming x NPK fertilization) did not influence the copper content in cereal grain.

Key words: copper, liming, mineral fertilization, spring barley, winter wheat.

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WPŁYW WAPNOWANIA I NAWOŻENIA MINERALNEGO NA ZAWARTOŚĆ MIEDZI W ZIARNIE JĘCZMIENIA JAREGO (*HORDEUM VULGARE* L.) I PSZENICY OZIMEJ (*TRITICUM AESTIVUM* L.) UPRAWIANYCH NA GLEBIE LESSOWEJ

Abstrakt

Badano zawartość miedzi w ziarnie pszenicy ozimej i jęczmienia jarego uprawianych w latach 1986-2001 na glebie płowej wytworzonej z lessu, położonej na Podgórzu Rzeszowskim (południowo-wschodnia Polska). Rośliny uprawiano na stałym polu nawozowym w 4-letnim zmianowaniu z zastosowaniem nawożenia mineralnego NPK Mg i NPK Mg i Ca. Doświadczenie założono metodą bloków losowanych z podblokami. Pierwszym czynnikiem było wapnowanie (A), drugim – niezależnie od wapnowania zróżnicowane nawożenie mineralne (B). Nawożenie mineralne NPK stosowano na tle stałego nawożenia Mg oraz Mg i Ca (wapnowanie). Podstawowy poziom nawożenia ($N_1P_1K_1$) jęczmienia jarego wynosił: 80 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} i 120 kg K_2O ha^{-1}, natomiast pszenicy ozimej: 90 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1} i 100 kg K_2O ha^{-1}. Doświadczenie obejmowało 4 zmianowania: w latach 1986-1989 – ziemniaki, jęczmień jary, kapustę pastewną, pszenicę ozimą, a w latach 1990-1993, 1994-1997, 1997-2001 – ziemniaki, jęczmień jary, słonecznik pastewny, pszenicę ozimą. Wapnowanie zastosowano w latach: 1985, 1989, 1993 (4 t ha^{-1} CaO). Doświadczenie obejmowało 14 obiektów nawozowych w 4 powtórzeniach. Zawartość miedzi w roślinach oznaczono metodą FAAS, po mineralizacji próbek roślin w mieszaninie HNO_3:$HClO_4$:H_2SO_4, w proporcji 20:5:1. Wapnowanie wpłynęło na zmniejszenie zawartości miedzi w ziarnie jęczmienia jarego, nie wpłynęło w sposób statystycznie istotny na zawartość miedzi w ziarnie pszenicy ozimej. Nawożenie mineralne wpłynęło na zawartość miedzi w ziarnie pszenicy ozimej, obniżając zawartość Cu w ziarnie po zastosowaniu zwiększonego nawożenia P na tle stałego nawożenia NK. Zaobserwowano, że interakcja wapnowania i nawożenia mineralnego nie wpływała na zawartość miedzi w ziarnie zbóż.

Słowa kluczowe: miedź, wapnowanie, nawożenie mineralne, jęczmień jary, pszenica ozima.

INTRODUCTION

Copper is a natural component of all soils, in which its content depends mainly on lithogenic and pedogenic factors, with the highest Cu accumulation recorded in clay sedimentary rocks (KABATA-PENDIAS 1996). Copper in soil can also originate from atmospheric precipitation, volcanic ash, dust created by wind erosion and from mineralization of biological substance (NICHOLSON et al. 2003). The copper content in soil is also shaped by anthropogenic factors. Intensive agronomy, including cultivation of high-yielding crops which require ample nutrition and fertilization, or increased yields of main and catch crops, contributes to the higher removal of copper from soils (GEMBARZEWSKI 2000, KOTECKI, KOZAK 2004, NOWAK, ZBROSZCZYK 2004, RABIKOWSKA, PISZCZ 2004). As well as being an essential element for living organisms, copper is highly toxic given it appears in excess (RUSZKOWSKA, WOJCIESKA-WYSKUPAJTYS 1996). NPK and calcium fertilizers, used for soil de-acidification, are a source of trace elements (GORLACH, GAMBUŚ 1997, KANIUCZAK 1998). On the other hand, they may elevate or reduce the phytoavailability of copper by changing the pH of soil (MERCIK, STĘPIEŃ 2000, BRAVIN et al. 2009).

The uptake of copper by cereals can also be influenced by a dose and chemical form of nitrogen (Tills, Alloway 1981, Kumar et al. 1990) and phosphorus (Singh, Swarup 1982, Gunes et al. 2009) used in fertilizers. The copper content in cereal grains most often appears within a narrow range, is frequently random in character and barely depends on the Cu content in soil (Nan et al. 2002) or on soil properties – texture, organic matter, available P, K, Mg, or pH_{KCl} (Bednarek et al. 2008, Nan et al. 2002). The copper content is often more strongly correlated with a cereal species than cultivation conditions and soil management (Ciołek et al. 2012, Nambiar 1976). For example, genotypes of cereal crops with a higher protein content are more likely to present copper deficiency than those with relatively less protein in grain (Nambiar 1976).

The aim of this study has been to determine the influence of liming and mineral NPK fertilization (with constant magnesium fertilization) on the copper content in grain of spring barley and winter wheat grown in a four-crop rotation system established on podzolic soil developed from loess.

MATERIAL AND METHODS

In 1986-2001, a study on the effects of liming (A) and mineral fertilization (B) on the copper content in grain of winter wheat and spring barley grown in four-crop rotation, was carried out on a static fertilization field in Krasne near Rzeszów, in the Rzeszów Foothills (SE Poland). The podzolic soil (*Haplic luvisol*) on which the experiment was set up on was developed from loess and represented the texture of silt loam. Prior to the experiment, the soil was tested to be very acid in the plough humus layer (Ap) pH_{KCl} 3.92 and in the enrichment layer (Bt) pH_{KCl} 3.89. It was low in available phosphorus, potassium and magnesium. The total copper content of 9.10 mg kg^{-1} was within normal range, and copper forms soluble in HCl 1 mol dm^{-3} represented approximately 30% of the total copper content in soil – 2.70 mg kg^{-1} (Kaniuczak 1998).

The experiment was set up in a random sub-block design with four replicates. The first variable was liming (A_2) or its absence (A_1), while the second one consisted of mineral fertilization variants (B) with constant magnesium nutrition, regardless of liming. The following crops were grown in the rotation system: potato, spring barley, fodder sunflower, and winter wheat; in the 1986-1989 rotation, fodder cabbage replaced sunflower. Four crop rotations were included in the experiment, during which winter wheat was grown in 1987, 1991, 1995 and in 1999, while spring barley was produced in 1989, 1993, 1997 and in 2001.

The basic doses of mineral fertilizers ($N_1P_1K_1$) against constant magnesium nutrition were as follows: spring barley: N_1 = 80 kg N, P_1 = 43.6 kg P, K_1 = 99.6 kg K ha^{-1}, winter wheat: N_1 = 90 kg N, P_1 = 34.9 kg P, K_1 = 83.0 kg K ha^{-1}. The constant magnesium fertilization doses applied before sowing in each experimental sub-block in 1986-1993 were: 24.1 kg Mg ha^{-1} under potato, spring barley and winter wheat, and 72.4 kg Mg ha^{-1} under the fodder crops. From 1994, the magnesium dose was reduced to 24.1 kg Mg ha^{-1}, applied under all rotation crops. Liming with 4 t CaO ha^{-1} was performed in 1985, 1989, 1993 and in 1997, i.e. prior to establishing the experiment and after the harvest of crops completing the rotation. Mineral NPK fertilizers were applied as ammonium nitrate, triple superphosphate, potassium salt KCl (58%), magnesium sulfate, and CaO or $CaCO_3$.

The copper content in the applied mineral fertilizers varied but averaged 2.6 mg kg^{-1} in ammonium nitrate, 20.0 mg kg^{-1} in triple superphosphate, 10.5 mg kg^{-1} in potassium salt and 17.0 mg kg^{-1} in calcium carbonate (Kaniuczak 1998).

Plant material samples were collected after winter wheat and spring barley harvest. In dry plant material, copper was determined with the FAAS technique, having digested the samples in a hot mixture of concentrated acids $HClO_4$, HNO_3, and H_2SO_4 (in the 20:5:1 ratio) in a Tecator digestion system.

The results were statistically processed using two-factor variance analysis (liming, mineral NPK fertilization) and calculating the Tukey's intervals (LSD) at the significance level of $p = 0.05$.

RESULTS AND DISCUSSION

The copper content in barley and wheat grain from the fertilization treatments is shown in Tables 1 and 2. The average copper content in grain of spring barley grown on limed soil (4.82 mg kg^{-1} d.m.) was statistically significantly lower than in barley from non-limed plots (5.37 mg kg^{-1} d.m.). The copper content tended to decrease in grain from cereals provided with mineral fertilization, especially with higher N and P doses. This tendency occurred both with and without liming. The available literature most frequently indicates the ability to reduce copper phytoavailability to plants by liming. Gorlach and Gambuś (1991) as well as Kabata-Pendias (1996) reported that soil liming contributed to reduced phytoavailability of copper in most of the analyzed plants, particularly on soils containing higher levels of the element than on soils with its natural quantities. In earlier research (Kaniuczak 1998), liming only slightly increased the content of total copper in soil (7.97 mg kg^{-1}) as compared to non-limed soil (7.73 mg kg^{-1}). At the same time, it also resulted in the reduction of soluble copper, which is potentially available to plants. This undesirable development in limed soil such as a lower content of soluble copper can be confirmed by lower copper concentrations in spring barley grain: the largest found in $N_2P_2K_2$ (from 5.98 mg kg^{-1} to 4.65 mg kg^{-1}) and $N_{1.5}P_{1.5}K_{1.5}$ treatments (from 5.73 mg kg^{-1} to 4.85 mg kg^{-1}). Gorlach et al. (1983) indicated that liming did not cause any major chan-

Table 1

Content of copper in grain spring barley (mg kg^{-1} d.m.) cultivated on loessial soil (mean from 4 years)

Treatments of fertilizers (B)	A_1		A_2		Mean (B)
	mean	range	mean	range	
$N_0P_0K_0$	5.50	4.4-6.5	5.07	4.7-6.3	5.29
$N_0P_1K_1$	5.65	4.7-6.1	4.82	5.0-6.3	5.24
$N_{0.5}P_1K_1$	5.60	5.1-5.8	5.02	4.4-6.9	5.31
$N_1P_1K_1$	5.45	4.4-5.7	4.67	3.9-6.3	5.06
$N_{1.5}P_1K_1$	5.30	3.9-5.8	4.50	3.5-6.4	4.90
$N_1P_0K_1$	5.50	5.0-6.1	5.02	4.5-6.9	5.26
$N_1P_{0.5}K_1$	5.20	5.3-6.1	5.27	4.2-6.7	5.24
$N_1P_{1.5}K_1$	4.33	4.5-6.1	4.75	4.0-5.8	4.54
$N_1P_1K_0$	4.83	4.0-5.4	4.82	4.0-6.3	4.83
$N_1P_1K_{0.5}$	5.36	4.1-5.6	4.62	3.9-6.1	4.99
$N_1P_1K_{1.5}$	5.20	4.6-5.6	4.70	4.1-5.9	4.95
$N_{0.5}P_{0.5}K_{0.5}$	5.50	4.4-6.6	4.70	4.0-6.7	5.10
$N_{1.5}P_{1.5}K_{1.5}$	5.73	5.0-6.0	4.85	4.1-6.8	5.29
$N_2P_2K_2$	5.98	4.6-6.2	4.65	4.2-6.9	5.32
Mean of (A)	5.37	-	4.82	-	-
LSD	$A^{*} = 0.36$, B = ns AB = ns				

A_1 – fertilization NPK + Mg constans, A_2 – fertilization NPK +Mg, Ca constans,
LSD – lowest significant difference for: A – liming, B – mineral fertilization (irrespective of liming), AB – interaction between liming and mineral fertilization, * significant at $p = 0.001$, ns – differences not significant

ges in the uptake of copper by grasses, although it distinctly impeded the absorption of copper by other plants. Pikuła and Stępień (2007) observed that an improved soil reaction expressed by an increase in pH in the range from 4 to 6 lowered the content of Cu in grain more strongly on sandy than on medium heavy soil. However, the Cu accumulation decline was relatively low in comparison with other heavy metals (Zn and Cd).

Mineral nutrition (B), regardless of liming, did not raise the copper content in grain, as compared to the control. However, there was a trend (at times ambiguous) for grain to have less copper in response to higher nitrogen and phosphorus fertilization rates combined with the constant nutrition using other components.

No interaction (insignificant AB LSD) appeared between liming and mineral fertilization in shaping the copper content in spring barley grain. Nevertheless, the copper content in grain was lower in all fertilized and limed plots than in non-limed ones (Table 1). An earlier experiment (Kaniuczak 1992*b*) performed on brown soil developed from loess showed some decre-

Table 2

Content of copper in grain winter wheat (mg kg^{-1} d.m.) cultivated on loessial soil (mean from 4 years)

Treatments of fertilizers (B)	A_1		A_2		Mean (B)
	mean	range	mean	range	
$N_0P_0K_0$	3.82	2.7-4.5	3.72	2.4-4.7	3.77
$N_0P_1K_1$	4.35	2.7-6.1	4.97	4.1-6.1	4.66
$N_{0.5}P_1K_1$	4.40	3.0-6.0	3.77	2.9-5.0	4.09
$N_1P_1K_1$	3.95	2.9-5.3	3.90	3.2-5.3	3.93
$N_{1.5}P_1K_1$	3.80	2.5-5.3	4.15	3.3-5.2	3.98
$N_1P_0K_1$	3.87	3.2-5.0	3.80	3.5-5.0	3.84
$N_1P_{0.5}K_1$	3.87	2.2-5.0	4.45	3.7-5.8	4.16
$N_1P_{1.5}K_1$	3.00	2.6-3.6	3.07	2.9-3.6	3.04
$N_1P_1K_0$	3.77	3.0-4.7	4.05	2.6-6.3	3.91
$N_1P_1K_{0.5}$	3.20	2.7-3.4	3.15	2.5-3.5	3.18
$N_1P_1K_{1.5}$	3.77	3.5-3.9	4.00	2.7-5.7	3.89
$N_{0.5}P_{0.5}K_{0.5}$	4.20	3.5-4.8	4.30	3.2-5.7	4.25
$N_{1.5}P_{1.5}K_{1.5}$	3.60	3.1-3.9	2.97	2.1-3.9	3.29
$N_2P_2K_2$	3.85	3.3-4.2	3.20	2.5-4.2	3.53
Mean of (A)	3.82	-	3.82	-	-
LSD	A = ns, B* = 1.51 AB = ns				

A_1 – fertilization NPK + Mg constans, A_2 – fertilization NPK +MgCa constans, LSD – lowest significant difference for: A – liming, B – mineral fertilization (irrespective of liming), AB – interaction between liming and mineral fertilization, * significant at $p = 0.05$, ns – differences not significant

ase in the copper content in spring barley grain, which could have resulted from increasing doses of nitrogen and phosphorus with constant PK and NK nutrition. Results achieved by Rabikowska (2000) on barley did not verify that mineral fertilizers had a consistent impact on Cu concentrations in grain.

The copper content in winter wheat grain is illustrated in Table 2. Liming did not remarkably affect the content of the element in the grain. The average copper content of 3.82 mg kg^{-1} remained on the same level both in limed and non-limed objects. Mineral fertilization (B), regardless of liming, raised the grain Cu content higher in the variant without nitrogen nutrition ($N_0P_1K_1$) than with a 1.5-fold increased dose combined with constant phosphorus fertilization NK ($N_1P_{1.5}K_1$). The highest rise in copper content, relative to the control, were observed for $N_0P_1K_1$ (up to 4.66 mg kg^{-1}) and $N_{0.5}P_{0.5}K_{0.5}$ (to 4.25 mg kg^{-1}) variants. These objects were not balanced in terms of N, P, and K doses, and in some cases phosphorus fertilization dominated over the nitrogen rate. Under such conditions, more copper was taken up by winter wheat. Although the research carried out by Gorlach and Gambuś (1997) showed no obvious dependence between the phosphorus and copper content in phosphate fertilizers, these authors suggest that phosphate fertilizers are a major source of copper (average content 13.9 mg Cu kg^{-1}, Unifoska 42.7 mg Cu kg^{-1}) to plants. The triple superphosphate used in the present experiment (Kaniuczak 1998) had an average content of copper equal 20.0 mg kg^{-1}, which was much higher than the Cu levels in ammonium nitrate (2.6 mg kg^{-1}) and potassium salt (10.5 mg kg^{-1}).

The results from an experiment run by Kaniuczak (1992*a*) on brown soil developed from loess indicate a decrease in the Cu content in winter wheat grain in plots supplied with increasing N doses (0-135 kg ha^{-1}) and constant PK fertilization, as well as an increased copper content resulting from the increasing doses of N (from 0 to 90 kg ha^{-1}) at a fixed ratio of other nutrients. In field experiments, Kotecki and Kozak (2004) demonstrated that increasing doses of phosphorus (from 0 to 78 kg P ha^{-1}) affected the copper content and its uptake by winter wheat. Singh and Swarup (1982) ran a pot experiment on winter wheat and concluded that phosphorus fertilization as well as nutrition with phosphorus and low nitrogen rates reduced the copper content in grain, because such fertilization regimes created favorable conditions for copper to bind within soil in the form of $Cu_3(PO_4)_2$. The application of higher nitrogen doses reduced the inhibitory effect of phosphorus on the accumulation of copper in grain. In the current study, the lowest copper concentrations in grain were found mostly in the plots with a dose of phosphorus higher than nitrogen ($N_1P_{1.5}K_1$).

The average copper content in the grain of wheat grown on different soils with balanced NPK fertilization and with no micronutrient nutrition varies between 2.3 and 4.6 mg kg^{-1} (Kulczycki, Grocholski 2004) and depends more strongly on a variety than on the level of mineral nutrition (Mikos, Styk 1989). Kaniuczak et al. (1996) determined the copper content in the range of 2.1-4.7 mg kg^{-1} in winter wheat grain originating from production fields situated in the region of the Rzeszów Foothills and characterized by different NPK levels. Several reserachers (Rachoń, Szumiło 2009, Ciołek et al. 2012) indicate that the Cu content is more dependent on a cereal variety than a cultivation system – organic and conventional farming (Ciołek et al. 2012). Also, conservation tillage compared to plough cultivation and stubble catch crop farming significantly raised the copper content in wheat grain (Kraska 2011).

The research conducted by Mercik et al. (2004) revealed that the copper content in winter wheat is hardly dependent on soil properties (C_{org} content and soil pH), although it could be significantly increased, up to 10.6 mg kg^{-1} (7.7 mg kg^{-1} for non-fertilized objects) after a treatment of soil nutrition with this element. Including the total copper content in soil and other physicochemical soil properties in a stepwise regression analysis leads to unsatisfactory predictions of the copper content in wheat grain (Nan et al. 2002, Właśniewski 2000).

The research carried out by Nowak et al. (2004) showed that the changeable soil moisture during the grain filling stage had a stronger influence on the copper content in winter wheat grain than the implementation of different crop protection techniques. Complete chemical protection of spring barley contributed significantly to the copper content, same as higher nitrogen nutrition; besides, significant differences between spring barley cultivars were detected (Nowak, Zbroszczyk 2004). Rabikowska and Piszcz (2004) observed a reduction in the Cu level in grain of wheat and barley due to increasing doses of mineral nitrogen, which in part may be explained by the dilution effect, i.e. higher yields under higher nitrogen doses. Tills and Alloway (1981) as well as Kumar et al. (1990) reported the effect of various nitrogen forms (NH_4^+, NO_3^- and NH_4NO_3) on the copper uptake by wheat and barley. Nitrogen fertilizers containing ammonium limited the copper accumulation more than those in the form of NO_3^- and NH_4NO_3. Singh and Swarup (1982) showed a significant effect of phosphorus (especially at low nitrogen doses) on the absorption of copper as $Cu_3(PO_4)_2$ in soil, which significantly reduced the concentration of Cu in winter wheat grain. The reduced Cu concentration in wheat due to phosphorus fertilization did not result from the dilution effect or inhibited translocation from roots to shoots, but from the reaction of positively charged Cu ions with negatively charged phosphate anions in soil (Gunes et al. 2009).

The average copper content in winter wheat grain (3.82 mg kg^{-1}) and spring barley (4.82-5.37 mg kg^{-1}) determined in our study is within the range of Cu concentrations in cereal grain found in Poland, which – according to Kabata-Pendias (1996) – is 2.6-6.5 mg kg^{-1} with an average concentration of 3.80 mg kg^{-1}, and – according to Moroń et al. (1992) – 4.1 mg kg^{-1} in wheat grain. Barley and wheat grain satisfies the requirements of food (standard 20 mg kg^{-1}) and feed cereals (25-50 mg kg^{-1}) (Kabata-Pendias et al. 1993). *The Commission Regulation (EC) No 1881/2006 of 19 December 2006* does not specify the maximum level of copper contamination in foodstuffs. High copper levels in cereals and cereal products rarely cause poisoning. In general, literature indicates serious copper deficiency in food rations, in which cereal products are an important source of Cu (Marzec 1996).

According to Moroń et al. (1992), the copper content in grain is a genetically coded trait, which is more difficult to change than in other parts of a plant. The metal is hardly released from soil; it accumulates mainly in roots of plants; in a contaminated environment, there is a slight increase in its content in grain (Bednarek et al. 2008, Kucharczyk, Moryl 2010). The monitoring studies conducted in parts of Poland affected by the copper industry, that is in the Głogów-Legnica copper district (Dobrzański et al. 2003) and around the Turów mine and power plant (Kucharczyk, Moryl 2010), show that the copper content in grain of wheat, within the range of 4.3-5.1 mg kg^{-1}, are low and do not pose any ecological of toxicological threat.

CONCLUSIONS

1. Liming did not have any statistically significant influence on the copper content in winter wheat grain; however, it decreased the copper content in spring barley grain.

2. Mineral fertilization, regardless of liming, did not significantly affect the copper content in spring barley grain, although it slightly diversified its content in winter wheat grain and markedly reduced the Cu content in grain after the application of a 1.5-fold higher phosphorus dose in combination with constant NK nutrition.

3. The interaction between liming and fertilization was not confirmed to have affected the copper content in the grain of spring barley and winter wheat. However, the copper content was generally lower in grain of spring barley originating from limed objects.

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CONTENT OF COPPER, IRON, MANGANESE AND ZINC IN TYPICAL LIGHT BROWN SOIL AND SPRING TRITICALE GRAIN DEPENDING ON A FERTILIZATION SYSTEM

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Abstract

The impact of mineral fertilization with or without multi-component fertilizers on the content of microelements in soil and spring triticale grains was investigated in field trials, in 2009-2011. The experiment was carried out on 8 fertilizing treatments with three replications, which included two varieties of spring triticale: Andrus and Milewo. The content of available zinc and manganese was higher on plots cropped with the cultivar Andrus and nitrogen fertilization with urea or with urea and ammonium nitrate. It was also found out that the content of available manganese, zinc and iron in the analyzed soils was within the natural average range. A higher content of manganese and zinc in grains was detected after the application of multi-component fertilizers. Nitrogen fertilization at a dose of 120 kg·ha^{-1} together with Azofoska and Ekolist resulted in an increase in the iron content in cv. Andrus. The regression analysis between the content of the analyzed microelements in soil and in triticale grains revealed a significant increase in the iron, manganese and zinc content in grains together with an increase in the content of these elements in soil under cv. Milewo. With respect to the zinc content in soil and in grain from this variety, the coefficient of determination was the closest to the coefficient of a linear correlation ($R^2 = 0.9105$). It was shown that an increase in the content of microelements in soil was not always accompanied by an increase in the content of these elements in spring triticale grains.

Key words: spring triticale, fertilization, microelements.

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ZAWARTOŚĆ MIEDZI, ŻELAZA MANGANU I CYNKU W TYPOWEJ LEKKIEJ GLEBIE BRUNATNEJ I W ZIARNIE PSZENŻYTA JAREGO W ZALEŻNOŚCI OD SYSTEMU NAWOŻENIA

Abstrakt

W latach 2009-2011 w doświadczeniu polowym badano wpływ nawożenia NPK stosowanego bez nawozów wieloskładnikowych lub łącznie z nimi na zawartość mikroelementów w glebie i ziarnie pszenżyta jarego. Doświadczenie obejmowało 8 obiektów nawozowych w 3 powtórzeniach z uwzględnieniem 2 odmian pszenżyta jarego: Andrus i Milewo. Stwierdzono, że gleba zawierała więcej przyswajalnego cynku i manganu w przypadku nawożenia azotem w formie mocznika lub mocznika i saletry amonowej w uprawie odmiany Andrus. Stwierdzono ponadto, że zasobność badanych gleb w przyswajalny mangan, cynk i żelazo kształtowała się w granicach ich zasobności na poziomie średnim. W ziarnie zaobserwowano przede wszystkim większe nagromadzenie manganu i cynku po zastosowania nawozów wieloskładnikowych. Nawożenie azotem w dawce 120 kg ha^{-1} łącznie z azofoską i ekolistem przyczyniło się również do zwiększenia zawartości żelaza w ziarnie odmiany Andrus. Analiza regresji między zawartością badanych mikroelementów wykazała istotny wzrost zawartości żelaza, manganu i cynku w ziarnie w miarę zwiększania się zasobności gleby w uprawie pszenżyta jarego odmiany Milewo. W przypadku zawartości cynku w glebie i w ziarnie tej odmiany, współczynnik determinacji był najbliższy współczynnikowi korelacji liniowej (R^2 = 0,9105). Wykazano, że wraz ze zwiększeniem się zawartości mikroelementów w glebie nie zawsze następował wzrost zawartości tych składników w ziarnie pszenżyta jarego.

Słowa kluczowe: pszenżyto jare, nawożenie, mikroelementy.

INTRODUCTION

Our interest in the content of microelements in the environment is driven mainly by the wish to attain an appropriate quality of soil and agricultural produce. As a life-sustaining habitat, soil plays the principal role in the growth of plants, but it also influences the development of humans and animals. Any excess or deficit of microelements has a negative impact on the physiological processes in plants (Grzyś 2004, Spiak 2000). Iron, zinc, copper and manganese are regarded to be as essential elements in nutrition (Knapowski et al. 2009). In the body, they activate numerous enzymes responsible for the metabolism of cells (Grzyś 2004, Knapowski et al. 2010).

In Poland, the natural concentration of microelements in soil is highly diverse within provinces and regions (Dąbkowska-Naskręt et al. 2006, Lipnicki 2009). The current level of soil abundance indicates a high share of areas with low copper concentrations (Lipnicki 2009). The content of other elements is on a moderate level. Numerous studies have shown that the phytoabsorption in soil and grains is conditioned by many factors, such as soil reaction, abundance of nutrients, absorbable forms of elements, fertilization, species and varieties of plants (Kalembasa et al. 2009, Diatta 2008, Diatta, Grzebisz 2006, Jakubus 2006, Ścigalska et al. 2000). The levels of microelements in plants are indirectly influenced by a dose and method of fertilization with other components, particularly with nitrogen (Diatta, Grzebisz 2006, Sienkiewicz et al. 2009, Wei et al. 2006). Intensive agronomic technologies involved in the cultivation of high-yielding plants, which have high nutritional and fertilizing demands, as well as the target increase in crop yields have led to the depletion of these microelements in soils.

Foliar fertilization is the most popular way to supply microelements in plant production. Simultaneous application of essential elements and microelements produces grain crops with the quality characteristics which enhance their value as food and feeds (Sztuder 2009). Direct feeding of plants by spraying is faster and more effective than absorption of nutrients from soil (Wójcik 2004). This method of plant nourishment is particularly suitable when soils are deficient in microelements and during the phases of intensive growth of plants. It is also necessary to supply microelements in doses appropriate for plants and sustainable for the environment.

The content of microelements in grain is a quality descriptor applied in the context of its value for human and animal consumption. It contributes to the composition of a daily nutritional ration (Knapowski et al. 2009).

In Poland, the rising interest in foliar fertilization with microelements is encouraged by the high percentage of light acid soils with a low and average content of absorbable forms, and by the low concentration of these elements in plants. Cereals are among the plants most sensitive to deficiencies of microelements. However, rational fertilization may replenish the loss of these elements in soil.

The aim of this study was to determine to what degree soil and foliar fertilization with multi-component fertilizers influences the content of zinc, manganese, copper and iron in soil and their content in spring triticale grain.

MATERIAL AND METHODS

In 2009-2011, a field trial was carried out at the Teaching and Research Centre of the UWM in Tomaszkowo. The experiment was set up on typical brown soil with the texture of light clay class III b with the silt subsoil. The soil was acidic, with a low content of organic carbon (C_{org} 7.71 g kg^{-1}), an average content of available zinc, manganese and iron and a low copper content (Table 1).

The experiment was set up in randomized blocks with 3 replications. The size of a single plot was 6.25 m^2, of which 4.0 m^2 were harvested. Spring

Table 1

The content of C_{org}, Cu, Fe, Mn and Zn in the soil before the experiment

pH KCl	$C_{org.}$ (g kg^{-1})	Content of available microelements (mg kg^{-1} in d.m.)							
		Cu		Fe		Mn		Zn	
		actual	wealth	actual	wealth	actual	wealth	actual	wealth
5.0	7.71	2.1	low	1100	mean	182	mean	14.5	mean

triticale was sown in the following densities: cv. Andrus 282.1 kg ha^{-1} and cv. Milewo 237.9 kg ha^{-1}. Both varieties sown in rows spaced 10.5 cm from one another. Winter triticale was the preceding crop in both cases. In all the experimental objects, fertilization with phosphorus and potassium was identical. A dose of 30.2 kg·ha^{-1} of phosphorus (P) in the form of 46% triple superphosphate and 83.1 kg ha^{-1} of potassium (K) in the form of potassium salt were used. Nitrogen fertilization and the supplementation of basic fertilization were applied according to the scheme (Table 2).

Table 2

Scheme of the field experiment

Treatment	Total N fertilization (kg N ha^{-1})	Fertilizer type and application time (kg ha^{-1} dose)		
		before sowing	tillering stage [BBCH 23-29]	stem elongation stage [BBCH 31-32]
1	80	-	urea (40)	urea (40)
2	80	-	urea (20) Azofoska (20)	urea (40)
3	80	-	urea (40)	urea 40*
4	80	-	urea (40)	ammonium nitrate (32) + ekolist* (8)
5	120	ammonium nitrate (40)	urea (40)	urea (40)
6	120	ammonium nitrate (40)	urea (40) zofoska (20)	urea (40)
7	120	ammonium nitrate (40)	urea (40)	urea (40)*
8	120	ammonium nitrate (40)	urea (40)	ammonium nitrate (32) + ekolist*(8)

*foliar fertilization

Two recommended multi-component fertilizers containing basic essential elements and the composition of selected microelements were used in the experiment (Table 3).

Table 3

Composition of the applied multi-component fertilizers

Type of multi-component fertilizer	N	P	K	Mg	S	Cu	Zn	Mn	Fe	Mo	B
Ekolist (g dm^{-3})	120.0	-	65.0	20	5	5.00	2.50	0.50	1.00	0.02	5.00
Azofoska (%)	13.6	1.83	15.9	2.71	9.2	0.18	0.045	0.27	0.17	0.04	0.045

The agronomic technologies applied to spring triticale were carried out as required.

The meteorological conditions, i.e. rainfalls, presented as the annual averages for the years when the experiment was conducted, differed from the values measured in the previous years (Table 4). The precipitation in April was less than half the average value for the previous years. In May, June and July the average precipitation was higher by 51.4, 28.8 and 46.7%, respectively, than the means from the previous years.

Table 4

Meteorological conditions in the investigadet period

Month / Period	April	May	June	July	August	Average Apr-Aug
Temperature (°C)						
2009-2011	8.9	12.5	16.1	19.4	18.2	15.0
1961-2005	6.9	12.8	15.9	17.8	17.7	14.2
Precipitation (mm)						
2009-2011	15.2	78.6	101.1	110.2	65.6	74.1
1961-2005	35.7	51.9	78.5	75.1	66.1	61.5

The soil sampled after harvest was dried, ground in a china mortar and passed through a sieve with 1mm mesh. The soil reaction (pH) was determined by the potentiometer method in 1 M KCl dm^{-3}. The microelements soluble in 1M HCl dm^{-3} were extracted at a chemical agricultural station, according to the total method, i.e. by shaking a soil sample with hydrochloric acid at the 1:10 ratio for 1 hour (Gembarzewski, Korzeniowska 1996)

The samples of grains were ground in a WŻ-1 laboratory-type mill and then mineralized in a heated mixture of HNO_3 and $HClO_4$ acids in the 3:1 ratio.

After soil extraction and grain mineralization, the content of Cu, Zn, Mn and Fe was determined in the solutions using the flame technique on an atomic absorption spectrometer.

The statistical calculations were performed according to a two-way analysis of variance, which was consistent with the experimental mathematical model, i.e. randomized blocks. Apart from basic statistical parameters, statistically homogenous groups were determined with the Tukey's range test at $\alpha = 0.05$. The relationships between the content of microelements in soil and their content in grains were also investigated. All statistical analyses and calculations were performed with MS Office Excel and Statistica software.

RESULTS AND DISCUSSION

The pH values (in KCl solution of a concentration 1 M) of the tested soil samples collected after the harvest of spring triticale were within the range of 4.75 and 5.04 for cv. Andrus and from 4.79 to 4.96 for cv. Milewo (Table 5). Under the experimental conditions, i.e. with small differences in pH values, the soil reaction did not influence the concentration of microelements, as reported by Diatta (2008) and Wei et al. (2006).

Table 5

Absorbable content of Cu, Fe, Mn and Zn in soil (the average in 2009-2011)

Variety	Object	pH	Cu	Fe	Mn	Zn
			(mg kg^{-1} of d.m.)			
Andrus	1	4.83 ± 0.24^{ab}	2.1 ± 0.18^{a}	1200 ± 156^{ab}	158 ± 18.9^{abc}	12.1 ± 1.81^{ab}
	2	$4.88 \pm 0{,}34^{bc}$	2.3 ± 0.27^{a}	1300 ± 118^{b}	153 ± 16.8^{ab}	12.2 ± 1.58^{ab}
	3	5.04 ± 0.50^{d}	1.9 ± 0.17^{a}	1100 ± 132^{ab}	177 ± 26.5^{d}	13.8 ± 1.10^{e}
	4	4.87 ± 0.58^{bc}	1.9 ± 0.26^{a}	1300 ± 182^{b}	150 ± 13.5^{a}	12.8 ± 2.17^{bcd}
	5	4.94 ± 0.20^{c}	1.9 ± 0.21^{b}	1200 ± 132^{ab}	161 ± 22.5^{abc}	12.6 ± 1.38^{abc}
	6	4.75 ± 0.28^{a}	2.0 ± 0.12^{a}	1110 ± 165^{ab}	165 ± 14.8^{bcd}	11.9 ± 2.02^{a}
	7	4.94 ± 0.39^{c}	2.1 ± 0.27^{a}	1150 ± 113^{ab}	169 ± 27.4^{cd}	13.5 ± 1.62^{de}
	8	4.95 ± 0.59^{cd}	2.2 ± 0.24^{a}	1060 ± 94^{a}	167 ± 18.4^{cd}	13.2 ± 1.18^{cde}
Milewo	1	4.91 ± 0.43^{bc}	1.9 ± 0.19^{a}	1200 ± 144^{a}	151 ± 19.6^{a}	12.2 ± 1.83^{a}
	2	4.82 ± 0.59^{ab}	2.1 ± 0.18^{a}	1100 ± 143^{a}	162 ± 22.7^{bc}	12.2 ± 1.34^{a}
	3	4.96 ± 0.34^{c}	2.3 ± 0.25^{a}	1000 ± 140^{a}	158 ± 19.0^{ab}	12.9 ± 0.91^{ab}
	4	4.83 ± 0.58^{ab}	2.3 ± 0.32^{a}	1200 ± 108^{a}	163 ± 22.8^{bc}	12.2 ± 1.58^{a}
	5	4.79 ± 0.95^{a}	2.2 ± 0.26^{a}	1200 ± 204^{a}	165 ± 14.8^{bcd}	13.5 ± 1.48^{ab}
	6	4.83 ± 0.19^{ab}	1.9 ± 0.28^{a}	1050 ± 136^{a}	172 ± 10.3^{d}	14.0 ± 1.12^{b}
	7	4.79 ± 0.48^{ab}	2.1 ± 0.27^{a}	1300 ± 182^{a}	165 ± 21.4^{bcd}	13.5 ± 2.16^{ab}
	8	4.96 ± 0.35^{c}	2.3 ± 0.41^{a}	1150 ± 138^{a}	168 ± 23.5^{cd}	13.4 ± 1.85^{ab}
Average for varieties						
Andrus		4.90 ± 0.64^{b}	$2.\ 0 \pm 0.32^{a}$	1178 ± 153^{a}	163 ± 21.2^{a}	12.8 ± 2.18^{a}
Milewo		4.86 ± 0.68^{a}	2.2 ± 0.24^{a}	1150 ± 184^{a}	163 ± 27.7^{a}	13.0 ± 1.68^{a}
Average for fertilization						
1		4.87 ± 0.53^{b}	2.0 ± 0.28^{a}	1200 ± 168^{ab}	154 ± 20.0^{a}	12.2 ± 1.71^{a}
2		4.85 ± 0.72^{b}	2.2 ± 0.24^{a}	1200 ± 204^{ab}	158 ± 17.4^{ab}	12.2 ± 1.59^{a}
3		5.00 ± 0.60^{c}	2.1 ± 0.27^{a}	1050 ± 126^{a}	168 ± 25.2^{c}	13.4 ± 2.01^{c}
4		4.85 ± 0.63^{b}	2.1 ± 0.31^{a}	1250 ± 175^{b}	157 ± 22.0^{ab}	12.5 ± 1.50^{ab}
5		4.87 ± 0.88^{b}	2.1 ± 0.29^{a}	1200 ± 192^{ab}	163 ± 22.4^{bc}	13.1 ± 2.10^{bc}
6		4.79 ± 0.62^{b}	2.0 ± 0.26^{a}	1080 ± 151^{ab}	169 ± 28.7^{c}	13.0 ± 2.08^{bc}
7		4.87 ± 0.73^{b}	2.1 ± 0.36^{a}	1225 ± 196^{b}	167 ± 23.4^{c}	13.5 ± 1.89^{c}
8		4.96 ± 0.59^{c}	2.3 ± 0.32^{a}	1105 ± 166^{ab}	168 ± 21.6^{c}	13.3 ± 2.13^{bc}

± standard error of the mean (SEM)
a, *b*, *c*, … homogenous groups

It was found that the content of absorbable fractions of zinc, copper, manganese and iron in soil after the harvest of spring triticale was differentiated (Table 5). According to Diatta and Grzebisz (2006), depending on the type of soil, nitrogen fertilizers such as $(NH_4)_2SO_4$, $CO(NH_2)_2$ and $NH_4NO_3 \cdot \cdot CaCO_3$ affect the buffer capacity of soil, hence altering the concentration of some microelements. Rutkowska et al. (2009) reports that nitrogen fertilization reduces the reaction of soil and causes an increase in the content of iron, manganese, zinc and copper. Other authors (Sienkiewicz et al. 2009) claim that manure fertilization significantly influences the accumulation of microelements in soil. In our experiment, a higher content of manganese and zinc was found only under the influence of nitrogen fertilization in the form of urea or urea with ammonium nitrate in the cultivation of cv. Andrus. The content of zinc in the cultivation of cv. Andrus increased as a result of the supplementation with Azofoska.

The highest increase in the zinc content (by 14.7%) was reported after the fertilization of cv. Milewo with the higher dose of nitrogen (120 kg ha^{-1}) and with Azofoska. A significant increase in the manganese content (by 11%) was observed in the cultivation of cv. Milewo fertilized with Azofoska, regardless of the nitrogen dose.

It was also found that the content of manganese, zinc and iron in the tested soil samples ranged within their natural average concentrations, while the content of copper was on a low level regardless of the type of fertilization.

Kastori et al. (2006), Szabó and Fodor (2006) showed that the accumulation of microelements, particularly zinc and manganese, in triticale or winter wheat grains and in sunflower or maize seeds largely depended on soil abundance. In our experiment, the content of the analyzed elements in spring triticale grains was differentiated and, similarly to reports by other authors (Wójcik 2004, Sienkiewicz et al. 2009, Sztuder 2009, Ścigalska et al. 2000, 2011), depended on the type of fertilization.

The average content of copper in grain ranged between 2.26 mg·kg^{-1} and 2.80 mg·kg^{-1} for cv. Andrus and from 2.44 mg kg^{-1} to 3.27 mg kg^{-1} for cv. Milewo (Table 6). A higher content of copper was observed under the influence of foliar and soil nitrogen fertilization at a dose of 80 kg ha^{-1}. The application of Azofoska resulted in cv. Andrus grain having 12.4% more copper than grain from triticale fertilized with urea at a dose of 80 kg ha^{-1}.

Nitrogen fertilization at a dose of 120 kg ha^{-1} together with Ekolist and Azofoska supplied during the BBCH 31-32 phase stimulated the iron accumulation in grains from cv. Andrus (up to 27.8 mg kg^{-1} and 28.0 mg kg$^{-1.}$). In cv. Milewo, the highest content of iron was reported only under the impact of nitrogen fertilization at a dose of 120 kg ha^{-1}.

In the cultivation of triticale fertilized with the higher dose of nitrogen (120 kg ha^{-1}), the beneficial effect of Azofoska supplied during the BBCH 23-29 phase on the content of manganese was observed for the cultivar Andrus. This type of fertilization affected the content of zinc in cv. Andrus variety grains and manganese and zinc in cv. Milewo grains. This experiment has indicated that foliar fertilization with liquid preparations is one of the most cost-efficient agrotechnical procedures (Sztuder 2009).

In the studies carried out by Kastori et al. (2006) on the cultivation of

Table 6

Content of Cu, Fe, Mn and Zn in triticale grains (the average in 2009-2011)

Variety	Object	(mg kg^{-1} of d.m.)			
		Cu	Fe	Mn	Zn
Andrus	1	2.26 ± 0.23^{a}	24.1 ± 2.17^{e}	32.2 ± 3.54^{b}	30.6 ± 2.45^{d}
	2	2.80 ± 0.39^{f}	21.1 ± 2.95^{c}	34.5 ± 2.41^{c}	28.9 ± 3.46^{a}
	3	2.72 ± 0.29^{e}	21.8 ± 1.74^{d}	31.2 ± 2.81^{ab}	29.1 ±3.49^{a}
	4	2.50 ± 0.20^{c}	19.3 ± 1.93^{a}	30.0 ± 2.70^{a}	29.1 ± 2.32^{a}
	5	2.44 ± 0.34^{b}	20.5 ± 2.66^{b}	31.6 ± 4.11^{b}	29.7 ± 4.45^{b}
	6	2.50 ± 0.22^{c}	27.8 ± 2.50^{g}	35.3 ± 5.29^{c}	32.3 ± 3.55^{e}
	7	2.49 ± 0.17^{c}	25.8 ± 3.09^{f}	31.3 ±3.76^{ab}	30.3 ± 2.70^{c}
	8	2.56 ± 0.33^{d}	28.0 ±1.63^{g}	34.6 ± 3.81^{c}	30.4 ± 3.34^{cd}
Milewo	1	3.14 ±0.40^{e}	25.8 ± 2.06^{e}	29.9 ± 3.59^{b}	30.8 ± 2.16^{a}
	2	2.80 ± 0.31^{c}	24.6 ± 3.44^{b}	29.7 ± 2.97^{b}	30.6 ± 3.67^{a}
	3	3.27 ± 0.26^{f}	24.5 ± 1.48^{b}	27.8 ± 3.61^{a}	31.3 ± 2.50^{b}
	4	2.84 ± 0.42^{d}	25.0 ± 3.50^{c}	27.6 ± 3.04^{a}	30.6 ± 2.75^{a}
	5	2.44 ± 0.22^{a}	26.8 ± 3.75^{f}	30.5 ± 1.83^{d}	35.5 ± 4.26^{d}
	6	2.55 ± 0.17^{b}	23.9 ± 1.91^{a}	32.6 ± 32.6^{e}	38.6 ± 3.86^{e}
	7	2.45 ± 0.34^{a}	27.5 ± 1.65^{g}	30.3 ± 3.33^{cd}	34.7 ±1.39^{c}
	8	2.79 ± 0.33^{c}	25.3 ± 3.04^{d}	30.2 ± 2.72^{c}	34.8 ± 3.82^{c}
Average for varieties					
Andrus		2.53 ± 0.43^{a}	23.6 ± 3.30^{a}	32.6 ± 3.90^{a}	30.1 ± 5.11^{a}
Milewo		2.64 ± 0.39^{b}	23.8 ± 3.09^{b}	32.3 ± 5.49^{b}	33.4 ± 5.01^{b}
Average for fertilization					
1		2.70 ± 0.38^{e}	25.0 ± 3.74^{e}	31.1 ± 5.28^{c}	30.7 ± 4.30^{c}
2		2.80 ± 0.33^{f}	22.9 ±2.97^{b}	32.1 ± 3.53^{d}	29.8 ± 4.46^{a}
3		3.00 ± 0.48^{g}	23.2± 3.24^{c}	29.5 ± 4.72^{b}	30.2 ± 5.13^{b}
4		2.67 ± 0.29^{d}	22.2 ± 3.10^{a}	28.8 ± 4.90^{a}	29.9 ± 5.37^{a}
5		2.44 ± 0.41^{a}	23.7 ± 3.78^{d}	31.1 ± 4.04^{c}	32.6 ± 4.24^{d}
6		2.53 ± 0.33^{c}	25.9 ± 2.58^{f}	34.0 ± 4.07^{e}	35.5 ± 5.32^{e}
7		2.47 ± 0.37^{b}	26.7 ± 3.73^{g}	30.8 ± 4.62^{c}	32.5 ± 4.87^{d}
8		2.68 ± 0.30^{d}	26.7 ± 3.99^{g}	32.4 ± 4.54^{d}	32.6 ± 5.54^{d}

± standard error of the mean (SEM)
a, *b*, *c*, … homogenous groups

triticale on Chermozem soil and fertilization with foliar supply of microelements, a significant correlation between the dose of fertilizer and the content of elements in grains was observed.

An increase in the content of microelements in soil was not always accompanied by an increase in the content of these elements in spring triticale grains. Analysis of regression confirmed these relations (Figures 1 - 8). Our

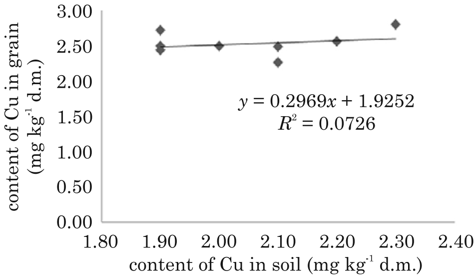


Fig. 1. The relationship between Cu content in cv. Andrus grains and in soil

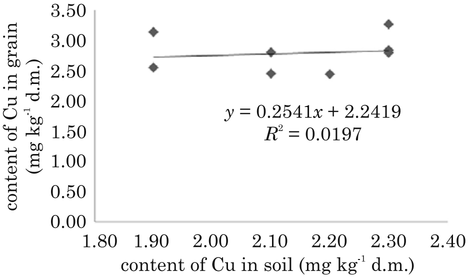
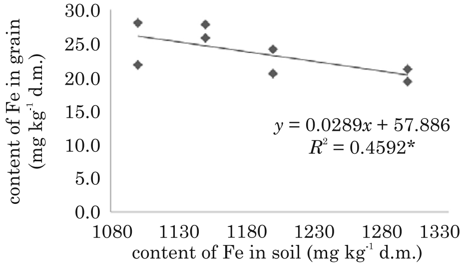
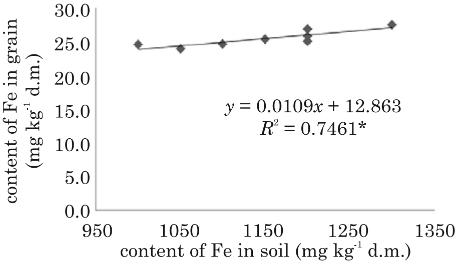


Fig. 2. The relationship between Cu content in cv. Milewo grains and in soil

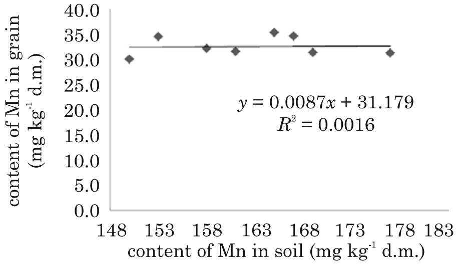


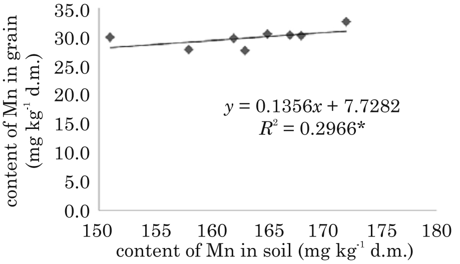


* coefficient of determination (R^2) significant at $p = 0.05$

Fig. 3. The relationship between Fe content in cv. Andrus grains and in soil

Fig. 4. The relationship between Fe content in cv. Milewo grains and in soil





* coefficient of determination (R^2) significant at $p = 0.05$

Fig. 5. The relationship between Mn content in cv. Andrus grains and in soil

Fig. 6. The relationship between Mn content in cv. Milewo grains and in soil

analysis of the relationships between the content of microelements in soil and in triticale grains revealed a significant increase in the content of iron, manganese and zinc in grains together with their increase in the soil in the plots croped with the cultivar Milewo. Regarding the zinc content in soil and in grain from cv. Milewo, the coefficient of determination was the closest to the coefficient of linear correlation ($R^2 = 0.9105$). It was also found that the content of iron in grain from cv. Andrus decreased along with an increase in the content of this element in soil ($R^2 = 0.4592$).

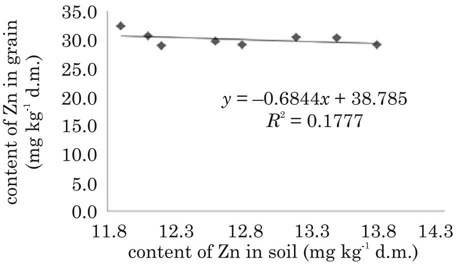
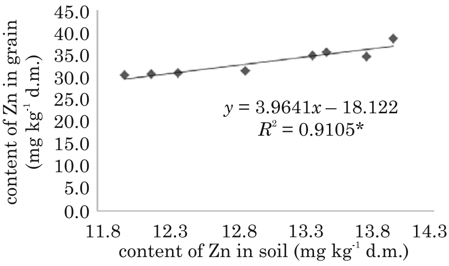


Fig. 7. The relationship between Zn content in cv. Andrus grains and in soil



* coefficient of determination (R^2) significant at $p = 0.05$

Fig. 8. The relationship between Zn content in cv. Milewo grains and in soil

CONCLUSIONS

1. Fertilization resulted in a significant increase in the content of available forms of manganese and zinc in soil, particularly after the application of higher doses of nitrogen with Ekolist and Azofoska.

2. In most cases, a dose of 120 kg N ha^{-1} supplemented with multi-component fertilizers, i.e. Azofoska and Ekolist increased the content of iron and manganese in spring triticale grains.

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BORON CONCENTRATIONS IN GROUNDWATER INTENDED FOR CONSUMPTION FROM INTAKES LOCATED IN NORTHERN POLAND

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Abstract

The studies on boron concentrations were carried out on the samples collected from the groundwater intakes located in Northern Poland in the Lower Vistula Valley and in the Starogard Lakeland with diversified hydro-geological conditions and depths. The 57 samples of water for assays were collected from the following intakes: Tczew "Park" and Tczew "Motława", Gniew, Wielkie Walichnowy, Małe Walichnowy, and Pelplin. The deepest drillings extended to 180 m (cretaceous stages), out of which 20% comprised quaternary formations up to 123 m deep. The analysis of water samples in these regions was performed from 2009-2011. The geological architecture of these areas was identified based on the legal documentation on water utilization submitted by the operators of the water intakes. The Polish sanitary regulations specify that the maximum content of boron in drinking water is 1.0 mg dm^{-3}. That it was found in the waters mesoregion Starogard Lakeland may be assumed to be good in the context of chemical status, because in all of the 24 water samples tested, the boron concentration did not exceed the threshold value. In the Lower Vistula Valley mesoregion in 17 samples out of 33 tested, the level of boron exceeded 1.0 mg dm^{-3}, which means that prior to consumption these waters require treatment to reduce the concentration of this element to the permissible limits. Slightly higher concentrations of boron (on average 1.63 mg dm^{-3}) were detected in water deposited in the cretaceous formations situated in Wielkie Wachlinowy and Małe Wachlinowy. It was also found that the content of boron in groundwater depended on the nature of geological layers from which the tested water samples were collected. The statistical analysis demonstrates that the aquifer stages exerted a significant impact on the content of boron in the tested water samples and the differences between them are significant.

Key words: boron, groundwater, aquifer stages, water quality assessment.

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STĘŻENIE BORU W WODACH PODZIEMNYCH POBIERANYCH DO SPOŻYCIA Z UJĘĆ W PÓLNOCNEJ POLSCE

Abstrakt

Badania stężenia boru w wodach podziemnych pobieranych z ujęć położonych w północnej Polsce prowadzono w regionach Doliny Dolnej Wisły i Pojezierza Starogardzkiego, charakteryzujących się zróżnicowanymi warunkami hydrogeologicznymi i głębokością ich zalegania. Próbki wody do analiz pobierano z ujęcia Tczew „Park" i Tczew „Motława", Gniew, Wielkie Walichnowy, Małe Walichnowy i Pelplin. Najgłębsze wiercenia na tym obszarze osiągnęły głębokość 180 m (piętra kredy), z czego 20% obejmowały formacje czwartorzędowe o głębokości do 123 m. Analizę 57 próbek wody z ww. regionów przeprowadzono w latach 2009-2011. Budowę geologiczną badanych regionów rozpoznano na podstawie operatów wodnoprawnych udostępnionych przez eksploatatorów ujęć. Maksymalną zawartość boru w wodach pitnych polskie przepisy sanitarne określają na 1,0 mg dm^{-3}. Wykazano, że wody mezoregionu Pojezierza Starogardzkiego można uznać za dobre pod względem stanu chemicznego, ponieważ we wszystkich 24 badanych próbkach wody zawartość boru nie przekroczyła wartości granicznej. W mezoregionie Doliny Dolnej Wisły aż w 17 próbkach, na zbadane 33, poziom boru przekroczył 1,0 mg dm^{-3}, co oznacza, że wody te przed przeznaczeniem do konsumpcji wymagają uzdatniania do poziomu dopuszczalnej jego zawartości. Nieco większą zawartość boru (średnio 1,63 mg dm^{-3}) stwierdzono w wodach utworów kredy w miejscowościach Wielkie Walichnowy i Małe Walichnowy. Stwierdzono również, że zawartość boru w wodach podziemnych była zależna od rodzaju pokładów geologicznych, z których pochodziła badana woda. Analiza statystyczna potwierdziła, że piętra wodonośne miały duży wpływ na zawartość boru w badanych wodach, a różnice między nimi należy uznać za istotne.

Słowa kluczowe: bor, piętro wodonośne, wody gruntowe, ocena jakości wody.

INTRODUCTION

Water is one of the most important components of the environment in which the organisms live. Therefore, pollution of the aquatic environment causes a variety of major changes that are generally unfavourable to humans. Hence, it is important to constantly monitor water quality in order to undertake timely preventive and remedial measures (Koc et al. 2006, Koc et al. 2007, Czekała et al. 2011, Wons et al. 2012, Papciak et al. 2013).

In nature, boron is found mainly as borosilicates and tourmalines in sediment rocks. In many lakes it is present as borax and in some natural water sources as boric acid. Boron compounds are transferred into water with industrial liquid wastes and communal sewage (Woods 1994). According to Kabata-Pendias and Pendias (1999), anthropogenic emission of boron into the environment may also originate from coal combustion, the chemical industry and rocket fuels. The concentration of boron in drinking water does not usually exceed 1 mg dm^{-3}, but also higher concentrations are detected in water from natural sources. The concentration of boron in groundwater ranges from below 0.3 mg dm^{-3} to over 100 mg dm^{-3}. The average content of boron in groundwater in Italy and Spain is 0.5-1.5 mg dm^{-3}, and in the Netherlands and United Kingdom it amounts to 0.6 mg dm^{-3}. Approximately 90% of water samples collected in Denmark, France and Germany contained boron in concentrations below 0.3 to 0.1 mg dm^{-3} (WHO 1998, Haberer 1996). In Turkey, in areas where borax mining is concentrated, the content of boron in water ranged from 2.0 to 29.0 mg dm^{-3} (Cöl, Cöl 2003). In Eastern Europe, high concentrations of boron were detected in some highly mineralized natural waters in Romania (20 mg dm^{-3}), Georgia (to 10 mg dm^{-3}), Slovakia (up to 9.48 mg dm^{-3}) and Slovenia (5.5 mg dm^{-3}). In hydrogeochemical conditions typical of groundwater in Poland, the boron concentration ranges from 0.01 to 0.5 mg dm^{-3} (WHO 2009). Local concentrations of boron in water may be associated with both an elevated content in surrounding geological formations and the presence of other contaminations such as fluorides (Koc et al. 2006, Queste et al. 2001).

Boron is a micro-component, which together with calcium is involved in the process of bone formation and prevents caries. Apart from its natural metabolic functions, it has been shown that it induces multiple pathological changes. It has been found that boron intoxication in humans causes convulsions, anaemia, vomiting, diarrhoea and skin inflammation. The organs that are exposed to immediate damage include the central nervous system, gastrointestinal tract, skin and mucous membranes, liver, and kidneys (Culvar, Hubbard 1996). The permissible limits of boron concentration in water have been thus specified and the national standards for drinking water quality are based on the guidelines by the World Health Organization (WHO 1998) and requirements set in the EU and Polish regulation 98/83/EC (*EU Council Directive* ... 1998, WHO 1998, *Regulation* … 2007).

MATERIALS AND METHODS

The studies on boron concentrations were carried out on the samples collected from the groundwater intakes located in Northern Poland in the Lower Vistula Valley and on Starogard Lakeland with diversified hydro-geological conditions (Koc et al. 2010). The deepest drillings extended to 180 m (cretaceous stages), out of which 20% comprised quaternary formations up to 123 m deep. The analysis of 57 water samples in these regions was performed from 2009-2011. The geological architecture of these areas was identified based on the legal documentation on water utilization submitted by the operators of the water intakes. Boron in water was determined with the methods specified in the Polish standards (POLISH STANDARD 2006). The samples of water for assays were collected from the following intakes: Tczew “Park” and Tczew “Motława”, Gniew, Wielkie Walichnowy, Małe Walichnowy, and Pelplin. The data assembly was characterized with descriptive statistics. Environmental and technological data were used to calculate correlative relations and a one -way analysis of variance (Anova) with Duncan’s test at $p \leq 0.05$. Mathematical and statistical processing of the results was performed with the package of statistical procedures included in Statistica 7.1 software (StatSoft).

RESULTS AND DISCUSSION

Boron concentration in water is determined by multiple factors. Hydro-geological conditions, i.e. depth of location, are important in the case of groundwater because they are associated with types of rocks with variable boron contents. In this context, it should be emphasized that strong correlations exist between the content of fluorite and boron (Queste et al. 2001). The present studies included hydro-geological conditions and depth of water location, which was described in detail in another publication (Wons et al. 2012). The statistical analysis demonstrates that the aquifer stages exerted a significant impact on the content of boron in the tested water samples (Table 1, Figure 1) and the differences between them are significant. The highest amounts of boron (ranging from 0.90 to 1.63 mg dm^{-3}, on average 1.28±0.20 mg dm^{-3}) were detected in the cretaceous aquifer stage (K). They were significantly higher than the content B in water in the tertiary stage – T (on average 0.89±0.07 mg dm^{-3}) and the quaternary stage – Q (0.24±0.06 mg dm^{-3}).

Such significance was also demonstrated for the tertiary and quaternary (TQ) water stages that were predominant on the intake Tczew "Motława" (0.41±0.08 mg dm^{-3}) as well as for the cretaceous-tertiary-quaternary stages (KTQ) on the intake Tczew "Park" (0.44±0.08 mg dm^{-3}). The diversification of boron content in water from the last two stages (TQ and KTQ) was statistically insignificant, which indicates a similarity of water properties on these intakes.

The analysis of geological cross-sections of the wells on both intakes (Koc et al. 2006, 2007, Wons et al. 2012) indicates a substantial similarity of geological formation located there. In general, waters of the Starogard Lakeland mesoregion that encompasses the intakes Tczew "Motława, Tczew "Park" and Pelplin, had a lower concentration of boron (Table 1) at 0.36±0.11mg dm^{-3} in

Table 1

Statistical description of boron concentrations (mg B dm^{-3}) in groundwater in 2009-2011

Region	Place	Place				Region		
		aquifer stages	*n*	average	SD	range	average	SD
Lower Vistula Valley	Małe Walichnowy	K	8	1.28	±0.20	0.90 - 1.63	1.17	±0.25
	Wielkie Walichnowy	K	16	1.28				
	Gniew	T	9	0.89	±0.07	0.79 - 0.99		
Starogard Lakeland	Tczew Motława	TQ	9	0.41	±0.08	0.31 - 0.53	0.36	±0.11
	Tczew Park	KTQ	7	0.44	±0.08	0.32 - 0.55		
	Pelplin	Q	8	0.24	±0.06	0.17 - 0.36		

SD – Standard Deviation

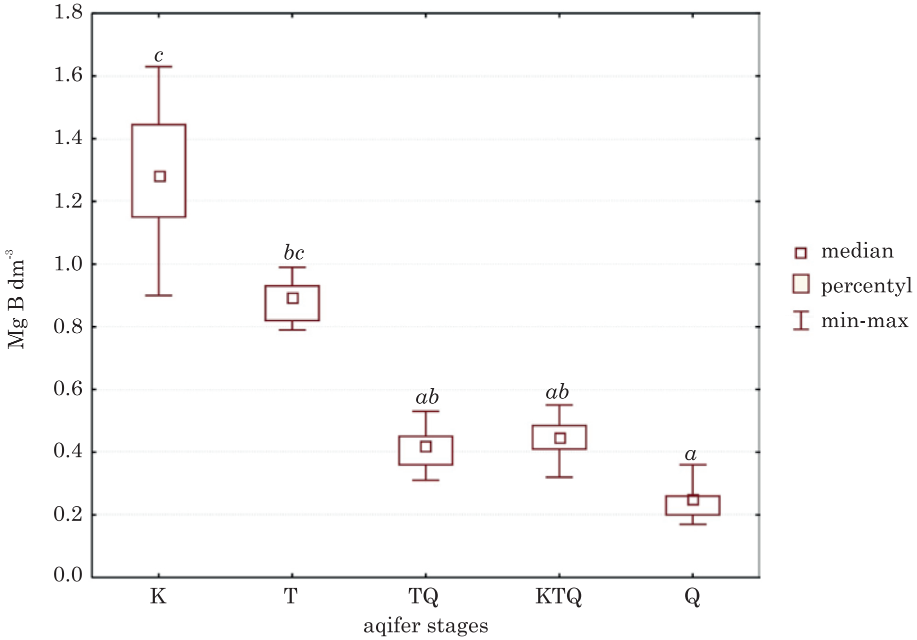

Fig. 1. Homogenous groups of boron content in groundwater and the margin mean values depending on the aquifer stages

comparison with 1.17±0.25 mg dm^{-3} in waters in the Lower Vistula Valley mesoregion.

Differences in boron content were also found between the investigated monitoring stations (Figure 2). Highly significant differences were found mainly between the locations in the individual mesoregions. This data confirms a previously formulated thesis on higher boron content in water from the stations in Małe Walichnowy and Wielkie Walichnowy and Gniew situated in the Lower Vistula Valley mesoregion. The average content of boron on the monitoring stations in both mesoregions was entirely different since the differences in boron content found between them were statistically insignificant.

Considering the value ranges and nearly four times higher average abundance of groundwater in boron from the intakes situated in the Lower Vistula Valley mesoregion, it is assumed that these differences result from a geological dissimilarity which influences the geochemical nature of drainage basin. It is associated with the presence of borates in both soil and in rocks; when they are released, mainly as borates from these formations, they may migrate to water layers. Moreover, a process of mixing of water from different aquifer layers cannot be excluded. In addition, it is possible that geochemical processes occur in groundwater layers and they may elevate or decrease the content of different elements and thus change the quality of groundwater (Barth 1988, Edmunds et al. 2003). It is important in the case of boron, as waters that are abundant in this element may pose a risk when

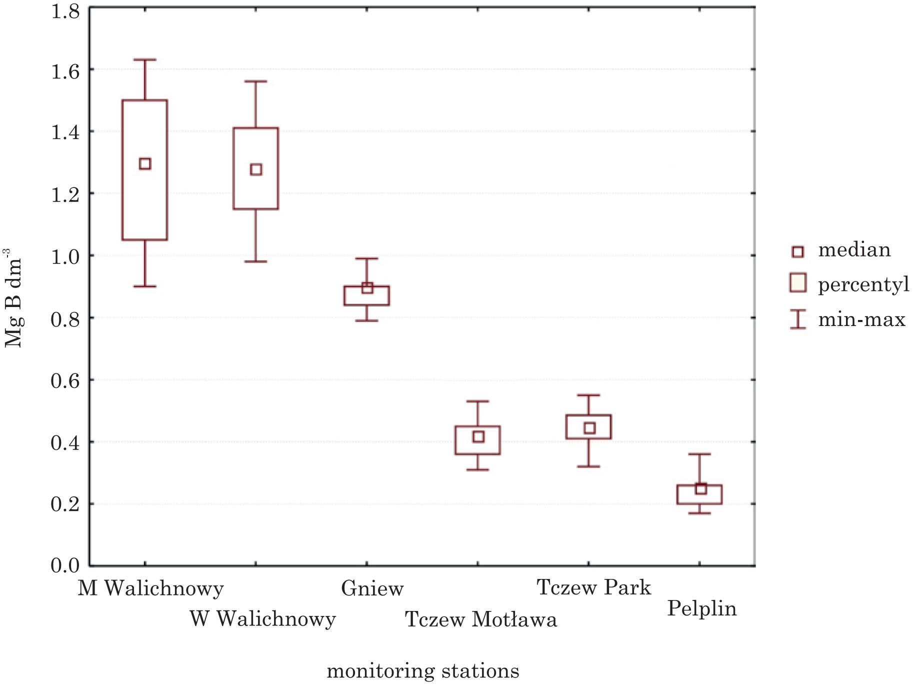

Fig. 2. Homogenous groups of boron content in groundwater and the margin mean values depending on the monitoring stations

drunk or used to irrigate fields or plants (Vousta et al. 2009). In addition, the authors emphasize the narrow range of concentrations between a deficiency in boron and the toxicity of this element.

Taking into consideration the criterion of boron content (1.0 mg dm^{-3}) for an evaluation of the quality of groundwater, it was found that waters in the mesoregion Starogard Lakeland may be assumed to be good in the context of chemical status (*Regulation* ... 2007, Yazbeck et al. 2005). This results from the fact that in all tested water samples, its concentration did not exceed the threshold value. The results were unlike for waters in the Lower Vistula Valley mesoregion. In 17 samples (app. 51%), out of 33 tested, the level of boron exceeded 1.0 mg dm^{-3}, which means that prior to consumption these waters require treatment to reduce the concentration of this element to the permissible limits.

CONCLUSIONS

1. The concentration of boron in groundwater extracted for consumption on Starogard Lakeland and in the Lower Vistula Valley was diversified depending on the exploited aquifer layers.

2. That it was found in the waters mesoregion Starogard Lakeland may be assumed to be good in the context of chemical status, because in all water samples tested, the boron concentration did not exceed the threshold value.

3. It was shown that a substantial proportion of water samples taken in the Lower Vistula Valley mesoregion (app. 52%) exceeded the lowest permissible concentration for boron set at 1.0 mg dm^{-3}.

4. A high concentration of boron was detected in the water samples collected in Wielkie Walichnowy and Małe Walichnowy and the quantitative range of concentrations ranged from 0.90 to 1.63 mg dm^{-3}.

5. The highest content of boron was measured in water extracted from the cretaceous stages and it thus requires treatment prior to consumption.

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THE CONTENT OF SELECTED MACROELEMENTS IN THE DRY WEIGHT OF PERMANENT GRASSLAND SWARD, GRASS YIELDS AND ITS AGRICULTURAL VALUE

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Abstract

Permanent grassland is a valuable source of valuable natural raw material for production of fodders. The fodder value of sward depends on the grass yield volume, agricultural value (UVN), content of minerals, etc.

The present paper discusses the results of a study conducted in 2009-2011, on permanent grassland situated in the valley of the Por River. Samples of the plant material were taken before the first cut from selected, representative areas. They were used to assess, with basic analytical and statistical methods, the yielding, agricultural value (UVN) and chemical composition of hay. The content of P, K, Ca and Mg was determined in the dry weight of the sward by Flame Atomic Absorption Spectroscopy *(*FAAS), while the total content of N was established by the Kjeldahl's method. For easier interpretation of the results, the classified syntaxons (Matuszkiewicz 2006) were ranked into higher syntaxonomic units.

The present research showed a fairly good content of P, Mg and Ca in the analyzed dry weight of plants, enough to satisfy the nutritional demand of ruminants. However, the total content of N and K in the plant samples was insufficient. The agricultural value (UVN) of the dry weight of plants and the yield volume of the examined phytocenoses were varied, which was statistically verified.

The high value of variability coefficients also testifies to the considerable differentiation of all the analyzed features within particular syntaxonomic units.

Key words: total nitrogen, phosphorus, magnesium, calcium, yielding, agricultural value, meadow sward.

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ZAWARTOŚĆ WYBRANYCH MAKROELEMENTÓW W SUCHEJ MASIE RUNI TRWAŁYCH UŻYTKÓW ZIELONYCH ORAZ ICH PLONOWANIE I WARTOŚĆ ROLNICZA

Abstrakt

Trwałe użytki zielone stanowią cenne źródło naturalnych pełnowartościowych surowców do produkcji pasz. Wartość paszową runi określa się m.in. pod względem zawartości składników mineralnych, wartości rolniczej (LWU) oraz wysokości plonu.

W pracy przedstawiono wyniki badań przeprowadzonych w latach 2009-2011 na trwałych użytkach zielonych w dolinie rzeki Por. Na wytypowanych, reprezentatywnych powierzchniach, przed zbiorem pierwszego pokosu, pobrano próby materiału roślinnego. Stanowiły one materiał do oceny plonowania, wartości rolniczej (LWU) i składu chemicznego siana. W suchej masie runi określono zawartość P, K, Ca, Mg metodą płomieniowej atomowej spektrofotometrii absorpcyjnej (FAAS) oraz ogólną zawartość N metodą Kjeldahla. W celu łatwiejszej interpretacji wyników sklasyfikowane syntaksony (Matuszkiewicz 2006) uszeregowano w nadrzędne jednostki syntaksonomiczne.

Wykazano dość dobrą pod względem potrzeb żywieniowych przeżuwaczy zawartość P, Mg oraz Ca w analizowanej suchej masie roślinnej, jednak ogólna zawartość N i K w badanych próbach roślinnych była niewystarczająca. Wartość rolnicza (LWU) suchej masy roślinnej oraz wysokość plonów badanych fitocenoz kształtowały się na zróżnicowanym poziomie, co potwierdziły analizy statystyczne.

Wysoka wartość współczynników zmienności świadczy również o znacznym zróżnicowaniu wszystkich analizowanych cech w obrębie poszczególnych jednostek syntaksonomicznych.

Słowa kluczowe: azot ogólny, fosfor, potas, magnez, wapń, plonowanie, wartość rolnicza, ruń łąkowa.

INTRODUCTION

The chemical value of fodder obtained from meadows and pastures is determined by the floristic composition of sward (Kozłowski 1996, Trzaskoś 1998, Kiryluk 2005, Sabiniarz, Kozłowski 2009), the developmental stage and parts of plants (Falkowski et al. 2000, Mastalerczuk 2007), patterns of adjacent plant groups, and even by individual species (Wesołowski et al. 2009, Wesołowski et al. 2011).

A significant effect on the level of mineral elements in plants is exerted by environmental factors such as the weather during the plant growing season (Wiśniowska-Kielian, Kasperczyk 1999), plant available elements in the soil (Niedźwiecki et al. 2009), habitat humidity and the groundwater level (Stępień, Pawluczuk 2011).

Numerous studies have shown strong relationships between the occurrence of mineral elements in the meadow soil and herbage and the use of a given habitat (Wiśniowska-Kielian, Kasperczyk 1999, Kasperczyk, Szewczyk 2007, Mastalerczuk 2007, Kolczarek et al. 2008, Baryła et al. 2009).

Grassland productivity is affected by the climatic and soil conditions, the type of a plant community as well as the mineral and organic fertilization

(Kasperczyk, Szewczyk 2007). Significant impact on the agricultural value (UVN) of grassland sward is produced by the quantitative and qualitative differences in the floristic composition during the plant growing season in the successive years of meadow use (Falkowski et al. 1996).

The content of nutrients, especially macroelements like N, P, K, Ca and Mg, affects the biological value of grassland sward, which makes good raw material for hay production (Kulik 2009, Sabiniarz, Kozłowski 2009).

The purpose of the present paper is to assess the content of total nitrogen, phosphorus, potassium, magnesium and calcium in the dry weight of selected grassland sward syntaxonomic units and the agricultural value (UVN) as well as the yield volume.

MATERIAL AND METHODS

In 2009-2011, a study was conducted on permanent grassland in the valley of the Por River (south-eastern Poland). Based on phytosociological records taken by the Braun-Blanquet method, plant communities and associations were distinguished, which were then classified using the syntaxonomic system by Matuszkiewicz (2006). Plant samples (150) were taken, each from an area of 1 m^2, in order to determine the fodder value of the analyzed meadows and pastures before the first cut of hay. They were cut from plant patches characteristic for particular phytocenoses. The samples were dried and then used to estimate the yielding, make botanical and gravimetric analyses and determine the chemical composition of hay.

Botanical and gravimetric analyses were the basis for calculations of the utility value number according to Filipek (1973). In the dry weight of the sward, the content of P, K, C and Mg was determined by Flame Atomic *Absorption* Spectroscopy (FAAS) while the total content of N was assayed by the Kjeldahl's method.

In order to facilitate the interpretation of the results, the distinguished plant communities and associations were ordered into superior syntaxonomic units, namely associations *Phragmition* and *Magnocaricion*, orders *Molinietalia* and *Arrhenatheretalia*, class *Scheuchzerio-Caricetea nigrae* and order *Trifolio fragiferae-Agrostietalia stoloniferae*. For the distinguished taxons, the analyses determined the content of P, K, Ca, Mg and total nitrogen as well as the utility value (V) and size of the yield in the dry weight of the sward.

The results were analyzed statistically using SAS 9.2 Enterprise Guide 4.2. The following methods of statistical analyses were applied: analysis of variance - Anova, the Tukey's HSD test, the Levene's Test for Homogeneity of Variances, the Welch's Anova and the Pearson's correlation coefficients with the analysis of statistical significance by Student's *t*-tests.

RESULTS AND DISCUSSION

Our comparative analysis of the means from different environments of the examined variables was based on a one-factor analysis of variance at the level of significance $\alpha = 0.05$.

Because certain traits presented considerable variability, deviations were suspected from the assumptions derived from Anova regarding the variance homogeneity of the compared groups. That was confirmed by analyses of the Levene's tests on variance homogeneity, which is why parallel calculations were done using the Welch's method to perform Anova, which does not require assuming variance homogeneity (Table 1).

Table 1

The one-factor variance analysis, Levene's tests and Welch's variance analysis of the N, P, K, Ca and Mg, hay yield and the agricultural value between the studied syntaxonomic units

Number of samples - 150	One-factor analysis of variance Anova			Levene's test		Welch's Anova	
Dependent variable	F	Pr.> F	$p = 0.05$	F	Pr.> F	F	Pr.> F
Total N	1.120	0.352	>	3.630	0.004	2.730	0.041
P	1.340	0.252	>	1.070	0.381	1.640	0.189
K	2.090	0.071	>	1.000	0.418	2.940	0.034
Mg	1.480	0.201	>	0.610	0.691	1.470	0.237
Ca	1.050	0.391	>	0.600	0.702	0.740	0.602
UVN	52.30	<.0001	<	2.640	0.027	65.41	<.0001
Hay yield	6.770	<.0001	<	3.050	0.013	12.38	<.0001

Variance analysis is fairly resistant to deviations from variance homogeneity, which was confirmed by the results of both methods (very close to the value of test functions F and the corresponding calculated probabilities).

Although Anova can establish whether groups differ significantly, it cannot state which groups (objects) differ in a statistically significant manner due to the mean values. That is the reason why T-Tukey's tests (HSD) were carried out at the previously adopted level of significance $p = 0.05$ so as to determine homogeneous groups (statistically homogeneous ones). The means followed by the same letter constitute a so-called group of homogenous means, which are the ones that do not significantly differ from each other. The absolute value of the difference of the means from the samples is higher than the so-called least significant difference LSD. Therefore, it can be stated that they are statistically significant (Table 2). The mean total nitrogen concentration in the studied meadow hay ranged from 15.6 g kg^{-1} of to 18.4 g kg^{-1} dry weight (DW). The smallest amount of this element was found in the plant dry weight of the communities from the order *Trifolio fragiferae-Agro-*

Table 2

Descriptive statistics concerning the content and differentiation of the analyzed macroelements, hay yield and agricultural value of the syntaxonomic units

Syntaxonomic unit	Number of samples (*n*)	Means	Variability coefficient Vc (%)	Tukey grouping
Total N (g kg^{-1} DW)				
*Phragmition**	9	15.9	9.6	A
*Magnocaricion**	26	18.4	17.4	A
*Trifolio fragiferae-Agrostietalia stoloniferae***	8	15.6	16.0	A
*Molinietalia caeruleae***	45	18.4	24.6	A
Arrhenatheretalia Pawł.**	49	18.2	19.1	A
*Scheuchzerio-Caricetea nigrae****	13	18.1	12.0	A
LSD – 0.441				
P (g kg^{-1} DW)				
*Phragmition**	9	2.40	27.8	A
*Magnocaricion**	26	2.22	30.6	A
*Trifolio fragiferae-Agrostietalia stoloniferae***	8	2.26	41.8	A
*Molinietalia caeruleae***	45	2.55	46.5	A
*Arrhenatheretalia***	49	2.09	40.6	A
*Scheuchzerio-Caricetea nigrae****	13	2.65	20.8	A
LSD – 0.113				
K (g kg^{-1} DW)				
*Phragmition**	9	18.3	25.4	A
*Magnocaricion**	26	14.8	31.6	AB
*Trifolio fragiferae-Agrostietalia stoloniferae***	8	16.4	18.1	AB
*Molinietalia caeruleae***	45	15.3	25.1	AB
*Arrhenatheretalia***	49	15.1	27.5	AB
*Scheuchzerio-Caricetea nigra****	13	12.0	23.4	B
LSD - 0.491				
Mg (g kg^{-1} DW)				
*Phragmition**	9	2.14	17.0	A
*Magnocaricion**	26	2.13	32.8	A
*Trifolio fragiferae-Agrostietalia stoloniferae***	8	1.91	56.6	A
*Molinietalia caeruleae***	45	2.47	31.9	A
*Arrhenatheretalia***	49	2.53	30.5	A
*Scheuchzerio-Caricetea nigrae****	13	2.45	29.4	A
LSD – 0.094				
Ca (g kg^{-1} DW)				
*Phragmition**	9	9.48	38.5	A
*Magnocaricion**	26	8.12	36.2	A
*Trifolio fragiferae-Agrostietalia stoloniferae***	8	7.60	50.9	A
*Molinietalia caeruleae***	45	8.01	41.8	A
*Arrhenatheretalia***	49	7.70	37.7	A
*Scheuchzerio-Caricetea nigrae****	13	9.83	39.7	A
LSD – 0.393				

Explanations: * – association, ** – order, *** - class *stietalia stoloniferae* and the association *Phragmition*. It is assumed that the dry weight of sward should contain about 20 g kg^{-1} of total nitrogen (Falkowski et al. 2000). The concentration of total nitrogen reported herein is below the values considered as optimal ones.

Our comparative analyses demonstrated no statistical differences between the arithmetic means. Quite low variability coefficients also indicate little differences in the total nitrogen concentration within particular syntaxonomic units (Tables 1, 2), except for communities of humid habitats from the order of *Molinietalia caeruleae*, where statistically significant variability was observed (variation coefficient = 24.6%).

The scientific literature finds considerable differences in the content of total nitrogen in plants' dry weight depending on the floristic composition of sward and the conditions within the habitat in which a given plant communities grows (Kozłowski 1996, Baryła, Kulik 2006, Mastalerczuk 2007, Wesołowski et al. 2009, Stępień, Pawluczuk 2011, Wesołowski et al. 2011).

The chemical analyses on the availability of phosphorus in fodders from the examined grassland, from the distinguished syntaxonomic units of the Por River Valley, imply slight deficiency of this element in the plant's dry weight. According to Falkowski et al. (2000), the content of phosphorus in dry hay which is optimum for animals should range from 2.8 to 3.6 g kg^{-1}. Out of all the studied plant samples, the highest concentration of phosphorus was determined for the fodder from sedge-moss peat bogs from the class *Scheuchzerio-Caricetea nigrae* (2.65 g kg^{-1} DW), whereas the lowest one was found for fresh habitats from the order *Arrhenatheretalia* (2.09 g kg^{-1} DW).

The one-factor analysis of variance (Table 1) and Tukey grouping (Table 2) did not show any statistically significant differences in the content of phosphorus in the plant dry weight of the examined syntaxonomic units. On the other hand, the highest statistical variability within the syntaxon was observed in the communities of humid habitats, especially *Molinietalia caeruleae*, while the lowest in *Scheuchzerio-Caricetea nigrae* (Table 3).

Previous studies showed that environmental factors such as soil moisture (Baryła, Kulik 2006) and unstable atmospheric conditions including long droughts (Falkowski et al. 2000), have a considerable effect on phosphorus accumulation in plants. The analyzed plant communities of the Por River Valley were characterized by distinctly different hydrological conditions. However, the results of statistical analyses did not confirm the observations reported elsewehere,

The mean concentration of potassium ranged from 12.0 to 18.3 g kg^{-1} DW. For animal nutrition, the right concentration of this element in hay is 17 g K kg^{-1} DW. (Falkowski et al. 2000). The lowest K concentration was found in the phytocenoses from the class *Scheuchzerio-Caricetea nigrae*, while the highest one was in the associations of *Phragmition*. Comparative analyses confirmed that the potassium content in the other syntaxons did not show any statistically significant differences (Tables 1, 2). In addition, a

Table 3

Descriptive statistics concerning hay yield and agricultural value of the syntaxonomic units

Syntaxonomic unit	Number of samples (*n*)	Means	Variability coefficient Vc (%)	Tukey's grouping
UVN				
*Phragmition**	9	2.90	35.7	CD
*Magnocaricion**	26	1.63	77.3	D
*Trifolio fragiferae-Agrostietalia stoloniferae***	8	5.16	10.1	B
*Molinietalia caeruleae***	45	4.16	41.7	BC
*Arrhenatheretalia***	49	6.83	13.7	A
*Scheuchzerio-Caricetea nigrae****	13	2.98	44.7	CD
				LSD – 1.594
Hay yield (t ha^{-1})				
*Phragmition**	9	3.38	24.2	A
*Magnocaricion**	26	2.47	33.3	AB
*Trifolio fragiferae-Agrostietalia stoloniferae***	8	1.69	66.5	BC
*Molinietalia caeruleae***	45	2.20	42.3	B
*Arrhenatheretalia***	49	2.25	27.9	B
*Scheuchzerio-Caricetea nigrae****	13	1.22	33.8	C
				LSD – 0.962

Key – cf. Table 2

high variability coefficient (Vc =31.6%) was observed, which points to a wide range of variability in the concentration of potassium in plant samples of *Magnocaricion* Koch 1926. Results of studies presented by researchers (Wesołowski et al. 2009, Stępień, Pawluczuk 2011, Wesołowski et al. 2011) on the content of macroelements in rush vegetation confirm considerable differentiation in the content of potassium in sward from excessively humid habitats. It is believed that the potassium content in permanent grassland sward is a species-specific genetic trait (Falkowski et al. 2000).

The magnesium concentration in the dry weight of the analyzed plant samples, in respect of the aforementioned syntaxonomic units of permanent grassland of the Por River Valley ranged from 1.9 to 2.5 g kg^{-1} DW. Falkowski et al. (2000) suggest that from the point of view of animal feeding, the dry weight of plants should contain 2 g of magnesium. Thus, the analyzed hay in most cases meets the requirements set for good quality fodders.

The one-factor analysis of variance Anova did not show any statistically significant differences in the concentration of magnesium in the plant dry weight among the distinguished syntaxonomic units of the Por River Valley. While considering variability coefficients within selected syntaxonomic units, it was observed that the biggest differences appeared in plant patches from the order of *Trifolio fragiferae-Agrostietalia stoloniferae* (Vc = 56.6%), while the smallest ones were typical of *Phragmition* (Vc = 17.0%).

In general, hay produced in Poland contains an insufficient amount of magnesium (Falkowski et al. 2000). Deficiency of this element in hay from excessively humid habitats was shown for example by Wyłupek (2006), Wesołowski et al. (2009), Stępień, Pawluczuk (2011), Wesołowski et al. (2011). On the other hand, Niedźwiecki et al. (2009), who studied the chemical composition of grassland vegetation of Western Pomerania, and Sabiniarz and Kozłowski (2009), who analyzed Czersk grasslands in the aspect of fodders, found out magnesium concentrations in grassland hay close to the optimum.

According to Mastalerczuk (2007), more cuts in a season significantly decrease the amount of magnesium ion in the fodder. Hence, it can be supposed that extensive grassland management dominating in the Por Valley positively affected the content of magnesium in the analyzed plants.

The mean content of Ca in all samples of the analyzed aerial biomass exceeded the optimum value of 7 g kg^{-1} DW (Falkowski et al. 2000). The highest concentration of this element was observed in the dry weight of grassland sward of phytocenoses of the class *Scheuchzerio-Caricetea nigrae* (9.8 g kg^{-1} DW), whereas the lowest one was in the dry weight of samples from plant communities of the order *Trifolio fragiferae-Agrostietalia stoloniferae* (7.6 g kg^{-1} DW). Results of chemical analyses testify to considerable bio-availability of calcium ions to plants. In other regions of Lublin Province, the availability of this element to plants is insufficient (Borowiec, Urban 1992, Urban et al. 2003).

Lack of statistically significant differences in the content of calcium in the plant dry weight between syntaxonomic units can be observed on the basis of grouping the arithmetic means using the *post-hoc* tests. On the other hand, the analysis of variability coefficients within the distinguished syntaxonomic units found out quite remarkable dispersion of results. The broadest spectrum of variability was observed in the dry weight of phytocenoses of the order *Trifolio fragiferae-Agrostietalia stoloniferae* (Vc = 50.9%)**,** while the smallest one appeared in high-sedge rushes *Magnocaricion* (Vc = 36.2%) – Table 2.

In different regions of Poland, the content of Ca in the dry weight of plant communities remains within fairly broad limits. Researchers observe both excess and deficiency of calcium in the plant dry weight referring to the feeding needs of animals (Kozłowski 1996, Trzaskoś 1998, Baryła et al. 2009, Kulik 2009, Sabiniarz, Kozłowski 2009, Wesołowski et al. 2009, Wesołowski et al. 2011).

The statistical analyses find out that the hay of phytocenoses of the Por River Valley was characterized by different agricultural values (UVN). The applied Tukey's procedure made it possible to group the studied syntaxonomic units into four homogenous groups (A, B, C, D). As these group "overlap", it was observed that the highest nutritive value is found for the fodder from plant communities of fresh habitats of the order *Arrhenatheretalia*, slightly lower for hay from the order *Trifolio fragiferae-Agrostietalia stoloniferae*, while the lowest one was for the dry weight of plant samples of plant communities of humid habitats of the association *Magnocaricion*. The dry weight of plants of the other syntaxonomic units was characterized by an average level of the agricultural value (Table 3).

The grassland was characterized by considerably differentiated yielding, which was confirmed statistically by variance analysis. According to the established homogenous groups, the highest yields of dry weight were from the phytocenoses of the association *Phragmition*, whereas the lowest ones were achieved from the communities of the class *Scheuchzerio-Caricetea nigrae* (Tables 1, 3).

Our analysis of the Pearson's linear correlation coefficient pointed to relationships between certain analyzed features of plant communities from the Por River Valley. Weak ($r < 0.5$) and directly proportional correlations (a positive coefficient of correlation) were found between the content of N total and P ($r = 0.240$), Mg and N total ($r = 0.268$), Mg and P ($r = 0.260$), Mg and Ca ($r = 0.308$). A positive relationship occurs between the hay yield of the 1st cut and its agricultural value (UVN) – Table 4.

Table 4

Pearson's correlation coefficient r with the analysis of significance using t-Student tests p between the selected features

Specification	Total N	P	K	Mg	Ca	UVN	Hay yield
Total N r =	1.000	0.240**	0.067	0.268**	-0.086	-0.014	-0.081
Calculated probability =	-	0.007	0.461	0.003	0.347	0.875	0.375
P r =	-	1.000	-0.092	0.260**	-0.036	-0.076	-0.131
p =	-	-	0.313	0.004	0.693	0.402	0.147
K r =	-	-	1.000	-0.027	0.118	0.032	0.100
p =	-	-	-	0.764	0.192	0.725	0.272
Mg r =	-	-	-	1.000	0.308**	0.115	0.011
p =	-	-	-	-	0.001	0.204	0.907
Ca r =	-	-	-	-	1.000	-0.145	0.038
p =	-	-	-	-	-	0.109	0.680
UVN r =	-	-	-	-	-	1.000	0.188*
p =	-	-	-	-	-	-	0.038
Hay yield r =	-	-	-	-	-	-	1.000
p =	-	-		-		-	

* Significant when ≤0.01,

** Significant when $p \le 0.05$

CONCLUSIONS

The above results of chemical and statistical studies of the dry weight of permanent grassland of the Por River Valley justify the conclusion that the content of the basic mineral elements in the analyzed plant samples was fairly differentiated.

The dry weight of the examined plant samples was characterized by quite good availability of phosphorus, magnesium and calcium, which ensures adequate nutritional effects. On the other hand, the availability level of total nitrogen and potassium in fodder implied small N deficiency in the dry weight of plants.

Variability coefficients are characterized by a high value, which testifies to considerable differentiation of the availability of phosphorus, potassium, magnesium and calcium in the dry weight of particular plant patches of the analyzed syntaxonomic units. In contras, the content of total nitrogen in the plant dry weight should be considered as moderately stable.

The agricultural value (UVN) of fodder from different plant communities of the Por River Valley and the volume of obtained yields were characterized by considerable statistical variability, inluding differences within particular syntaxons and mutual comparisons of the arithmetic means.

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REVIEW PAPERS

BIOFORTIFICATION – PROMISING APPROACH TO INCREASING THE CONTENT OF IRON AND ZINC IN STAPLE FOOD CROPS*

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Abstract

Micronutrient deficiencies have increased over recent decades due to the general depreciation of the quality of poor people's diet, both in developed and developing countries. The deficiencies of iron (Fe) and zinc (Zn) are a critical public health problem worldwide, with the negative impact on health, lifespan and productivity. Biofortification is an agricultural approach that can improve human nutrition on a global scale. Agronomic biofortification is considered a short-term and complementary strategy, but economic analyses suggest that genetic biofortification is the most effective strategy for increasing dietary Fe and Zn intakes of vulnerable populations. Enrichment of cereal grains by breeding is a high-priority area of research, and an effective strategy among other approaches, e.g, fortification, supplementation and food diversification. This review discusses the role of Fe and Zn in plant nutrition, the potential strategies for developing Fe and Zn biofortified crops and their importance in human nutrition.

Key words: metal homeostasis, ferti-fortification, breeding crops, micronutrient malnutrition, anaemia, bioavailability.

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BIOFORTYFIKACJA – OBIECUJĄCY SPOSÓB ZWIĘKSZANIA ZAWARTOŚCI ŻELAZA I CYNKU W PODSTAWOWYCH ROŚLINACH UPRAWNYCH

Abstrakt

Obecnie niedobory Fe i Zn stanowią istotny problem dotyczący zdrowia publicznego, są bowiem przyczyną negatywnego wpływu na zdrowie, średnią długość życia i przyrost naturalny. Biofortyfikacja agronomiczna jest to metoda, która może poprawić żywienie człowieka na całym świecie. Analizy ekonomiczne wskazują, że genetyczna biofortyfikacja jest najbardziej skuteczną strategią zwiększenia spożycia Fe i Zn, natomiast agronomiczna biofortifikacja może być podejściem komplementarnym, i to w krótkim czasie. Strategia genetyczna jest obszarem priorytetowym w badaniach zboża, i okazuje się być bardziej skuteczna niż metody fortyfikacji, suplementacji lub zróżnicowania środków spożywczych. W pracy omówiono rolę Fe i Zn w odżywianiu roślin, potencjalne strategie rozwoju upraw z wykorzystaniem biofortyfikacji Fe i Zn oraz znaczenie tych upraw w żywieniu człowieka.

Słowa kluczowe: homeostazy, biofortyfikacja, niedożywienie, mikroelementów, niedokrwistość, biodostępność.

INTRODUCTION

Iron deficiency induced anaemia and Zn deficiency are major public health problems worldwide, to which children are particularly. The major cause of malnutrition is poor quality diet, mainly lacking in animal products. Consequently, populations that consume few animal foods may suffer from a high prevalence of several micronutrient deficiencies. At the same time, the proportion of the global population suffering from micronutrient malnutrition (MNM) has increased because modern plant breeding has been historically oriented toward high agronomic yield rather than the nutritional quality, producing a lower density of minerals in many crops. It has been suggested that 4.5 billion people worldwide are affected by deficiencies of Fe, vitamin A and I; Zn is of increasing concern. MNM diminishes motivation and development, consequently impairing mental and cognitive abilities and finally reducing the productivity and potential of entire societies (Bouis, Islam 2011, Modestine et al. 2012).

Iron and zinc are essential elements for human health, required for the activity of many enzymes involved in major metabolic pathways. International research programs have been undertaken to enhance Zn and Fe nutrition; significant benefits are found through initiatives such as supplementation, fortification, food diversification and biofortification. Fortification has an important role in tackling malnutrition, but it is highly dependent on funding and restricted to urban areas. By contrast, biofortification is a prominent strategy that can increase levels of micronutrients in crops. However, to biofortify plants it is necessary to achieve a clear molecular understanding of micronutrient homeostasis (Lungaho et al. 2011).

Agronomic biofortification and genetic biofortification represent complementary agricultural approaches. It is claimed than application of Zn and Fe fertilizers is a short-term solution, complementary to plant breeding. There is convincing evidence that especially foliar application of Zn fertilizers is effective in improving Zn in grain. By contrast, Fe fertilization is less effective in the enrichment of grains, expensive and dangerous to the environment. In addition, it appears that improving the nitrogen nutritional status of plants promotes the accumulation of Fe and Zn in grain (CAKMAK 2010, WHITE, BROADLEY 2011, MURGIA et al. 2012, SPEROTTO et al. 2012).

Genetic biofortification offers a sustainable and low-cost way to provide micronutrients to people in developing countries. Breeding nutrient-rich staple food crops is indeed the main goal of different international consortia, whose aim is to reduce MNM through biofortification programs. Variability is exploited to produce Fe and Zn-rich crops. Also, this study is amended by inclusion of the information on retention after processing, bioavailability from the diet, daily consumption and required amounts of plant food that have measurable effects on the nutrient status. It is thought that Fe and Zn bioavailability to a human organism can be improved by increasing the dietary factors that enhance absorption, by decreasing the factors that inhibit absorption, or by increasing the micronutrient content of a diet (CAKMAK 2010, BOUIS et al. 2011, WHITE, BROADLEY 2011, MURGIA et al. 2012).

Until now, Fe deficiency is counteracted mainly by food fortification and supplementation. With respect to Zn, there is strong evidence supporting the beneficial impact of these strategies, especially Zn supplementation of children. However, economic analyses suggest that biofortification is the most practical, lasting, and cost-effective strategy for increasing the dietary Fe and Zn intake by vulnerable populations (HESS, BROWN 2009, GIBSON 2012, BHULLAR, GRUISSEM 2013). This review describes the roles of Fe and Zn in plant and human nutrition. Particular attention is paid to the importance of the molecular pathway that directs specific steps in networks of micronutrient homeostasis, to strategies for production of Fe and Zn-rich crops by biofortification, and to the importance of their increased bioavailability through diets among human populations.

THE ESSENTIAL ROLE OF IRON AND ZINC IN PLANTS

There are two criteria defined for an element to be essential for plant growth. Either a plant is unable to complete a normal life cycle in the absence of an element, or when the said element is part of some essential plant constituent or metabolite. In the traditional classification of nutrients and beneficial elements, Fe and Zn are included in the category of micronutrients essential for biological systems (HELL, STEPHAN 2003, BARKER, PILBEAM 2007).

Like other organisms, plants require Fe to complete their life cycle. This micronutrient occurs in multiple redox states, readily accepting and donating electrons, thus being able to serve as a cofactor for several plant proteins that participate in crucial metabolic pathways. The function of Fe relies mostly on the reversible redox reaction of Fe^{2+} and Fe^{3+} ions, its ability to form octahedral complexes with various ligands and to vary its redox potential in response to different ligands of the environment. This redox potential (Fe^{2+}/Fe^{3+}) enables its use, in the form of heme or Fe sulphur clusters, in a number of protein complexes, especially those involved in electron transfer. However, excess Fe is toxic as both Fe^{2+} and Fe^{3+} can act as catalysts in the formation of noxious reactive oxygen species (ROS), which are potent oxidizing agents able to damage DNA, proteins and lipids. Therefore, Fe homeostasis in the whole organism must be balanced to supply enough Fe for cell metabolism and to avoid excessive, toxic levels. Because Fe represents one of the most versatile metals in biology with numerous cellular functions, its deficiency is among the most serious problems worldwide (CURIE, BRIAT 2003, HELL, STEPHAN 2003, BASHIR et al. 2011).

Zinc is the second to iron most abundant intracellular metal. Zinc is an essential micronutrient required by both animals and plants as a structural constituent of proteins or a regulatory co-factor of enzymes involved in many biochemical pathways. For example, many proteins contain Zn prosthetic groups (zinc finger, zinc twist) and around 300 enzymes require Zn as a cofactor. Actually, Zn is the only metal found in all six enzyme classes. In addition, it has been estimated that several proteins are capable of binding Zn and some of these Zn-binding proteins are transcription factors needed for gene regulation. Many cells secrete Zn as a signaling molecule, including cells in the immune and nervous systems. Zinc is also required for the structural and functional integrity of biological membranes and for the detoxification of highly aggressive free radicals. In general, Zn deficiency is a well-documented problem in food crops, causing a decrease in both yield and nutritional quality of crops. Therefore, regions with Zn-deficient soils around the world are typically characterized by a widespread Zn deficiency in humans (BROADLEY et al. 2006, CAKMAK 2008, GOMEZ-GALERA et al. 2010, LEE et al. 2011).

COMPLEX REGULATION NETWORK OF IRON AND ZINC IN PLANTS: MECHANISMS OF THE CONTROL OF UPTAKE AND DISTRIBUTION

Although essential minerals are generally abundant in soils, most of them largely occur in forms that are not easily available to plants. In order to satisfy the demand for minerals whilst avoiding their possible toxic effects, plants have evolved a complex regulation network controlling mineral homeostasis. Our knowledge of the molecular pathway which controls specific steps in the network of mineral homeostasis, although still undeveloped, is growing rapidly. This information is expected to improve crop yield, crop nutritional value and food safety, which are aspects of major global concern. Today, the lack of knowledge about this mechanism is an obstacle to devising approaches for biofortification, i.e. genetic engineering of staple crops to accumulate additional bioavailable nutrients in edible parts (Ghandilyan et al. 2006, Walker, Connolly 2008).

Metal homeostasis is a function of an organism that regulates its internal metal environment (in cells and organelles) so as to maintain a stable and constant condition. This process is based on well-controlled metal uptake, translocation, redistribution and sequestration mechanisms. In general, strategies of plants to facilitate metal uptake include the modification of the environment to increase bioavailability, the up-regulation of high-affinity transporters and the activation of intra-cellular and inter-cellular pathways for subsequent distribution. Under metal excess, non-specific metal uptake from soil is unavoidable and plants activate mechanisms for intracellular chelation and compartmentalization (Puig, Peñarrubia 2009, Hassan, Aarts 2011). The regulation of metal homeostasis is mainly mediated by membrane transporters. In many cases, transcriptional control of these transporters contributes to homeostasis. However, too little is known about post-transcriptional and post-translational control of transporter activity. In addition, whereas much is still to be learned about Zn, we are now closer to attaining the full understanding of Fe homeostasis (Pilon et al. 2009).

Uptake of Fe and Zn in Plants

Soil is the main source of metals for plants. Metals can be inaccessible in soil, primarily when present in the insoluble form, which is particularly common at higher pH of alkaline soils. To overcome such inaccessibility of some metals, non-graminaceous plants rely primarily on a reduction-based strategy of uptake (strategy I), whereas gramineous plants (grasses) more commonly use a chelation-based strategy (strategy II), both depending on the environment characteristics, in order to efficiently incorporate and systemically distribute micronutrients (Palmer, Guerinot 2009, Jeong, Guerinot 2009).

In the case of Fe uptake, plants are classically divided into two groups, according to their strategy to obtain Fe from the environment: (1) strategy I, those acquiring Fe^{2+} after reduction by Fe^{3+} reductases and (2) strategy II, those secreting phytosiderophores (PSs) that bind Fe^{3+} for subsequent acquisition of the Fe^{3+}–chelate. However, latest results emphasize the importance of the environment in Fe acquisition (Jeong, Guerinot 2009, Puig, Peñarrubia 2009).

Many plants use strategy I for metal uptake because metal transporters have specific affinity for a particular oxidation state. For example, whenever Zn is always found in the +2 oxidation state, under physiologically relevant conditions, Fe needs to be reduced for its uptake into the plant. The Fe uptake by Strategy I involves the release of protons to increase the solubility of Fe, via H^+-ATPases of the root plasma membrane, upon Fe deficiency. This reduction is performed by Fe-deficiency inducible plasma membrane-bound Fe^{3+}reductase. After acidification, Fe^{3+} is reduced to Fe^{2+} by membrane-bound ferric reductase oxidase (FRO). Once Fe^{3+} is reduced, Fe^{2+} is transported into the root by Fe-regulated transporter (IRT), a member of the Zn regulated transporter (ZRT)-IRT-like protein (ZIP) family (GHANDILYAN et al. 2006, JEONG, GUERINOT 2009). On the other hand, the Fe uptake by strategy II employs the release of chelators known as PSs into the rhizosphere, to bind Fe^{3+} for transport into the plant. PSs are synthesized from methionine and are compounds of the mugineic acid family (MAs) that form stable Fe^{3+}–chelates in soil. PSs, like MAs, are released to chelate Fe^{3+} and the resulting PS–Fe^{3+} complexes are then moved into the roots, via the Yellow Stripe-like (YSL) transporters, named after the YSL1 PS transporter of maize (ROBERTS et al. 2004, JEONG, GUERINOT 2009).

Although the molecular mechanisms for Zn uptake are not completely understood, it has been suggested that plants may also use Strategy II to obtain Zn from soil. Graminaceous plant species respond to Fe and Zn deficiency by exudation of PSs, increasing the availability of these metals. Thus, PS–Fe^{3+}or PS–Zn^{2+} complexes are transported from the rhizosphere to the root for uptake. The MAs family plays a major role in Fe acquisition, and contributes to the acquisition of Zn by these plants. Although, the IRT transporter has been established as the major Fe uptake system from the soil, IRT is also responsible for uptake of Zn. Genetic engineering approaches have been applied to increasing plant tolerance to low-Zn soils. For example, overexpression of known Zn transporters from Arabidopsis to barley can increase the plant Zn uptake and seed Zn content. These results show the contribution of molecular genetics tools to manipulating Zn and Fe efficiency in crops and the potential for enrichment of the food supply with these metals (VERT et al. 2002, HELL, STEPHAN 2003, ZUO, ZHANG 2008).

Distribution of Fe and Zn in plants

Metals must be transported throughout the plant, from the uptake into the roots up to the tissues where they are required. Once within the root epidermal cell, ions can move through symplastic passages, from the epidermis to the pericycle, to be loaded into the xylem. From the xylem, metals are transported to the aerial organs of the plant (via the transpiration stream), to shoot tissue or across the plasma membrane into leaf cells. Seeds are not fed by the transpiration stream and must rely on the phloem for nutrients. In addition, developing leaves do not have fully differentiated xylem and must receive the necessary metals through the phloem (KERKEB, CONNOLLY 2006, JEONG, GUERINOT 2009).

Plants have a wide range of transporters, whose exact function is not known yet. As for the Fe and Zn uptake and distribution, there are several identified transporters (Figure 1). As mentioned previously, ZRT/IRT-like

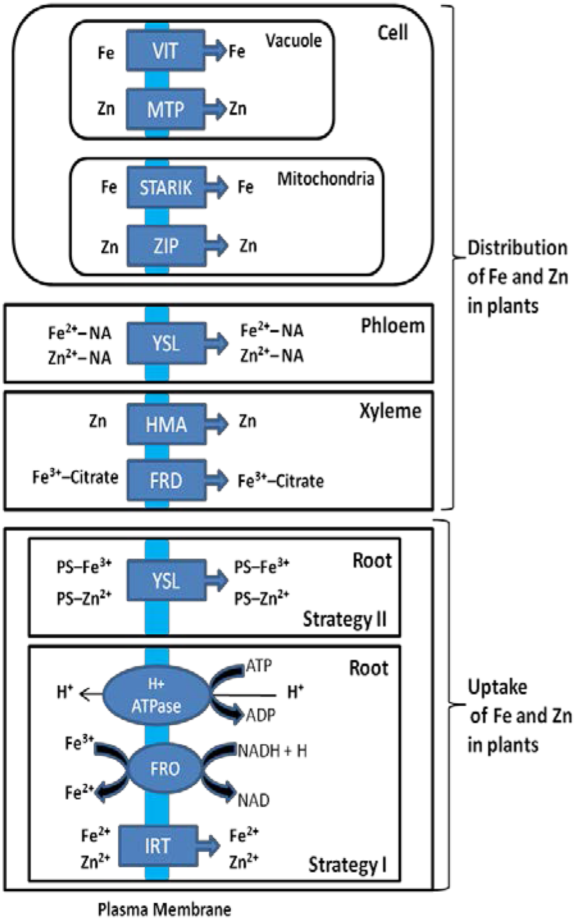

Fig. 1. Metal transports in plants. Non graminaceous plants use a reduction-based strategy I of uptake, whereas gramineous plants use a chelation-based strategy II. In non graminaceous plants, acidification of the soil by an ATPase and reduction of Fe^{3+} by FRO contributes to increase uptake of Fe^{2+} and Zn^{2+} into the root. In gramineous plants, Fe^{3+} and Zn^{2+} are also uptake as PS chelates by YSL transporters in the epidermis and Fe can also be taken up by IRT. Metals can then travel through the symplastic space to the vasculature. Transport into the xylem involves members of the HMA family and the citrate effluxer FRD. YSLs may also translocate metals to the phloem, where they can then be delivered to the above-ground organs. Intracellular Fe and Zn efflux to the mitochondria could take place by STARIK1 and ZIP transporters, respectively. Once metals have reached their target destinations, they needto be stored in vacuoles. Vacuole is an essential metal storage compartment in seeds, Zn and Fe are transported into the vacuole by the MTP and VIT1 transporter, respectively. Blue boxes represent the metals transporters. NA, nicotianamine. PS, Phytosiderophore

protein (ZIP)-like transporters are needed for the uptake of Fe and Zn into roots. With respect of the uptake by Strategy II plants, Fe is taken up as a PS-metal chelate through Yellow Stripe-like (YSL) transporters into roots, while Zn appears to be taken up through ZIP-like transporters (IRT), as in

Strategy I plants. Once in the roots, both minerals will be transferred to the xylem for further distribution towards the aerial organs by various transporters (Vert et al. 2002, Roberts et al. 2004, Ghandilyan et al. 2006).

Xylem. In order to avoid handling toxic free metals during translocation throughout the plant, they are transported in the chelated form with other molecules. The chelator candidates that load Fe into the xylem are citrate and nicotianamine (NA). Depending on the Fe-chelate complex formed, different transport systems are involved in distributing Fe throughout the plant. For example, NA may act an an Fe chelate and facilitate its symplastic translocation to the xylem parenchyma (Kerkeb, Connolly 2006). However, the pH of the xylem favors the chelation of Fe to citrate rather than NA, and it is known that Fe exists as Fe^{3+}–citrate chelates in the xylem (Durrett et al. 2007, Palmer, Guerinot 2009). Because Fe^{3+}–citrate is the major form of Fe present in xylem exudates, citrate is thought to be involved in long distance Fe transport from roots to shoots (Jeong, Guerinot 2009). Once Fe is chelated, the resulting Fe^{3+}–citrate complexes are removed into the xylem, via transporters such as FRD gene. FRD (ferric reductase defective), a citrate transporter localized in the plasma membrane of the pericycle and the vascular cylinder, has been shown to efflux citrate into the xylem and is required for Fe transport to the shoot (Durrett et al. 2007). Thus, Fe is thought to be unloaded from the vasculature into developed tissue through yet-unknown mechanisms (Ghandilyan et al. 2006, Palmer, Guerinot 2009). On the other hand, the heavy-metal ATPase (HMA) genes, which are expressed in vascular tissue, appear to be the most likely candidates to transport Zn into the xylem (Hussain et al. 2004, Ghandilyan et al. 2006). Zn is effluxed into the xylem for long-distance transport by HMA (HMA2 and HMA4), which localize to the plasma membrane of the root and shoot vasculature. The *hma2, hma4* mutants show decreased shoot Zn and increased root Zn, which supports the role of HMA2 and HMA4 in xylem loading. HMA4 is also identified as a gene with increased expression in the Zn hyperaccumulator *Arabidopsis halleri* (Hussain et al. 2004, Palmer, Guerinot 2009).

Phloem. Metal delivery to developing tissues requires transport through the alkaline phloem, with metals probably bound to chelators such as nicotianamine (NA). Fe^{2+} and Zn^{2+} are thought to be transported not only in the xylem, but especially in the phloem transport as NA chelates. The transporters involved in phloem loading are thought to include members of the YSL group (a subfamily of the oligopeptide transporter OPT family), and the NA–metal complexes are most probably transported across plasma membranes by these transporters (Ghandilyan et al. 2006). YSLs may translocate metals to the phloem, from which they can be delivered to the seed. One of more thoroughly described members of this subfamily is YSL1, which localizes to the shoot vasculature as well as the siliques, pollen grains and the developing seeds (Palmer, Guerinot 2009). In *Arabidopsis*, the YSL family (YSL1 and YSL3) as well as the oligopeptide transporter OPT3 are implicated in metal delivery from the vascular tissues to developing seeds (Stacey et al. 2008). Furthermore, it is suggested that YSL proteins could participate in both xylem-to-phloem metal transport in young growing tissues and phloem-to-xylem exchange in roots. Interestingly, the function and transport of other metal ligands including mugineic acid, histidine and phytate have been recently reviewed (Puig, Peñarrubia 2009).

Cell. Once transported to the proper tissue, metals must be distributed on the subcellular level to ensure sufficient amounts to the necessary cell compartments. Storage and buffering of Fe at the subcellular level are crucial mechanisms that allow plants to cope with Fe deficiency and toxicity. Organelles such as vacuoles and plastids play a key role in the intracellular compartmentalization or storage of Fe. In plastids, the ferritins can store an important fraction of cellular Fe, and these proteins play various roles related to Fe homeostasis during development or in response to environmental stresses (Briat et al. 2007, Palmer, Guerinot 2009).

Because electron transport chains and primary carbon metabolism require Fe, organelles such as chloroplasts and mitochondria are the most important sites for Fe utilization in the cell (Pilon et al. 2009). For example, nearly 90% of Fe in the plant is localized to the chloroplast, where it is required for use in the electron transport chain and the synthesis of chlorophyll, heme and Fe-S clusters; Fe is also transported into the mitochondria to function in same process (Kim, Guerinot 2007, Palmer, Guerinot 2009). It is believed that Fe efflux from *Arabidopsis* mitochondria could occur by the ABC-type transporter STARIK1 (STA1) protein (homolog of the yeast ATM1p: ABC Transporter Mitochondria 1 protein) located at the inner membrane (Briat et al. 2007), but not all Fe transporters involved in mitochondria and chloroplasts are identified yet (Palmer, Guerinot 2009). In addition, Zn and Fe are used in the chloroplast as cofactors for superoxide dismutases (SODs), preventing cellular damage by the reactive hydroxyl radical species. Zinc is most likely transported by a ZIP that localizes to the mitochondria, but as of yet no ZIP transporters have been assigned this function (Palmer, Guerinot 2009).

Regarding metal transports into the vacuole, this organelle is an essential metal storage compartment in seeds. The vacuole functions during early seedling development as an initial store of metals. Zinc has been shown to be transported into the vacuole by members of the MTP (metal tolerance protein) family, also referred to as CDF (cation diffusion facilitator) proteins (Gustin et al. 2009). Advances concerning Fe fluxes across the tonoplast of *Arabidopsis* seeds demonstrate than mobilization of Fe stored in the seed allow young germinating seedlings to develop during the initial heterotrophic phase. Transporters identified in both vacuolar Fe influx and efflux have been shown to be essential for germination and seedling development (Briat et al. 2007). Iron is transported into the vacuole by the transporter VIT1 (Vacuolar Iron Transport 1), which is critical for localization of Fe into the seed. Remobilization of Fe from the vacuole is thought to be mediated by the actions of NRAMP3 and NRAMP4, which are upregulated under Fe deficiency (Lanquar et al. 2005, Palmer, Guerinot 2009).

It is important to mention than the main strategies that the plant uses to combat metal toxicity are sequestration and chelation to carrier metals. Consequently, in addition to sequestration within the vacuole, Fe has been shown to be stored in plastids in ferritin, a protein nanocage that can store up to 4,500 atoms of Fe^{3+} in its interior as an Fe oxide mineral (Hintze, Theil 2009). In animals, ferritin is the primary storage form for Fe, but recent work has suggested that in *Arabidopsis* the role of ferritin is solely to deal with excess Fe and prevent oxidative damage (Ravet et al. 2009). Therefore, most plants use ferritin primarily to detoxify Fe rather than as a major storage unit. However, some plants use ferritin as a storage unit, and an exciting new study has shown that oceanic diatoms use ferritin to safely store Fe for later use (Marchetti et al. 2009, Palmer, Guerinot 2009).

PROGRESS TO IMPROVE THE IRON AND ZINC CONTENTS IN CROP PLANTS BY BIOFORTIFICATION STRATEGIES

Fe and Zn deficiencies are a well-documented public health issue and an important soil constraint to crop production. The developed world has made great attempt to alleviate MNM through diversification of diets, food fortification, improved public health care and supplementation. Whereas these strategies have been effective in industrialized countries, they have met with limited success in developing countries because these are often too expensive and difficult to sustain (Cakmak 2010, Lungaho et al. 2011). Alternatively, an agricultural approach that can be widely applied to overcome MNM is biofortification, i.e. a process of increasing the level and/or bioavailability of essential nutrients in crops. This is a relatively new strategy than involves the improvement of agronomic characteristics and the nutritional content of crops through agronomy, plant breeding and biotechnology (Petry et al. 2010, White, Broadley 2011).

Agronomic biofortification

Agronomic biofortification (ferti-fortification: fertilizer applications) and plant breeding (genetic biofortification) represent complementary agricultural approaches (Cakmak 2008, White, Broadley 2011). It is considered than application of Zn- and Fe-containing fertilizers is a short-term solution and complement to plant breeding. Published data provide convincing evidence that soil and especially foliar applications of Zn fertilizers are effective in improving grain concentration of Zn. For example, increasing bioavailable Zn levels via Zn fertilization has been shown for pea, navy bean and wheat

(Cakmak et al. 2010, Zhang et al. 2010). In addition, the Harvest Zinc Fertilizer Project has found that foliar application of Zn fertilizers to wheat can significantly increase Zn concentration in the grain; therefore, it is believed that - depending on the extent of Zn deficiency in soils - Zn fertilizers can contribute to better yield of cereal crops (Bouis, Islam 2011).

By contrast, due to the rapid conversion of Fe into unavailable forms when applied to calcareous soils and the poor mobility of Fe in phloem, soil and/or foliar Fe fertilization appears to be less effective than Zn fertilization in enrichment of grains (Cakmak 2008, Zhang et al. 2010). Moreover, some work has shown that plants did not respond to foliar Fe fertilization in terms of grain Fe concentration, such as application of various inorganic and chelated Fe fertilizers that remain ineffective for increasing grain Fe concentration. However, it appears that nitrogen (N) nutritional status of plants plays a critical role in biofortification of cereal grain. Improving the N nutritional status of plants by higher N-fertilizer applications promoted accumulation of Fe and Zn in grain (Cakmak 2010, White, Broadley 2011).

In general, agronomic biofortification of food crops (for certain essential micronutrients) can be used as an effective agricultural tool to improve human nutrition of people in the developing world. The best example of biofortification of food crops using Se fertilizers comes from Finland; this was a documented study where a whole country participated. Since 1984, the addition of selenate to NPK fertilizers for use on crops and pastures was the first method tested to increase the entire population's Se status. Conversely, ferti-fortification could not be a long-term sustainable approach in developing countries. For example, Fe fertilization is even more complicated, as Fe has a strong tendency towards insolubility, unless used in large quantities or when expensively chelated to organic molecules. Foliar applications, which improve yields of plant grown in Fe-deficient soils and increases Fe level in crops is another strategy, but when applied regularly, are costly and constitute potential dangers to the environment (Broadley et al. 2006, Bouis, Welch 2010, Murgia et al. 2012, Sperotto et al. 2012).

Genetic biofortification

On the other hand, crop biofortification by breeding offers a sustainable and low-cost way to provide micronutrients to people in developing countries. Breeding of nutrient-rich staple food crops is indeed the main goal of different international consortia, which aim to reduce MNM through different biofortification programs (White, Broadley 2011, Murgia et al. 2012). Three primary conditions have been identified to make biofortification successful. They are (1) a biofortified crop must have high nutrient density combined with high yields and high profitability, (2) this crop must be shown to be efficacious and effective in reducing micronutrient malnutrition in humans, and (3) the crop must be acceptable to both farmers and consumers in target regions where people are afflicted with MNM (Bouis, Welch 2010).

HarvestPlus, a component of the CGIAR Research Program on Agriculture for Improved Nutrition and Health, leads a global effort to develop and deliver biofortified staple food crops with most limiting nutrients in the diets of the poor: vitamin A, Zn and Fe. This interdisciplinary program works with experts in more than 40 countries. The main target crops studied are bean (Fe/Zn – DR Congo/Rwanda), cassava (Vitamin A - DR Congo and Nigeria), maize (Vitamin A – Zambia), pearl mille (Fe/Zn – India), rice (Zn/Fe – Bangladesh/India), sweet potato (Vitamin A – Uganda/Mozambique), wheat (Zn/Fe – India/Pakistan). This program also supports initial studies of the following crops: banana/plantain (vitamin A), lentil (Fe/Zn), potato (Fe/Zn) and sorghum (Zn/Fe). Most biofortified crops are still in the development pipeline. However, one biofortified staple food crop that has been successfully released is the orange sweet potato (OSP), conventionally bred to combat vitamin A deficiency in regions of Africa where sweet potato is a staple food crop (Bouis, Islam 2011).

Breeding new plant genotypes for high grain levels of Fe and Zn is the most cost-effective strategy. A broad range of Fe and Zn content in grains of major crops has been associated with genotypic variation. Such variability is exploited through breeding programs to produce Fe- and Zn-rich crop varieties. Also, research to assess and enhance genetic variability for Fe and Zn has been amended with the information on retention after processing, bioavailability from the diet, consumption per day, and amount needed in plant food to have measurable effects on nutrient status (Cakmak 2010, Murgia et al. 2012). Thus, HarvestPlus sets the preliminary “minimum” target levels for micronutrient content using gross assumptions about staple food intake (g day^{-1}); bioavailability (% of nutrient absorbed); losses of the target nutrient with processing (milling, storage and cooking); and the proportion of the daily nutrient requirement that should be achieved from the additional amount of micronutrient in the staple food (Bouis et al. 2011). Target Zn concentrations set by the HarvestPlus program are (content as dry weight: µg g^{-1}): 28 in polished rice, 38 in wheat grain, 38 in maize, 66 in pearl millet, 56 in bean, 34 in cassava root, and 70 in root of sweet potato (Bouis et al. 2011). These target concentrations are considered to be conservative, and have been exceeded in breeding lines of rice, wheat and maize (White, Broadley 2011). In the same way, target levels for Fe content of biofortified staple food crops set by this program are (content as dry weight: µg g^{-1}):15 in polished rice, 59 in wheat grain, 60 in maize, 88 in pearl millet, 107 in bean, 45 in cassava root, and 85 in root of sweet potato. The program identified five crops (pearl millet, beans, wheat, rice and potato) with the greatest potential for Zn and/or Fe biofortification (Bouis et al. 2011). It is important to mention that some otheer recent research supports the hypothesis of micronutrient dilution in cultivars over time. For example, wild and primitive wheat represent a better and more promising genetic resource for high Zn content. Collections of wild emmer wheat showed impressive genetic variation and the highest levels Zn (14-190 mg kg^{1}); also, some accessions show simultaneously both very high levels of Zn (up to 139 mg kg^{-1}) and Fe (up to 88 mg kg^{-1}) in seeds (Cakmak 2008).

Plant foods (seeds and grains) contain antinutrients [phytic acid (PA) and polyphenols (PP)] that can reduce the bioavailability of dietary Fe and Zn to humans. Dietary substances that promote/enhance the bioavailability of micronutrients [prebiotics] in the presence of antinutrients are also known, whose levels are controlled by genetic and environmental factors. These compounds, than affect Fe and Zn absorption from the human diet, must be considered when work with biofortificated crops. For example, in the last decade one of the biofortification approaches for improving Fe absorption in humans has been the isolation of low phytate (lpa) genotypes of crops to improve Fe bioavailability. But a recent study on women whose diet had a lower level of either PA or PP level in common beans, has demonstrated only a modest positive influence of the tested factors on Fe absorption. This result implies that the priority should be to breed for high Fe concentration rather than low PA content. Dietary PA and PP are associated with desirable human health benefits, PP has diverse beneficial properties (antineoplastic, antioxidant and anti-inflammation) and PA is a broad spectrum antineoplastic agent, thus it is suggested that these compounds might protect against cancer in humans (Pixley et al. 2011, Murgia et al. 2012). On the other hand, increasing Fe availability can help to treat anaemia, but there is evidence that it can also promote pathogen growth. Thus, an optimum Fe status is by and large achieved safely in developed countries. However, in developing countries, the widespread incidence of infectious diseases and the consequent host-pathogen competition for Fe complicate any effort at designing guidelines for daily Fe requirements. Crops biofortified with prebiotics (such as inulin) have the potential to avoid the ‘Fe paradox’ caused by the host-pathogen competition for Fe, by favoring amelioration of gut health and gut-associated immune defense. Fruit and vegetables have significant levels of inulin and there is some evidence of stimulatory effects of inulin on dietary Fe absorption in animals. Therefore, it is argued that an increase of the content of Fe and prebiotics in edible parts of plants is expected to improve health, whereas the reduction of PA in crops valuable for human diet might be less beneficial in developed countries than in developing countries exposed to endemic infections (Murgia et al. 2012).

Biotechnology biofortification

Finally, in the absence of genetic variation in the micronutrient content among varieties, transgenic approaches can be a valid alternative for biofortification. Two distinct approaches are used to improve mineral content: (a) increase the efficiency of uptake and transport into edible tissues and (b) increase the amount of bioavailable mineral accumulation in the plant. Engineering strategies to increase the plant mineral content are concentrated primarily on Fe and Zn, which are more frequently deficient in human diets (Palmgren et al. 2008, Hirschi et al. 2009).

Key genes of metabolic pathways, uptake, translocation and re-translocation that influence the accumulation in harvested plant parts have been identified. For example, nicotianamine (NA) is an important chelator that can control metal accumulation in seeds, thus manipulation of cellular NA levels is another approach for improving Fe and Zn contents in plants. Recent studies have shown that enhanced expression of a rice-nicotianamine synthase gene (OsNAS3) results in an increase of these metals in both vegetative tissues and mature seeds (LEE et al. 2009).

Overexpression of ferritin (Fe storage protein of bacteria, animal and plant cells) represents a possible transgenic approach to enhancing the Fe concentration in edible parts of plants. Eukaryotic ferritins consist of 24 subunits, which assemble in a cage-like structure able to sequester or release Fe upon demand, thus representing a dynamic 'Fe-reservoir' that can be the predominant mechanism of Fe storage in seeds of many plants (MURGIA et al. 2012). The study on transgenic rice expressing an Fe storage protein, ferritin, showed up to threefold higher Fe in transgenic brown rice grains (38.1±4.5 mg kg^{-1}) compared to untransformed grains (11.2±0.9 mg kg^{-1}) (AIZAT et al. 2011).

Maize has been altered using transgenes to increase Fe bioavailability, e.g. DRAKAKAKI et al. (2005) generated transgenic maize expressing, both an Aspergillusphytase and soybean ferritin, in the kernel. In the most active transgenic line, up to 95% of the phytate was degraded and a 50% increase in the Fe level of the grain was observed. Fe bioavailability was evaluated using an *in vitro* digestion/Caco-2 cell model and demonstrated that phytase expression was directly correlated with Fe bioavailability and uptake (LUNGAHO et al. 2011). In addition, it was possible to increase the Fe content in polished rice more than six-fold by transferring two plant genes: nicotianamin synthase (nicotianamin) and ferritin. Their synergic action allows the rice plant to absorb more Fe from the soil and store it in the rice kernel: nicotianamin binds the Fe temporarily and facilitates its transportation in the plant and ferritin acts as a storage depot for Fe in both plants and humans. The genes are controlled in such a way that nicotianamin is expressed throughout the rice plant, but ferritin only in the rice kernel; this prototype works well in a greenhouse, without possible negative effects (GRUISSEM 2010).

It is important to mention that those are some examples of biotechnology showing the positive impact on human nutrition by reducing Fe malnutrition via biofortification. Most of the work being done to biofortify staple food crops relies on traditional plant breeding techniques. Only for those nutrients that cannot be bred by conventional methods, some organizations are conducting preliminary research to determine what role transgenics can play in breeding biofortified crops. However, it is claimed that only a national agricultural research program of each country can help to make dissemination decisions. For example, as a policy, HarvestPlus will not distribute transgenics ever developed under its auspices to any country that does not have biosafety regulatory systems in place or does not wish to adopt transgenic varieties (HARVESTPLUSFAQ 2012, http://www.harvestplus.org/content/faq).

IMPACT OF IRON AND ZINC BIOFORTIFICATION ON HUMAN NUTRITION AND HEALTH

Mineral nutrient deficiencies are a worldwide problem, which is directly correlated with poverty and food insecurity. MNM can affect all age groups, but young children and women of reproductive age tend to be among those most at risk of developing micronutrient deficiencies. MNM has many adverse effects on human health, not all of which are clinically evident (Benton 2008). It is estimated that 2 billion people suffer from MNM, due to the lack of critical micronutrients such as vitamin A, Zn, and Fe in the diet. This situation impairs the mental and physical development of young populations (lower IQ, stunted growth and blindness). In addition, MNM reduces the productivity of adults due to an increased risk of illness and reduced work capacity, which has profound implications for economic development (Bouis, Islam 2011). Worldwide, the three most common forms of MNM are Fe, vitamin A and iodine deficiency, followed by the shortage of micronutrients such as Zn, folate, calcium, proteins and vitamins, but Fe deficiency is the most prevalent (Benton 2008, WHO 2012. www.who.int/nutrition /topics/ida/en/index.html).

The major cause of MNM is a poor quality diet, mainly lacking in animal products (micronutrient-rich foods such as meat, fish, poultry, eggs, milk and dairy products). Consequently, populations that consume few animal source foods may suffer from a high prevalence of several micronutrient deficiencies simultaneously. Therefore, a balanced diet would be the best way to prevent MNM, but very often people have no access to appropriate food. At the same time, the proportion of the global population suffering from MNM has increased because modern plant breeding has been historically oriented toward high agronomic yield rather than the nutritional quality, producing a lower density of minerals in many crops (Rana et al. 2012).

Iron. Micronutrients are essential elements needed in small amounts for adequate human nutrition. The mineral Fe is essential to human well-being and an adequate supply of Fe helps to prevent Fe deficiency, a prevalent health concern of the entire world. Most of Fe in the human body is present in erythrocytes as haemoglobin, where its main function is to carry oxygen from the lungs to tissues. Iron is also an important component of various enzyme systems, such as cytochromes, involved in oxidative metabolism. This micronutrient is generally stored in the liver as ferritin and as haemosiderin. Iron differs from other minerals because the Fe balance in a human body is regulated by absorption only as there is no physiological mechanism for its excretion (Benton 2008, Hurrel, Egli 2010).

Iron deficiency is the result of a long-term negative Fe balance and severe stages of deficiency cause anaemia. Anaemia is defined as a low blood haemoglobin concentration; haemoglobin values that indicate anaemia have been defined for various population groups by the WHO. This is the most widespread nutritional disorder in the world and the only nutrient deficiency with significant prevalence in industrialized countries. Two billion people are anaemic (over 30% of the world's population) due to Fe deficiency, and this situation is frequently exacerbated by infectious diseases in resource-poor areas. Malaria, HIV/AIDS, hookworm infestation, schistosomiasis, and tuberculosis are important factors contributing to the high prevalence of anaemia (WHO, 2012, www.who.int/nutrition/topics/ida/en/index.html).

In general, Fe deficiency affects more people than any other condition, constituting a public health condition of epidemic proportions. Fe deficiency and anaemia reduce the work capacity of individuals and entire populations, bringing serious economic consequences and obstacles to development of affected countries. Low dietary diversity and inadequate daily intake are the main reasons for the widespread occurrence of Fe deficiency in human populations, affecting a large number of children and women in developing countries. Main consequences of Fe deficiency include mental retardation, decreased immune function, reduction of work capacity and increased mortality of mother and child at birth (WHO 2012. www.who.int/nutrition/topics/ida/en/index.html).

Based on intake data and isotope studies, Fe bioavailability has been estimated to be in the range of 14-18% for mixed diets and 5-12% for vegetarian diets, and these values has been used to generate dietary reference values for all population (Hurrell, Egli 2010). The daily recommended Fe intake for human ranges between 8 and 18 mg day^{-1} depending on age and gender, with recommended 30 mg day^{-1} for pregnant women. However, a large number of people in the world do not have the privilege of enriching their diets enough to allow this recommended intake (Aciksoz et al. 2011, Bhullar, Gruissem 2013).

It is important to mention than recent data refutes the use of haemoglobin as the sole indicator of Fe deficiency since this information is influenced by other factors. Therefore, prevalence of Fe deficiency can be evaluated on the basis of age, sex, race, socioeconomic status and regional variances. Newer measurements to diagnose Fe deficiency in population studies include biomarkers such as serum ferritin, transferrin saturation, free erythrocyte protoporphyrin, and C-reactive protein (Beard et al. 2006).

The causes of Fe deficiency vary significantly during different stages of life, according to socioeconomic status, but these differences in the Fe status are also related to dietary constituents. Dietary Fe intake, as well as other dietary constituents that influence food Fe absorption such as inhibitors and enhancers play a more important role than the Fe content of the diet (Hurrell, Egli 2010). For example, populations consuming diets rich in meat and ascorbic acid, both facilitators of Fe absorption, tend to have less Fe deficiency than populations with diets rich in inhibitors of Fe absorption (phytates and polyphenols). Diets high in fiber and/or Ca have a negative effect on Fe absorption, but to a lesser extent than those rich in inhibitors. Diets containing lower Ca content, taken with food that enhances absorption, do not have an inhibitory effect on absorption, compared to high Ca-containing diets. Until now, the interaction between all these dietary constitutes is difficult to integrate and further research is needed to gain better understanding of the relationship between dietary patterns and Fe supply (Fleming et al. 1998, Roughead et al. 2002, Hurrell, Egli 2010).

Improvement of Fe deficiency has been practiced mainly through food fortification and supplementation programs to help overcome the rising Fe deficiency statistics around the world (Schonfeldt et al. 2010). Although these strategies have been in place for many years, food-based approaches to improve Fe status in the general population are considered more sustainable. Development of Fe-rich varieties of staple food crops may benefit almost every population in the world owing to the simple production and spread of Fe-rich crops, and such nutrition-rich foods are safe for consumption. Because the majority of people suffering from micronutrient malnutrition often do not have access to supplementation strategies or to diversifying their diets, biofortification could become the most sustainable approach to improvement of micronutrients nutrition across generations in resource-poor, rural households in low-income countries (Brown et al. 2009, Gibson 2012, Bhullar, Gruissem 2013). However, more Fe studies are needed to facilitate the development of a sustainable food-based approach to combat Fe deficiency (Schonfeldt, Hall 2011).

Some unresolved Fe bioavailability issues concern the forms of Fe found in food. It is generally accepted that of the two primary forms of Fe found in food, heme Fe is more available for absorption than non-heme Fe. Although non-heme Fe forms a greater portion of the total Fe in foods, its absorption is low and affected by many factors such as the Fe status of the host, enhancing and inhibiting substances, factors consumed prior to/or with the meal and solubility in the intestine. Heme Fe, although mostly consumed in smaller amounts, is two to three times more bioavailable (15-35%) than non-heme Fe (2-20%) and it is less affected by other dietary factors. For these reasons, the total Fe content of food needs to be further investigated and the heme- and non-heme fractions reported to facilitate development of a sustainable food-based approach to combat Fe deficiency (Schonfeldt, Hall 2011). In addition, the mechanism by which Ca inhibits Fe absorption, the nature of the meat factor, and the influence of vitamin A, carotenoids, and nondigestible carbohydrates on Fe bioavailability from mixed diets needs clarification. The Fe status of the individual is the dominant factor that determines Fe bioavailability, and other host-related factors, such as inflammation (obesity is an inflammatory disorder related with the reduction of Fe bioavailability) may also play an important role. Actually, the consumption of Fe-fortified foods and the bioavailability of Fe-fortification compounds vary widely, so the contribution of fortification Fe to the bioavailability factors is difficult to estimate. In general, it is a priority to study the Fe bioavailability factors to combat Fe deficiency; the situation than depends on the consumption of meat, fruit, vegetables, processed foods, Fe-fortified foods and Fe-biofortificated foods (Hurrell, Egli 2010).

Zinc. Zn deficiencies in humans occur as a consequence of an inadequate dietary intake. Factors that decrease absorption include dietary inhibitors, such as phytate or certain types of fiber, drugs, and interactions between essential nutrients. Meat is also the best food source of bioavailable Fe and Zn, so in vegetarian populations and developing countries, Fe and Zn deficiencies usually coexist (Graham et al. 2012).

The importance of Zn as an essential nutrient for adequate human health is well known. Adequate Zn nutrition is essential for human health because of zinc's critical structural and functional roles in multiple enzyme systems that are involved in gene expression, cell division and growth and immunologic and reproductive functions. As a consequence, Zn deficiency affects children's physical growth and raises the risk and severity of a variety of infections. About 30% of the world's population is Zn deficient, but infants and young children are probably the most vulnerable. Pregnant and lactating women are also likely to be very susceptible to Zn deficiency, and there is an urgent need for more information on the implications of low Zn status in these particular population groups (Lowe et al. 2009, Yakoob et al. 2011). The most important negative effects of Zn deficiency involve immune competence, subnormal growth and reproductive function. Some of the adverse health consequences of Zn deficiency vary with age: diarrhoea, dermatitis and neurobehavioural disturbances are common during infancy, whereas skin changes, anorexia, impaired taste acuity, growth retardation and recurrent infections are more frequent in children. During adolescence, delayed sexual maturation and abnormalities in skeletal growth and mineralization have been described, and among the elderly, chronic non-healing leg ulcers and recurrent infections occur (Tesan et al. 2011, Gibson 2012).

Inadequate intakes of dietary Zn can arise from low intakes of Zn *per se*, poor bioavailability, or a combination of these dietary factors. Zn is the mineral most abundant and easily absorbable from animal proteins, whereas consumption of vegetable and cereals decreases its absorption due to binding of Zn to phytates, the only substantial dietary factor that inhibits Zn absorption, especially when diets are low in flesh foods (Yakoob et al. 2011, Gibson 2012). Although mineral bioavailability is complex, phytate-to-mineral molar ratios can be used as a qualitative measure of their bioavailability in human food. Indeed, most variation in Zn bioavailability is explained by phytate:Zn contents in human diet. In fact, this ratio in food is considered to be a good indicator of Zn bioavailability. The efficiency of Zn absorption from a diet ranges from about 15% to 35%, depending largely on the amount of Zn consumed and the presence of dietary phytate (Gibson 2012). The current recommendations for dietary Zn intake in adults range from 7 mg d^{-1} (UK Reference Nutrient Intake) to 11 mg d^{-1} (US Recommended Dietary Allowance).This broad range reflects in part the variation in requirements due to differences in the bioavailability of Zn from different diets and the difficulties associated with estimating the requirements for optimal health. High levels of dietary calcium can inhibit Zn absorption, especially in the presence of phytates. Unlike Fe, Zn absorption is neither inhibited by phenolic compounds, nor enhanced by vitamin C. Until now, the influence of these risk factors for Zn deficiency has been difficult to integrate, thus further research is needed to evaluate the bioavailability of Zn from usual diets to gain better understanding of the relationship between dietary patterns and Zn supply (LOWE et al. 2009, GOMEZ-GALERA et al. 2010). Zn deficiency is now widely recognized as a leading risk factor for morbidity and mortality. Zn deficiency has been estimated to be responsible for approximately 4% of the worldwide burden of morbidity and mortality in children less than five years of age. On 2009, the IZiNCG (International Zinc Nutrition Consultative Group) Steering Committee reexamined the latest strategies to control Zn deficiency and to enhance Zn nutrition. They agrue that potential strategies to combat Zn deficiency are supplementation, fortification, dietary diversification/modification and biofortification, the choice depending on the magnitude of risk, life-stage group, and scenery. Zn supplementation is recommended for treating acute diarrhoea, and to prevent stunted growth, diarrhoea, pneumonia, and mortality in high-risk children. Zn fortified cereals are appropriate for urban households, whereas dietary diversification/modification and biofortification are suitable for the rural poor. For the maximum impact, interventions should be integrated with effective public health programs that address underlying causes of Zn deficiency (GIBSON 2012).

There is good evidence supporting the beneficial impact of Zn interventions, especially of zinc supplementation. Zn supplementation trials conducted over the last few decades in children from developing countries have demonstrated the positive benefits of improved Zn status, including better growth rates and reductions in the incidence of various infectious diseases; also, therapeutic Zn supplementation reduces the duration and severity of diarrhea (BROWN et al. 2009).

Less information is available on the impact of Zn fortification programs and of dietary intervention strategies, although the available evidence suggests that both of these approaches should enhance Zn status. Food fortification or the addition of nutrients to food in a higher level to that found originally, are considered a cost-effective strategy to improve the micronutrient status of a population. The available studies clearly show that Zn fortification can increase dietary Zn intake and total daily Zn absorption. Despite the positive effect of Zn fortification on the total Zn absorption, only a few studies have found positive impacts of Zn fortification on the serum Zn concentrations or functional indicators of Zn status. Thus, additional research is needed to determine the impact of Zn fortification, with or without other micronutrients, in populations at risk of Zn deficiency (HESS, BROWN 2009). Fortification with Zn has been limited and generally confined to infant formula milks, complementary foods, and ready-to eat breakfast cereals (HENNESSY-PRIEST et al. 2008).

Finally, dietary modification and/or diversification refer to the use of strategies to improve to access and use of Zn-rich foods. There are several strategies that can be used and although some are considered long-term strategies, they are very sustainable. Breastmilk is an important potential source of bioavailable Zn for infants, so promotion of breastfeeding programs than support adequate Zn nutrition of young children are needed. Other interventions to increase the availability, accessibility, and consumption of animal-source foods or to increase the Zn content of plant-source foods or to increase Zn absorption from these foods should all enhance the Zn statusof the consumers. However, rigorous evaluations of large-scale dietary approaches are still lacking. In the future, provided the health benefits are confirmed, biofortification could become the most sustainable approach to improve Zn nutrition across generations in resource poor rural households in low-income countries (Brown et al. 2009, Gibson 2012).

The recent WHO publication provided information on how to determine appropriate levels of fortification for conventional food fortification programs, and similar approaches can be used for establishing desirable levels of biofortification. Moreover, specific guidelines have now been published on the recommended levels of Zn and Fe fortification of cereal flours. The recommendations for Zn are based on estimates of the ability of mixed diets to meet human physiological requirements for absorbed Zn, using information on the amount of flour consumed by the population, the degree of milling commonly practiced, and the amounts of Zn and phytate consumed in the rest of the diet. By contrast, the recommendations for Fe fortification are based mainly on the experience of prior intervention programs, using different types of fortificants, because predicting Fe absorption is complicated by the fact that it is regulated according to individual Fe status and affected by multiple inhibitors and facilitators of absorption. Nevertheless, the proposed levels of conventional fortification with both Zn and Fe can be compared with proposed breeding targets for biofortification in given populations (Brown et al. 2010).

CONCLUSIONS

MNM arising from Zn and Fe deficiency is a continuing and serious public health problem in the world. The developed world has made great attempt in alleviating MNM through diversification of diets, fortification and supplementation. Although food fortification has played an important role in resolve MNM problem, this strategy has two great disadvantages: it is usually dependent on funding and restricted to urban areas. Increasing the micronutrient density of staple crops, or biofortification, can improve human nutrition on a global scale. It can be achieved by agronomic fertilization; however, this is not a long-term sustainable approach in developing countries, some fertilizers (Fe) are costly and dangerous to the environment. By contrast, genetic biofortification has multiple advantages, principally that the benefits reach the total population. Therefore, international agricultural researches are implementing programs to develop biofortified staple crops. It is well known that biofortification through plant breeding is considered as a promising and cost-effective approach for diminishing MNM, either as a stand-alone solution or in combination with supplementation and fortification. Yet some work remains to explain genetic control and molecular mechanisms affecting the accumulation of Zn and Fe in grain. Further, evidence suggests that nitrogen (N) nutritional status of plants can have a positive impact on root uptake and the deposition of Fe and Zn in seed.

Conventional breeding is the primary focus of programs to enhance staple food crops with sufficient levels of Fe and Zn, to meet the needs of at-risk populations in the world. But it is our priority to study the bioavailability factors that depend on the consumption of meats, vegetables, processed foods, fortified and biofortified foods. It is claimed that Fe and Zn bioavailability can be improved by increasing dietary factors that enhance absorption, by decreasing factors that inhibit absorption, or by increasing their content of the diet. Today, the interaction of these dietary constituents is difficult to integrate and further research is needed to understand the relationship between dietary patterns and supply of micronutrients.

Until now, the potential strategies to combat Fe and Zn deficiencies in human are fortification, supplementation, dietary diversification and biofortification, their choice depending on the magnitude of risk, life-stage group, and scenery. The recent WHO publication provided information on how to determine appropriate levels of fortification for conventional food fortification programs, and specific guidelines about the recommended levels of Zn and Fe for cereal flours. These proposed levels of fortification will help to compare and support the studies of proposed breeding targets for biofortification with both Zn and Fe, to understand the connection between dietary patterns and micronutrient supply. Furthermore, most economic analyses suggest that genetic biofortification is more effective than other programs for increasing dietary Fe and Zn intakes of vulnerable populations.

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IRON IN MEDICINE AND TREATMENT

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Abstract

Being a component of many proteins and enzymes, iron is an essential microelement for humans. However, this element can also be toxic when present in excess because of its ability to generate reactive oxygen species. This dual nature imposes a strict regulation mechanism of the iron concentration in the body. In humans, systemic iron homeostasis is mainly regulated on the level of intestinal absorption. A patient diagnosed with excess iron in the body should be treated safely and effectively. And the therapy should be consistent with the treatment of concurrent diseases.

On the other hand, iron deficiency is one of the most common disorders affecting humans. Iron-deficiency anaemia continues to represent a major public health problem worldwide, being prevalent among pregnant women, where it represents an important risk factor for maternal and infant health.

A problem detecetd relatively recently and therefore not fully clarified yet is the iron therapy in patients with restless legs syndrome (RLS). RLS is a common neurological condition defined clinically as the urge to move the legs. Reduced brain iron is strongly associated with restless legs syndrome. RLS can also be a consequence of iron deficiency in the body.

This review will focus on iron as an element whose abnormal metabolism or deficiency in the body can lead to diseases e.g. anaemia, restless legs syndrome and iron overload. Here we will describe methods of therapy, paying particular attention to the types and dosages of medications.

Key words: iron deficiency, anaemia, iron overload, restless legs syndrome, pregnancy.

ŻELAZO W MEDYCYNIE I LECZNICTWIE

Abstrakt

Żelazo jest podstawowym mikroelementem organizmu ludzkiego, stanowi bowiem istotny element wielu białek i enzymów. Jednak pierwiastek ten może wykazywać działanie toksyczne, gdy występuje w nadmiarze, ze względu na jego zdolność do generowania reaktywnych form tlenu. Ten podwójny charakter żelaza narzuca ścisłą regulację stężenia żelaza w organizmie. U ludzi homeostaza ustrojowa żelaza jest głównie regulowana na poziomie wchłaniania jelitowego. Pacjent, u którego zdiagnozowano nadmiar żelaza w organizmie, powinien być poddany bezpiecznemu i skutecznemu leczeniu, które jest zgodne z terapią współistniejących schorzeń.

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Niedobór żelaza jest jednym z najczęstszych zaburzeń dotykających ludzi. Niedokrwistość spowodowana niedoborem żelaza nadal stanowi istotny problem zdrowia publicznego na całym świecie. Szczególnie dotyczy kobiet w ciąży, stanowiąc istotny czynnik ryzyka dla zdrowia matki i dziecka.

Stosunkowo nowym i nie do końca wyjaśnionym zagadnieniem jest terapia żelazem chorych na zespół niespokojnych nóg (RLS). Jest to stan neurologiczny klinicznie określany jako przymus poruszania nogami. Z zespołem niespokojnych nóg związana jest ściśle redukcja żelaza w mózgu. RLS może być również konsekwencją niedoboru żelaza w organizmie.

W pracy omówiono nieprawidłowy metabolizm żelaza lub jego brak w organizmie, co może prowadzić do ww. jednostek chorobowych. Opisano również metody terapii, zwracając szczególną uwagę na rodzaj i wielkość dawki proponowanych leków.

Słowa kluczowe: niedobór żelaza, niedokrwistość, nadmiar żelaza, zespół niespokojnych nóg, ciąża.

IRON METABOLISM

Iron is an essential component of the human body. It is used for synthesis of haemoglobin, muscle myoglobin and enzymes. The iron daily requirements range from 1 mg to 3 mg. The requirements are met with the daily dietary intake of 12-15 mg. (Heeney et al. 2004). Under normal conditions, approximately 1-2 mg of iron per day enters the body *via* the enterocytes of the proximal small intestine. Absorption of nearly all dietary iron takes place in the proximal duodenum and includes the following steps:

1) reduction of iron from the ferric state (III) to the ferrous state (II) – this happens in the presence of ascorbic acid, cysteine and glutathione;
2) apical uptake by enterocytes followed by transcellular trafficking;
3) basolateral efflux by the ferrous iron transporter ferroportin (FPN) (Camaschella, Strati 2010).

Newly absorbed iron is released into the circulation and binds to the serum protein transferrin. Most absorbed iron is transported in the bloodstream bound to the glycoprotein transferrin. Transferrin is a carrier protein that plays a role in regulating the transport of iron from the site of absorption to virtually all tissues. The proper level of serum transferrin is 20-120 µg l^{-1} for women and 30-300 µg l^{-1} for men. Transferrin binds only two iron atoms. Normally, 20-45% of transferrin binding sites are filled. Approximately 3 mg of iron circulates bound to transferrin. Transferrin-bound iron is taken up by cells by transferrin receptor 1 (TfR1)-mediated endocytosis. Most of the transferrin-bound iron in the circulation is destined for developing erythrocytes of the bone marrow, where it is taken up at a rate of approximately 21 mg of iron per day, and used in the production of haemoglobin. About 65–70% of body iron exists in this form in circulating red blood cells. Old or damaged red blood cells are removed from the circulation by the macrophages of the reticuloendothelial (RE) system, where iron is released from haemoglobin and either stored in the intracellular iron storage protein ferritin, or released back into the circulation as transferrin-bound iron. The cells of the RE system release about 21 mg of iron per day, thus replacing the amount taken up by the bone marrow (Anderson et al. 2007).

The main systemic regulator of iron absorption and macrophage release is hepcidin. This 25 amino acid liver peptide negatively interacts with and degrades ferroportin in response to iron overload and inflammation. In this way, iron absorption and macrophage release are blocked to reduce circulating iron (Camaschella, Strati 2010).

Approximately 10–15% of body iron is present in such proteins, with up to 80% of this element found in muscle cell myoglobin. The remaining 20% of body iron is present as storage iron, predominantly located in the macrophages of the RE system and the hepatocytes of the liver (Anderson et al. 2007). The liver is a major storage organ of iron, in which excess iron is stored as ferritin and hemosiderin (Kahgo et al. 2008)

The iron turnover is extremely limited. Iron metabolism occurs largely in a closed system, because the losses are small. Under physiological conditions, the daily turnover of iron is 35 mg, meanning that this amount of iron leaves plasma: 32 mg leaves the pool of erythropoietic iron, 1 mg of iron is lost in urine, sweat, bile, exfoliating the epidermis and intestinal epithelium, 1 mg of iron is incorporated into myoglobin and heme enzymes. Simultaneously, 35 mg of iron returns to the plasma during the day: 21 mg of iron from the erythrocytes which broke up, 11 mg comes from erythropoietic iron pool, 1 mg comes from the extravascular space, 1 mg comes from storage and 1 mg is absorbed from the gastrointestinal tract (Pasternak 2000)

The proper concentration of iron in the blood plasma is 12.5-26.6 μmol l^{-1}. Under physiological conditions, normal parameters for iron excretion from the body are: 3.58 μmol^{-1}day for urine and 17.9 μmol^{-1} day^{-1} for stool (Pasternak 2000).

TREATMENT OF IRON DEFICIENCY

Iron deficiency is one of the most common disorders affecting approximately 2 billion people all over the world. Iron-deficiency anaemia represents a major public health problem worldwide. It is especially common among women of childbearing age because of pregnancy and menstrual blood loss. Another groups of patients include those with other sources of blood loss, malnutrition, or gut malabsorption (Alleyne et al. 2009).

Alleyne et al. (2009) classified the causes of iron deficiency anaemia into 4 different categories related to the consumption or loss of iron. This classification is presented in Table 1. In the majority of cases, iron-deficiency causes anemia is avoidable and reversible by increasing iron supplementation or reducing iron loss (Alleyne et al 2009).

Iron absorption is a reliable process. Under normal conditions, the loss of 1 mg of iron through the microbleeding or sloughing of intestinal epithelial

Table 1

Causes of iron deficiency in adults

Causes	Examples
Increased iron loss	acute hemorrhage alimentary respiratory urinogenital dermal chronic or occult hemorrhage menstruation inflammatory cancer hemolysis blood donation
Decreased iron in diet	vegetarian diet malnutrition dementia psychiatric illness
Decreased iron absorption	antacid therapy or high gastric pH celiac disease inflammatory bowel disease partial gastrectomy
Increased iron requirements	pregnancy lactation

cells is precisely balanced by the absorption of the same amount. Importantly, iron absorption can increase severalfold after iron depletion. However, when the absorptive capacity of the small intestine (which increases to a maximum of 2-4 times above normal) is exceeded by iron loss over a prolonged period of time, the result is iron deficiency anaemia. The fact that iron deficiency anaemia occurs only after depletion of total iron contained in the body is also relevant (RIMON et al. 2006).

Iron therapy is recommended to nearly all patients with anaemia caused by iron deficiency. Iron is available in many different doses and formulations and there are various ways of its administration. Various iron preparations are available in the forms of tablets, capsules, drops, and syrups in different dose sizes. Slow release iron reduces the side effects of iron therapy. Iron salts such as sulphate, gluconate, lactate, fumarate and succinate are absorbed to about the same extent. Iron carbonate, phosphate and citrate are poorly absorbed. Iron preparations generally contain 1 of 3 iron salts (BEUTLER 2006). The amount of elemental iron available in different formulations varies greatly, e.g. ferrous sulphate involves 20% elemental iron, i.e. a 325 mg tablet contains about 65 mg of elemental iron. The recommended daily dose of elemental iron for adults suffering from iron deficiency ranges from 150 to 200 mg. This approach entails prescribing 1 ferrous sulphate tablet three times daily because each tablet contains approximately 60 mg of elemental iron (ALLEYNE et al. 2009).

The recommended daily dose of elemental iron for patients with iron deficiency anaemia ranges from 120 mg to 200 mg. Oral iron preparations are best taken on an empty stomach or between meals to increase absorption. If patients suffer from symptoms from the gastrointestinal tract, the formulations may be administered after a meal, though it may reduce its absorption. There are multiple variables that may enhance or inhibit the absorption of iron. Differences in absorption are caused by the requirement of acidity in the duodenum and upper jejunum for iron solubility. For iron released beyond these sites, the alkaline environment reduces absorption. The products inhibiting iron absorption are coffee, tea, milk, cereals, dietary fiber, multivitamin or dietary supplements containing calcium, zinc, manganese, or copper, antacids, H2 blockers, and proton pump inhibitors, quinolones and tetracycline antibiotics. The products facilitating iron absorption include vitamin C, acidic foods, eg, tomato sauce, nonenteric, coated iron tablets, fasting ingestion of iron supplements (Alleyne et al. 2009).

The second important issue is administration of this element to patients with iron deficiency anaemia for a longer period of time. Typically, therapy should take 2-3 months for haemoglobin to return to normal levels. However, in patients with anaemia, iron levels in the body are low and should be supplemented. Iron supplementation usually requires 4-6 months (Rimon et al. 2006).

Iron is most often given orally, although for those with serious absorptive abnormalities, it can be administered intravenously (Rimon et al. 2006). The role of intravenous iron in clinical medicine is poorly understood and underused in the treatment of iron deficiency and anaemia in chronic diseases. Iron administered parenterally was considered dangerous and used only in extreme situations, or when oral iron was not tolerated. It was mainly based on poorly characterized anaphylactic reaction to the high-molecular-weight dextran preparation (Imferon), which was the only formulation available for intravenous administration. It is now known that intravenous iron, as opposed to oral iron, increases erythropoiesis in dialysis patients, therefore it is routinely used (Auerbach et al. 2007).

Currently, four parenteral iron preparations are available: low-molecular-weight iron dextran, iron saccharate, ferric glucontate and high-molecular-weight iron dextran. The most serious adverse events have been associated with the high-molecular-weight iron dextran (Imferon, which is no longer available and the current preparation, Dexferrum) and are rare (<1:200 000) with low-molecular-weight iron dextran or the two iron salts: gluconate and citrate (Chertow et al. 2004, 2006). A single infusion of the total dose of low-molecular-weight iron dextran is most convenient and cost effective in patients with uncomplicated iron deficiency. The replacement dose is calculated, diluted in normal saline and infused over 4 hours. The dose, in mg of iron, is calculated as follows:

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$$D_{Fe} = 0{,}136 \cdot m \cdot \left[\left(\frac{C_{Hb} \cdot 100}{14{,}8} \right) - 100 \right],$$

where:

m – body weight (kg),

C_{Hb} – haemoglobin concentration (g dL^{-1}).

An intravenous test dose of 25 mg of the diluted solution is required. If no adverse events occur within 1 h, the remaining solution can be administered. The administration of methylprednisolone before the test and after the infusion decreases the incidence of myalgias and arthralgias. The total intravenous dose is most appropriate for iron deficiency because of pregnancy, menometrorrhagia, surgical blood loss, and gastric bypass and for those patients with uncomplicated iron deficiency who are non-compliant with or intolerant of oral iron (Rimon et al. 2006).

Unfortunately, one of the main problems in the iron treatment is a reaction from the digestive system. The reported side effects include abdominal pain, nausea, vomiting, abdominal pain, diarrhoea and constipation. These ailments can cause a decrease in food intake. Abdominal discomfort, nausea, vomiting, changes in bowel movements, black stools are much more common in patients taking large doses of iron (150 mg elemental iron).

This problem can be eliminated by administering smaller doses of iron for an extended period of time. Rimon et al (2005) argue that this type of therapy is equally effective. Moreover, they speculate that low doses of iron can replace the commonly used higher doses (Rimon et al. 2005).

Another important question concerns controlled release of iron from the preparation. Controlled-release formulations were given for several, large groups of patients and their actions were compared with that of ferrous sulphate. The findings demonstrated fewer side effects in patients receiving controlled-release formulations of iron. However, there is a theoretical contraindication for the use of controlled-release iron preparations. It is associated with the place of iron absorption - iron is absorbed in the early parts of the duodenum and not in the final one, where it is released from sustained-release preparations (Alleyne et al. 2009).

IRON THERAPY IN RESTLESS LEGS SYNDROME

Restless legs syndrome (RLS) is a common neurological condition clinically defined by an urge to move the legs, improvement during movement, worsening while at rest, and worsening in the evening and night (Allen 2003). Reduced brain iron is strongly associated with restless legs syndrome (RLS). The most consistent pathologic finding in RLS is reduced brain iron and alterations in iron related proteins (Connor et al. 2003, 2008).

RLS is also considered to be a consequence of systemic iron deficiency (Allen 2003).

Recent research suggests that the underlying pathophysiology of RLS involves the central nervous system iron homeostatic deregulation. Cerebrospinal fluid ferritin is lower in RLS cases, and imaging studies show reduced iron stores in the striatum and red nucleus. Most importantly, pathologic data in RLS-autopsied brains show reduced ferritin staining, iron staining, and increased transferrin stains, but also show reduced transferrin receptors. No pathology aside from iron abnormalities has been identified. It is important to find the reduced transferrin receptor, because globally reduced iron stores would normally upregulate transferrin receptors. Therefore, it appears that primary RLS has reduced intracellular iron indices secondary to a perturbation of homeostatic mechanisms that regulate iron influx and/or efflux from the cell (Ondo 2005).

The mechanisms by which low intracellular iron subsequently manifests RLS symptomatology are not well understood. Dopaminergic systems are strongly implicated in RLS. Dopamine agonists (DA) most robustly treat RLS symptoms, and dopaminergic functional brain imaging studies inconsistently show modest abnormalities. There are several potential interactions between iron and dopamine systems:

- iron is a cofactor for tyrosine-hydroxylase, which is the rate-limiting step in the production of dopamine,
- iron is a component of the dopamine type-2 (D2) receptor,
- iron is necessary for Thy1 protein regulation.

Thy1 regulates vesicular release of monoamines, including dopamine. It also stabilizes synapses and suppresses dendritic growth. This hypothesis, therefore, states that both presynaptic and postsynaptic dopaminergic anatomy is intact, but the actual junction itself is dysfunction. This is most consistent with the functional imaging studies and clinical responses seen in RLS patients (Ondo 2005).

The first study on oral iron therapy in patients diagnosed with RLS was conducted in 1994 by O'Keeffe et al. (1994). They divided the patients into three groups based on serum ferritin levels: the ferritin level <18 mcg L^{-1} (1 mcg L^{-1} = 1 μg L^{-1}), ferritin between 18 and 45 mcg L^{-1} and ferritin between 45 and 100 mcg L^{-1}. All patients (15 persons) were treated for 2 months; a single dose of ferrous sulphate was 200 mg administrated 3 times a day. The greatest benefits were reported in patients with the lowest concentration of serum ferritin (<18 mcg L^{-1}) (O'Keeffe et al. 1994). Davis et al. (2000) conducted further studies using a randomized, double-blind study protocol. The authors found no benefit of treatment with iron (Davis et al. 2000). The issues that complicated Davis and co-authors' study was related to the kinetics of iron absorption from the gastrointestinal tract. An iron absorption curve is exponential, characterized by very high absorption of iron in the lower limit of serum ferritin (<18 mcg L^{-1}). Poor absorption of iron is observed at a concentration of ferritin above 100 mcg L^{-1}.

The percentage of iron absorbed is probably less than 1-2% in patients with ferritin level in serum >100 mcg L^{-1}. Therefore, oral administration of iron in patients with RLS and with high ferritin levels (as was the case in the study by Davis et al.) requires very high doses of iron and long-term (6-12 months) treatment (Earley 2009).

Oral iron supplements are commonly recommended for RLS but are largely ineffective due to poor absorption and poor tolerability at required doses. Controlled trials of oral iron for RLS in subjects with normal serum iron indices did not show efficacy, nor did it increase serum iron level (Wang 2009). Intravenous iron dextran has been shown to increase brain iron content. Surprisingly, only a few reports have ever presented data on the clinical effect of high dose intravenous iron for RLS (Ondo 2010). However, high molecular weight iron dextran has higher rates of anaphylaxis compared to iron sucrose or sodium ferric gluconate (Chertow 2006). A low molecular iron dextran reduces the risk of anaphylaxis, but there is no data presenting its efficacy in patients with RLS. All intravenous preparations are able to increase serum iron; however, iron dextran is retained longer by macrophages compared with other preparations, which can be important, as theoretically iron therapy for RLS presumably requires the CNS (central nervous system) iron accumulation, which might take days rather than the shorter time allowed by other iron preparations. Patients usually report a delay of at least three days before observing any benefit, which would also suggest that iron requires extended transport into the brain. Iron access to the brain is extensively regulated and not completely understood (Connor 2008). It may require a large serum iron overload extended over a period of time to shunt iron into the brain, which might not be achieved with oral iron or other intravenous iron preparations. Iron dextran has been proven to increase brain iron based on imaging studies (Earley et al. 2003).

IRON OVERLOAD

Iron overload can occur in various diseases, including hereditary haemochromatosis, thalassaemia, sickle cell disease, myelodysplastic syndromes (MDS), and some rare anaemias. In these conditions, increased iron absorption and/or transfusional loading can lead to iron accumulation in the key organs, which may result in iron-toxicity-related organ damage, as well as increased morbidity and mortality. Iron overload is characterized by excessive iron deposition and subsequent injury and dysfunction of the heart, liver, anterior pituitary, pancreas and joints, and induces damage in the central nervous system (Barton 2007).

The main cause of this organ damage is due to the overproduction of ROS (reactive oxygen species) in the presence of excess iron. The production of ROS by iron is mainly through the Fenton reaction, which eventually forms hydroxyl radicals from superoxide or hydrogen peroxide (Crichton et al. 2002). Among ROS, the hydroxyl radical is the most toxic fraction and it targets carbohydrate, protein, and nucleic acids. It is known that the reaction of hydroxyl radicals with the nucleic acid base 8-hydroxyguanine is highly correlated with teratogenicity and carcinogenicity by oxidative stresses (Kohgo et al. 2008).

Another powerful ROS showing similar reactivity as the hydroxyl radical is lipid hydroxyl-peroxide: ROOH. In iron overload, lipid peroxidative products such as malondialdehyde and 4-hydroxy-2-nonenal are increased, which form the radicals ROO-(alkyl oxyradical) and RO-(alkoxyradical). These lipid-based radicals possess longer half lives than hydroxyl radicals, and also have a stronger capacity for chronic cell toxicity and DNA damage (Kohgo et al. 2008).

Pathological conditions representing body iron overload are designated as iron overload syndromes. Iron overload syndromes are classified as genetic or secondary (Kohgo et al. 2008). Hereditary haemochromatosis is the most common genetic disorder (Yen et al. 2006), and its clinical manifestation is systemic iron deposition mainly in the liver, heart, brain and endocrine organs. Damage to these organs is considered to be a result of tissue injuries by iron-induced oxidative stresses (Pietrangelo 2007). In 1996, the causative gene was identified as HFE (human hemochromatosis protein) in the human chromosome 6 (Feder et al. 1996), and approximately 85% of patients with hereditary haemochromatosis have a homologous mutation of C282Y in their HFE gene. Thereafter, other genes such as hemojuvelin (HJV), TfR2, ferroportin, and hepcidin (HAMP) gene were identified (Franchini 2006).

Iron overload is commonly observed as a secondary condition. The most common condition occurs in patients who require long-term blood transfusions due to severe anaemias. This condition includes genetic disorders such as thalassaemia and SCD (sickle cell disease), and anaemia refractory to conventional treatments. In these patients, ineffective erythropoiesis and continuous accumulation of exogenous iron by transfusion are considered to be responsible for the iron overload. The resulting organ failures such as liver failure, cardiac failure, and severe diabetes mellitus affect patients' outcome (Andrews 1999). In addition to these classical conditions, there are many diseases that show mild iron deposition or deregulation of body iron distribution. Such conditions include chronic hepatitis C, alcoholic liver disease, non-alcoholic steatohepatitis, and insulin resistance, and iron is an important cofactor that modifies these disease conditions (Kohgo et al. 2008).

Since physiologic mechanisms to excrete iron are very limited, patients with iron overload and its complications need safe, effective therapy that is compatible with their coexisting medical conditions. Three licensed iron chelation drugs: one parenteral, two oral ones, are used in the treatment of iron overload (Barton 2007). Deferoxamine (DFO) is a hexadentate siderophore derived from Streptomyces pilosus. It is a chelating drug with high affinity for iron and aluminum ions. The drug is not absorbed from the gastrointestinal tract. Biological half-life in the first phase is approximately one hour, in the second phase - 6 hours. Since the half-life of DFO is very short, standard treatment involves a stringent infusion routine, which is necessary for optimal iron chelation and excretion. It is most commonly used orally in concentrations of 5-10%. Subcutaneous or intramuscular injections are performed in the doses dependent on patients` condition. Lack of patient compliance and physician dissatisfaction are major impediments to successful DFO therapy (Barton 2007).

Deferiprone (DFP; 1,2 dimethyl-3-hydroxypyrid-4-one) is an orally administered bidentate iron chelator. The drug is used to treat iron overload in patients with ß-thalassaemia, mainly when DFO is contraindicated or inadequate (Liu et al. 2002), Clinical studies have shown that a dose of 25 mg kg^{-1} body weight, administered three times a day, reduces the concentration of iron in the blood and prevents its deposition in tissues. However, the drug does not protect against damage to internal organs caused by high levels of iron. Studies have demonstrated that deferiprone, in recommended doses, is less effective than deferoxamine. DFP traverses cell membranes more readily than DFO. A standard dose of DFP is approximately equivalent to a standard dose of DFO, measured by urinary iron excretion (Wanless et al. 2002).

Deferasirox (4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl] benzoic acid) is an orally administered tridentate iron chelator that is indicated for the treatment of transfusion iron overload in patients over 2 years. Its main use is to reduce chronic iron overload in patients who are receiving long-term blood transfusions for conditions such as β-thalassaemia and other chronic anemias. The half-life of deferasirox is between 8 and 16 hours. This allows the administration of this drug once a day. Two molecules of deferasirox are capable of binding to 1 ion of iron, which is subsequently eliminated by faecal excretion. Its low molecular weight and high lipophilicity allows the drug to be taken orally. Together with deferiprone, deferasirox seems to be capable of removing iron from cells (cardiac myocytes and hepatocytes) and from the blood (Barton 2007).

Chronic iron overload due to blood transfusions leads to significant morbidity and early mortality unless adequate chelation therapy is administered. Deferoxamine is recommended for chelation therapy, but the problem lies in the fact that this substance has to be administered by prolonged subcutaneous or intravenous infusion. Deferasirox with a low toxicity profile has the advantage of being administered once a day (Vermylen 2008).

IRON AND PREGNANCY

Pregnancy is characterized by an increased iron requirement. Iron deficiency anaemia is still prevalent among pregnant women and represents an important risk factor for maternal and infant health. However, the degree of fetal iron deficiency is not always as severe as that in the mother. Iron transfer from the mother to the fetus is supported by a substantial increase in maternal iron absorption during pregnancy and is regulated by the placenta. Most iron transfer to the fetus occurs after the 30th week of gestation and likely involves placental expression of those proteins known to mediate systemic iron homeostasis (PAESANO et al. 2009).

In the second and third trimesters of human pregnancy, natural dietary iron absorption is increased fivefold and ninefold, respectively. The increased iron uptake is required for physiological needs of the fetus as well as for the compensation for loss of iron in maternal bleeding at delivery. Numerous studies have recommended iron supplementation of pregnant women (MILMAN 2008). The awareness concerning such hazards of iron medications as upper gastrointestinal tract erosion, pre-eclampsia, oxidative stress, haemoconcentration and gestational diabetes, has been increasing (WEINBERG 2010).

Supplementation has therefore been proposed to be restricted to those women who have moderate or low reserves of iron. Reserves can be determined by a serum ferritin assay either before or during early pregnancy. A value above 70 ng ml^{-1} would advise the person to forego iron supplementation. Women with values between 30 and 70 ng ml^{-1} should be recommended to take 40 mg ferrous iron daily. For those with serum ferritin below 30 ng ml^{-1}, the recommended dose would be 80 mg d^{-1} (MILMAN 2006).

Lactoferrin (LF) is an iron-binding glycoprotein abundantly found in exocrine secretions of mammals. LF is an important regulator of systemic iron homeostasis. Recent data suggest that this natural compound, capable of interacting with the most important components of iron homeostasis, may represent a valuable alternative to iron supplements in the prevention and cure of pregnancy-associated iron deficiency and iron deficiency anemia (PAESANO et al. 2008). The results of clinical trials carried out in 2006 by PAESANO et al. (2006) in pregnant woman suffering from iron deficiency and iron deficiency anaemia revealed the strong therapeutic potential of LF (PAESANO et al. 2006). This natural compound represents an efficient alternative to ferrous sulphate therapy. Ferrous sulphate restores only the haemoglobin concentration,but does not significantly increase the number of red blood cells, total serum iron and serum ferritin. The failure of ferrous sulphate therapy to increase the total serum iron concentration indicates that this therapeutic regiment fails in restoring iron transport from tissues to blood, differently from that observed after LF therapy. Independently of the pregnancy trimester, oral administration of 100 mg of LF (about 30% iron-saturated) twice a day before a meal increased the total serum iron and haemoglobin concentrations to a greater extent than that observed after oral administration of ferrous sulphate. In contrast to the administration of ferrous sulphate, LF oral administration did not result in any side effect (PAESANO et al. 2008).

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TOXICITY OF SALINOMYCIN AND NARASIN IN TURKEYS[1]

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Abstract

Coccidiosis continues to be one of the most serious diseases in poultry breeding, causing major economic losses in this industry. Many trials are conducted to prevent and control this disease. However, only a few medications are approved for use in the prevention and treatment of coccidiosis. Ionophore coccidiostats (e.g. salinomycin and narasin) act by altering the transmembrane movement of monovalent or divalent ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Rb^+, Cs^+), resulting in altered ionic gradients and disturbed physiological processes in coccidia. In broiler and turkey breeding, these agents are used for nearly the whole fattening period. Ionophore coccidiostats have a narrow safety margin. Their toxicity is probably due to a disturbed ion balance or to oxidative damage. Although ionophore coccidiostats are considered to be relatively safe for target animals, there are numerous reports of poisoning cases caused by these medications in birds. This paper summarizes the current state of knowledge on the toxicity of salinomycin and narasin in turkeys. It reviews the data concerning the symptoms, mortality rate and possible causes of poisoning with these agents. Moreover, the paper discusses the legal regulations regarding the use of these drugs in poultry.

Key words: ionophore coccidiostats, salinomycin, narasin, toxicity, turkeys.

TOKSYCZNOŚĆ SALINOMYCYNY I NARAZYNY U INDYKÓW

Abstrakt

Kokcydioza jest nadal uznawana za jedną z najważniejszych chorób w hodowli drobiu, stanowiąc główną przyczynę strat ekonomicznych w przemyśle drobiarskim. Podejmuje się wiele działań na rzecz zapobiegania i kontroli tej choroby. Do profilaktyki i terapii kokcydiozy zatwierdzono tylko kilka leków. Mechanizm działania kokcydiostatyków jonoforowych (np. salinomycyny i narazyny) jest związany z zakłóceniem transbłonowego transportu monowalentnych

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i dwuwalentnych jonów (Na^+, K^+, Ca^{2+}, Mg^{2+}, Rb^+, Cs^+), prowadzącym do zmiany gradientu jonowego i wtórnego zaburzenia procesów fizjologicznych u kokcydiów. U brojlerów i indyków leki te podawane są przez prawie cały okres tuczu. Kokcydiostatyki jonoforowe mają wąski margines bezpieczeństwa. Ich toksyczność jest związana z zaburzeniem równowagi jonowej lub uszkodzeniami oksydacyjnymi. Chociaż kokcydiostatyki jonoforowe są uznawane za stosunkowo bezpieczne dla zwierząt docelowych, w literaturze można znaleźć liczne doniesienia na temat zatruć tymi lekami u ptaków. W artykule przedstawiano bieżący stan wiedzy na temat toksyczności salinomycyny i narazyny u indyków, a także dane dotyczące objawów, upadków i możliwych przyczyn zatruć wywołanych tymi lekami. Omawiano również regulacje prawne w zakresie ich stosowania.

Słowa kluczowe: kokcydiostatyki jonoforowe, salinomycyna, narazyna, toksyczność, indyki.

INTRODUCTION

Coccidiostats were discovered at the early 1950s and described as a new class of chemotherapeutics in 1964 (Moore, Pressman 1964). New regulations introduced in the UE member states, which allow contamination of turkey fodder with ionophore coccidiostats like salinomycin and narasin, can be justified although the acceptable amounts set for these compounds as undesirable substances do not seem to be on an optimum level. This creates the risk of poisoning among older turkeys due to contamination of non-target feed stuffs even though the permissible threshold of an undesirable substance has not been exceeded. Moreover, the use of all antimicrobial growth promoters was prohibited in the European Union as of 2006. The consequences of this step on the incidence of *Eimeria* related diseases or the prevalence of *salmonella* and *campylobacter* are essentially unknown (Johansen et al. 2007).

COCIDIOSTATIC IN GENERAL

Currently, there are several substances possessing coccidiostatic properties, which can be divided into two groups. The first one consists of chemical compounds of natural origin, which are products of actinobacterial fermentation from *Sterptomyces* spp. and *Actinomadura* spp. They include narasin, monensin, lasalosid, maduramycin, salinomycin and *semduramicin* (Mehlorn 2008). The second group consists of synthetic coccidiostats, which vary in their mode of action, biological activity and chemical structure, e.g. robenidine (from the guanidine group), decoquinate (from quinolones), diclazuril (from benzeneacetonitriles), halofuginone (from quinolines) and nicarbazin.

Coccidiostats from the first group (of natural origin) are referred to as ionophore antibiotics. As such, they have found application in veterinary medicine, mainly as fodder additives used for the prevention and treatment of coccidiosis, a disease caused by protozoas *Eimeria (Report from the*

Commission (WE)... 2008, *Community Register...* 2012). There are seven main pathogenic species identified: *Eimeria* in poultry (*E. necatrix, E. mitis, E. brunetti, E. acervulina, E. tenella, E. maxima and E. praecox*) and other types peculiar to turkeys (*E. meleagrimitis, E. adenoides, E. gallopavonis and E. dispersa*). As well as poultry, coccidiosis may affect cattle, sheep, pigs and rabbits (OLEJNIK, SZPRENGER-JUSZKIEWICZ 2007, KOUTOULIS et al. 2013).

A wide spectrum of effects against all pathogenic types of *Eimeria* spp. which appear in poultry ensures adequate control of these protozoa (i.e. sporozoites *Eimeria tenella* die after 12-24 hour incubation in a liquid with salinomycine and monensin). However, their widespread use induces cases of immunity to these medications, first observed in the USA and Europe during the early 1980s (MEHLORN 2008). Currently, there are strains resistant to all ionophore coccidiostats. Noteworthy is the cross resistance in this group of medicines, i.e. when microorganisms gain resistance to one antibiotic, simultaneously they become resistant to other antibiotics from the same group (EFSA... 2008).

THE MODE OF ACTION

Ionophores modify the permeability of biological membranes by forming lipid soluble, dynamically reversible cation complexes, which transport cations across membranes. Each ionophore group has its own, typical, inorganic ion selectivity pattern. The mechanism of action of ionophore coccidiostats is related to their binding in lipophilic substances with monovalent or divalent ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Rb^+, Cs^+), causing perturbation that depends on the grade of transport concentration through cell membranes, which in consequence leads to quick depletion of intracellular energy stores and cell death (EFSA... 2008).

Salinomycin selectively facilitates the transmembrane exchange of sodium and potassium. As a result of this uncontrolled movement, ion gradient and concentration across the cell membrane are altered and the physiological processes in coccidia are deranged (RIZVI et al. 2008).

COCCIDIOSTATICS IN PRACTICE OF FEED FORMULATION

In the European Union, conditions for approval of coccidiostats and their application are regulated by the European Union legislature and laws of particular countries (*Council Regulation (EEC)...* 1990, *Regulation (EC)...* 2003, *Act on Animal ...* 2006, *Regulation on the European Parlament (WE)...* 2009, *Community Register...* 2012). Coccidiostats are used as fodder additives in poultry and rabbit breeding (ESFA... 2004b, *Report from Commission (WE)...* 2008, *Community Register...* 2012). Currently, 27 pharmaceutical products are authorized in the EU member states for the prevention of coccidiosis in one or more animal species, but only 9 are allowed to be used in turkey breeding: Elancoban (monensin sodium), Elancogran (monensin sodium), Coxidin (monensin sodium), Cycostat (robenidine hydrochloride), Cygro (maduramicin ammonium), Avatec (lasalocid A sodium), Halofuginone (halofuginone hydrobromide), Clinacox 0.2 (diclazuril), Clinacox 0.5 (diclazuril) (*Community Register...* 2012). An approval document sets the minimum and maximum level in which coccidiostats can be used as feed additives in the animal's diet. It may specify the animal species as well as the species categories (for example chickens for fattening and chickens reared for egg laying) and in some cases withdrawal periods (*Council Regulation (EEC)...* 1990, EFSA... 2008).

In broiler chicken, turkey and rabbit breeding, coccidostats are used for almost the entire fattening period, with a 0-, 1-, 3- or 5-day (depending on the substance) withdrawal period before slaughter (*Report from the Commission (WE)...* 2008). Medicated substances are added to 86% of starter/grower fodders for broilers, 97% of starter/grower for turkeys, 45% of fodders for rabbits and 15% of fodders for young hens which start the egg yield period (Olejnik et al. 2009). The application of ionophore coccidiostats in laying hens' fodder of finisher type is forbidden.

In accordance with the European Commission's Regulation and European Food Safety Authority (*Regulation (WE)...* 2007, ESFA... 2004a, EFSA... 2008), salinomycin is used as sodium salt in the product called Sacox 120 microGranulate. The description of the use of fodder with sodium salinomycin should contain the warning: 'Salinomycin is dangerous to horses and turkeys', as well as some additional information like 'This additive in the fodder contains ionophore; when used with certain medicinal substances (like tiamulin) it can be contraindicated.' Therefore, such fodders can be used, as recommended, only for broilers provided the salinomycin content does not exceed a dose of 60-70 mg kg^{-1}, chickens reared for laying until the age of 12 weeks with the salinomycin content no more than 50 mg kg^{-1} and rabbits reared for fattening with the salinomycin content which does not exceed a dose of 20-25 mg kg^{-1} of the fodder. Despite the prohibition of the use of salinomycin in fodder additives for turkeys, cases of intoxication with this and other coccidiostat are still reported.

Salinomycin in the form of sodium salt is an authorized feed additive in non-target feed following unavoidable carry-over. For equine species, turkeys, laying birds and chickens reared for laying (over > 12 weeks), its acceptable amount is 0.7 mg kg^{-1} of feed regarding feeds with 12% water content (*Commission Regulation (EU)...* 2011).

The above assumption has been made because a certain level of contamination of fodders is impossible to avoid due to economical and technological reasons. It has been established that the content of coccidiostats including salinomycin in fodders for animals other than target species should not exceed the amount of 1-3% of the highest concentration for target animals. The level of 1% is accepted in the fodders for laying chickens and dairy animals as well as for the species which are considered to be sensitive, namely equines and turkeys. The accepted limit of the content of salinomycin in fodders for animals different than the target animals arises from an arbitrary assessment of a possible level of contamination of fodders. As the acceptable concentration (mg/kg) of this coccidiostatic in the case of broilers is the dose of 70 mg kg^{-1} of the fodder, 1% is a dose of 0.7 mg kg^{-1} of salinomycin in the fodder. This quantity is the amount of undesirable substance that is accepted by the legislator. However, it does not mean that the acceptable amount of salinomycin which appears in the fodder cannot cause intoxication in turkeys. Researchers and clinical veterinarians know of the falls of older turkeys (20 weeks) after feeding with the fodder in which salinomycin appeared in the amount of 0.26 mg kg^{-1}. This supports the suggestion that the acceptable amount of salinomycin as an undesirable fodder additive should be lower, especially in the case of older turkeys (over 12 weeks old), which are more sensitive to this coccidiostatic.

SIGNS OF INTOXICATION

Signs of intoxication, including cardiovascular effects (raised blood pressure and myocardial degeneration), anorexia, weakness, ataxia and paralysis, are reported in various non-target animal species (EFSA... 2008). These signs correlate with the mode of action of ionophores and occur also in target animal species at dose levels exceeding the maximum authorized level. Animals particularly sensitive to salinomycin are turkeys, pre-ruminant calves and cattle, horses, cats and dogs, less pigs. In dogs, signs of neurotoxicity was observed experimentally. Toxicity is described in some non-target animal species at salinomycin concentrations in feed below the maximum level authorized for chickens for fattening, hence reflecting the significant species difference and a small margin of safety of sodium salinomycin used as a coccidiostat. The Panel on Contaminants in the Food Chain (CONTAM) concluded that ingestion of the maximum authorized level of salinomycin in poultry feed of 70 mg kg^{-1} feed may cause intoxications and constitute a health risk for several non-target animal species (EFSA... 2008).

ANALYSIS OF SALINOMYCIN IN PREMIXES AND ANIMAL FEEDS AND TOXICOSIS

All substances from the ionophore coccidiostat group have a narrow safety margin, i.e. there is a small gap between a therapeutic and a toxic dose. Some of them, such as salinomycin, narasin, maduramycin and – to a lesser degree – monensin, may be toxic to animals. Ionophore coccidiostats can be ordered in terms of increasing toxicity as follows: salinomycin < lasalosid < narasin < monensin < maduramycin (OEHME, PICKRELL 1999). Because equines and turkeys are very sensitive to salinomycin, its application in these species is forbidden. Salinomycin causes bad food intoxication in turkeys. Its symptoms are decreased fodder and water intake, growth inhibition, diarrhoea, emaciation, sleepiness, lack of motor coordination, muscle impairment, sultriness and lying on the sternum with legs extended forward. Intoxication caused by these coccidiostats often ends up in falls (REECE 1988). Moreover, severe intoxication with ionophores appears when these substances are given with other medicines administered to poultry. Such poisoning cases are noted among birds after a combined application of salinomycin with tiamulin (FOWLER 1995, MEHLORN 2008). Other researchers proved that the application of salinomycin in the amount of 44 or 66 mg kg^{-1} in fodder causes a higher mortality rate among 32-week turkey cocks (13 out of 20 of the examined birds were dead) than among 7-week turkey cocks (one out of 84 examined birds was dead) (POTTER et al. 1986). Thus, older turkeys are evidently more sensitive to salinomycin than younger ones. Higher toxicity of salinomycin among older turkeys was confirmed by data included in the Anticoccidial Compendium – Table 1 (FOWLER 1995). In another examination, a fall of 400 30-week birds in a flock consisting of 700 turkeys occurred within seven days from the beginning of feed application with salinomycin at a dose of 50 mg kg^{-1} of fodder (GRIFFITHS et al. 1989). Among the dead birds, no anatomopathological features were noted and histopathological examinations disclosed segmental muscle necrosis. The toxic effect of salinomycin is also described in 10- and 13-week turkeys fed fodder for chickens, which included

Table 1

Toxicity of salinomycin in turkeys

Age	Dose	Effect
Turkeys 0-12 week	40 ppm	no effect level
12 + week	20 ppm	no effect level
Adult females	10-15 ppm	no effect level for adult breeding females
Growing	< 50 ppm	no negative effects
Adult	< 50 ppm	dyspnoea, ataxia, death
Breeders	15-30 ppm	< 16% mortality
Fatteners	60 ppm	12-14% mortality
14 week old	16 ppm	dyspnoea, some mortality/hot
Young ages	22 ppm	depressed growth
Older ages	22 ppm	growth prevented or decreased

salinomycin at a dose of 60 mg kg^{-1} of fodder (Harries 1991). First symptoms of food poisoning such as loss of appetite, muscle impairment and falls appeared on the second day of feeding with this feed. In a flock of 10-week turkeys consisting of 3000 birds, 1200 birds died, and in a flock of 13-week turkeys consisting of 300 birds, 75 birds were dead. Similar observations were made by other researchers (Andreasen, Schleifer 1995), who found that due to feed contamination with salinomycin at a dose of 13 mg kg^{-1} of fodder (an assembly line on which cock fodder had been prepared was not cleaned properly) 180 birds died in a flock consisting of six-hundred 48-week turkeys. Degeneration and necrosis of skeletal muscles were asserted in histopathological examination.

SALINOMYCIN - ANTICOCCIDIAL ACTIVITY

Salinomycin, like other polyether ionophores, is effective against sporozoites as well as early and late asexual stages of coccidia in the intestines of chickens. The biological activity is based on the ability of ionophores to form lipid soluble and dynamically reversible complexes with ions (preferably the alkaline ions K^+ and Na^+). Salinomycin encloses the cation in a hollow ball, in the centre of which the cation is fixed and immobilised. It functions as a carrier of ions, mediating an electrically-neutral exchange of cations across the membranes. The resultant changes in transmembrane ion gradients and electrical potentials often produce profound effects on cellular function and metabolism that can lead to the death of coccidia (EFSA... 2008).

SALINOMYCIN - ANTIBACTERIAL ACTIVITY

Salinomycin shows selective antibacterial activity when applied in a concentration range of 0.5 to 16 mg L^{-1}. It is effective against many Gram -positive bacteria species, but *Enterobacteriaceae* are resistant. The minimum inhibitory concentration of salinomycin for common intestinal bacterial species such as *Enterococcus faecalis*, *E. faecium*, *Staphylococcus* spp. and *Clostridium perfringens* is between 0.5 and 16 mg L^{-1}. Inhibitory concentrations of salinomycin to susceptible bacterial strains are thus lower than the concentration in supplemented feed (EFSA... 2008).

DRUG-DRUG INTERACTION

Drug-drug interaction with salinomycin (used as a feed additive) and tiamulin (used therapeutically against infections with *Mycoplasma* spp.)

is frequently reported and results in up to 60% mortality in some poultry herds (Lin 1995). Moreover, serious poisoning with ionophore antibiotics may appear in cases of their delivery with other medicines administered to poultry. Such intoxication was noted among birds after combined application of salinomycin with tiamulin (Fowler 1995, Mehlorn 2008). The interaction between these medicines hinders the process of biotransformation of salinomycin and its accumulation in the birds' system, particularly in livers (Mézes et al. 1992, Fowler 1995, Mehlorn 2008). It is know that tiamulin (and also valnemulin, a related pleuromutilin) is a potent inhibitor of the activity of hepatic cytochrome P450 enzymes. Subsequently, the latter accumulate following daily ingestions, and signs of toxicity occur due to a relative overdose (Witkamp et al. 1995, Szucs et al. 2004, EFSA... 2008).

Earlier studies on the fattening of male chickens (28 day-old) fed with a diet containing 60 mg kg^{-1} salinomycin with and without experimental treatment with tiamulin given intraoesophageally (50 mg kg^{-1} b.w.) showed that the hepatic malondialdehyde concentration rose in the salinomycin-treated group, indicating lipid peroxidation. At the same time, glutathione concentrations and glutathione peroxidase activity decreased rapidly and these effects preceded clinical signs of toxicity in the animals and indicated that salinomycin and tiamulin exerted a synergistic effect in affecting the antioxidant (glutathione) system (Mézes et al. 1992, EFSA... 2008).

EGGS

Several studies show that salinomycin was detected in eggs from laying hens that were treated with salinomycin sodium in their feed (Kan, Petz 2000, Rizvi et al. 2008). When hens were fed with the doses of 30, 60, 90 or 150 mg kg^{-1} of salinomycin sodium for 14 days, their eggs contained salinomycin in amounts of <10, 80, 110 and 200 µg kg^{-1} in the egg white, and 1400, 2000, 2800 and 3700 µg kg^{-1} in the yolk, respectively. However, in hens treated with a dose of 60 mg kg^{-1} in their feed for 7 days, the excretion of salinomycin with eggs resulted in concentrations of 50 µg kg^{-1} in the egg whites, and 1500 µg kg^{-1} in the yolks. In another study, hens given 60 mg kg^{-1} for 5 days had salinomycin concentrations of <10 µg kg^{-1} in the egg white and 220 mg kg^{-1} in the yolk (EFSA... 2008). Another feeding trial showed that the accumulation of salinomycin in eggs was 3.3 µg kg^{-1} egg per mg kg^{-1} feed (Kennedy et al. 1998). Mean egg salinomycin concentrations did not exceed 60 µg kg^{-1} at any time (EFSA... 2008).

EXCRETA

Salinomycin is extensively metabolised by chicken and unchanged salinomycin represents only a very small fraction of total radioactive residues in the excreta. More than twenty metabolites have been separated and identified from the excreta, each representing less than 10% of the total salinomycin-derived compounds (EFSA... 2008).

NARASIN

A similar problem appears in the case of narasin, which according to the community legislator (*Regulation (WE)...* 2006) can be used as a fodder additive in a product called Monteban. The document informs (analogously to salinomycin) that the same information should be included for this narasin-enriched fodder as for Sacox. As indicated, the fodder is intended for broilers of any age. The dose of narsin in Monteban is 60-70 mg of active ingredient/kg of complete compound feed stuff.

Although narasin, as well as other ionophore coccidiostats, is considered to be effective and safe for target animals, the literature describes numerous examples of birds poisoned with narasin (BRAUNIUS 1985). The main reason for intoxication is wrong application, e.g. overdosing or incorrect fodder mixing (NOVILLA 1992, FOWLER 1995, OEHME, PICKRELL 1999). Symptoms of intoxication and a high death rate (up to 32%) were observed in a flock of 3000 turkeys aged 11 weeks, which had been fed with fodder containing 42.8 mg kg^{-1} of narasin (SALYI et al. 1988). The falls of birds took place during the first week after the first symptoms of poisoning such as incoordination, dyspnoae and diarrhoea. The post mortem revealed gastritis, degeneration of kidneys and pulmonary congestion. The symptoms of poisoning and falls were also noted among turkey cocks aged over 12 and 18 weeks which had been fed with fodder containing 70 mg kg^{-1} dose of narasin (DAVIS 1983). Falls of turkeys were also observed after the intake of a dose of 25-40 mg of narasin kg^{-1} in fodder given for 3 days. A much higher death rate was observed among turkeys at the age of 18 and 22 weeks when compared to 8-week-old birds – Table 2 (FOWLER 1995).

In turkeys, narasin is an authorized feed additive in non-target feed following unavoidable carry-over. The admissible amount of narasin is 0.7 mg kg^{-1} of feed and its content in feed was calculated same as for salinomycin (*Commission Regulation (EU)...* 2011).

In conclusion, salinomycin and narasin intoxication may create serious health and production problems in poultry keeping. This issue should be discussed and veterinarians have to educate breeders about the current problem caused by contaminated or toxic feeds.

Table 2

Toxicity of narasin in turkeys

Age	Dose	Effect
Turkeys 11 week old	43 ppm	- 30% mortality < 7 days
	28 ppm	- 30% mortality in both the above there was also slight monensin contamination.
Adult males	20 ppm/10 day	- no negative effects
8 week		- 7.2% mortality
18 week	25/40 ppm /3+ day	- 84% mortality
22 week		- 69% mortality anorexia, ataxia, paralysis, drooping, wings, dyspnoae, diarrhea.
18 week old males	70 ppm	- 30% mortality: reduced feed consuption
Adult females		weight gain, incoordination

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EFFECTS OF MINERAL DEFICIENCY ON THE HEALTH OF YOUNG RUMINANTS

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Abstract

Twenty-two elements have been identified as essential to the growth and health of animals. They include 7 macroelements and 15 microelements, which play four key functions in the body: structural, physiological, catalytic and regulatory. Mineral deficiencies can result from low quality feed, impaired absorption or assimilation in the body or increased demand for minerals during intensive growth, pregnancy and lactation. Mineral-deficient feed and diets with an unbalanced mineral content impair the growth and development of young animals, decrease appetite, lower nutrient absorption, decrease immunity and increase susceptibility to contagious diseases. This paper discusses the consequences of low levels of macronutrients and micronutrients that are required for the optimal growth of calves, lambs and kids, including calcium, phosphorus, magnesium, selenium, cobalt, iron, zinc, copper, sodium, potassium and chloride.

Key words: calves, lambs, kids, microelements, macroelements, deficiency.

WPŁYW NIEDOBORU PIERWIASTKÓW MINERALNYCH NA ZDROWIE MŁODYCH PRZEŻUWACZY

Abstrakt

Przyjmuje się, że 22 pierwiastki są niezbędne do prawidłowego funkcjonowania organizmów zwierzęcych. Należy do nich 7 makro- i 15 mikroelementów, które w organizmie pełnią 4 zasadnicze funkcje: strukturalną, fizjologiczną, katalityczną oraz regulacyjną. Przyczyną niedoboru pierwiastków mineralnych może być niewłaściwa jakość paszy, nieodpowiednia absorpcja lub asymilacja pierwiastków w organizmie lub zwiększone zapotrzebowanie zwierzęcia na składniki mineralne (okres wzrostu, ciąża, karmienie potomstwa). Niedobór pierwiastków mineralnych w paszy lub ich nieodpowiedni stosunek przyczynia się w głównej mierze do: zahamowania wzrostu i rozwoju młodych zwierząt, zmniejszenia apetytu oraz gorszego wykorzystania składników pokarmowych lub spadku odporności i większej zapadalności na choroby zakaźne. Celem artykułu jest omówienie skutków niedoboru najbardziej istotnych makro- i mikroelementów, takich jak: wapń, fosfor, magnez, selen, kobalt, żelazo, cynk, miedź, sód, potas oraz chlor, niezbędnych do prawidłowego rozwoju cieląt, jagniąt i koźląt.

Słowa kluczowe: cielęta, jagnięta, koźlęta, mikroelementy, makroelementy, niedobór.

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INTRODUCTION

The advancements made in molecular biology in the late 20th century contributed to our understanding of the functions and transformations of mineral elements in living organisms and the complex mechanisms responsible for the transport of trace elements across cell membranes (MAYLAND, SHEWMAKER 2001). The minerals found in body tissues and fluids of adult animals originate mainly from exogenous sources and constitute approximately 4% of the animal's body weight. Subject to their content in the body, minerals are generally divided into macroelements with concentrations higher than 50 mg kg^{-1} BW and trace elements or microelements with concentrations below 50 mg kg^{-1} BW. A list of 22 elements essential to animal life was developed in 1981. It included 7 macroelements: calcium, phosphorus, potassium, sodium, chloride, magnesium and sulphur, and 15 microelements: iron, iodine, zinc, copper, manganese, cobalt, molybdenum, selenium, chromium, tin, vanadium, fluoride, silicon, nickel and arsenic (BEDNAREK, BIK 1994). Macronutrients and micronutrients play four key roles in the body: structural, physiological, catalytic and regulatory. The structural function involves elements that build organ and tissue structures (calcium, magnesium, phosphorus, silicon in bones and teeth, phosphorus and sulphur in muscle proteins). The physiological function is responsible for the supply of electrolytes to body fluids and tissues in order to regulate osmotic pressure, maintain the acid-base balance, regulate membrane permeability and nerve impulse transmissions (sodium, potassium, chloride, calcium, magnesium). The catalytic role of minerals is probably the most important function. Macronutrients and micronutrients act as catalysts in enzyme and endocrine systems; they can act as coenzymes to initiate enzyme and endocrine functions, and they can constitute integral and specific structural elements of metalloenzymes and hormones. In living organisms, mineral elements are also responsible for cell replication and differentiation. Zinc influences transcription, whereas iodine is a component of thyroxine, a hormone responsible for thyroid function and energy processes (SUTTLE 2000).

In ruminants, mineral deficiency can impair or even inhibit metabolic pathways required for normal body function, and produce clinical symptoms of different intensity. Severe macroelement or microelement deficiencies are manifested by symptoms corresponding to the function of the deficient element in the body, thus contributing to an accurate diagnosis of the health problem. In a minor deficiency, the symptoms are non-specific, often transient and difficult to diagnose due to low intensity. Mineral deficiency generally leads to impaired immunity, inhibited growth, reproductive disorders and lower productivity in animals. In ruminants, subclinical deficiencies are observed more frequently than severe ones, hence the interest in the role of minerals in animal production on behalf of breeders, feed manufacturers, veterinary practitioners as well as researchers.

Mineral deficiencies can result from low quality feed, impaired absorption or assimilation of minerals in the body or increased demand for minerals during intensive growth, pregnancy and lactation.

CALCIUM AND PHOSPHORUS

Calcium (Ca) is the most abundant mineral in the body, and 99% of this element is found in bones. Calcium is essential to key body processes such as ossification, blood coagulation, cardiac rhythm control, cell membrane permeability, nerve and muscle excitation, activation and secretion of hormones and enzymes (Soetan et al. 2010). Phosphorus (P) is the second most abundant mineral in animals, and 80% of this element is located in bones and teeth. Phosphorus works together with calcium in ossification processes, and is a constituent of energy rich compounds and nucleic acids. Calcium and phosphorus levels in the body are regulated by vitamin D3 and parathyroid hormones: parathormone and calcitonin (Sobiech et al. 2010). Phosphorus deficiency, which may be exacerbated by low vitamin D levels, contributes to changes in the skeletal system and development of rickets in young animals. In most cases, vitamin D deficiency results from a poor diet and insufficient exposure to sunshine (UV radiation induces the conversion of vitamin D into active metabolites). Phosphorus deficiency is caused by a diet low in this element, which distorts the Ca:P ratio (1.5-2:1), inhibits Ca and P absorption and their transport to the skeletal system (Iqbal et al.2005). According to the literature, cattle are more sensitive to phosphorus deficiency than sheep (Dittmer, Thompson 2010). Defective bone mineralization accompanied by inhibited necrosis of cartilaginous cells and osteoblast penetration leads to the loss of bone elasticity and bone deformation. Another consequence is the compensatory proliferation of cartilaginous tissue and inhibited bone elongation. Fluorosis can also contribute to rickets. Fluoride stimulates bone formation, increases the demand for calcium and aggravates vitamin D deficiency (Kurland et al. 2007).

Inhibited growth and allotriophagy are among the earliest symptoms of rickets. The disease may be accompanied by the characteristic thickening of epiphyses and circumferential rib segments, which leads to the formation of the rachitic rosary. In animals, rickets may also result in curvature of legs, impaired mobility, lameness and recumbence. Inhibited dental development and enamel loss are also observed (Dittmer, Thompson 2010).

MAGNESIUM

Magnesium (Mg) is the second most abundant intracellular cation in mammals after potassium. Bones and muscles are the main magnesium pools in the body. Magnesium plays vital roles in nearly all physiological processes and participates in many cellular metabolic pathways. It activates nearly 30 enzymes and participates in the metabolism of carbohydrates, nucleic acids and proteins. Magnesium stabilizes DNA structure and influences RNA transcription as well as the formation of ribosomal subunits. The presence of Mg^{2+} ions is required in all processes involving ATP. The ability of magnesium to stabilize cell membranes is one of its most important functions (Soetan et al. 2010). Magnesium and calcium remain in a dynamic equilibrium, and a higher intake of magnesium than calcium can stilt the bone growth (Zimmermann et al. 2000). Magnesium improves potassium absorption and protects cardiac muscle cells and neurons against free radicals and toxic substances. It activates classical and alternative complement pathways (McCoy, Kenney 1992).

In adult ruminants, particularly in animals fed green fodder, even a minor magnesium deficiency can lead to nerve and muscle excitation, and a further drop in Mg concentrations increases the risk of hypomagnesemic-tetany (Martens, Schweigel 2000). In young ruminants, hypomagnesemia affects sucklings and animals fed solely milk or milk replacers deficient in magnesium. Other factors that increase the demand for magnesium or lower its availability, such as exercise, low temperature, decreased appetite/fasting and diarrhoea, also raise the risk of the disease (Robson et al. 2004). In calves, hypomagnesemictetany affects mostly rapidly growing animals at the age of 1.5 to 4 months, but in animals with bowel inflammations, the disease may occur as early as the second or third week of life. The first symptoms of nerve and muscle excitation are observed after several days or weeks of hypomagnesemia. They include anxiety, twitching of the ears, bulging eyes, jerking movements of the head, kicking at the abdomen, stiff and unsteady gait (Naik et al. 2010). The above stage is not always observed, and it is followed by contraction episodes lasting 5 to 20 minutes during which the animal lies on its side with stiffly stretched limbs, makes rowing movements, salivates profusely, makes chewing motions and grinds its teeth. The head and the neck are thrown backward (*opisthotonus*), the eyes are bulged, the heart rate and the respiratory rate are significantly elevated. After each episode, the animal remains weak, may exhibit tremor and signs of allotriophagy. Contractions may reappear 1-2 days later, and they can lead to death (Soni, Shukla 2012).

SELENIUM

In higher animals, selenium (Se) is closely linked with vitamin E and sulphur-containing amino acids. The physiological significance of selenium can be attributed to its presence in more than 30 selenoproteins, most of which are enzymes. Glutathione peroxidase (GSH-Px), one of the major selenoproteins, protects haemoglobin and fatty acids against oxidation and scavenges free radicals. Other selenoproteins include iodothyroninedeiodinase, which regulates the conversion of thyroxine (T_4) to 3,5,3'-tri iodothyronine (T_3), thioredoxin reductase, which works with electrons from NADPH to reduce oxidized thioredoxin in a catalytic reaction that transfers the reducing capacity from thioredoxin to cell proteins, selenoprotein P, which protects endothelial cells, and selenoprotein W, which protects myoblasts against oxidative stress and participates in muscle differentiation and development (Żarczyńska et al. 2013). Selenium influences immune processes. Selenium compounds participate in humoral immune responses and increase the concentrations of immunoglobulin M (Maggini et al. 2007). Selenium deficiency is generally associated with a low selenium content of soils. High environmental concentrations of sulphur, an antagonist of selenium, further decrease selenium concentrations. Selenium deficiency affects all animal species, but ruminants, in particular sheep and goats, seem to be at a higher risk.

Nutritional muscular dystrophy (NMD), also known as white muscle disease, is the most common disorder caused by selenium and vitamin E deficiency. NMD involves hyaline degeneration of skeletal muscles in various regions of the body, including the diaphragm, heart muscle and tongue (Beytut et al. 2002). The disease is most often diagnosed in healthy calves, lambs and kids younger than 6 months. The most common symptoms include an incorrect posture with widely spread limbs, hunched up spine, stilted gait and recumbency. The disease affects mostly thigh and crus muscles. Changes in tongue muscles prevent sucking, swallowing and lead to milk discharge through the nostrils. Pathological processes spread to the diaphragm and the heart muscle, leading to dyspnoea, higher respiratory rate, pulmonary murmur and cough (Ghany-Hefnawy, Tortora-Perez 2009). NMD affecting the cardiac function leads to mass animal deaths (up to 90% mortality), but it is far less lethal when only muscles are affected (Ramirez-bribiesca et al. 2005). Young animals with hyposelenosis are more susceptible to respiratory and gastric infections. Lower body gains are also reported (Aleman 2008).

COBALT

Cobalt (Co) catalyzes biochemical reactions in various metabolic pathways, including hydration, hydrogenation and desulphurization. This element participates in the biosynthesis of nucleic acids and stimulates the production erythropoietin in the kidneys, which is required for healthy erythropoiesis in living organisms. Cobalt activates gluconolactonase and participates in the pentose-phosphate pathway, an important process of tissue oxidation, which is independent of the Krebs cycle. Glucose oxidation is most intensive in the mammary gland (Kennedy et al. 1994). Half of the cobalt supplied to the rumen is used to synthesize vitamin B_{12}, which contains up to 4.5% cobalt and is therefore referred to as cobalamin. In ruminants, cobalt is stored in small amounts mainly in the liver, kidneys and heart, and its concentrations range from 3.0 to 220.0 μg g^{-1} of fresh tissue weight (Sivertsen, Plassen 2004). Cobalt is essential for the optimal development and function of ruminal microflora, and ruminants, in particular lambs, sheep, goats, calves and adult cattle, are most susceptible to cobalt deficiency (Sharman et al. 2008).

The symptoms of cobalt deficiency are relatively non-specific. Low levels of cobalt contribute to changes in the composition of ruminal microflora and function, which inhibit the decomposition of cellulose and transformation of pyruvic acid, disrupts the production of methane, ammonia and volatile fatty acids and contributes to chronic indigestion and metabolic disorders. In lambs, clinical symptoms of cobalt deficiency generally appear after weaning in the grazing season (summer to autumn). Early stages of the disease are accompanied by a higher feed conversion ratio and decreased appetite. Growth is inhibited, and symptoms of wasting may be observed. In extreme cases, animals look starved (Sharman et al. 2008). Cobalt deficiency leads to hyperchromic macrocytic anaemia (Digest 2007). It lowers the immune function in ruminants, in particular resistance to parasitic infections of the gastrointestinal tract, and decreases the ability of neutrophils to kill bacteria (Schwarz et al. 2000). Other symptoms include poor coat condition and skin changes. Severe cobalt deficiency can also be manifested by the nervous system disorders, mostly depression, aimless wandering and pushing against obstacles (Fisher, MacPherson 1991).

IRON

Iron (Fe) is essential for the maintenance of systemic homeostasis. Iron is the fourth most abundant element on the earth, but environmental reactions such as oxidation prevent its full biological use (Mohri et al. 2004). Iron compounds play various roles in the body, the major ones being oxygen transport (haemoglobin) and storage (myoglobin). As well as regulating phagocytosis and immunoglobulin production, iron-containing enzymes participate in immune responses by conditioning the non-specific immunity of acute phase proteins, transferrin, haptoglobin and lactoferrin (Walter 1997, Benito, Miller 1998). The mechanisms that regulate Fe levels are based on iron absorption, but they do not evacuate excess iron from the body. Iron has to be reduced to Fe^{+2} in order to be absorbed in the intestines. Under physiological conditions, iron absorption from food is controlled by iron levels in the body. Iron uptake increases when its concentrations in body tissues decrease, whereas iron buildup inhibits absorption. When enterocytes are absorbed by the mucous membrane, iron is oxidized to Fe^{+3}, which binds to apoferritin to produce ferritin. Excessive iron concentrations prevent binding with apoferritin and lead to the formation of haemosiderin, an insoluble iron-storage complex. The highest iron concentrations are found in haemoglobin, followed by porphyrin respiratory enzymes, ferritin, myoglobin and cytochromes. Iron released from degraded erythrocytes is reused and stored.

In young ruminants, the risk of anaemia posed by iron deficiency is not as high as in piglets, where the rate of erythropoiesis and conversion of fetal to adult haemoglobin is very high and requires iron supplementation. Subclinical anaemia, which leads mainly to inhibited growth, is rarely observed in suckling ruminants. Clinical symptoms generally subside after the introduction of a solid diet (Heidarpour, Bami et al. 2008). In young ruminants, the risk of iron deficiency is determined by iron concentrations stored in the liver and spleen during fetal development. Iron reserves are depleted in the first 3-4 weeks of life.

Anaemia is the most common manifestation of iron deficiency, and although it is generally subclinical, it can be aggravated by secondary diseases resulting from a weaker immune response and lower efficiency of circulatory and respiratory systems. Clinical symptoms are intensified when the Fe deficit cannot be compensated. In calves, the first symptoms are non-specific, including loss of appetite, inhibited growth, apathy, fatigue and allotriophagy, and they appear at the age of approximately 2 months. Mucosal pallor, increased respiratory rate and heart rate are noted in severe cases. In young animals, iron deficiency rarely leads to death, and cases of mortality are observed mostly in animals with inflammations of the respiratory (bronchi, lungs) tract or the gastrointestinal (diarrhea) system. In older ruminants, sideropenia can result from high dietary concentrations of Fe antagonists, such as phosphates and divalent ions, including copper, manganese, zinc, cobalt, selenium and nickel ions, which impair iron absorption in the proximal portion of the small intestine (Benito, Miller 1998).

Iron deficiency is rarely reported in traditional ruminant production systems, but it is noted in calves that are fed only milk or milk replacers with a low Fe content to obtain “white veal”. The above leads to acute anaemia with haemoglobin concentrations of up to 50% of the physiological norm, muscle pallor and an underdeveloped gastrointestinal system. In those animals, the contents of forestomachs and the entire alimentary tract account for only 1-3% body weight. The production of calves for white veal has been banned in Poland and other countries.

COPPER

Copper (Cu) is found in many enzymes and plays a very important regulatory role in biochemical processes. Copper is a component of two key aerobic metabolism enzymes: cytochrome C oxidase and superoxide dismutase.

The discussed element influences the prooxidative-antioxidative balance and exerts antioxidative effects. Copper participates in the formation of myelin sheaths surrounding nerve fibers, haematopoiesis, and in skeletal development; it regulates the synthesis of collagen, elastin, melanin and catecholamines; it influences the color and waviness of the hair coat. Copper stimulates iron absorption and metabolism (HOSTETLER et al. 2003). In ruminants, Cu is absorbed partially in forestomachs with the involvement of microflora, and in the stomach and the small intestine, where copper ions are bound by metallothioenins, low-molecular-weight proteins with a high content of cysteine residues (GOONERATNE et al. 1989). In calves, Cu absorption is high at 70-80%, but it decreases to >1.0–10.0% after the forestomach development (SPEARS 2003). Copper is accumulated already during the fetal development. GOONERATNE and CHRISTENSEN (1989) observed significantly higher copper concentrations in the livers of cattle fetuses than in the mothers. The Cu content of the liver in a fetus was found to increase during pregnancy (HOSTETLER et al. 2003).

Ruminants' sensitivity to copper-deficient feed decreases with age because copper is particularly important to rapidly growing calves, lambs and kids. Primary copper deficiency is observed mainly in regions where the copper content of soil is low or where the analyzed element is not available to plants. Grass and green fodder from such pastures and fields are unable to meet the animals' demand for copper, and young individuals are not supplied with adequate amounts of Cu in milk. Secondary copper deficiency occurs when the presence of copper antagonists, such as molybdenum, sulphur, cadmium, lead, zinc, calcium and iron, inhibits the availability of copper from feed. Diseases that inhibit the availability of dietary copper, in particular gastrointestinal disorders, can also contribute to secondary Cu deficiency.

Copper plays a multitude of roles in the body, and its deficiency produces a variety of symptoms, which is why Cu deficiency in calves is known under different names, such as "falling disease" in Australia and "pine" in Scotland. In lambs, clinical symptoms also vary in different regions of the world. In Great Britain, copper deficiency is manifested by swayback, in Australia – by anaemia and low wool quality, whereas in New Zealand – by bone mineralization disorders. In young cattle, the most common symptoms of primary and secondary copper deficiency include inhibited growth and development, bone abnormalities such as osteophytes on distal metacarpal and metatarsal growth plates, which lead to stiffness of the fetlock joint and steppage gait. Calves are also affected by lameness and transient hind limb ataxia (which subsides after rest) accompanied by sudden falls and/or dog-sitting position, progressing weakness that leads to wasting and allotriophagy (in particular dirt eating). Changes in the coat color and hypochromic macrocytic anaemia are more frequently noted in adult cattle (GOONERATNE et al. 1989). BANTON et al. (1990) identified two forms of copper deficiency in lambs and kids: congenital (swayback) and late (enzootic ataxia). The congenital form develops already in the fetus, and clinical symptoms are visible at birth. They include recumbency, weakness, head shaking, tremor and ataxia. The symptoms of nervous system disorders are caused by an absence of or damage to white matter in cerebral hemispheres. Gelatinous malacic foci and cavitation are observed in the white matter. Demyelination is noted in motor pathways of white matter in the spinal cord. The late form occurs in lambs and kids at the age of 1 week to 6 months, and it is characterized by incoordination, ataxia and paresis of hind limb. Anatomopathological changes are limited to enlarged brainstem and spinal cord neurons, and cerebellar changes have also been reported in young goats (Banton et al. 1990).

ZINC

Zinc (Zn) is a component or an activator of nearly 300 enzymes and it plays a variety of biological roles in the body (Al-Saad et al. 2010, Miao et al. 2013). It is a structural element of superoxide dismutase, alkaline phosphatase, carbonic anhydrase, lactate dehydrogenase, RNA and DNA polymerases (Kavas et al. 2013). Zinc is essential for hormonal functions, including the growth hormone (GH), thyroid stimulating hormone (TSH), glucagon, insulin, follicular stimulating hormone (FSH), luteinizing hormone (LH) and adrenocorticotropic hormone (Alves et al. 2012). The discussed element influences the immune response, the development and activity of neutrophils and NK cells, and lymphocyte gene expression (Kincaid 1999, El-Far 2013).

Similarly to other microelement deficiencies, Zn deficiency can be both primary, when milk and feed do not supply animals with sufficient quantities of the element, and secondary, when feed contains adequate levels of zinc, but its availability from feed is reduced by Zn antagonists (copper, magnesium, calcium, phosphates, divalent iron compounds) and amino acid deficiency. In cattle, mostly Holstein-Friesian, Aberdeen-Angus, Simmental and Shorthorn breeds, impaired zinc absorption may be an autosomal recessive congenital defect (lethal trait A46) (Yuzbasiyan-Gurkan, Bartlett 2006).

Zinc deficiency has been studied extensively in calves and sheep. Mild deficiency is accompanied by non-specific symptoms such as impaired appetite, higher feed conversion ratio and lower weight gains. Delayed testicular development and hypogonadism leading to oligospermia and testosterone deficiency were observed in bull calves with low zinc levels (Bedwal, Bahuguna 1994). Severe Zn deficiency may contribute to skin changes, including peeling, scabbing, itching and hair loss. These changes are initially observed around the eyes, lips, nostrils, on the neck and at the back of the neck, on limbs (in particular on the inner side), udder skin, scrotum skin, in the area of the rectum and vulva. Swelling may appear in the affected areas. This type of Zn deficiency may also be accompanied by inflammations of oral and nasal cavities with excessive salivation, swelling of the gums and teeth grinding (Machen et al. 1996, Hosnedlová et al. 2007). The above changes are symptoms of parakeratosis, a disorder which is probably caused by impaired protein synthesis and activation of Zn-dependent enzymes that are essential for carbohydrate, lipid and nucleic acid metabolism (Al-Saad et al. 2010, Alves et al. 2012). A severe form of the disease is observed in calves with inherited lethal trait A46, where skin changes are accompanied by atrophy of the thymus and significant impairment of T cell-dependent immune response. In the absence of dietary zinc supplementation, the affected calves usually die at the age of 4-6 months (Yuzbasiyan-Gurkan, Bartlett 2006).

SODIUM, CHLORIDE AND POTASSIUM

Sodium and potassium ions are the major cations responsible for the maintenance of physiological blood pH values in animals. Their content differs considerably in intracellular and extracellular fluids (plasma and extracellular, extravascular fluid). Potassium concentrations are higher than Na concentrations inside cells, whereas the reverse is observed in plasma, where Na accounts for 90% of all mineral elements. Sodium and potassium regulate osmotic pressure in body tissues. Different concentrations of Na and K ions on the two sides of the cell membrane contribute to the maintenance of stable resting membrane potential and nerve conduction. Na and K ions affect muscle contractility and tension. Physiological Na concentrations support monosaccharide and amino acid absorption. Bicarbonates and Cl ions constitute the major anions in plasma and interstitial fluid, and Cl ions play a key role in maintaining the acid-base balance in the body. Chloride is a component of hydrochloric acid that is produced by parietal cells for digesting nutritive compounds. Acid production and Cl absorption increase in the perinatal period. In lambs, the amount of hypochloric acid produced in the abomasum increases rapidly and lowers abomasal pH from 7 on day 142 of fetal development to 3 in 2-day-old infants (Gilloteau et al. 2009).

The discussed macroelements are supplied mainly with feed, including milk and milk replacers in young animals and solid feed in adult individuals. The quality and quantity of drinking water are also important. Salt licks can compensate for Na and Cl deficiency resulting from poor quality feed. Na, K and Cl are excreted mainly by the kidneys with urine, and they are evacuated in smaller amounts *via* the gastrointestinal system and the skin. Physiological levels of the discussed macroelements, mostly Na and K, are regulated by the nervous system, including the hypothalamus and the renin-angiotensin-aldosterone (RAA) system, and hormones, including thyroid hormones, and atrial natriuretic factors (Dratwa et al. 2004).

In calves, stress factors such as high stocking density, heat stress and transport, increase the demand for electrolytes. The above can be attributed to the elevated secretion of ACTH from the pituitary gland and higher release of aldosterone and corticosterone from the adrenal cortex. Aldosterone is the key hormone that regulates Na absorption in the kidneys and Na secretion with urine (Fitzsimons 1998). The discussed hormones also increase water and Na uptake through the angiotensin II neuropeptide. In calves, sodium deficiency intensifies stereotypies, which are altered behavioral responses to stress. Phillips et al. (1999) demonstrated that increased Na concentrations in feed improve feed and water uptake, increase body gains and reduce stereotypies such as cross-sucking in calves. The use of electrolyte solutions is recommended to reduce stress before animal handling operations such as transport (Schaefer et al. 1997).

Na, K and Cl deficiencies resulting from low concentrations of dietary macroelements are rarely reported in young ruminants. In calves, lambs and kids, the first symptoms of Na and Cl deficiency include allotriophagy, sparse hair coat, low body gains and lower feed uptake. Other symptoms involve abnormal licking behavior, dirt eating, drinking own or other animals' urine. In ruminants, electrolyte deficiency can be exacerbated by infectious and non -infectious diseases with diarrhoea (Bazeley 2003). Symptoms of dehydration, including weaker skin tension, deep eye sockets, tachycardia and decreased diuresis, are also observed (Guzelbektes et al. 2007). Water and electrolyte deficit treatments generally improve animals' health condition and eliminate clinical symptoms (Naylor 1990, Constable 2003).

CONCLUSIONS

In young ruminants, macroelement and microelement deficiencies contribute to metabolic disorders and clinical changes in individuals and in the herd. They impair the immune response and increase susceptibility to disease, in particular contagious diseases (diarrhoea, respiratory disorders). The mineral content of feed should be regularly monitored to improve animal health and promote healthy growth and development of animals.

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