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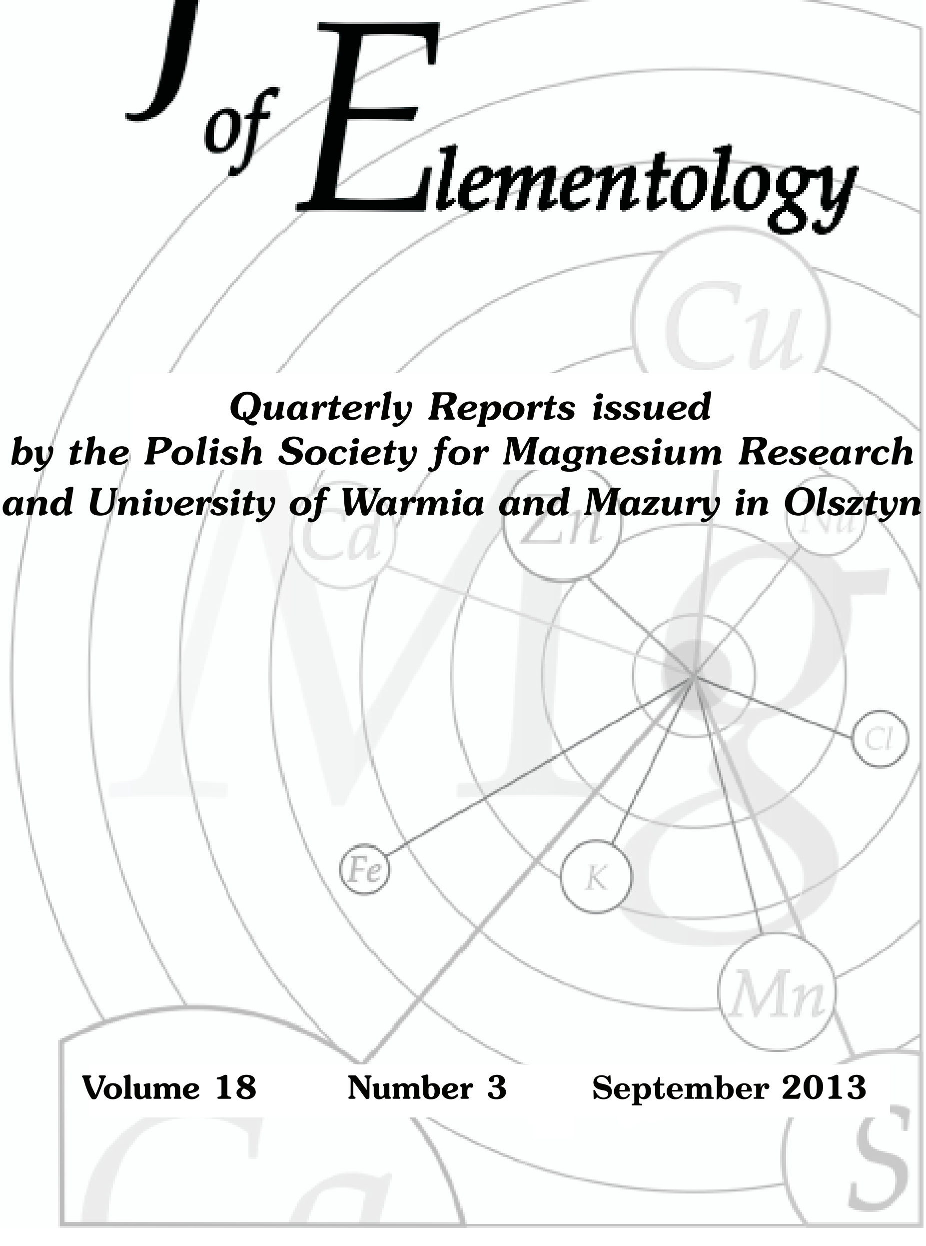

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Editorial Office

University of Warmia and Mazury in Olsztyn
Chair of Agricultural Chemistry and Environment Protection
Oczapowskiego Street 8, 10-719 Olsztyn, Poland
phone:+48 89 5233231
jelementol@uwm.edu.pl

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ORIGINAL PAPERS

EVALUATION OF LICHENS AS BIO-INDICATORS OF METAL POLLUTION

Ali Aslan[1], Hasan Gurbuz, Kenan Yazici[2], Arzu Cicek[3],
Metin Turan[4], Sezai Ercisli[5]

[1]Department of Biology, Atatürk University, 25240 Erzurum, Turkey
[2]Department of Biology, Karadeniz Technical University,
61080 Trabzon, Turkey
[3]Applied Research Centre for Environmental Problems, Anadolu University,
26200 Eskishir, Turkey
[4]Department of Soil Science, Atatürk University, 25240 Erzurum, Turkey
[5]Department of Horticulture, Atatürk University, 25240 Erzurum, Turkey

Abstract

The objectives of this study have been to determine the impact of the distance from a combustor of a cement plant (downwind direction) and duration of exposure to pollution on the bioaccumulation of metals by four lichen species. Nickel, cadmium, chromium, copper and lead accumulated in lichen thalli, with the highest accumulation occurring at 50 m of the cement plant and upon prolonged exposure. In contrast, the concentrations of Al were not consistently affected by the distance from the plant or the duration of exposure. *Pseudevernia furfuracea* was most effective as an indicator of cement dust pollution. We concluded that transplantation of *Pseudevernia furfuracea* on trees or shrubs can be an easy and cost-effective means of Ni, Cd, Cr, Cu and Pb pollution monitoring.

Key words: cement plant pollution, enrichment factor, heavy metal pollution, lichens.

Sezai Ercisli, Department of Horticulture, Atatürk University, 25240 Erzurum, Turkey, e-mail: sercisli@gmail.com, tel.: +90 442 2312599; Fax: +90 2360958

OCENA POROSTÓW JAKO BIOLOGICZNYCH WSKAŹNIKÓW ZANIECZYSZCZENIA METALAMI

Abstrakt

Celem badań było określenie wpływu odległości od komory spalania w cementowni (przy dominującym kierunku wiatru) oraz długotrwałości wystawienia na zanieczyszczenie na bioakumulację metali przez cztery gatunki porostów. Najwyży poziom akumulacji w plechach porostów takich metali, jak nikiel, kadm, chrom, miedź i ołów stwierdzono w odległości 50 m od cementowni i przy długotrwałym narażeniu na zanieczyszczenie. Natomiast stwierdzone zawartości Al w porostach nie były jednoznacznie powiązane z badanymi czynnikami. *Pseudevernia furfuracea* okazał się najbardziej skutecznym wskaźnikiem zanieczyszczenia powodowanego przez cementownię. Wyniki badań dowodzą, że obsadzenie drzew lub krzewów porostami z gatunku *Pseudevernia furfuracea* może stanowić łatwą i tanią metodę monitorowania zanieczyszczenia przez Ni, Cd, Cr, Cu i Pb.

Słowa kluczowe: zanieczyszczenie pochodzące z cementowni, czynnik wzbogacenia, zanieczyszczenie metalami ciężkimi, porosty.

INTRODUCTION

The cement industry generates cement dust, which contains metals such as Cd, Cr, Cu, Ni and Pb (AL-KHASMAN, SHAWABKEH 2006). Although cement factories usually stand far from city centers, their surroundings suffer from pollution. SCHUHMACHER et al. (2009) demonstrated that cement dust and associated chemicals can spread over a large area with wind and rain, accumulating in lichens, plants, animals and soils, located downwind from a cement plant.

Lichens have been used as indicators of air pollution and biosorption (YAZICI, ASLAN, 2006, CICEK et al. 2008, BINGOL et al. 2009). Owing to their peculiar anatomical, morphological and physiological characteristics, lichens are one of the most valuable biomonitors of atmospheric pollution (BATTAL et al. 2004). They can be used as sensitive indicators to estimate biological effects of pollutants by recording changes in a given community, and as accumulative monitors of persistent pollutants, which can be evaluated by assaying their trace element content. Many epiphytic lichen species are sensitive to air pollutants and have been used widely to detect changes in air quality (SHOWMAN 1988). As a bioindicator method, lichen mapping is useful because the fieldwork can be completed by one person without expensive equipment. In order to be a good indicator, a given lichen species should be expected togrow at every site (SHOWMAN 1988), and species should have been widespread in an area submitted to assessment before it became affected by air pollution (CALVELO, BACCALA 2009).

Our objectives were to evaluate the effectiveness of four lichen species as bioindicators of air pollution from the cement industry. Specifically, we studied metal accumulation in four lichen species exposed to air emissions (1) at some distance from the cement plant (50, 100 and 200 m), (2) for the duration of exposure equal four, eight, and twelve months. Two other factors analysed were (3) heavy metals originating from the factory or from natural parent material, according to the enrichment factor, and (4) the reliability of the tested lichens as biomonitoring tools for the evaluation of levels of atmospheric pollutants.

MATERIAL AND METHODS

The study area is located in eastern Turkey (N 39° 55´ 31′′, E 40° 40’ 12″). The region has a continental climate, characterized by hot, dry summers and cold, snowy winters. Eastern Anatolis is the coldest region in Turkey and has a very short vegetation period. Relative humidity averages 60%. The soil is classified as Vertisol according to the USA taxonomy, with parent materials mostly consisting of volcanic, marn and lacustrin transported material. The major type of plant cover is steppe. Forests are located in the higher parts of mountains in the north and northeast. Woodland communities include *Pinus sylvestris. Picea orientalis, Fagus orientalis, Quercus petrea, Juniperus oxycedrus, Abies nordmanniana, Ulmus minor,* and *Fraxinus excelsior* species and conifers, mostly at altitudes of 700-2500 m. The Aşkale cement plant is located 55 km west of the town of Erzurum. It has been operating in the area since 1974.

Study area and climatic condition

Four lichen species were collected from ten different provinces in Eastern Anatolia, Turkey, in July 2008. The species, identifed using various keys (Aslan 2000, Aslan et al. 2002), were *Cetraria islandica* (L.) Ach., *Lobaria pulmonaria* (L.) Hoffm., *Pseudevernia furfurace*a (L.) Zopf., and *Usnea longissima* Ach. Lichen species were transplanted on trees on the cement plant premises, placed 50, 100 or 200 m downwind from the combustion unit of the plant, in 10 replications. Both soil and lichens samples were taken four, eight and twelve months after the transplantion of the lichens into the area. Twenty surface (0-10 cm) soil and 20 lichens samples were taken at each sampling time.

Soil Analysis

Soil samples were air-dried, crushed and passed through a 2-mm sieve prior to chemical analysis. Total Cu, Pb, Ni, Al, Cr and Cd were determined after wet digestion using a HNO_3-HCl acid mixture 10 ml (1:3 v/v) according to Mertens (2005a,b), using a Bergof Speedwave MWS-2 microwave (Bergof Speedwave Microwave Digestion Equipment MWS-2) and an Optima 2100 DV Inductively Couple Plasma spectrophotometer (Perkin-Elmer, Shelton, CT, USA).

Lichen Analysis

Lichen samples were oven-dried at 68°C for 48 h and ground to pass through a 1-mm sieve. Total Cu, Pb, Ni, Al, Cr and Cd were determined after microwave wet digestion using a HNO_3-H_2O_2 acid mixture (2:3 v/v) according to Mertens (2005a,b) in a Bergof Speedwave MWS-2 microwave (Bergof Speedwave Microwave Digestion Equipment MWS-2) (Brodo et al. 2001), and an Optima 2100 DV Inductively Couple Plasma spectrophotometer (Perkin-Elmer, Shelton, CT, USA).

Statistical analysis

Data gathered from individual exposure times (four, eight, and twelve months) were subjected to analysis of variance (ANOVA) and mean separation using Duncan's test at $P<0.05$ of SPSS 13.0 statistical program,with the distance (50, 100 and 200 m) as a fixed effect and replications as random effects.

RESULTS AND DISCUSSION

Soil transition and essential, non-essential and basic metal content

Essential (Cu), non-essential (Ni, Cr, Cd), and basic metal (Al and Pb) concentrations in the soil samples varied with the distance (50, 100 or 200 m) from the combustor of the cement plant (predominant wind direction). It was demonstrated that cement fumes increased the content of all of the metals, especially non-essential and basic ones. There were statisitically significant differences between the distances in respect of the total element concentration (Table 1). The total Cu, Ni, Cr, Cd, Al and Pb content near the cement plant soils (50 m) was considerably higher than in samples taken far away from the cement plant (100 and 200 m) – Table 1. The levels of the determined metals decreased rapidly with the distance, reaching the background amounts at a distance of 100 m. In general, Cu, Ni, Cr, Cd and Pb were more abundant in the topsoil (0-10 cm) than the deeper soil layers (10-30 cm) (data not given). These metals originating from industrial operations are distributed in soil by the atmospheric deposition as a function of the distance.

Lichen transition and essential, non-essential and basic metal content

It was shown that cement fumes raised the content of non-essential and basic metals in lichens. Essential (Cu), non-essential (Ni, Cr, Cd) and basic metal (Al and Pb) concentrations in lichen samples varied with the distance from the combustor of the cement plant and duration of exposure. There were statisitically significant differences between the distance and duration

Table 1

Mean (n=10) and ranges for the descriptive parameters of 50 soil samples (0-10 cm depth) from 50, 100 or 200 m downwind from the combus-tion unit of the plant before the lichens transplant

		Unpolluted area		Polluted area					
Parameter	units	mean	range	mean 50 m	range	mean 100 m	range	mean 150 m	range
General soil properties									
pH		7.8 ^{b*}	7.20-8.10	8.2 ^{a}	7.25-8.70	7.96 ^{ab}	7.15-8.40	7.82 ^{b}	7.10-8.14
EC	(ms cm^{-1})	366 ^{b}	310-430	405 ^{a}	320-460	394 ^{ab}	325-445	370 ^{b}	310-420
OM	(%)	1.2 ^{c}	0.8-2.2	1.4 ^{a}	0.7-2.8	1.4 ^{a}	0.6-2.6	1.3 ^{b}	0.4-1.9
$CaCO_3$	(%)	9.1 ^{c}	6.8-23.5	13.6 ^{a}	6.0-30.5	11.4 ^{b}	6.4-24.7	8.9 ^{c}	6.4-20.5
CEC	(cmolc kg^{-1})	43.5 ^{ab}	33.5-69.7	48.2 ^{a}	35.5-74.3	42.8 ^{b}	30.5-60.2	40.8 ^{c}	31.4-58.7
Essential transition metals									
Cu	(mg kg^{-1})	3.4 ^{c}	2.2-5.2	7.8 ^{a}	2.2-10.8	6.2 ^{b}	2.4-8.4	4 ^{c}	2.0-6.1
Non-essential transition metals									
Cd	(mg kg^{-1})	10.1 ^{d}	6.8-15.9	24.9 ^{a}	7.4-60.6	18.6 ^{b}	7.04-48.4	13.2 ^{c}	7.22-27.5
Cr	(mg kg^{-1})	121 ^{d}	98-155	197 ^{a}	114-205	154 ^{b}	102-196	134 ^{c}	98-172
Ni	(mg kg^{-1})	31 ^{d}	27-44	68 ^{a}	28-74	51 ^{b}	22-63	34.8 ^{c}	23-42
Basic metals									
Pb	(mg kg^{-1})	0.6 ^{d}	0.2-2	2.3 ^{a}	0.2-4	1.8 ^{b}	0.2-3.1	1.2 ^{c}	0.2-2.8
Al	(mg kg^{-1})	10 940 ^{c}	9 760-21 456	11 430 ^{a}	9 760-23 423	11 000 ^{b}	9 620-20 300	10 900 ^{b}	9 500-20 000

*Means followed by different letters are significantly different (α</0.05) tested in rows.

of exposure variants in respect of total element concentrations (Figures 1, 2). *Pseudevernia furfuracea* was the most effective indicator of cement dust pollution. Nickel, Cd, Cr, Cu and Pb accumulated the highest in lichen thalli within 50 m of the plant and 12-month period of exposure, while the concentrations of Al were not consistently affected by the distance from the plant and duration of exposure.

Concentrations of some essential (Cu), non essential (Ni, Cd, Cr,) and basic (Pb) metals in soil and in lichen species near the cement plant were higher than in samples taken far away from it. They decreased rapidly with the distance from the road, and reached the background level within the 100 m transect. The relationship between the content of metals in soil and in lichens versus the distance from the cement plant can be described using a power function (Figures 1, 2). But cement fumes did not affect the basic metal (Al) content in lichens and the mean differences for this element were not statistically significant.

The above findings provide evidence that the cement plant and industrial waste incinerator are sources of metals that influence the elemental composition of the nearby topsoil. In the study area, higher concentrations of Ni, Cd, Cr, Cu and Pb may be explained by rock crushing during cement production. Cement is made through the reaction of crushed and ground calcareous rocks (limestone or chalk) and argillaceous rocks (clay or shale) at high temperatures.

The distribution pattern of the total concentration of an element can be used to judge whether the enrichment with that element is caused by the fume pollution, farming practice or natural parent material. The enrichment factor (EF) ($EF_{soil}= (M/Fe)_{soil}/(M/Fe)_{control}$) is also used as an index to distinguish whether a given element in soil originates from natural or anthropogenic sources. According to OLIVIA and ESPINOSA (2007), the enrichment factor value >2 is considered as a critical level of enrichment contributed mainly by anthropogenic inputs.

Solid particles can have a negative effect on the air quality, and cement manufacturing is among the industries responsible for particle pollution. Although cement factories are generally established far from city centers, local areas are affected negatively. Cement dust spreads over a large area through wind, rain, etc., accumulates in and on plants, animals and soil, and can be very harmful to human health (AYVAZ 1992).

Agricultural soils receive metals mainly from fertilizers, manure, pesticides, wastewater and other scattered point pollution sources such as industries, traffic emissions, incineration facilities, etc. The geographical distribution of copper in the investigated area is mainly dominated by the cement plant emissions. The lowest value was measured in the most distant sites from the cement plant, where it fell down to 5.6 mg kg^{-1} dry lichen (Figure 1). This proves that anthropogenic activities are the main source of the metal in soil.

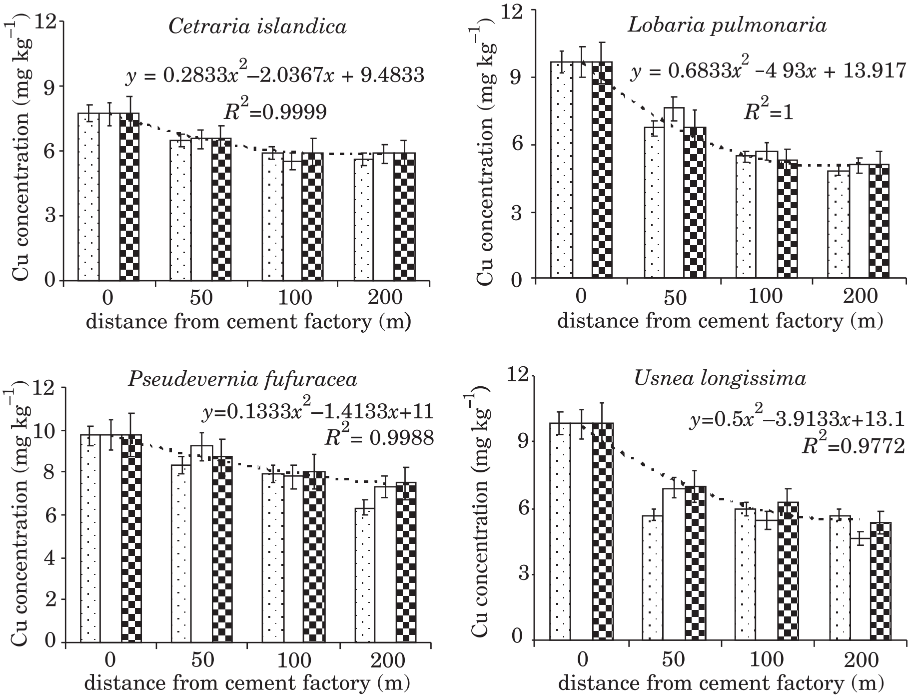


Fig. 1. Impact of location (distance relative to a cement factory) and duration of exposure on the Cu (essential transition metals) content of four lichen species

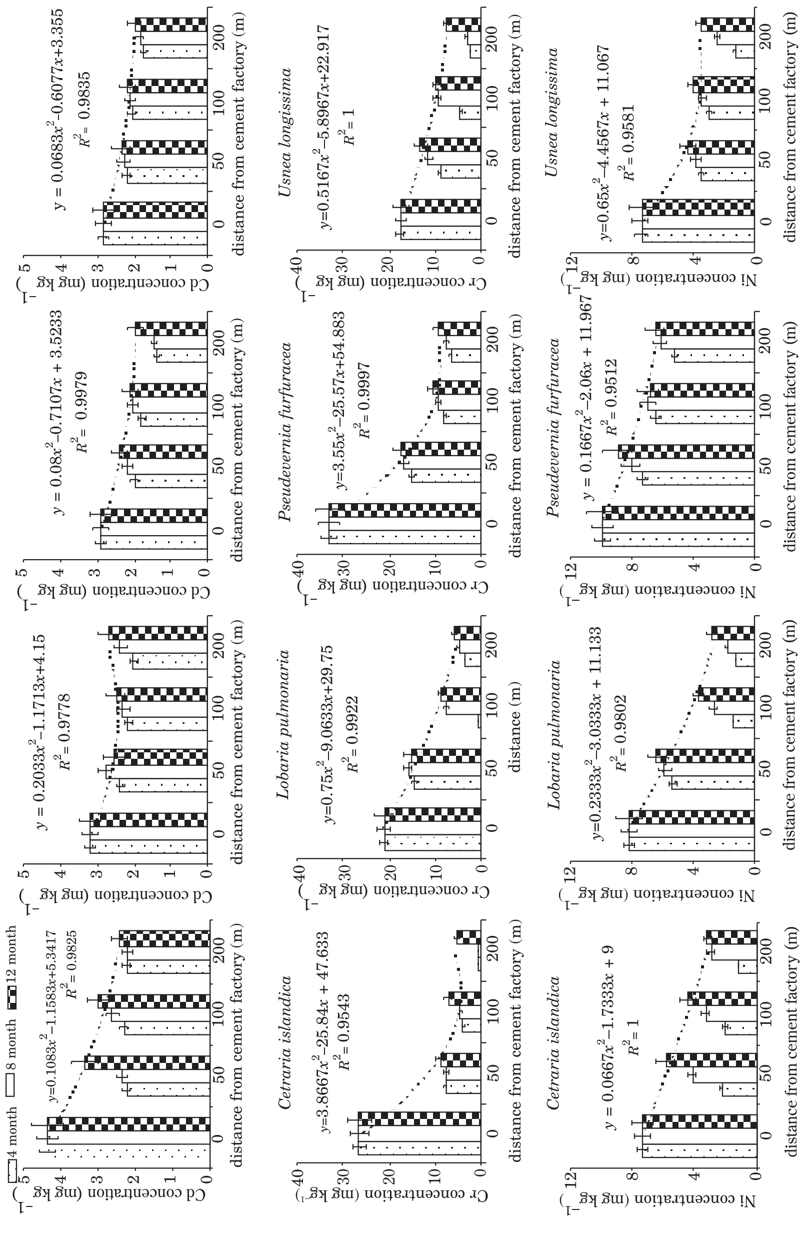
Copper is a trace element in most of soils. It is an essential element for plants, animals and people, but has also been implicated as a toxic element to all organisms. According to the WHO, the copper content in soil ranges between 2 and 60 mg kg^{-1} (WHO, 1998).The toxic concentration was set at 130, 70 and 100 mg kg^{-1} by the ICRCL (1987), ERD (1999) and Efroymson et al. (2007), resepectively, In the Netherland, UK, Hong Kong, and Turkey, the governments defined such levels at 36 mg kg^{-1}, 27 mg kg^{-1}, 24.8 mg kg^{-1}, and 140 mg kg^{-1}. Accordingly, the concentation of total Cu of all lichen samples (ranged from 5.6-7.73 mg kg^{-1}) in this study area was not toxic.

This study, however, has demonstrated that the main source of Cu pollution in the analysed soil was the cement plant, and the affected area was only in the direct vicinity to the plant (Figure 1). There were correlations beetwen the soil and lichen Cu content versus the distance (50, 100 or 200 m) from the combustor of the cement plant, and the Cu concentrations in the polluted soil and lichens revealed similar trends. The Cu content in lichens determined in our study was higher than reported by Bajpai et al. (2010) for north India. On the other hand, the Cu content in the polluted soil determined in our study was higher than observed by Al-Khasman and

Shawabkeh (2006) in Jordan but lower than obtained by Thornton (1991) in London, Paterson et al. (1996) in Aberdeen, Wong et al. (1996) in Hong Kong, Li et al. (2001) also in Hong Kong, and Banat et al. (2005) in central Jordan.The relationships between the content of the metal in soil or in lichens and the distance from the cement plant can be described using a power function. The coefficient of determination for the fitted regression model was significant (r^2 = 0.99). The EF values of Cu ranged from 1.40 to 2.2 and increased nearer the cement plant (Figure 1). The EF value of Cu can be a good criterion for distinguishing the pollution from cement plant emissions. The highest Cu uptake was detected by *Pseudevernia furfuracea* species at 12-month period. This finding supports the claim that the cement plant emissions contributed to the Cu pollution. Moreover, Cu accumulated in the investigated area at an accumulation rate similar to that of Cd, both of the metals being the main pollutants from the cement plant's emissions.

Chromates are among allergens that are unavoidably widespread in the environment. Because chromate salts are used in the machine industry and cement forms contain a certain amount of chromate, chromium sensitivity is considered an industrial problem (WHO 1998). Chromium is a trace element in most of soils. It is not an essential nutrient of plants, but is a trace essential element for animals and people. It is also considered toxic, especially of its Cr (VI) form. The toxic concentrations were set at 100 mg kg^{-1} by Anon (1983), while Paterson et al. (1996) set the threshold at 24 mg kg^{-1}, and the Ball et al. (1991) claimed it was 84 kg^{-1}. The concentration of the soil total Cr in the study area ranged from 0.6 to 26.5 mg kg^{-1} (134 1-197 mg kg^{-1} in Table 1) . Thus, the soil Cr content data showed that the study area was polluted with Cr. The Cr content in the polluted soil and lichens had similar trends in respect to the distance (50, 100 or 200 m) from the combustor of the cement plant (Figure 2). The Cr content in lichen tissues determined in our study was higher than obtained by Tuncel and Yenisoy-Karakas (2003) in Western Anatolia, Jeran et al. (1996) in Slovenia, Steinnes et al. (1992) in Norway and Bajpai et al. (2010) in north India, but lower than achieved by Sloof and Wolterbeek (1991) in the Netherlands. On the other hand, the Cr content in the polluted soil determined in our study was higher than reported by Al-Khasman and Shawabkeh (2006) in Jordan, Thornton (1991) in London, Paterson et al. (1996) in Aberdeen, Wong et al. (1996) in Hong Kong, Li et al. (2001) in Hong Kong, Banat et al. (2005) in Central Jordan and Calzoni et al. (2007) in Italy. The Cr pollution of arable lands was mainly from the cement plant emission, with the atmopsheric deposition being the major source. The total concentration of Cr in the analysed soil decresed with an increase in the distance from the cement plant, while thee total Cr content in lichen tissues rose with the increasing duration of exposure. These correlations proved that the predominant pollution source of Cr in this area was the cement plant's emission and the affected area was only the immediate surroundings of the main cement plant facilities (Figure 2). The relationship between the content of Cr in soil or lichens and the distance from the cement plant can be described using a power function. The coefficient of determination for the fitted regression model was significant ($r^2 = 0.99$). The EF values of Cr ranged from 1.20 to 1.6 and increased nearer to the cement plant (Figure 2). The EF value of Cr can be a good critical value to distinguish the pollution from cement plant emission. The highest Cr uptake was detected by the *Pseudevernia furfuracea* species. This justifies the claim that emmissions from the cement plant are the principal contributor to the local Cr pollution. This also support the thesis that Cr was accumulated in the study area, and the accumulation rate was similar to that of Cd, both of the metals being the main pollutants from the cement plant. In the cement industry, the linings for rotaries contain chromium, which could be liberated by wear and friction, thus becoming a source of chromium in the soil and lichen samples (ANON 1983).

Cadmium is a trace element in most of soils. It is not an essential nutrient of plants, but is a toxic element to plants, animals and human beings. Cadmium is emitted into the atmosphere from natural sources, mainly basalt rocks, and from anthropogenic sources. Metallurgy (drying of zinc concentrates and roasting, smelting and refining of ores) is the largest source of anthropogenic atmospheric cadmium emissions, followed by waste incineration and other sources, including the production of batteries, fossil fuel combustion and generation of dust by industrial processes such as cement manufacturing (YAMAGATA 1970). Generally, cadmium is found in lower concentration than other metals in soil. In the investigated area, the high levels of Cd in lichens are associated mainly with emissions from the cement industry. According to ELLIS and REVITT (1982), zinc and cadmium may be derived from the mechanical abrasion of vehicles and also associated with tyre wear. Cadmium is also a common component of leaded petrol, diesel oil and even unleaded petrol (HUANG et al. 1994). Cadium pollution is associated with the wearing out of tyres and brakes, and with industrial fumes (BALL et al. 1994). The toxic concentrations were set at 1 and 3 mg kg^{-1} by ANON (1983), while BANAT et al. (2005) quote the amount of 5 mg kg^{-1}, and the municipalities of London, and Hong Kong sets target level at 1 and 2 mg kg^{-1} (THORNTON 1991, LI et al. 2001). The concentration of the total Cd in the analysed soil ranged from 2.0 to 4.3 mg kg^{-1} (13.2 to 24.9 in Table 1). Thus, the data showed that the study area suffered from Cd pollution. The cadmium pollution of arable lands was mainly due to the cement plant's emissions; the contribution of Cd from the atmospheric deposition prevailed (BAJPAI et al. 2010). The Cd content in the polluted soil and lichens showed similar trends in respect to the distance (50, 100 or 200 m) from the combustor of the cement plant. The Cd content in lichens determined in our study results was higher than obtained by TUNCEL and YENISOY-KARAKAS (2003) in Western Anatolia, JERAN et al. (1996) in Slovenia, or FREITAS et al. (1999) in Portugal. Moreover, the Cd content in the polluted soil determined in


4 month
8 month
12 month
Cetraria islandica
y=0.1083x^2−1.1583x+5.3417
R^2 = 0.9825
Lobaria pulmonaria
y = 0.2033x^2−1.1713x+4.15
R^2 = 0.9778
Pseudevernia furfuracea
y = 0.08x^2−0.7107x + 3.5233
R^2 = 0.9979
Usnea longissima
y = 0.0683x^2−0.6077x+3.355
R^2 = 0.9835
Cd concentration (mg kg^−1)
0
1
2
3
4
5
Cetraria islandica
y=3.8667x^2−25.84x + 47.633
R^2 = 0.9543
Lobaria pulmonaria
y=0.75x^2−9.0633x+29.75
R^2 = 0.9922
Pseudevernia furfuracea
y=3.55x^2−25.57x+54.883
R^2 = 0.9997
Usnea longissima
y=0.5167x^2−5.8967x+22.917
R^2 = 1
Cr concentration (mg kg^−1)
0
10
20
30
40
distance (m)
Cetraria islandica
y = 0.0667x^2−1.7333x + 9
R^2 = 1
Lobaria pulmonaria
y=0.2333x^2−3.0333x + 11.133
R^2 = 0.9802
Pseudevernia furfuracea
y = 0.1667x^2−2.06x + 11.967
R^2 = 0.9512
Usnea longissima
y=0.65x^2−4.4567x + 11.067
R^2 = 0.9581
Ni concentration (mg kg^−1)
0
4
8
12
0
50
100
200
distance from cement factory (m)

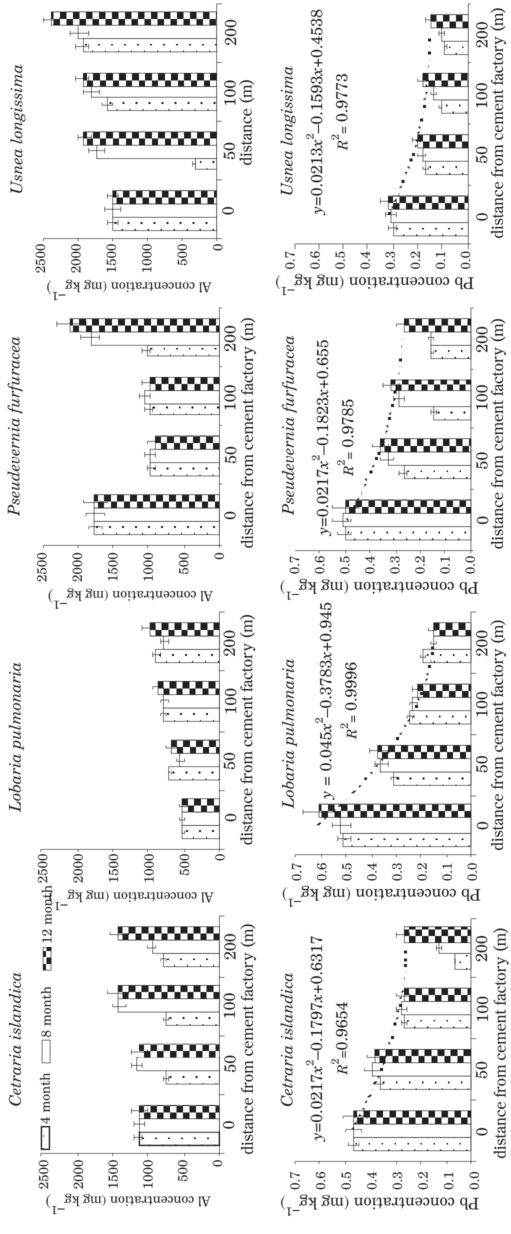


Fig. 2. Impact of location (distance relative to a cement factory) and duration of exposure on Cd, Cr and Ni (non-essential transition metals) and Al and Pb (basic metal) content of four lichen species

our study was higher than reported by Al-Khasman and Shawabkeh (2006) in Jordan, Thornton (1991) in London, and Banat et al. (2005) in Central Jordan.

The concentration of the total Cd in soil in the study area decresed at larger distances from the cement plant, while the total Cd content in lichen species rose with the longer exposure time. These facts proved that the dominant source of pollution with Cd in this area's soil was the cement plant's emission and the affected area was limited to the nearest environs of the plant (Figure 2). The relationship between the content of Cd in soil or lichens and the distance from the cement plant can be described using a power function. The coefficient of determination for the fitted regression model was significant (r^2 = 0.97). The EF values of Cd ranged from 1.40 to 2.5 and increased with nearer to the cement plant (Figure 2). The EF value of Cd can be a good critical value to distinguish the pollution from cement plant emission. The highest Cd uptake was detected by *Cetraria islandica* species. This justifies the conclusion that emmissions from the cement plant contribute to the Cd pollution, but without more sophisticated calculations it is impossible to weigh out how much of it comes fromm the cement plant. The achieved data give a warning about the pollution with Cd in the analysed area.

Nickel is a trace element in soils. It is an essential element for animals and some plants. Some reports found it was richer in soils near cement plants, but no direct evidence has been produced to conclude that the said enrichment came from cement plants' emissions (Monaci et al. 2000, Olivia, Espinosa 2007). However, it was found that fossil fuel emissions contribute to nickel contamination The toxic concentration was set at 70, 38, 75 and 300 mg kg^{-1} by ICRCL (1987), ERD (1999), Anon (1983) and Efroymson et al. (2007), respectively, while the Netherland government sets their target level at 35 mg kg^{-1} (Lijzen et al. 2002, Anon 2007). The concentration of the total Ni in all the lichen samples in the study area ranged from 1.2 to 9.3 mg kg^{-1} (Figure 2).

The Ni content in the polluted soil and lichens had similar trends in respect to the distance (50, 100 or 200 m) from the combustor of the cement plant. The Ni content in lichen tissues determined in our study was lower than obtained by Bajpai et al. (2010) in North India. On the other hand, the Ni content in the polluted soil determined in our study results was higher than obtained by Calzono et al. (2007) in Italy. This proves that the source of Ni pollution in the analysed soil was mainly the emission from the cement plant, and the affected area was the plant's immediate surroundings (Figure 2). The relationship between the content of Ni in soil or lichens and the distance from the cement plant can be described using a power function. The coefficient of determination for the fitted regression model was significant (r^2 = 0.95). The EF values of Ni ranged from 1.20 to 2.1 and increased nearer to the cement plant (Figure 2). The EF value of Ni can be a good critical value to distinguish the pollution from the cement plant's emissions. The highest Ni uptake was detected by *Pseudevernia furfuracea* species at the 12-month sampling period. This justifies the thesis that emssions from the cement plant contribute to the local Ni pollution.

Aluminum is the third richest element in soil, but it is not an essential nutrient for most plants. Although it was supposed to be a traffic pollutant, as it was found in cars' emissions due to being used in catalytic mufflers (LIJZEN et al. 2002), or in fumes emitted by car manufacturing plants, there is no evidence to prove that these sources pollute soils (MONACI et al. 2000). The Al content in the analysed soil and lichens had similar trends in respect to the distance (50, 100 or 200 m) from the combustor of the cement plant. The Al content in lichens determined in our study was lower than obtained by TUNCEL and YENISOY-KARAKAS (2003) in Western Anatolia, and by BAJPAI et al. (2010) in North India, but higher than reported by STEINNES et al. (1992) for Norway. The soil's total Al concentration decreased with an increase in the distance from the cement plant (Figure 2). The highest Al uptake was determined by *Usnea longissima*, and but all of the EF values were in a narrow range (0.9 to 1.0), which was due to the excessively high Al concentration in soil and the fact that the cement plant was not the main source of Al pollution. When the elemental composition of lichen tissues was compared to the sampling time, the highest Al content in lichens was determine at the 12-month period (Figure 2).

Lead is a trace element in soil, known for its toxicity to organisms. It is a component of leaded petrol, diesel oil and even unleaded petrol (<0.015 g L^{-1}). It is implicated as a main pollutant from traffic and industrial activities (SWIETLICKI et al. 1996, JANSSEN et al. 1997). The toxic concentration was set at 500 and 813 mg kg^{-1} by the ICRCL (1987), while EFROYMSON et al. (2007) and ERD (1999) set it at 50 and 120 mg kg^{-1}, respectively, and the governments in London, Hong Kong and Turkey set their target level at 85 mg kg^{-1}, 30 mg kg^{-1}, 93 mg kg^{-1} and 300 mg kg^{-1} (ANON 1983, THORNTON 1991, LI et al. 2001, LIJZEN et al. 2002, ANON 2007). The concentration of total Pb in the study area ranged from 0.1 to 1.8 mg kg^{-1} (1.2 to 2.3 in Table 1). Thus, the data from soil analyses showed that the Pb pollution was not severe. The concentration of the total pB in soil in the study area decresed with an increase of the distance from the cement plant, while an increase in the total Pb content in lichen species occurred with the increasing sampling time. This proved that the source of Pb pollution of soil in this area was mainly the cement plant, and the affected area was only the land near to the main cement plant facilities (Figure 2). The relationship between the content of Pb in soil or lichens and the distance from the cement plant can be described using a power function. The coefficient of determination for the fitted regression model was significant ($r^2 = 0.97$). This could be attributed to the fact that thee cement industry requires substantial amounts energy for the process and production of cement, which is supplied by burning fossil fuel. Another source of Pb pollution could be the traffic in and around the cement plant (ICRCL 1987). The Pb content in the polluted soil and lichen had similar trends in respect to the distance (50, 100 or 200 m) from the combustor of the cement plant. The Pb content in lichens determined in our study was lower than obtained by Bajpai et al. (2010) in north India. Moreover, the Pb content in the polluted soil determined in our study was lower than obtained by Al-Khasman and Shawabkeh (2006) in Jordan, Thornton (1991) in London and Calzoni et al. (2007) in Italy. The EF values of Pb ranged from 1.20 to 2.1 and increased closer to the cement plant (Figure 2). The EF value of Pb can be a good critical value to distinguish the pollution from a cement plant's emissions. The highest Pb uptake was detected by *Pseudevernia furfuracea* species at a 12-month sampling period. The Pb concentration reached a constant level over 100 m distance from the cement plant. This justified the claim that the cement plants could contribute to Pb pollution.

CONCLUSIONS

The data obtained in this study demonstrate that metal concentrations in urban soils can be used as a powerful geochemical indicator for monitoring the impact of anthropogenic activity, provided that the background levels have been correctly interpreted and established. The distribution of metals in the analysed soil indicated that the area had been affected by anthropogenic activity, in particular by the cement industry, leading to a high accumulation of heavy metals compared with the natural background levels. The distribution of the metal concentration of the soil in the study area indicated that the cement industry together with other industrial activities were responsible for most of the metal pollution, as the highest metal concentrations were found close to the cement factory. The conclusion regarding anthropogenic influences was further corroborated by analyses of soil and lichen samples collected around the cement factory at sites chosen according to the prevailing wind direction. The results of the statistical analysis and distribution of the pollutant metals suggested that cement emissions represented the most important source of contaminants for the investigated area. The contamination of soils near the cement lant with heavy metals emmited by the factory is rarely detected at a distance of over 200 m, but closer than 100 m from the factory soil is found to be contaminated with Ni, Cd, Cr, Cu and Pb. Further away from the cement plant, the EF factor reached values that proved the contamination was not predominantly from the cement emission. They included the critical value of about 1.5. In conclusion, it can be said that an appropriate and safe distance of over 200 m from a cement plant should be selected for residential purposes in order to avoid contamination with Ni, Cd,Cr, Cu and Pb. In our studz, this was the distance where the background levels of Ni, Cd, Cr, Cu and Pb were almost reached. The coefficient of determination for the fitted regression model was significant (r^2 = 0.95). This implies that the Ni, Cd, Cr, Cu and Pb content of soil and lichens was strongly dependent on the distance from the cement plant.These findings suggest that the lichen species *Pseudevernia furfuracea* and *Usnea longissima* present in the region can be used to monitor pollution originating from a cement plant origin, that particularly *Pseudevernia furfuracea* is a very good indicator of such contamination. Because lichens grow very slowly and are rarely encountered in polluted areas, lichen transplantation seems to be a solution. Transplantation of *Pseudevernia furfuracea* could be an easy and cost-effective means of air pollution monitoring and could provide valuable data for undertaking preventive measures. In this work, our goal was to contribute to lichen studies, which are of great importance for bio-monitoring, and this article disucsses metal pollution originating from a cement plant, using structural features of lichens. Further studies should be conducted, including determinations of pollution in different soil profile depths near cement plants and of the hiperaccumlator capacity of lichen species across the region.

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MANGANESE MOBILITY IN SOILS UNDER THE IMPACT OF ALKALINE DUST EMISSION

Halina Dąbkowska-Naskręt, Hanna Jaworska

Chair of Soil Science and Soil Protection
University of Technology and Life Sciences in Bydgoszcz

Abstract

Manganese is required by plants for photosynthesis. It is also is a key activator in a number of critical processes in plant tissues. Moreover, it has been proven that Mn is an essential element for animals and people.

Manganese deficiency may occur in soils that are sandy alkaline or calcareous. The objective of the study was to asses the long-term impact of alkaline cement dust, emitted by the Lafarge Cement Plant in Bielawy (Kujawy region) on manganese availability in soils in the vicinity of the source of emission.

The mobility of Mn was estimated by DTPA-extraction of soil samples. It was stated that DTPA-extractable Mn was in the range of 0.81-12.07 mg kg^{-1} i.e. below the recommended limits for the proper plant growth. The lowest content was detected in soils adjacent to the cement plant, with the highest pH due to the most intensive alkaline dust emission. Cement dust acts like a liming substance and affects manganese mobility.

Key words: manganese, mobility, cement dust, alkalization.

MOBILNOŚĆ MANGANU W GLEBACH BĘDĄCYCH POD WPŁYWEM PYŁÓW ALKALICZNYCH

Abstrakt

Mangan jest niezbędny dla roślin w procesie fotosyntezy oraz jako aktywator wielu procesów zachodzących w tkankach roślinnych. Stwierdzono także, że jest on istotnym pierwiastkiem dla zwierząt i człowieka.

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prof. dr hab. Halina Dąbkowska-Naskręt, Chair of Soil Science and Soil Protection, University of Technology and Life Sciences, 85-029 Bydgoszcz, Bernardyńska 6, e-mail: dabkowska@utp.edu.pl

Niedobór manganu występuje w glebach piaszczystych, węglanowych lub o odczynie alkalicznym. Celem badań była ocena wieloletniego wpływu emisji pyłu cementowego o odczynie alkalicznym emitowanego przez Zakłady Cementowe Lafarge S.A. w Bielawach (Kujawy) na dostępność manganu w glebach w sąsiedztwie źródła emisji.

Mobilność manganu oznaczono przez ekstrakcję próbek gleby roztworem DTPA. Stwierdzono, że zawartość DTPA – ekstrahowanego Mn była w zakresie 0.81-12.07 mg kg^{-1}, tj. poniżej wartości deficytowych dla roślin. Pył cementowy działa na gleby jak materiał wapnujący i wpływa na mobilność manganu.

Słowa kluczowe: mangan, mobilność, pył cementowy, alkalizacja.

INTRODUCTION

Manganese is an essential trace element which occurs in varying amounts in all tissues. It is a constituent or activator of enzymes in plants and animals. This micronutrient is required for photosynthesis and is a key activator in a number of critical metabolic processes in plants (Yu, Rengel 1999). Moreover, Mn is important in the development of resistance in plants to root and foliar diseases of fungal origin, lowering the inoculum potential of soil-borne pathogens. Animals need Mn for neural development (Froslie 1990). It has also been proven that Mn is an essential element for humans (Van Campen 1991). It was reported that Mn^{+2} ions protect biological components and living cells from oxidative damage (Varani et. al. 1991).

Manganese deficiency occurs in soils that are alkaline, calcerous, saline or sandy. The concentration of available Mn is also related to the cropping system or the way land is maintained (Żarczyński et al. 2011). Yields of crops grown on calcerous soils are frequently limited by Mn deficiency resulting from a low Mn availability rather than a low Mn content in the soil.

Antropogenically alkalized soils may suffer from a low or deficit level of this micronutrient in an available form. Long-term exposure to alkaline dust pollution from a cement plant has resulted in soil alkalization (Cieśla et al. 1994). The problem of pollution is caused by the production, handling and transportation of cement. Heavy metals are among the best-known substances emitted during the process of cement manufacture. Furthermore, alkaline dust has a harmful effect on the soil environment. Air pollutants generated during the cement manufacturing process consist primarily of particulates from raw and finished materials as well as by-products of fuel combustion. The raw material – a combination of calcareous rock (limestone) and aluminosilicate material is brought to temperatures up to 1450°C to decarbonate the calcareous material and to produce cement clinker. Portland cement is a fine powder comprised of calcium oxide (60 to 67%) silicon oxide (17 to 25%) aluminium trioxide (3 to 8%) and ferric oxide (0-5%). The cement and lime industry is among the industries generating most dust (Farmer 1993).

The objective of the present study has been to asses the long-term impact of alkaline cement dust on the mobility of manganese in soils adjacent to a cement plant. The Lafarge Cement S.A. plant, located in Bielawy (Kujawy region), has been chosen for the study. The plant is a serious source of environmental dust pollution.

The scarcity of published information about the Mn status in soils contaminated with cement dust encouraged the above study undertaken in an attempt to fill this gap in our knowledge.

MATERIAL AND METHODS

The analyzed location lies in the rural area of Kujawy region. The research material comprised samples of sandy soils, taken at different distances from the pollution emitter, i.e. the Lafarge Cement S.A. plant in Bielawy (Figure 1). Soils were sampled from two depths (0-20 cm and 20-40 cm) at six sites located in different distances from the source of dust emission. The soil material was sampled once a year for six years (2006-2011).

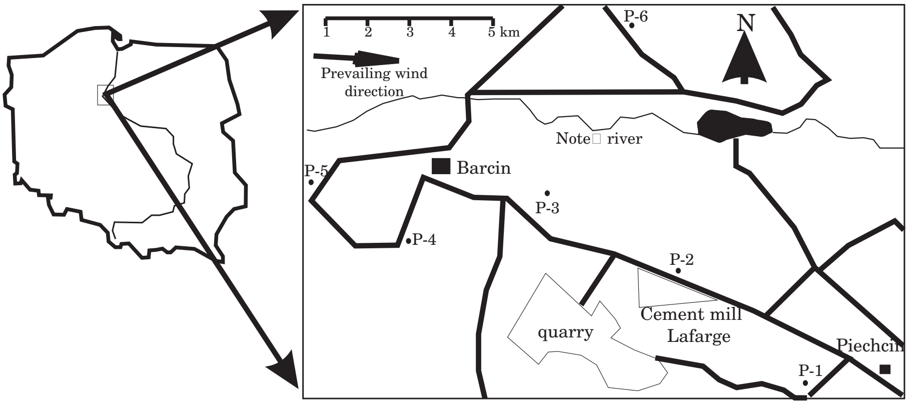

Fig. 1. Location of soil samples

The soil samples were air dried and crushed to pass through a 2 mm mesh sieve. The soil's pH was measured in a 1:2,5 (w:v) soil to water suspension using a glass electrode pH-meter.

The soil samples were digested in a mixture of HCl and HNO_3 concentrated acids for the determination of total amounts of manganese. Manganese availability to plants was estimated by extracting with DTPA-solution, according to Lindsay and Norvell (1978). The concentrations of Mn in solutions were measured by atomic absorption spectrometry on a PU 9100 X Philips instrument. All soil samples were analyzed in triplicate.

The statistical analysis of the results was performed with a Statistica 8.0 PL computer programme. The probability level of $\alpha = 0.05$ was selected to establish statistical significance.

RESULTS AND DISCUSSION

Table 1 provides information about some general characteristics of the soils examined in this study. The texture of the soils varied only a little and all the samples were classified as sandy soil. The content of soil organic carbon was generally low (less than 12.0 g kg^{-1}) ranging from 7.6 to 14.0 g kg^{-1}, except for sample P2.

The pH of the soils differed depending on the location and sampling period, varying from 5.49 to 8.83 (Table 2). The highest pH was detected in samples in the direct vicinity of the cement plant (site P2). The elevated alkaline pH occurred each year of the study (2006-2011) and was observed in surface and subsurface samples from site P2.

The natural soil pH is in the range 5.0-6.2 (CIEŚLA et. al. 1994). Thus, differences in the pH of these soils compared to their originally slightly acidic pH correspond to differences in the H^+ concentration equal ~ 5.000 fold (~ 3.5 pH units).

Table 1

Selected properties of soils

Sample	Depth (cm)	Corg (g kg^{-1})	$CaCO_3$ (%)	Mn total (mg kg^{-1})
P1	0 - 20	11.9	0.33	272.2
	20 - 40	8.50	0.29	243.3
P2	0 - 20	14.1	6.80	319.1
	20 - 40	8.20	4.60	346.4
P3	0 - 20	11.5	-	261.0
	20 - 40	10.0	-	276.1
P4	0 - 20	10.7	-	218.1
	20 - 40	9.10	-	229.4
P5	0 - 20	11.7	1.80	168.9
	20 - 40	10.2	1.30	144.1
P6	0 - 20	8.50	-	163.1
	20 - 40	7.60	-	140.6

Table 2

Changes in DTPA extracted Mn and concentration of (H^+) in soils during the 6 years of observations

Sample	Depth (cm)	pH H_2O	SD	DTPA- Mn (mg kg^{-1})	SD**
P1	0 - 20	6.32 - 8.05 * 7.22	0.59	1.6 - 6.27 * 3.81	1.73
	20 - 40	6.51 - 7.34 8.18	0.56	1.27 - 4.52 3.02	1.18
P2	0 - 20	7.94 - 8.52 8.12	0.22	1.05 - 11.5 3.78	3.84
	20 - 40	8.10 - 8.83 8.30	0.26	1.02 - 10.9 3.26	3.79
P3	0 - 20	5.89 - 6.72 6.19	0.30	4.23 - 8.62 5.68	1.73
	20 - 40	5.90 - 6.72 6.30	0.36	2.60 - 7.10 4.81	1.73
P4	0 - 20	6.01 - 6.42 6.23	0.16	2.67 - 7.70 5.53	1.95
	20 - 40	5.98 - 6.58 6.27	0.26	1.08 - 12.0 6.22	4.07
P5	0 - 20	6.20 - 7.80 7.38	0.62	0.83 - 10.0 5.25	4.15
	20 - 40	6.08 - 7.78 7.42	0.61	0.81 - 6.40 2.66	2.03
P6	0 - 20	5.49 - 6.54 6.06	0.40	1.60 - 3.40 2.60	0.71
	20 - 40	5.76 - 6.96 6.22	0.47	1.07 - 6.10 3.01	1.94

*minimum – maximum
mean value
**SD – standard deviation

Dust falling on the soil caused a shift in the soil pH to the alkaline side due to carbonates present in emitted particles. Calcium carbonate was detected in samples located in the direct vicinity of the emitter (Table 1). In soils adjacent to the cement plant, the content of $CaCO_3$ ranged between 0.33% to 6.8% in surface sample and from 0.29% to 4.6% in subsurface samples, typically increasing in the subsequent years. The soil sampled at site P-2 had the pH above 7.94 up to 8.52 (mean value 8.12) in the surface and 8.10 up to 8.33 (mean 8.30) in the subsurface horizon. In soils lying in the same region but unaffected by the cement plant, pH values were in the range 5.0-6.2. Some previous research on soils near the Lafarge Plant in Bielawy showed that the parent material did not contain $CaCO_3$ (Cieśla et al. 1994).

Much smaller changes were observed in soils located further from the cement dust emission point (sites P2-P4 and P6). Calcium carbonate in acid soils being under the impact of industrial emission is described as the tracer of the pollution lead by the cement industry (Zerrougi, Sbaa 2008).

The total Mn concentrations in soils varied from 140.6 to 346.5 mg kg^{-1} and DTPA-extractable Mn was in the range 0.81-12.07 mg kg^{-1} (Table 2). The lowest content of DTPA-Mn was detected in site P2 with the highest pH. A simple linear correlation analysis showed few significant correlations between soil properties and DTPA extractable manganese. Significant correlation was obtained between organic matter and DTPA-extractable manganese (r = 0.4273, α = 0.05) in the studied soils. The soil organic matter content has been associated with an increased Mn availability in numerous experiments (Moraghan, Mascagni 1991, Mc Bride 1989). The effect of organic matter has been attributed to formation of complexes.

Critical levels for DTPA-extractable Mn range from 2.5 to 5.0 mg kg^{-1} (Moraghan, Mascagni 1991). Extractable Mn showed no correlation with the pH (or exactly with the concentration of H^+ ions) in the examined soils. However, such a correlation was obtained for soil sampled in the direct vicinity of cement plant (site P2; Figure 1). A significant (r = 0.6211) statistical correlation was observed between [H+] concentration and DTPA extractable Mn in P2 soil (Figure 2). For this site, significant correlations between Corg and DTPA-Mn as well as between the $CaCO_3$ concentration and DTPA extractable Mn (r = 0.499, r = 0575, respectively). were obtained for six years of the observation period (Figures 3 and 4). Cement dust rich in $CaCO_3$ acts like a liming substance and affects the plant availability of manganese in soils from the site adjacent to the cement plant.

A negative trend in response to the alkaline contaminant was observed in almost all the soil samples and in all the years of the study. It was the most evident for site P2 (Figure 1).

The Mn deficit to plants arises from complex interactions of many factors and is related to soil properties, plant-specific factors and their interactions at the soil-root interface (Tong et al. 1997, Thomson et al. 2005).

Manganese deficiency may occur as a primary condition, where the soil (and its parent material) is poor in manganese (like sandy soils), or in a secondary form, associated with an alkaline pH of the soil. Several authors (Wei et al. 2006, Mc Bride 1989) have found that pH has the strongest effect on the availability of Mn. The mobility of amphoteric cations (Mn) decreases when soil undergoes an alkaline treatment.

The two main forms of Mn is soils are soluble Mn(II) and hardly soluble Mn(IV). In well-aerated soils, Mn occurs as Mn(II) ions in the soil solution,

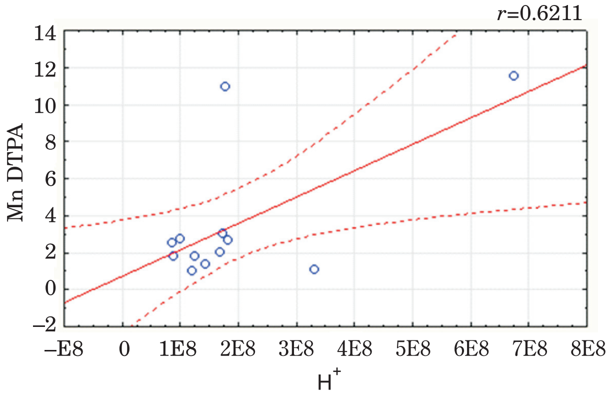

Fig. 2. Corelation between (H+) concentration and Mn-DTPA in P2 soil

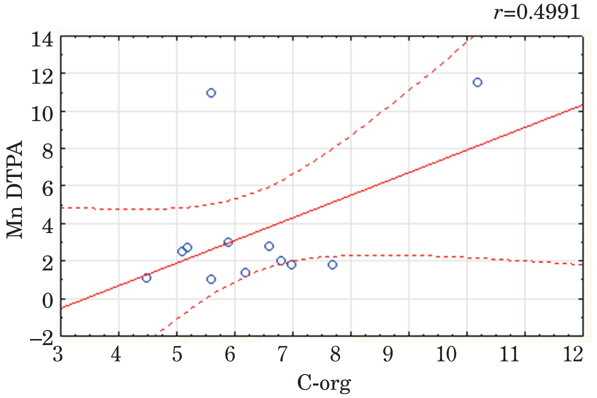

Fig. 3. Corelation between C-org. concentration and Mn-DTPA in P2 soil

Mn(II) adsorbed by organic matter and clay minerals or co-precipitates (Bartlett, Janes 1993). In calcerous soils with high soil pH, plant available Mn(II) is oxidized to Mn(IV) ions. Moreover, Norvell (1988) stated that when soil pH > 8, auto-oxidation of Mn(II) took place. Manganese oxides are very strong oxididizing agents with oxidation potentials higher than O_2 itself. The high soil pH in site P2 could have initiated the auto-oxidation process and reduced the manganese mobility.

Mobility and bioavailability of manganese are largely governed by the pH and redox conditions. An increase in the soil's pH lowers the mobility of this micronutrient due to the oxidation of soluble Mn(II) to hardly soluble Mn(IV) which occurs at about neutral pH (Norvel 1988). Other authors (Ponnamperuna 1986) reported that the amount of water-soluble manganese decreased to a tenth when the pH in soil increased from 7 to 8. The analyzed

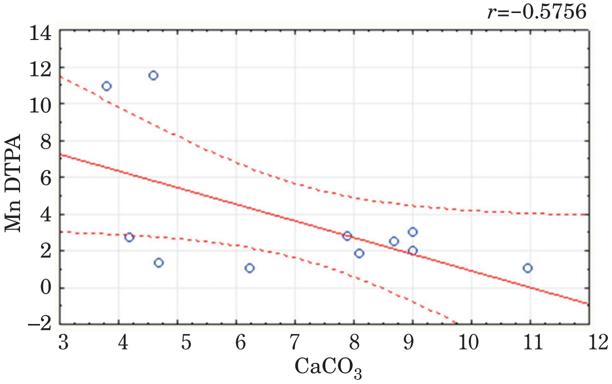

Fig. 4. Corelation between $CaCO_3$ content and Mn-DTPA in P2 soil

soils in the direct vicinity of the cement plant had a pH in these ranges, thus the impact of alkalization on the Mn mobility was the most profound there.

In such alkaline soil, nitrogen fertilization with some acid forming fertilizer like $(NH_4)_2SO_4$ is recommended, as it will acidify the soil solution, which in turn will enhance the mobility of Mn, B and Fe (Domagała-Świątkiewicz, Sady 2010). This is particularly important for crops sensitive to Mn deficiency.

CONCLUSIONS

1. The alkalization of the soil environment by cement dust complicates mineral nutrition due to disrupting good manganese availability in soils, particularly ones adjacent to the source of emission.

2. Cement dust rich in $CaCO_3$, acts like a liming substance and affects plant availability of manganese in soils in the vicinity of a cement plant.

3. Based on the metal extractability with DTPA, it was concluded that concentrations of plant-available manganese in cement dust affected soils were below the recommended limits due to alkalization of soils by cement particles.

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CONTAMINATION OF CEREAL PRODUCTS WITH LEAD AND CADMIUM AS A RISK FACTOR TO HEALTH OF THE POPULATION IN THE PROVINCE OF PODLASIE (*WOJEWÓDZTWO PODLASKIE*)*

Joanna Fiłon[1], Jolanta Ustymowicz-Farbiszewska[1], Jan Górski[2], Jan Karczewski[1]

[1]Department of Hygiene and Epidemiology
[2]Department of Physiology
Medical University of Białystok

Abstract

Lead and cadmium pose a serious threat to human health, hence their content in food products is regulated by the EU standards, also binding in Poland. Food contamination with these metals is an etiological factor of civilization diseases. In order to prevent such disorders, it is extremely important to assess the degree of environmental pollution with Pb and Cd, reflected by the level of these metals in food products. Determination of Pb and Cd in food products demonstrates exposure to the toxic effect of these metals and enables evaluation of the threat to human health in a given population. The aim of this study has been to estimate the health threat to the population of the Province of Podlasie (*województwo podlaskie*) due to Pb and Cd contamination of cereal products.

The following foodstuffs were analyzed: flour, groats, bread, pasta, rice, bran and soya products. They were collected in 13 administrative districts of the Province of Podlasie while monitoring health quality of food products. Pb and Cd concentrations were determined with the AAS method.

The highest Cd level was noted in pastas (0.058±0.0330 mg kg^{-1}) and the highest Pb level was determined in cuscus (0.120±0.0899 mg kg^{-1}). The lowest average Pb concentra-

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mgr Joanna Fiłon, Department of Hygiene and Epidemiology, Medical University of Białystok, Mickiewicza 2c, 15-089 Białystok, tel/fax: (85) 7485560, e-mail: joanna@umb.edu.pl

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tion was found in groats (0.042±0.0306 mg kg^{-1}) and Cd was the lowest in wholemeal bread (0.016±0.0106 mg kg^{-1}).

The average concentrations of Pb and Cd in cereal products did not exceed the permissible limits established by the Minister of Health. The permissible level of Pb was not surpassed until the level of the 90th percentyl (in couscous and soya products). An average intake of Pb and Cd was within the tolerated amount, corresponding to 10% PTWI and 12% PTWI, respectively. Thus, no threat to the health of the population in Podlasie was detected.

Key words: lead, cadmium, cereal products, atomic absorption spectrometry.

ZANIECZYSZCZENIE PRODUKTÓW ZBOŻOWYCH PB I CD JAKO CZYNNIK RYZYKA ZDROWOTNEGO LUDNOŚCI WOJEWÓDZTWA PODLASKIEGO

Abstrakt

Pb i Cd stanowią istotne zagrożenie dla zdrowia ludzkiego, i w związku z tym ich zawartość w żywności jest limitowana przez normy Unii Europejskiej obowiązujące w Polsce. Skażenie żywności tymi metalami jest czynnikiem etiologicznym chorób cywilizacyjnych. Z punktu widzenia profilaktyki tych schorzeń bardzo istotne jest poznanie stopnia skażenia środowiska Pb i Cd, czego odzwierciedleniem jest poziom tych metali w produktach spożywczych. Badanie zawartości Pb i Cd w produktach spożywczych umożliwia ocenę stopnia narażenia na ich toksyczne działanie i określenie zagrożenia zdrowia badanej populacji ludzkiej. Celem pracy była ocena zagrożenia zdrowia ludności woj. podlaskiego produktami zbożowymi zanieczyszczonymi Pb i Cd.

Materiał do badań stanowiły próbki mąki pszennej i żytniej, kasz, pieczywa białego i razowego, makaronów, otrębów, ryżu i produktów sojowych pobrane w 13 powiatach woj. podlaskiego w ramach monitoringu jakości zdrowotnej środków spożywczych i przedmiotów użytku. Stężenie Pb i Cd oznaczano metodą AAS.

Najwyższą zawartość Cd odnotowano w makaronach (0,058±0,0330 mg kg^{-1}), natomiast Pb – w kaszy kuskus (0,120±0,0899 mg kg^{-1}). Najniższą średnią zawartość Pb stwierdzono w kaszach (0,042±0,0306 mg kg^{-1}), a Cd w pieczywie razowym (0,016±0,0106 mg kg^{-1}).

Stwierdzono, że średnia zawartość Pb i Cd w badanych produktach zbożowych nie przekraczała limitu ustalonego w Rozporządzeniu Ministra Zdrowia. Przekroczenie dopuszczalnej zawartości Pb stwierdzono dopiero na poziomie 90-percentyla (w kaszy kuskus i produktach sojowych).

Wykazano, że średnie pobranie Pb i Cd mieści się w granicach pobrania tolerowanego przez organizm i wynosi odpowiednio 10% PTWI i 12% PTWI, co nie stwarza zagrożenia dla zdrowia ludności woj. podlaskiego.

Słowa kluczowe: ołów, kadm, produkty zbożowe, atomowa spektrometria absorpcyjna.

INTRODUCTION

Lead (Pb) and cadmium (Cd) are highly toxic elements, which are quickly absorbed from the alimentary tract. Afterwards, they easily pass through biological barriers and accumulate in internal organs. Even small amounts of Pb and Cd may cause metabolic disorders (Kozielec et al. 2002, Malara et al. 2002, Wojciechowska-Mazurek et al. 2008, Medyńska A. et al. 2009, Dobrzański et al. 2009, Winiarska-Mieczan 2009). Lead is the cause of many diseases, including cancer of the stomach, ovaries, kidneys and leukemia; it also causes irreversible damage to the nervous system. Cadmium is responsible for decalcification and deformation of bones, myatrophy and anosmia, impotence and hypertension; it has also been classified as carcinogenic to humans by the IARC (Starska et al. 1996, Kozielec et al. 2002, ATSDR 2007, 2008, Fortier et al. 2008, Iavicoli et al. 2009, EFSA 2009, 2010, Trojanowski et al. 2010, Kaniuczak et al. 2011, Nowak et al. 2011).

Lead and cadmium pose a serious threat to human health, hence their content in food products is regulated by the EU standards, also binding in Poland. Food contamination with these metals is an etiological factor of civilization diseases. It is extremely important for prophylaxis to know the extent of environmental pollution with Pb and Cd, reflected in the levels of these metals in food products. Determination of Pb and Cd in food products enables one to estimate the exposure to their toxic action and therefore to assess the health risk in particular human populations. Nowadays, due to environmental pollution, all food products are contaminated with Pb and Cd. Since Poland accessed the European Union, only these two metals have been taken into consideration for the assessment of food contamination (Wojciechowska-Mazurek et al. 2003, Wójcik-Stopczyńska 2003, Bączek-Kwinta et al. 2011).

The objective of this study has been to determine whether consumption of cereal products by residents of Podlasie threatens their health due to Pb or Cd contamination.

MATERIAL AND METHODS

The material for analysis included 148 samples of cereal products (wheat and wholemeal flour, cereals, groats, bran, white and wholemeal bread, pasta and soya products) collected in 13 districts of the Province of Podlasie while monitoring health quality of food and other household products.

The sample collection procedures complied with the binding standards. Validated analytical methods were applied, in line with the EU legal criteria for the methods used for official food testing (*Commission Directive 2001/22/EC of 8 March 2001, Directive of Minister of Health of 30 April 2004.* Journal of Law No 120 item 1257). Certified reference materials were used for quality control. The recovery was 98% for Pb and 96% for Cd, and the coefficient of variation was 4.2% and 3.6%, respectively. Concentrations of Pb and Cd were determined by atomic absorption spectrophometry (AAS).

The contamination of cereal products with Pb and Cd was determined with reference to the binding Polish norms (*Directive of Minister of Health of 13 January 2003*). The intake of these metals with cereal products was estimated according to the data published by the Statistical Office in Białystok concerning consumption of all the analyzed groups of products (GUS 2008). The risk to human health posed by Pb and Cd in cereal products was evaluated by comparing their mean intake with the provisional tolerable weekly intake (PTWI).

The results were statistically analyzed using the software Statistica 7.1. The Duncan's test was applied to compare the significance of differences between the means depending on the type of cereal product. The level of significance was $p \leq 0.05$.

RESULTS AND DISCUSSION

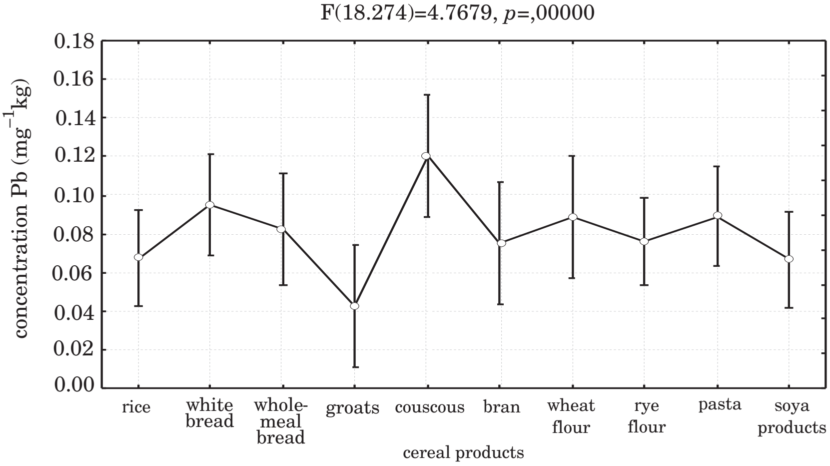
The level of Pb in cereal products available in Podlasie ranged from 0.013 mg kg^{-1} to 0.275 mg kg^{-1}, depending on the type of foodstuff. The mean Pb content was 0.080±0.0545 mg kg^{-1}, median – 0.061 mg kg^{-1}; 90% of results did not exceed 0.125 mg kg^{-1}.

The cereal products from Podlasie Province contained from 0.000 mg Cd kg^{-1} to 0.100 mg Cd kg^{-1}. The mean Cd content was 0.028±0.0217 mg kg^{-1}, median – 0.025 mg kg^{-1}; 90% of results did not exceed 0.059 mg kg^{-1}.

The results show distinct dispersion of the values of the elements studied within one assortment. The highest Pb content was noted in couscous (0.120 mg kg^{-1} ± 0.0899), with 90% of results not exceeding 0.250 mg kg^{-1}. The level of Pb was the lowest in barley groats (0.042 mg kg^{-1} ± 0.0306) and only 10% of results exceeded 0.085 mg kg^{-1}. These values were statistically significant (Figure 1).

The lowest mean amounts of Cd were noted in wholemeal bread and rye flour (0.016 mg kg^{-1} ± 0.0106, 0.018 mg kg^{-1} ± 00099, respectively), and only 10% of results were higher than 0.025 mg kg^{-1}. Statistical analysis revealed (Figure 1) a significantly higher Cd content in pasta (0.058 mg kg^{-1} ± 0.0330) and bran (0.039 mg kg^{-1} ±0.0181) as compared to other cereal products. In 10% of the examined pasta samples, the Cd content was 0.100 mg kg^{-1}.

The above findings are similar to those reported in the 1990s by BULIŃSKI et al. (1990, 1992), ILOW et al. (1999) and FALANDYSZ et al. (1987). The results are also consistent with later research by other Polish authors (KOT 2003, KOT, ZARĘBA 2007, WOJCIECHOWSKA-MAZUREK et al. 2008, KOT et al. 2009 MEDYŃSKA et al. 2009).



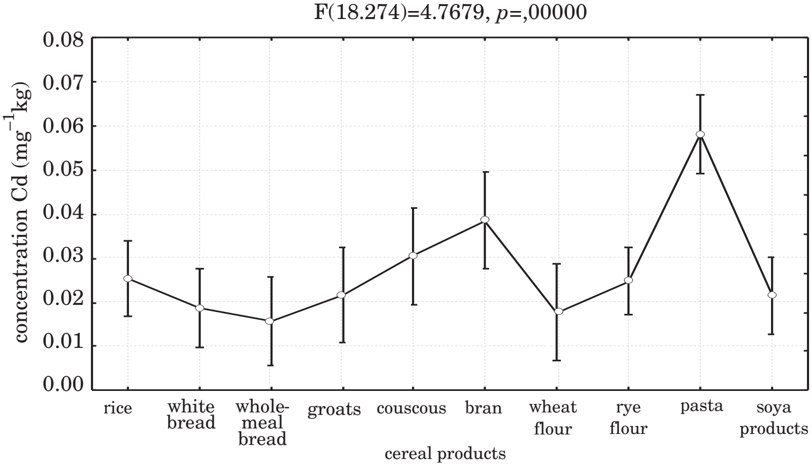


Fig. 1. Results of Anova analysis for the Pb and Cd content in cereal products (mg kg^{-1})

Smoczyńska et al. (1999) obtained higher levels of Pb and Cd in flours from southern Poland (0.104 mg kg^{-1} and 0.034 mg kg^{-1}, respectively), which was consistent with the data reported in the 1980s (Zawadzka et al. 1985).

The contents of toxic metals in Polish food products do not differ significantly from those reported from other countries. Only cereal products from Germany and Finland have lower Pb and Cd concentrations (Brüggemann, Kumpulainen 1995, Brüggemann et al. 1996, Tahvonen, Kumpulainen 1994, SCOOP 2004, Food Standards Agency 2004, 2007, EFSA 2009, 2010). By far, lower Pb levels were also revealed by the Polish food monitoring of 2004 supervised by the Department of Food Research, National Institute of Public Health – National Institute of Hygiene (NIZP-PZH) (Wojciechowska-Mazurek et al. 2008).

Rye flours contained more Pb as compared to wheat flours, whereas the Cd content was the highest in wheat flour. These results are supported by other authors in various parts of Europe and Poland (BRÜGGEMANN, KUMPULAINEN 1995, BRÜGGEMANN et al. 1996, BULIŃSKI et al. 1990, 1992, KOT, ZARĘBA 2007, KOT et al. 2009).

The mean Pb content in bread was similar to or higher than the Pb level in the flours from which the bread was produced, which is consistent with observations of other authors (BRÜGGEMANN, KUMPULAINEN 1995, KOT 2003, KOT, ZARĘBA 2007). This is explained by the effect of the technological process and addition of other ingredients during the production process. The Cd content in pasta was almost twice as high as in wheat flour. Worthy of note is the fact that white bread had more Pb and Cd than wholemeal bread. Most authors confirm higher concentrations of these elements in wheat bread (BULIŃSKI et al. 1990, 1992, KOT 2003, KOT, ZARĘBA 2007).

Contamination of cereal products with Pb and Cd was determined based on standards binding in Poland (*Directive of Minister of Health of 13 January 2003*). Results have been presented in Figure 2.

It was only at the 90th percentile that the content of Pb in couscous (125%) and soya products (112.5%) exceeded the standard limit established in the *Directive of the Minister of Health of 13 January 2003*. In 10% of the pasta samples, the level of Cd was on the borderline of the permitted limit. In other cereal products, the mean levels of Pb and Cd as well as the 90th percentile values did not exceed 62% of the permitted limit.

The temporary PTWI doses of Pb and Cd from all sources tolerated by a healthy human, established by the Joint FAO/WHO Expert Committee, are respectively 0.025 mg kg^{-1} and 0.007 mg kg^{-1} body weight (*Commission Regulation (EC) no 1881/2006 of 19 December 2006*). Taking into account the mean concentrations of Pb and Cd in the respective cereal products and their intake-related data, a weekly intake of these metals was determined for an adult person of 60 kg body weight. Results have been presented in Figure 3.

The calculated intake of Pb and Cd with cereal products is within the limits of tolerable intake, being 9.7% PTWI and 12% PTWI respectively, of which 47% (for Cd) and 85% (for Pb) come from bread.

Taking into account high intake of cereal products, especially bread, the levels of Pb and Cd should be considered elevated yet not health-threatening.

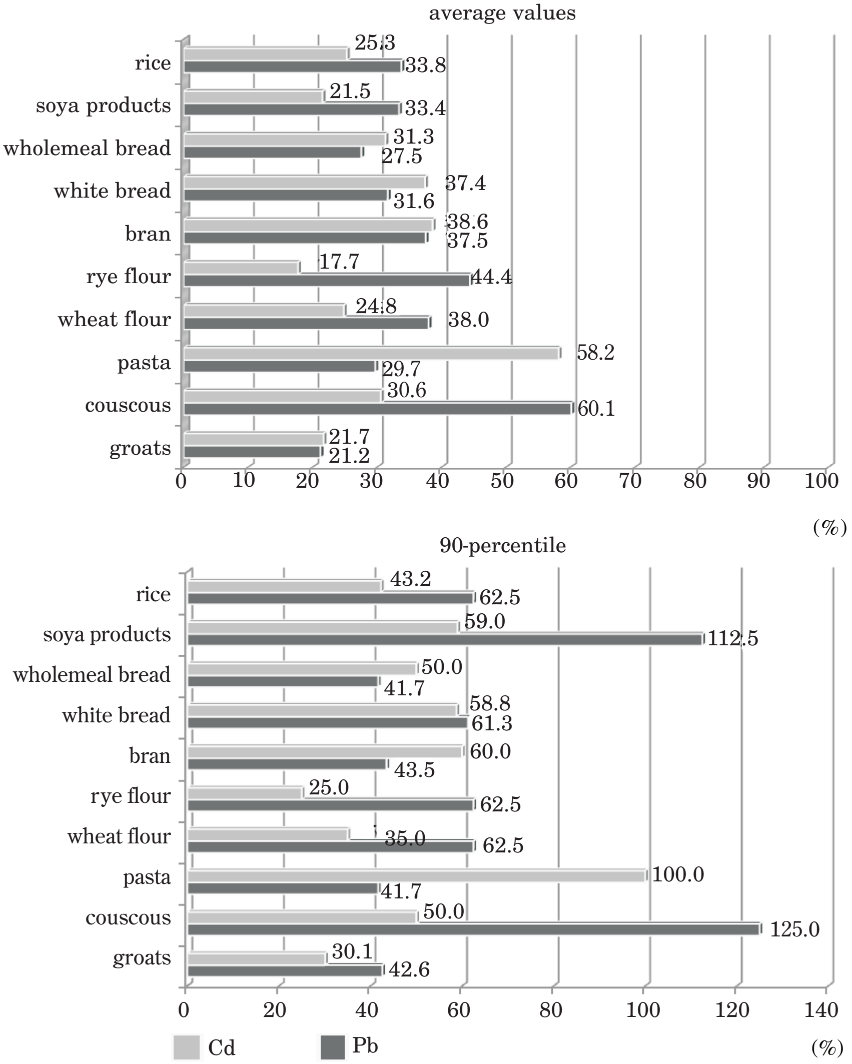


Fig. 2. Mean content and the 90th percentile values for Pb and Cd in respective cereal products from Podlasie Province with reference to the standards of the Ministry of Health

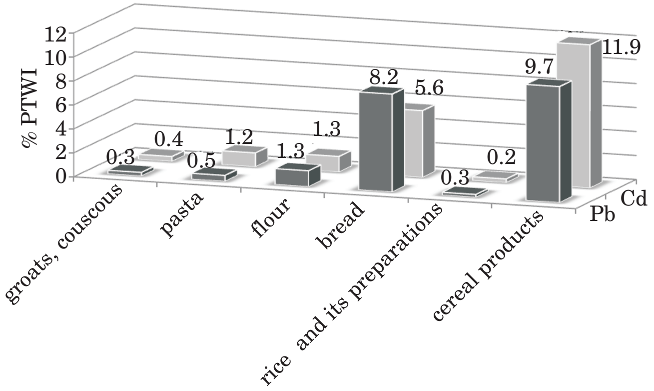


Fig. 3. Pb and Cd in respective cereal products from Podlasie Provincee with reference to PTWI

CONCLUSIONS

1. The mean levels of Pb and Cd did not exceed the limit values. The Pb content was found to be higher than the standard limit only at the 90th percentile (in couscous and soya products).

2. The intake of Pb and Cd with cereal products is within the limits of tolerable intake (10% PTWI and 12% PTWI, respectively) and does not threaten the health of the population in Podlasie Province.

3. Significant variations were found in Pb and Cd levels in the cereal products studied depending on the type of the product.

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LEAD CONTENT IN CEREAL PRODUCTS AS A POPULATION HEALTH THREAT MARKER: A CASE STUDY IN THE PROVINCE OF PODLASIE*

Joanna Fiłon, Jolanta Ustymowicz-Farbiszewska, Jan Karczewski

Department of Hygiene and Epidemiology
Medical University of Białystok

Abstract

Heavy metals, which are considered basic industrial and environmental pollutants, pose a threat to the human health if found in food products even in trace amounts. Lead may cause such conditions as functional disorders of the kidneys, liver or the circulatory system. It can also impede the biosynthesis of heme and cause disturbances in the metabolism of vitamin D and the following microelements: Fe, Cu, Zn Se and Ca.

The aim of the study was to assess the health threat and estimate risks from the presence of Pb in cereal products available in retail shops in the Province of Podlasie. The material consisted of samples of cereal products (flour, groats, bakery products, pasta and rice) taken at random from retail shops in Podlasie in 2004-2005 and 2010-2011. The lead concentration was estimated by using the atomic absorption spectrometry method (AAS). The content of Pb was analyzed depending on a product type and sampling period; the health threat was assessed with reference to the current Polish requirements. The results were statistically analyzed using Statistica 7.1 software, Duncan's test and Wilcoxon test.

The results revealed that the average content of Pb in the examined cereal products did not exceed the norms. During the analyzed period, a decrease in the Pb concentration in cereals was found. This may indicate an improvement in the quality of the environment, which can be measured by the content of Pb in cereal products. At the same time, owing to the significant role of cereal products in human nutrition, the lead content may also serve as a marker of health exposure.

Key words: lead, cereal products, atomic absorption spectrometry, health threat.

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mgr Joanna Fiłon, Department of Hygiene and Epidemiology, Medical University of Białystok, Mickiewicza 2c, 15-089 Białystok, tel/fax: (85) 7485560, e-mail:

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ZAWARTOŚĆ OŁOWIU W PRODUKTACH ZBOŻOWYCH JAKO MARKER NARAŻENIA ZDROWOTNEGO POPULACJI NA PRZYKŁADZIE WOJEWÓDZTWA PODLASKIEGO

Abstrakt

Metale ciężkie, zaliczane do podstawowych zanieczyszczeń przemysłowych i środowiskowych, obecne w żywności nawet w ilościach śladowych stanowią zagrożenie dla zdrowia ludzi. Pb może powodować m.in. zaburzenia funkcjonowania nerek, wątroby, układu krążenia, a także hamować biosyntezę hemu oraz zakłócać metabolizm witaminy D i mikroelementów, tj.: Fe, Cu, Zn Se i Ca.

Celem pracy była ocena zagrożenia zdrowotnego oraz oszacowanie ryzyka wynikającego z obecności Pb w produktach zbożowych dostępnych w sprzedaży detalicznej w woj. podlaskim oraz ocena dynamiki zmian zawartości tego pierwiastka w czasie.

Materiał do badań stanowiły próbki produktów zbożowych (mąki, kasz, pieczywa, makaronu i ryżu) pobranych losowo w placówkach handlu detalicznego w woj. podlaskim w latach 2004-2005 i 2010-2011. Stężenie Pb oznaczano metodą atomowej spektrometrii absorpcyjnej (ASA).

Analizowano zawartość Pb w badanych próbkach w zależności od rodzaju produktu i okresu pobrania próby oraz oceniano zagrożenie zdrowia, opierając się na obowiązujących w Polsce normach. Wyniki poddano analizie statystycznej z użyciem programu komputerowego Statistica PL 7.1, testu Duncana i testu kolejności par Wilcoxona.

Stwierdzono że średnia zawartość Pb w badanych produktach zbożowych nie przekraczała przyjętych norm. Średnia zawartość Pb w latach 2010-2011 była istotnie niższa w porównaniu z zawartością tego pierwiastka w latach 2004-2005. Wyniki te mogą świadczyć o poprawie stanu środowiska naturalnego, czego miernikiem może być zawartość Pb w produktach zbożowych. Jednocześnie ze względu na istotną rolę tej grupy produktów w żywieniu człowieka może on stanowić również marker narażenia zdrowotnego.

Słowa kluczowe: ołów, produkty zbożowe, atomowa spektrometria absorpcyjna, zagrożenie

INTRODUCTION

As a result of various human activities, heavy metals are widespread in the environment and permeate into food products, exposing people to their toxic effects. Heavy metals, which are considered basic industrial and environmental pollutant, pose a threat to the human health if found in food products even in trace amounts. This group includes both elements that are necessary for the body (e.g. microelements: Cu, Fe, Zn, Ni, Cr) and elements of an unknown physiological role (e.g. Cd, Hg, Pb, As) (WOJCIECHOWSKA-MAZUREK et al. 2008 , DUFAULT et al. 2009, WINIARSKA-MIECZAN 2009). Lead (Pb) is considered as particularly harmful for human health. As a microelement, it can be found in all types of biological material: soil, plants, water and live organisms. It is characterized by a high accumulation factor in the body. Moreover, it is quickly absorbed from the digestive tract and easily permeates across biological barriers. Lead is absorbed into the human body mainly by the digestive tract and respiratory system. As regards food in the digestive tract, it is absorbed in approximately 10% by adults and up to 50% by children. In the human body, lead from food first permeates into internal organs and then is accumulated in the osseous tissue, most commonly in joints, teeth, hair and the liver. The toxic effects of lead on the body include mainly functional disorders of the cardiovascular system, kidneys and nervous system (Tong et al. 2000, ATSDR 2007, Medyńska et al. 2009, EFSA 2010, Trojanowski et al. 2010, WHO 2011, Cielecka, Dereń 2011, Nowak et al. 2011).

The presence of free lead in tissues causes significant metabolic disturbances: retarded activation of certain enzymes, disturbances of protein synthesis and other metallic element balance. For example, lead may have an antagonistic effect or can compete with Se, Fe, Ca, Cu or Zn, which may disturb the cell's functions and impair the cellular antioxidative defense. Moreover, it has been shown that exposure to lead under experimental conditions as well and occupational or environmental exposure may lead to a decrease in the supplies of various antioxidative vitamins, especially ascorbic acid or a-tocopherol. A high intake of calcium and phosphorus in the diet causes a decrease in the amount of lead absorbed in the intestines. In contrast, ascorbic and citric acids intensify this process (Hsu, Guo 2002, Stohs, Bagchi 1995, Antonowicz et al. 1998, Peraza et al. 1998, Kozielec et al. 2002, ATSDR 2007, EFSA 2010, WHO 2011, Cielecka, Dereń 2011).

Cereal products play a crucial role in human nutrition, being a staple element of everyday diet. However, they are also one of the main sources of lead intake (approximately 33% of daily dose of this element) (Kot et al. 2011). Therefore, attempts to assess the level of lead contamination in these products are undertaken. Research provides data on amounts of metals in food products and their changes in time. The analysis of lead in cereal products enables assessment of consumers' exposure to its toxic effects.

The aim of the study was to assess the health threat and to estimate the lead presence in cereal products available in retail shops in the Province of Podlasie as well as to evaluate the dynamics of changes in the lead content in time.

MATERIAL AND METHODS

The material consisted of samples of cereal products collected randomly in retail shops in the Province of Podlasie in 2004-2005 and 2010-2011. The study included 251 cereal products: flour (wheat and rye), groats, brans, bakery products (white and wholemeal), pasta and rice.

After the mineralization of samples, levels of lead were determined by electrothermal AAS in a graphite cuvette with the Zeeman background correction, at 283.3 nm wavelength, using a Z-5000 Hitachi apparatus. The accuracy of determinations was verified on certified reference material NSC ZC73009 (flour). The recovery was approximately 97%, at the variation coefficient of 4.1%.

The lead content in samples was analyzed depending on a type of product and time of sampling. The level of lead contamination of food products was assessed according to the Polish norms (*Commission Regulation (EC) no 1881/2006 of 19 December 2006, Commission Regulation (EC) no 420/ 2011 of 29 April 2011*). Based on the data of the Statistical Office in Białystok (GUS 2007, 2011) on consumption of particular groups of products, the intake of the analyzed metal with cereal products was estimated. The health threat was estimated by comparison of the average lead intake with the values of the Provisional Tolerable Weekly Intake (PTWI) and Benchmark Dose Lower Confidence Limit; in children $BMDL_{01}$ = 0.50 μg kg^{-1} bw day^{-1} (neurotoxic effect), in adults $BMDL_{10}$=0.63 μg kg^{-1} b.w. day^{-1} (nephrotoxic effect) and $BMDL_{01}$ = 1.50 μg kg b.w. day^{-1} (cardiovascular disorders) (*Commission Regulation (EC) no 1881/2006 of 19 December 2006*, EFSA 2010, WHO 2011).

The results were statistically analyzed by means of Statistica PL software. The analysis of changes in the lead content in the examined products depending on a type of product and time of sampling was performed by univariate analysis of variance (Anova) with the use of Duncan test and Wilcoxon signed rank test. Statistical significance was considered as $p \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 presents the mean content of lead in cereal products, ranges, standard deviations, medians and 90th percentile values in particular years.

The products collected in Podlasie contained between 0.009 mg Pb kg^{-1} and 0.299 mg Pb kg^{-1}, depending on the type of cereal products and collection time.

The results show an evident dispersion of lead values within each group of products. In 2004, the highest mean lead content was observed in pasta (0.094 ± 0.04 mg kg^{-1}), in 2005 – in rye flour (0.125 ± 0.00 mg kg^{-1}), in 2010 and 2011 – in bran (0.256 ± 0.03 mg kg^{-1} and 0.211± 0.02 mg kg^{-1} respectively).

In 2004, the lowest mean lead content was observed in wholemeal bakery products (0.043± 0.00 mg kg^{-1}), in 2005 – in groat (0.037 ± 0.03 mg kg^{-1}), while in 2010 and 2011 – in white bakery products (0.025 ± 0.01 mg kg^{-1} and 0.28 ± 0,01 mg kg^{-1} respectively).

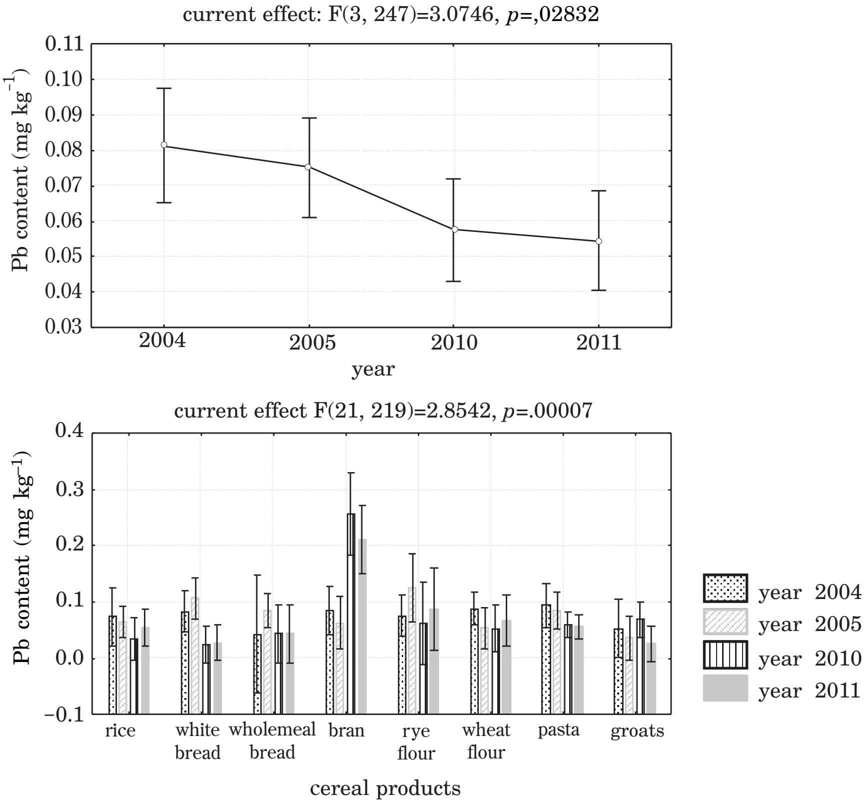
A decrease in the lead contamination in cereal products was observed during the analyzed period of time. The samples collected in 2004 and 2005 contained approximately twice as much lead as the samples from 2010 and

Table 1

Pb content in cereal products from the Province of Podlasie (mg kg^{-1})

Cereal products		2004 N = 52	2005 N = 67	2010 N = 64	2011 N = 68
Groats	$\bar{x}$±SD	0.053±0.03	0.037±0.03	0.069±0.11	0.026±0.02
	median	0.052	0.025	0.023	0.021
	range	0.022-0.085	0.013-0.097	0.011-0.375	0.012-0.067
	90th-percentile	0.085	0.097	0.179	0.041
Pasta	$\bar{x}$±SD	0.094±0.04	0.086±0.05	0.060±0.06	0.056±0.07
	median	0.125	0.125	0.046	0.036
	range	0.042-0.125	0.013-0.125	0.009-0.287	0.007-0.299
	90th-percentile	0.125	0.125	0.102	0.162
Wheat flour	$\bar{x}$±SD	0.089±0.04	0.054±0.04	0.053±0.09	0.067±0.06
	median	0.12	0.04	0.02	0.064
	range	0.022-0.125	0.017-0.120	0.009-0.228	0.018-0.157
	90th-percentile	0.125	0.12	0.228	0.157
Rye flour	$\bar{x}$±SD	0.075±0.04	0.125±0.00	0.062±0.00	0.087±0.02
	median	0.057	0.125	0.062	0.087
	range	0.032-0.120	0.125-0.125	0.060-0.064	0.073-0.102
	90th-percentile	0.12	0.125	0.064	0.102
Bran	$\bar{x}$±SD	0.085±0.00	0.063±0.03	0.256±0.03	0.211±0.02
	median	0.085	0.087	0.256	0.197
	range	0.084-0.087	0.013-0.087	0.234-0.278	0.197-0.240
	90th-percentile	0.087	0.087	0.278	0.240
White bread	$\bar{x}$±SD	0.083±0.04	0.107±0.07	0.025±0.01	0.028±0.01
	median	0.091	0.123	0.027	0.025
	range	0.028-0.120	0.017-0.206	0.013-0.038	0.013-0.044
	90th-percentile	0.12	0.206	0.037	0.041
Wholemeal bread	$\bar{x}$±SD	0.043±0.00	0.086±0.05	0.044±0.02	0.044±0.02
	median	0.043	0.123	0.046	0.046
	range	0.043-0.043	0.025-0.125	0.020-0.062	0.020-0.062
	90th-percentile	0.043	0.125	0.062	0.062
Rice and rice products	$\bar{x}$±SD	0.074±0.06	0.066±0.04	0.035±0.03	0.056±0.06
	median	0.074	0.06	0.026	0.032
	range	0.025-0.125	0.013-0.125	0.012-0.089	0.014-0.218
	90th-percentile	0.123	0.114	0.089	0.153
All cereal products	$\bar{x}$±SD	0.081±0.04	0.081±0.04	0.058±0.07	0.055±0.06
	median	0.084	0.084	0.029	0.034
	range	0.022-0.125	0.022-0.125	0.009-0.375	0.007-0.299
	90th-percentile	0.125	0.125	0.158	0.162

2011. The lead concentration in wholemeal bakery products and rye flour did not change in the years of sample collection. A considerable decrease (approximately two-fold) in the lead content was observed in pasta, white bakery products (statistically significant dependency) and rice. Statistical analysis (Figure 1) showed that the time of sample collection significantly affected the lead content in cereal products. The analysis of the results from particular cereal products (Figure 1) showed that the lead content in bran in 2010 and 2011 was significantly higher compared to other cereal products.



current effect: F(3, 247)=3.0746, *p*=,02832

current effect F(21, 219)=2.8542, *p*=.00007

Fig. 1. Results of Anova analysis for the Pb content in cereal products (mg kg^{-1})

These results may suggest that the natural environments is improving, and the rate of improvement could be measured by the lead content in cereal products. The lead content in cereal products could also be a marker of the health threat because cereals are a staple food.

The results are not significantly different from the data obtained in the 1990s by Buliński et al. (1990, 1992), Falandysz et al. (1987) and Ilow et al. (1999) or published later by other Polish authors (Kot 2003, Kot 2003, Kot, Zaręba 2007, Wojciechowska-Mazurek et al. 2008, Medyńska et al. 2009, Kot et al. 2011, Zając 2011).

In the south of Poland, for example, Smoczyńska et al. (1999) obtained higher results, as the reported mean lead content in flour reached 0.104 mg kg^{-1} compared to the present 0.034 mg kg^{-1}, and was in accordance with studies carried out in the 1980s (Nabrzyski, Gajewska 1984, Zawadzka et al. 1985).

The above results are higher than found in Germany (Brüggemann, Kumpulainen 1995, Brüggemann et al. 1996), Finland (Tahvonen, Kumpulainen 1994), the UK (Food Standards Agency 2004, Food Standards Agency 2007), or in most countries participating in the SCOOP programme (2004) as well as the data obtained by the Polish nationwide food monitoring in 2004 (Wojciechowska-Mazurek et al. 2008).

Our assessment of the lead content in cereal products (Figure 2) showed an excess of the Polish norms on two occasions, namely bran in 2010 and 2011 (*Commission Regulation (EC) no 1881/2006 of 19 December 2006, Commission Regulation (EC) no 420/2011 of 29 April 2011*). As regards the other cereal products, the mean lead content was below 63% of the threshold limit.

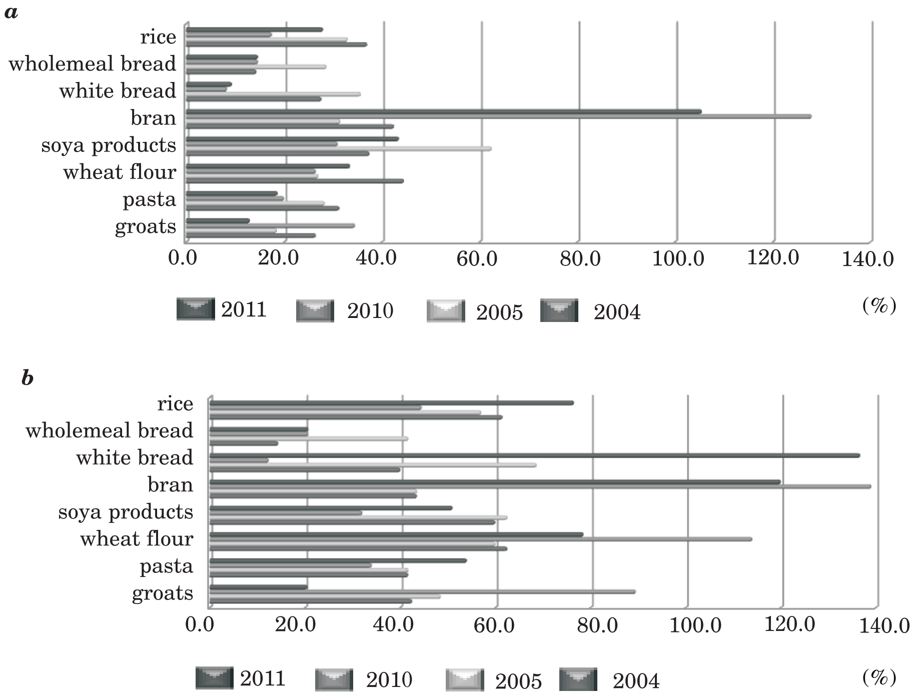


Fig. 2. Mean content (*a*) and the 90th percentile values (*b*) for Pb in cereal products from the Province of Podlasie with reference to the standards

The provisional tolerable weekly intake (PTWI) of lead from all the sources tolerated by the human body, established by the FAO/WHO Expert Committee, is 0.025 mg kg^{-1} of body weight (Commission Regulation (EC) No 1881/2006 of 19 December 2006). A weekly intake of these metals by an adult person weighing 60 kg was estimated based on both the mean lead content in particular cereal products and the data on their intake. The results are presented on Figure 3.

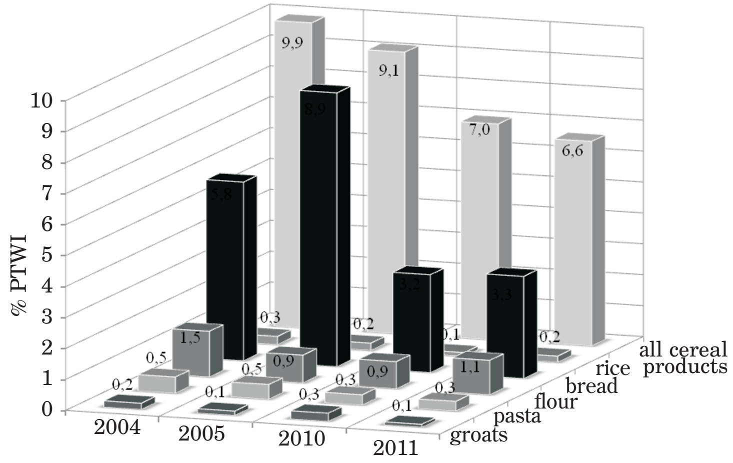


Fig. 3. Pb in cereal products from the Province of Podlasie with reference to the PTWI

According to the calculations, the lead intake with cereal products was within the amounts tolerable by the human body, that is less than 9.9% of the PTWI, of which 50% originated from bakery products.

In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) cancelled the weekly lead content tolerable by the human body (PTWI) of 0.025 mg kg^{-1} b.w. on the ground that it had failed to ensure health safety. Lower doses were determined, such as the Benchmark Dose Lower Confidence Limit (BMDL), with respect to a clearly determined effect on the human body (EFSA 2010, WHO 2011). The daily lead intake was below 56% of the $BMDL_{10}$ and 24% of the $BMDL_{01,}$ and thus the risk of developing functional disorders by the kidneys or cardiovascular system is very low. However, considering both the high intake of cereal products during the whole life and accumulation of the metal in the body, the level of lead may be estimated as being higher albeit not threatening the human health yet.

CONCLUSIONS

1. The mean lead content in the examined products (except bran in 2010-2011) was within the acceptable limits.

2. The lead intake with cereal products is within the limits tolerable by the human body (10% of the PTWI) and the lowest benchmark dose (56% $BMDL_{10}$ and 24% $BMDL_{01}$), which poses no threat to the health of residents of the Province of Podlasie.

3. A significant variety of the lead content in the examined cereal products, depending on the time of sample collection and type of product, was observed.

4. The mean lead content in 2010-2011 was significantly lower compared to 2001-2005.

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THE EFFECT OF SILICON APPLICATION AND TYPE OF SUBSTRATE ON YIELD AND CHEMICAL COMPOSITION OF LEAVES AND FRUIT OF CUCUMBER

Zbigniew Jarosz

Department of Cultivation and Fertilization of Horticultural Plants
University of Life Sciences in Lublin

Abstract

Silicon fertilization of plants improves yields and increases plant resistance to various stress factors. The use of silicon in plant fertilization is particularly justified in soilless culture. The aim of the present study, conducted in 2009-2010, was to determine the effect of root application of silicon in the form of silica sol and two growing media with a varying silica content on yield and chemical composition of fruit and leaves of the cultivar Unicum of greenhouse cucumber. Plants were grown in 10 dm^3 cylinders filled with sphagnum peat (I) or peat mixed with large-grained river sand at a ratio of 3:1 (v/v) (II). Four rates of silicon were applied in the study: 0, 250, 500 or 750 mg dm^{-3} Si. The study found a significant increase in cucumber fruit yield when plants were fertilized with silicon by root application at the rates of 500 and 750 mg dm^{-3} Si, compared to the control plants. Cucumbers grown in peat with the addition of sand (v/v 3:1) produced significantly fewer fruits compared to the culture in peat alone, but these fruits contained more total soluble solids. Significantly less calcium and more silicon were found in leaves of cucumber root-fertilized with silicon compared to the control plants. Fruits of cucumber plants fed with silicon by root application contained more dry matter, total soluble solids and silicon but less zinc and copper compared to fruits of the control plants.

Key words: peat, peat with sand, dry weight, TSS, nutritional status, macronutrients, micronutrients.

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dr Zbigniew Jarosz, DEng, Department of Cultivation and Fertilization of Horticultural Plants, University of Life Sciences in Lublin, Leszczyńskiego 58, 20-068 Lublin, Poland, e-mail: zbigniew.jarosz@up.lublin.pl

WPŁYW STOSOWANIA KRZEMU I RODZAJU PODŁOŻA NA PLONOWANIE ORAZ SKŁAD CHEMICZNY LIŚCI I OWOCÓW OGÓRKA

Abstrakt

Żywienie roślin krzemem poprawia plonowanie roślin oraz zwiększa ich odporność na różnorodne czynniki stresowe. Stosowanie krzemu w żywieniu roślin jest szczególnie uzasadnione w uprawach bezglebowych. Celem badań przeprowadzonych w latach 2009-2010 było określenie wpływu dokorzeniowego stosowania krzemu w formie zolu krzemianowego oraz dwu podłoży o zróżnicowanej zawartości krzemionki na plonowanie i skład chemiczny owoców i liści ogórka szklarniowego odmiany Unicum. Rośliny uprawiano w cylindrach o pojemności 10 dm^3 wypełnionych torfem wysokim (I) oraz torfem zmieszanym z gruboziarnistym piaskiem rzecznym w proporcji v/v 3:1 (II). W badaniach zróżnicowano dawki krzemu, stosując: 0; 250; 500 lub 750 mg dm^{-3} Si. W badaniach stwierdzono istotny wzrost plonu owoców ogórka po dokorzeniowym żywieniu roślin krzemem w dawce 500 i 750 mg dm^{-3} Si, w porównaniu z roślinami z obiektu kontrolnego. Ogórek uprawiany w torfie z dodatkiem piasku (v/v 3:1) wydał istotnie mniej owoców w porównaniu z uprawą w torfie, ale owoce te zawierały więcej ekstraktu. W liściach ogórka żywionego dokorzeniowo krzemem odnotowano istotnie mniej wapnia oraz więcej krzemu w porównaniu z roślinami kontrolnymi. Owoce ogórka żywionego dokorzeniowo krzemem zawierały więcej suchej masy, ekstraktu, krzemu oraz mniej cynku i miedzi w porównaniu z owocami roślin kontrolnych

Słowa kluczowe: torf, torf z piaskiem, sucha masa, ekstrakt, stan odżywienia, makroelementy, mikroelementy.

INTRODUCTION

The application of silicon in soilless culture of cucumber increases yield and improves the basic quality parameters of its fruit (Savant et al. 1999, Hogendorp 2008, Górecki, Danielski-Busch 2009). Numerous studies have also proven that silicon fertilization of plants improves plant resistance to stress factors, such as biotic stress (Liang et al. 2005, Kamenidou et al. 2008, Epstein 2009), salt stress (Zhu et al. 2004, Liang et al. 2006, Amirossadat et al. 2012), water stress (Kamenidou et al. 2008, Sacała 2009), and toxic metal excess (Mitani, Ma 2005, Liang et al. 2006).

The application of silicon is particularly justified in a soilless culture system, in which the roots of plants cannot use silicon resources from the soil solution (Kamenidou et al. 2008, Sonneveld, Voogt 2009). The effectiveness of silicon fertilization of plants is strictly dependent on the type of silicon fertilizer used and the applied rate of this element (Kamenidou et al. 2008, Górecki, Danielski-Busch 2009, Mohaghegh et al. 2010). The use of potassium or sodium metasilicates, waterglass oligomeric, as well as colloidal silica solutions is recommended for fertigation of plants grown in a soilless system, although the use of these materials has disadvantages too (Datnoff et al. 2001). Due to the risk of polymerisation in the nutrient solution and in the root environment, researchers continue efforts to find sources of silicon that would eliminate this adverse phenomenon during cultivation (Hogendorp 2008).

An appropriately selected medium composed of silicon-rich materials, which – under the influence of the conditions in the root environment - release to the rhizosphere plnat-available ortosilic acid, which can be an important source of silicon for soilless grown plants (Datnoff at al. 2001, Cornelis et al. 2011). Organic materials rich in this element and easily mineralized, such as rice hull and straw (Kamenidou et al. 2008, Yu et al. 2008), cereal straw (Ma and Takahashi 2002, Nurzyński et al. 2012) and composts (Kamenidou et al. 2008, Yu et al. 2008, Cornelis et al. 2011), are most frequently mentioned as a potential source of silicon for plants. Silicon available to plants can also be released from materials that are generally considered to be inert, such as rockwool, glass wool and perlite (Datnoff et al. 2001). Sand is another potential source of silicon available to plants; it is the material often added to mixed substrates (Olympios 1999, Raviw et al. 2002). However, the literature is short of more specific reports on this subject.

The aim of the present study was to determine the effect of different rates of silicon applied as silica sol and two types of substrate on yield and chemical composition of fruit and leaves of cv. Unicum greenhouse cucumber.

MATERIAL AND METHODS

In 2009-2010, a study was conducted in a greenhouse of the Department of Cultivation and Fertilization of Horticultural Plants, University of Life Sciences in Lublin. The experimental plant was cv. Unicum cucumber grown in 10 dm^3 cylinders filled with sphagnum peat (I) or peat mixed with large-grained river sand at a ratio of 3:1 (v/v) (II). Cucumber seeds were sown on 15. June 2009 and 11. June 2010 in general purpose peat growing medium. Seedlings were planted in their permanent place on 6. July 2009 and 5. July 2010 in the medium with a pH adjusted to 6.5. The experiment was carried out using a completely randomized design in 8 replications.

The plants were watered automatically by a drip-irrigation system from a deep well containing water with the following chemical composition (in mg dm^{-3}): 0.02 $N\text{-}NH_4$, 5.0 $N\text{-}NO_3$, 4.0 $P\text{-}PO_4$, 1.4 K, 120 Ca, 13.8 Mg, 32.0 $S\text{-}SO_4$, 9.5 Cl, 6.7 Si, 2.7 Na, 0.24 Fe, 0.018 B, 0.026 Mn, 0.038 Zn, 0.001 Cu, pH 7.44 and EC 0.71 mS cm^{-1}. The plants were fertilized regularly, every 8-10 days, with liquid nutrient solution prepared from the fertilizer Nutrifol green (Yara) and additionally with solution of calcium nitrate, potassium nitrate and magnesium sulphate (23% MgO). The amount of nutrients was the same in all treatments, adjusted according to the results of chemical analysis of the growing medium and to the nutritional requirements of cucumber (Sonneveld, Voogt, 2009). The rates of silicon were differentiated, consisting of 0, 250, 500 or 750 mg Si dm^{-3}. Silicon was supplied to the roots as a liquid solution of silica sol prepared in accordance with the recommendations Iler (1979), at five equal doses during the fertilization of the plants.

Fruit picking was carried out three times a week from 3 August to 19 August 2009 and from 4 August to 23 August 2010, each time determining the number and weight of fruits from particular plants. Due to their very good quality parameters, all fruits harvested from the treatments were classified as marketable yield. Plant protection treatments and cultivation were performed in accordance with the relevant recommendations.

Fruits were sampled for analysis in the middle of the fruiting period, while leaves were collected at the end of the experiment. Dry weight was determined in fresh fruits by the gravimetric method (PN-90/A-75101/03), while total soluble solids (TSS) were determined refractometrically. After leaves and fruits were dried (temp. 105°C), total nitrogen was determined using Kjeldahl's method. Following mineralization of the material in a mixture of nitric and perchloric acids at a ratio of 3:1 (v/v), phosphorus was determined colourimetrically with ammonium-vanadium-molybdate (Thermo, Evolution 300), while potassium, calcium, magnesium, iron, manganese, zinc and copper were assessed by AAS (Perkin-Elmer, Analyst 300). After ashing the material, the silicon content in leaves and fruits was determined by X-ray fluorescence (XRF) using an Axios spectrometer (Panalitycal).

The results were submitted to analysis of variance, using the mean values and employing Tukey's test to evaluate the significance of differences at α=0.05 level of significance. The presented results are two-year means.

RESULTS AND DISCUSSION

Silicon is an element that has a beneficial effect on plants, but crop plants differ radically in their ability to take up and accumulate this element (Epstein 1994, Savant et al. 1999, Epstein 2009). Numerous studies have proven that the application of silicon in fertilization of cucumber has a positive influence on the growth, development and yield of plants (Hogendorp 2008, Górecki, Danielski-Busch 2009, Mohaghegh et al. 2010).

The statistical analysis of the present results showed a significant increase in cucumber fruit yield in the treatments where silicon was root applied at the rates of 500 and 750 mg dm^{-3} compared to the control, in which plants were not supplied with silicon (Table 1). This increase was 8.60 and 5.89%, respectively. These results support earlier studies, in which silicon fertilization of plants resulted in a significant increase in cucumber yield (Górecki, Danielski-Busch 2009, Mohaghegh et al. 2010). In the research

Table 1

The effect of silicon dose (mg) and type of substrate on the yield of fruits (kg plant $^{-1}$), number of fruit per plant, dry weight (%) and total soluble solid (% fr. w.) in the fruit of cucumber

Substrate (A)	Silicon dose (B)	Year (C)	Yield of fruit	Number of fruit	Dry weight	TSS
Peat	0	2009	1.879	9.875	3.945	3.157
		2010	1.539	7.501	4.295	4.071
	250	2009	1.909	9.125	4.630	3.914
		2010	1.846	8.375	4.470	3.800
	500	2009	2.010	9.130	4.745	2.857
		2010	1.809	8.875	4.545	4.100
	750	2009	1.899	8.503	4.700	3.486
		2010	1.794	8.375	4.475	4.171
Peat + sand	0	2009	1.761	8.505	3.360	2.786
		2010	1.473	7.510	3.955	3.329
	250	2009	1.831	8.375	4.085	3.243
		2010	1.456	7.495	4.215	3.371
	500	2009	1.799	7.750	3.910	3.343
		2010	1.604	8.490	4.320	3.529
	750	2009	1.960	8.250	4.650	3.829
		2010	1.394	6.750	4.320	3.643
Mean A	peat		1.819	8.719	4.501	3.695
	peat + sand		1.660	7.891	4.102	3.834
Mean B	0 mg		1.663	8.344	3.939	3.336
	250 mg		1.727	8.243	4.350	3.582
	500 mg		1.806	8.562	4.380	3.457
	750 mg		1.761	7.969	4.536	3.782
Mean C	2009		1.875	8.687	4.253	3.327
	2010		1.604	7.922	4.349	3.752
$LSD_{0.05}$						
A			0.043	0.407	0.072	0.093
B			0.079	n.s.	0.138	0.173
C			0.152	0.563	0.075	0.090
A x B			0.183	n.s.	0.237	0.290

n.s.- not significant

of Górecki, Danielski-Busch (2009), an increase in yield of silicon-fed plants resulted from a significantly higher number of fruits per plant. In the present study, the number of fruits per plant in individual treatments did not differ (Table 1), and the recorded increase in yield should therefore be explained by the higher unit fruit weight.

A significant decrease (by 8.74%) in the fruit yield of cucumber plants grown in peat with the addition of sand (v/v 3:1) compared to those grown in peat alone is an interesting finding. This result should be related to the nutritional status of the examined plants (Table 2). Significantly less potassium (40.06 g kg^{-1} DW), calcium (79.02 g kg^{-1} DW) and magnesium (12.35 g kg^{-1} DW) occurred in leaves of cucumber grown in peat with the addition of sand compared to plants grown in peat alone. Poorer nutrition of plants grown in peat with sand could have been the reason for the poorer yield of plants growing in these treatments. Similar results were also obtained in an experiment on tomato grown in sand, compared to peat and rockwool (Jarosz 2006). The poorer yield of cucumber grown in peat with sand could also have resulted from an increase in the medium density and the consequent change in air and water conditions (Olympios 1999, Raviw et al. 2002).

The analysis of the results obtained in the present study showed a significantly higher content of dry matter and total soluble solids (TSS) in fruits of silicon-fertilized plants by root application compared to control plants (Table 1). These results corroborate earlier reports that proved the beneficial effect of the application of silicon in plant cultivation on the main quality parameters of fruits, including the content of dry matter and total soluble solids (Aziz et al. 2002, Hogendorp 2008). Significantly less dry matter (4.102%) and more TSS (3.834 % fr. w.) were recorded in fruits of cucumber grown in peat with the addition of sand compared to cucumber plants grown in peat alone.

Silicon is taken up by plants mainly as uncharged orthosilicic acid monomers (Ma, Takahashi 2002, Epstein 2009, Sacała 2009), with ion forms being less important in plant nutrition (Ma, Takahashi 2002, Matichenkov, Calvert 1999). Nevertheless, root fertilization with silicon significantly affects the uptake and distribution of nutrients in the plant (Epstein 1994, Aziz et al. 2002, Ma, Takahashi 2002, Epstein 2009). This is also confirmed by the results reported herein (Tables 2-3). It should be stressed that there was no significant effect of root-applied silicon on the phosphorus content in leaves and fruits of the analyzed plants. These results are contradictory to many reports, which demonstrate a beneficial effect of silicon fertilization on improved phosphorus nutrition of plants (Epstein 1994, Aziz et al. 2002). According to Matichenkov, Ammosova (1996), orthosilicic acid has the ability to release phosphorus from calcium, aluminium and iron phosphates, which are unavailable to plants, and it adsorbs aluminium hydroxides reducing their mobility; thereby, the chemical sorption of phosphates is reduced by 40-70% (Junior et al. 2010). However, all these relationships were observed

Table 2

The effect of silicon dose (mg) and type of substrate on the content of selected nutrients and silicon in leaves of cucumber (2009-2010)

Substrate (A)	Silicon dose (B)	N total	P	K	Ca	Mg	Si	Fe	Zn	Mn	Cu
		(g kg^{-1} d.w.)						(mg kg^{-1} d.w.)			
Peat	0	45.82	4.465	45.12	103.9	12.30	2.340	89.95	86.85	241.14	5.475
	250	46.15	3.543	45.10	87.05	14.35	5.530	113.41	98.87	318.21	5.820
	500	45.70	4.438	41.05	91.18	13.85	5.457	210.73	94.47	262.67	5.997
	750	44.62	3.920	38.97	78.37	12.65	6.887	95.24	91.70	263.14	5.577
Peat + sand	0	46.30	4.045	41.62	86.92	12.15	4.527	90.62	101.61	375.73	5.607
	250	45.87	4.990	40.61	79.75	12.45	4.850	97.07	103.84	339.67	5.955
	500	45.82	5.135	39.75	77.45	11.77	5.905	90.69	98.77	366.01	5.982
	750	46.30	4.495	38.25	71.97	13.02	6.690	102.70	89.12	375.54	5.702
Mean A	peat	45.57	4.091	42.56	90.29	13.29	5.054	127.39	92.97	271.18	5.717
	peat + sand	46.07	4.666	40.06	79.02	12.35	5.493	95.26	98.32	364.24	5.812
Mean B	0	46.06	4.255	43.37	95.42	12.22	3.434	90.29	94.24	308.42	5.541
	250	46.01	4.266	42.85	83.40	13.40	5.190	105.22	101.33	329.13	5.887
	500	45.76	4.786	40.41	84.62	12.81	5.681	150.68	96.62	314.31	5.990
	750	45.46	4.208	38.61	75.17	12.84	6.789	98.96	90.41	319.19	5.640
LSD$_{0.05}$											
A		0.310	0.466	0.771	2.736	0.687	n.s.	n.s.	4.315	9.738	n.s.
B		n.s.	n.s.	1.471	5.224	n.s.	1.601	n.s.	n.s.	15.59	n.s.
A x B		1.018	n.s.	2.518	8.938	n.s.	n.s.	n.s.	n.s.	31.82	n.s.

n.s.- not significant

Table 3

The effect of silicon dose (mg) and type of substrate on the content of selected nutrients and silicon in fruits of cucumber (2009-2010)

Substrate (A)	Silicon dose (B)	N total	P	K	Ca	Mg	Si	Fe	Zn	Mn	Cu
		(g kg^{-1} d.w.)						(mg kg^{-1} d.w.)			
Peat	0	37.75	5.541	31.60	5.495	2.218	0.723	48.52	33.55	34.72	9.300
	250	37.95	5.737	31.05	4.243	1.821	0.983	47.97	32.27	32.40	7.210
	500	37.01	5.958	31.42	5.398	2.164	1.115	44.07	29.90	32.85	7.460
	750	33.92	5.759	33.82	5.310	2.216	1.324	49.25	28.37	34.67	6.775
Peat + sand	0	40.02	6.320	30.55	4.400	2.020	0.907	50.42	40.45	40.55	9.437
	250	36.52	6.492	22.05	4.470	2.254	1.138	45.57	34.77	39.42	7.705
	500	38.01	6.331	23.90	4.795	2.238	1.130	44.22	34.62	39.30	7.447
	750	34.85	7.051	32.15	4.160	2.206	1.368	44.15	30.68	40.60	6.367
Mean A	peat	36.66	5.749	31.97	5.111	2.105	1.037	47.45	31.02	33.62	7.687
	peat + sand	37.35	6.549	27.16	4.456	2.179	1.135	46.09	35.13	39.97	7.739
Mean B	0	38.89	6.040	31.07	4.948	2.119	0.815	49.47	37.00	37.64	9.369
	250	37.24	6.114	26.55	4.356	2.037	1.058	46.77	33.52	35.91	7.457
	500	37.50	6.145	27.66	5.096	2.201	1.125	44.15	32.26	36.07	7.454
	750	34.39	6.296	32.99	4.735	2.221	1.346	46.70	29.52	37.64	6.572
LSD$_{0.05}$											
A		0.307	0.171	1.355	0.332	n.s.	n.s.	n.s.	1.415	1.226	n.s.
B		0.588	n.s.	2.588	0.634	n.s.	0.191	2.877	2.701	n.s.	0.398
A x B		1.005	0.558	4.428	1.085	0.357	n.s.	n.s.	n.s.	n.s.	n.s.

n.s.- not significant

under field conditions and seem related to the soil environment. In soilless culture, the effectiveness of silicic acid is of lesser importance, since the root environment of plants grown in growing media is free from excessive mobile forms of aluminium, iron and manganese, while phosphorus is regularly supplied in small doses throughout the whole growing period. It is also worth remembering that the effect of silicates used in plant fertilization on the uptake and allocation of nutrients in the plant is not fully explained and the conclusions are often contradictory. An example is the study of MA, TAKAHASHI (2002), who showed a decrease in the phosphate ion content in aerial parts of plants fertilized with orthosilicic anions, explaining this phenomenon by the competition in the uptake of these anions by plants. In turn, EPSTEIN (1994) thinks that the benefits resulting from silicon fertilization of plants, compared to phosphorus fertilization, primarily consist in regulating the uptake and allocation of phosphorus and zinc in plants by silicon.

The analysis of the results obtained in the present study showed a significantly lower calcium content, which ranged from 75.15 to 84.62 g kg^{-1} DW, in leaves of cucumber grown in the silicon-fertilized treatments (Table 2). Leaves of cucumber grown in peat with the addition of sand also contained significantly less calcium (79.02 g kg^{-1} DW) compared to plants grown in peat alone (90.29 g kg^{-1} DW). These results are in agreement with the findings of other authors who showed a reduction in the calcium content in plants fed with silicon (EPSTEIN 1994, CHEN et al. 2011). A decrease in the calcium content in silicon-fed plants can be caused by the fixation of this element by silicates in the root environment of plants as well as in the plant structures (MA, TAKAHASHI 2002). AZIZ et al. (2002) present a different view. These authors quote the results indicating that the application of silicon in rice growing caused a decrease in the content of nitrogen, potassium, iron and manganese as well as an increase in the content of calcium and magnesium in plants. The present study found a significant reduction in the leaf potassium content compared to the control treatments only in the treatments where plants were fertilized with silicon at the rates of 500 and 750 mg Si dm^{-3} (by 6.82 and 10.97%, respectively).

Cucumber is a species characterized by a moderate affinity for the uptake of silicon, accumulating from 0.5 to 1.5% Si in dry matter (HOGENDORP 2008). In this study, an amount from 4.850 to 6.690 g kg^{-1} DW of silicon was found in leaves of plants fertilized with silicon by root application, while the silicon content in fruits was 0.983 do 1.368 g Si kg^{-1} DW. A lower content of silicon in cucumber leaves and fruit was found by GÓRECKI, DANIELSKI-BUSCH (2009), who investigated the effectiveness of calcium and ammonium silicate as slow silicon release fertilizers in container cultivation. In turn, studying the effect of different silicon concentrations together with fertigation in rockwool-grown cucumber, SONNEVELD, VOOGT (2009) found from 350 to 1400 mmol Si kg^{-1} DW in leaves and from 25 to 100 mmol Si kg^{-1} DW in fruits.

A relatively high Si content in leaves and fruits of plants in the control treatments, in which this element was not used for root fertilization, needs to be stressed (Tables 2, 3). As reported by MA, TAKAHASHI (2002), water used for watering plants can be a source of available silicon for plants grown in a soilless system. Orthosilicic acid can also be released from a growing medium. LIU et al. (2011) showed the release of silicon forms available to plants from sand at an amount of 8-13 mg kg^{-1}, whereas RAVIW et al. (2002) reported an analogous value within the range of 8-10 mg kg^{-1}, depending on the plant growing conditions. In turn, DATNOFF et al. (2001) reports that sphagnum peat, which contains from 15 to 220 mmol kg^{-1} Si and releases this element during plant cultivation at an amount of *ca* 0.2 mmol dm^{-3}, can also be a source of silicon available to plants. However, the present study did not show significant differences in the silicon content in leaves and fruits of plants depending on the type of medium.

The statistical analysis of the results obtained in the present study showed significantly less zinc (29.52-33.52 mg kg^{-1} DW) and copper (6.572-7.457 mg kg^{-1} DW) in fruits of plants fertilized with silicon by root application compared to fruits picked from control plants. Similar results are presented in other reports, which confirms that silicon fertilization of plants significantly modifies the chemical composition of marketable plant organs (EPSTEIN 1994, SAVANT et al. 1999, MA, TAKAHASHI, 2002, EPSTEIN 2009).

CONCLUSIONS

1. The study demonstrated a significant increase in cucumber fruit yield when plants were fertilized by root application with silicon at the rates of 500 and 750 mg dm^{-3} Si, compared to control plants.

2. Cucumber grown in peat with the addition of sand (v/v 3:1) produced significantly fewer fruits compared to plants grown in peat alone, but these fruits contained more total soluble solids.

3. Significantly more silicon and less calcium were found in leaves of cucumber plants fertilized by root application with silicon, compared to control plants.

4. Fruits of cucumber plants fed with silicon by root application contained more dry matter, total soluble solids and silicon but less zinc and copper compared to fruits of control plants.

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CONTENT OF TOTAL PHOSPHORUS IN SOIL UNDER MAIZE TREATED WITH MINERAL FERTILIZATION AGAINST THE PHOSPHATASE ACTIVITY

Joanna Lemanowicz, Anetta Siwik-Ziomek, Jan Koper

Department of Biochemistry
University of Technology and Life Sciences in Bydgoszcz

Abstract

The aim of the paper was to determine changes in the content of total phosphorus and its selected forms in soil under maize treated with mineral fertilization. The activity of enzymes participating in transformations of this bioelement was also investigated.

In October 2008, soil was sampled from a field experiment set up at the Agricultural Experimental Station in Grabów nad Wisłą, conducted by the IUNG Institute in Puławy. The experiment involved the application of nitrogen in the form of ammonium nitrate at rates of 0, 50, 100, 150, 200 and 250 kg N ha^{-1} and concomitant P, K, Mg, Ca and S fertilization. The following determinations were made on the soil samples: the content of total phosphorus, phosphorus in organic compounds and available phosphorus as well as the activity of alkaline and acid phosphatases. The analysis of variance showed a significant effect of the experimental factors on changes in the content of total phosphorus and its selected forms. The content of available phosphorus was 55.85 mg P_{E-R} kg^{-1}, which classified the soil to class III, that is with a moderate content of this nutrient. The lack of phosphorus fertilization (KMgCaS variant) as well as the use of high nitrogen rates changed the soil phosphorus abundance class from average to low. The soil content of phosphorus in organic compounds was about 25% of the total phosphorus content, but when nitrogen fertilization had been applied at the rates above 100 kg N ha^{-1}, P_{org} decreased to about 15%.

The activity of phosphomonoesterases was the highest in soil from the treatments not fertilized with phosphorus (KMgCaS). A close dependence was found between the activity of acid phosphatase and the total phosphorus content or the content of its examined forms. The value of the coefficient of correlation between the AcP activity and the P_{E-R} content in soil was r=-0.83, p<0.05.

Key words: phosphorus, alkaline and acid phosphatases, mineral fertilization, soil.

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dr inż. Joanna Lemanowicz, Department of Biochemistry, University of Technology and Life Sciences in Bydgoszcz, 85-029 Bydgoszcz, Bernardyńska 6, Poland, phone: +48 (052) 374-95-55, e-mail: jl09@interia.pl

ZAWARTOŚĆ FOSFORU W GLEBIE SPOD UPRAWY KUKURYDZY NAWOŻONEJ MINERALNIE NA TLE AKTYWNOŚCI FOSFATAZOWEJ

Abstrakt

Celem pracy było określenie zmian zawartości fosforu ogółem oraz jego wybranych form w glebie spod uprawy kukurydzy nawożonej mineralnie. Zbadano również aktywność enzymów biorących udział w przemianach tego biopierwiastka.

Próbki gleby pobrano w październiku 2008 r. z doświadczenia polowego założonego na terenie RZD w Grabowie nad Wisłą przez IUNG w Puławach. W doświadczeniu zastosowano azot w postaci saletry amonowej w dawkach 0, 50, 100, 150, 200 i 250 kg N ha^{-1} oraz nawożenie P, K, Mg, Ca i S. W próbkach gleby oznaczono zawartość fosforu ogółem, związków organicznych i przyswajalnego oraz aktywność fosfatazy alkalicznej i kwaśnej. Analiza wariancji wykazała istotny wpływ zastosowanych czynników doświadczenia na zmiany zawartości fosforu ogółem i jego wybranych form. Zawartość fosforu przyswajalnego kształtowała się na poziomie 55.85 mg $P_{E\text{-}R}$ kg^{-1}, co klasyfikuje glebę do klasy III o średniej zawartości tego składnika pokarmowego. Brak nawożenia fosforem (kombinacja KMgCaS) oraz stosowanie wysokich dawek azotu spowodowało zmianę klasy zasobności gleby w fosfor ze średniej na niską. Zawartość fosforu związków organicznych w glebie stanowiła około 25% całkowitej zawartości fosforu, a przy nawożeniu azotem w dawkach powyżej 100 kg N ha^{-1} spowodowało zmniejszenie P_{org} do ok 15%.

Aktywność badanych fosfomonoesteraz była najwyższa w glebie z obiektów nienawożonych fosforem (KMgCaS). Stwierdzono ścisłą zależność między aktywnością kwaśnej fosfatazy a zawartością fosforu ogółem i jego badanych form. Współczynnik korelacji między aktywnością AcP a zawartością $P_{E\text{-}R}$ w glebie wyniósł r=-0.83, p<0.05.

Słowa kluczowe: fosfor, fosfataza alkaliczna i kwaśna, nawożenie mineralne, gleba.

INTRODUCTION

Phosphorus plays a key role in defining the nutrient richness and fertility of soils. It is also a crucial element, indispensable for sustaining proper functions of plants and obtaining high quantities and good quality of yields (Gaj, Grzebisz 2003). However, like nitrogen, phosphorus can trigger severe disorders in the natural environment, for example eutrophication of water bodies. *The Regulation of the Minister of the Environment* (2002) based on the Nitrate Directive (91/676/EEC) was the first step towards restraining the negative effect of agriculture on the environment. One of the more serious side-effects of agricultural production in Poland is excessive soil acidification (Kaczor 2002). The main factors responsible for undesirable soil acidification are unbalanced mineral fertilization treatments, especially excessive application of nitrogen fertilizers treated as a key yield-forming factor. Supplying plants with right proportions of nutrients is among the essential conditions assuring optimal crop yields (Kaczor, Łaszcz-Zakorczmenna 2009, Murawska, Spychaj-Fabisiak 2009, Lemanowicz 2011) However, the amount of a nutrient introduced into soil with fertilizer does not correspond to the amount up taken with the yield. The use of phosphorus by plants does not exceed 40%; the remaining amount is transformed into insoluble forms, unavailable to crops and often leached from soil (Mazur, Mazur 2010). The biogeochemical phosphorus cycle is sensitive to changes induced by agricultural treatments since most mineral fertilizers include this element. This is the reason why agronomic practice often changes the natural phosphorus cycling.

The aim of the paper was to evaluate the content of selected phosphorus forms and the activity of phosphatases in soil under maize treated with mineral fertilization.

MATERIAL AND METHODS

A long-term field experiment from which soil was sampled had been set up at the Agricultural Experiment Station in Grabów nad Wisłą, by the IUNG Institute of Soil Science and Plant Cultivation in Puławy. The soils at the Experimental Station in Grabów are Haplic Luvisols (PN-R-04033, 1998) classified as a very good rye complex. The experiment was conducted in a four-year rotation cycle: grain maize, spring barley, winter oilseed rape, winter wheat+intercrop.

A two-factorial experiment in a randomised block design was run in two replications. The first factor involved P, K, Mg, Ca and S fertilization at six levels: 1 – P K Mg Ca S, 2 – K Mg Ca S, 3 – P Mg Ca S, 4 – P K Ca S, 5 – P K Mg S, 6 – P K Mg Ca. The second factor consisted of nitrogen fertilization at the following rates: 0, 50, 100, 150, 200, 250 kg N ha^{-1}.

The following fertilizer forms were applied: phosphorus and potassium fertilizers containing sulphur such as single superphosphate and potassium sulphate in treatments with sulphur; phosphorus and potassium fertilizers which did not include sulphur such as triple superphosphate and high-percentage potassium salt in treatments without sulphur; dolomite containing 30% CaO and 10% Mg in treatments with Ca and Mg; lime was used at the amount of 200 kg CaO ha^{-1} on plots without Mg, and magnesium sulphate was supplied at the rate of 70 kg MgO ha^{-1} on plots without Ca. The rates of minerals applied in the experiment were as follows: 80 kg P_2O_5 ha^{-1}, 140 K_2O ha^{-1}, 70 MgO ha^{-1}, 200 CaO ha^{-1}. The S rate is a result of the sulphur amount introduced with adequate rates of P, K, Mg.

Soils samples from fields cropped with maize were collected in October 2008.

The following determinations were made on properly prepared samples of soil material:

- total phosphorus was isolated from soil (P_{tot}) with the method by Mehta et al. (1954);

- phosphorus in organic compounds (P_{org}) was calculated as the difference between the total P content determined in mineralized samples and the content of inorganic phosphorus determined in non-mineralized samples;
- content of available phosphorus ($P_{E\text{-}R}$) in soil was tested with the Egner-Riehm method – DL (Lityński et al. 1976);
- the activity of alkaline (AcP) [E.C. 3.1.3.1] and acid phosphatases (AcP) [E.C. 3.1.3.2] in soil was analysed with the Tabatabai and Bremner method (1969), which enabled the calculation of the AlP:AcP ratio, expressing the right soil reaction (Dick et al. 2000);
- pH in 1M KCl was checked using the potentiometric method.

The data thus achieved underwent an analysis of variance and the significance of differences between the means was defined with Tukey's test at the significance level p=0.05. The calculations were made with FR-ANAL -WAR software based on Microsoft Excel. Finally, the results were submitted to an analysis of simple correlation (p<0.05), which determined the degree of the dependence between respective features. The correlation analysis was made using Statistica for Windows Pl software.

RESULTS AND DISCUTION

The exchangeable acidity of soil measured in the humus horizon (Ap) ranged from 5.8 to 6.5 (Table 1), which means that the soil was slightly acid. The mineral fertilization applied exclusively in the experiment differentiated the soil reaction rather weakly. Janowiak et al. (2005) showed a decrease in soil pH by 1.7 unit following an application of 180 kg N ha^{-1}. According to Filipek (2001), nitrogen in the form of ammonium causes acidification of soil solution due to both nitrification and the uptake of NH_4^+ cations by the plant root system.

The total phosphorus content in the soil ranged from 0.271 to 0.389 g P_{tot} kg^{-1}, depending on the variant of mineral and nitrogen fertilization

Table 1

pH KCl of the soil

Mineral fertilization	Nitrogen (g kg^{-1})					
	0	50	100	150	200	250
P K Mg Ca S	6.4	6.4	6.3	6.3	6.1	5.9
K Mg Ca S	6.5	6.4	6.4	6.3	6.1	5.8
P Mg Ca S	6.4	6.3	6.3	6.1	6.0	5.9
P K Ca S	6.2	6.2	6.0	6.1	5.9	5.9
P K Mg S	6.1	6.1	6.0	5.9	5.8	5.6
P K Mg Ca	6.5	6.4	6.5	6.3	6.3	6.1

(Table 2). As reported by VERMA et al. (2005), in the soils fertilized with nitrogen alone, the P_{tot} content was much higher and remained at the level of 0.507 g P_{tot} kg^{-1}. The highest P_{tot} content (0.389 g P_{tot} kg^{-1}) was found in the soil with complete mineral fertilization (P K Mg Ca S variant) – Table 2. Increasing nitrogen rates decreased the content of total phosphorus in soil.

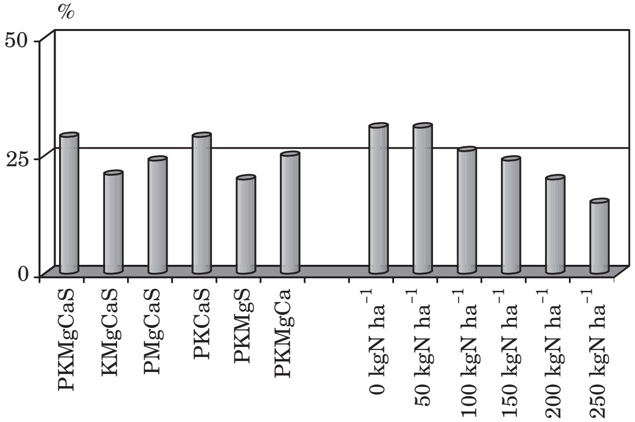
Table 2

The content of total phosphorus (P_{tot}), organic phosphorus (P_{org}) and available phosphorus ($P_{E\text{-}R}$) of investigated lessive soil depending on differentiated mineral fertilization (P, K, Mg, Ca, S) and increasing nitrogen doses

Specification		Phosphorus		
		total (g kg^{-1})	organic (g kg^{-1})	available (mg kg^{-1})
Mineral fertilization I factor	P K Mg Ca S	0.389	0.111	73.33
	K Mg Ca S	0.271	0.057	44.21
	P Mg Ca S	0.350	0.085	50.15
	P K Ca S	0.330	0.097	57.50
	P K Mg S	0.304	0.062	49.10
	P K Mg Ca	0.316	0.079	60.87
Nitrogen (kg ha^{-1}) II factor	0	0.366	0.113	77.83
	50	0.346	0.107	72.71
	100	0.340	0.089	59.96
	150	0.320	0.078	50.37
	200	0.305	0.062	42.58
	250	0.284	0.042	32.01
Mean		0.327	0.082	55.85
$LSD_{0.05}$				
I factor		0.003	0.003	1.614
II factor		0.003	0.003	1.614
Interaction				
I/II		0.008	0.008	3.955
II/I		0.008	0.008	3.955

Mineral fertilization alone, accompanied by increasing nitrogen rates, decreased the content of phosphorus in organic compounds in soil (Table 2). MCDOWELL, MONAGHAN (2002) found that nitrogen application at the rates of 200 kg N ha^{-1} and 400 kg N ha^{-1} resulted in a phosphorus decrease by 12% and 6%, respectively, compared with the control.

In most arable soils, phosphorus contained in organic bonds accounts for 20-50% of total P, but in the present research the share of organic phosphorus compounds in the total content of this nutrient was 15%-31%, depending on the fertilization (Figure 1). This result was attributed to the application of exclusive mineral fertilization, since in both natural and organic fertilizers nutrients occur mostly in organic compounds and are taken up by plants owing to microbiological and biochemical mineralization.



Rys. 1 Percentage of phosphorus in organic compounds in the total phosphorus concentration in the analyzed soil

The content of available phosphorus was 55.85 mg $P_{E\text{-}R}$ kg^{-1}, which classified the soil as class III, with an average content of this nutrient, according to PN-R-04023 (1996) (Table 2). On the other hand, a decrease in the $P_{E\text{-}R}$ content (44.21 mg $P_{E\text{-}R}$ kg^{-1}) was observed in the soil without phosphorus fertilization (K Mg Ca S variant), depressing the soil abundance from average to low. In the soil where maize was grown without Ca fertilization (P K Mg S variant), the $P_{E\text{-}R}$ content was 33% lower than in the variant with complete fertilization (P K Mg Ca S). The lack of liming, causing a lower soil pH, depressed the activation of soil phosphorus reserves by stimulating the transition of available forms into those hardly available to plants.

The variant without sulphur fertilization had a higher content of available phosphorus (60.87 mg $P_{E\text{-}R}$ kg^{-1}) than the other fertilization combinations except for complete mineral nutrition. As reported by FILIPEK and SKOWROŃSKA (2009), sulphur oxidation in soil increases the concentration of H^+ ions, which can lower the soil pH to 2-3, thus decreasing the P content in soil (SKWIERAWSKA, ZAWARTKA 2009, MAJCHERCZAK et al. 2013). Similarly, according to KACZOR, ŁASZCZ-ZAKORCZMENNA (2009), sulphur deficit in soil can limit the uptake of basic nutrients, including phosphorus, by plants. A lower supply of plants with sulphur limited the phosphorus uptake by plants, so that the soil from the sulphur unfertilized treatments was very rich in available phosphorus form.

Soil from the treatments with nitrogen fertilization was found to contain far less phosphorus available to plants, less by 33% on average for all the treatments, compared to the treatments not fertilized with this nutrient (Table 2), a result connected with the yield-stimulating effect of nitrogen and a resultant increase in the phosphorus uptake, accompanied by an increase in soil acidification caused by nitrogen fertilizers. In response to the increasing nitrogen rates, the soil changed its richness class from average to low. The lack of organic fertilization and unbalanced mineral fertilization decreased the content of the plant available phosphorus forms. Adding organic matter to soil alleviates the effects of unbalanced mineral fertilization and intensifies the processes of phosphate uptake by plants since FYM carbon releases phosphorus from organic bonds.

The statistical analysis confirmed a significant effect of the experimental factors on the changes in the activity of alkaline and acid phosphatase, the enzymes responsible for transformations of phosphorus in soil. In the soil taken up from the treatments where maize was fertilized without phosphorus (K Mg Ca S variant), the activity of both phosphatases was high (alkaline phosphatase 1.064 mM pNP kg^{-1} h^{-1}, acid phosphatase 2.293 mM pNP kg^{-1} h^{-1}).

According to ŻEBROWSKA et al. (2008), phosphorus deficit increases the production and secretion of extracellular acid phosphatases by plant roots to the substrate. No liming (P K Mg S variants) depressed the activity of alkaline phosphatase (0.844 mM pNP kg^{-1} h^{-1}). At the same time, a decrease in soil pH occurred in non-limed treatments (Table 1). The low activity of alkaline phosphomonoesterase could be due to the sensitivity of phosphatases to soil reaction changes (WITTMANN et al. 2004). Increasing nitrogen rates significantly affected the activity of soil phosphatases, for example an inhibition of the activity of alkaline phosphatase was observed. A decrease in the enzymatic soil activity as a result of its acidification is due to the destruction of ion and hydrogen bonds in the active centre of an enzymatic protein. In turn, the activity of acid phosphatase was increasing to the nitrogen rate of 250 kg N ha^{-1}, when it reached the highest value (2.169 mM pNP kg^{-1} h^{-1}) – Table 3. The enzymatic index of the soil pH was derived from the achieved values of the activity of acid and alkaline phosphatases (DICK et al. 2000). The value of the AlP:AcP ratio during the research was 0.312-0.741 (Table 3). The soil pH value considered optimal for the growth and development of plants ensures an adequate ratio of the AlP:AcP activity, namely 0.500. The value of the AlP:AcP ratio lower than 0.50 implies acid soil reaction and liming is recommended (DICK et al. 2000). Based on the enzymatic index of the soil pH calculated, it was noticed that the nitrogen rates above 100 kg N ha^{-1} as well as the lack of Ca fertilization (P K Mg S variant) (AlP:AcP <0.500) acidified the soil. The soil pH index can be used as an alternative method to determine soil pH and its changes (LEMANOWICZ, KOPER 2009, LEMANOWICZ 2011).

The activity of acid phosphatase was closely correlated with the content of total phosphorus and its forms. The value of the correlation coefficient between the activity of AcP and the $P_{E\text{-}R}$ content in soil was $r=-0.83$, $p<0.05$, which corroborates earlier reports by FUKUDA et al. (2001) and KIELISZEWSKA--ROKICKA (2001), showing that the activity of soil phosphatases is usually reversely proportional to the content of mineral phosphorus. In response to

Table 3

The activity of alkaline (AlP) and acid (AcP) phosphatases and the AlP:AcP ratio in the investigated lessive soil depending on differentiated mineral fertilization (P, K, Mg, Ca, S) and increasing nitrogen doses

Specification		Phosphatases (mM pNP kg^{-1} h^{-1})		AlP:AcP
		Alkaline	Acid	
Mineral fertilization I factor	P K Mg Ca S	0.909	1.558	0.583
	K Mg Ca S	1.064	2.293	0.464
	P Mg Ca S	0.871	1.690	0.515
	P K Ca S	0.877	1.741	0.504
	P K Mg S	0.844	1.835	0.459
	P K Mg Ca	1.001	1.914	0.523
Nitrogen (kg ha^{-1}) II factor	0	1.167	1.575	0.741
	50	1.137	1.630	0.698
	100	0.986	1.778	0.555
	150	0.860	1.882	0.473
	200	0.742	1.998	0.371
	250	0.676	2.169	0.312
Mean		0.928	1.838	
$LSD_{0.05}$				
I factor		0.011	0.034	
II factor		0.011	0.034	
Interaction				
I/II		0.026	0.083	
II/I		0.026	0.083	

the deficit of phytoavailable phosphorus, plants synthesize acid phosphatases, which stimulate transformations of organic phosphorus compounds into inorganic ones, directly available to plants. However, plant species differ in the amount and activity of the enzyme secreted. A significantly high coefficient of the correlation between the soil content of phosphorus in organic compounds and the available phosphorus form in soil was determined (r=0.95; p<0.05). Based on the significant values of the correlation coefficients between the activity of acid phosphatase and the content of phosphorus, it was concluded that the activity of this enzyme was a good parameter for characterization of soil, unlike the activity of alkaline phosphatase. According to YADAV and TARAFDAR (2001), the activity of phosphatases can be used as an index to determine the degree of mineralization of organic phosphorus compounds. At the same time, phosphatases could be used for diagnosing the content of inorganic phosphorus in soils.

CONCLUSIONS

1. Significant effect of mineral and nitrogen fertilization on changes in the content of total, organic and available phosphorus was determined. The highest content of phosphorus and its forms was shown in the soil with complete mineral fertilization composed of P K Mg Ca S and treated with 0 and 50 kg N ha^{-1}.

2. Unbalanced nitrogen fertilization without phosphorus nutrition decreased the content of the available form of phosphorus, changing the class of P richness of soil from average to low.

3. Determination of the activity of acid and alkaline phosphatases could aid long-term monitoring of the phosphorus status of soil and changes in soil abundance due to anthropogenic factors.

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ACCUMULATION OF COPPER IN SELECTED ELEMENTS OF A FOOD CHAIN IN A POND ECOSYSTEM

Marcin Niemiec, Barbara Wiśniowska-Kielian

Chair of Agricultural and Environmental Chemistry
University of Agriculture in Krakow

Abstract

The content of elements in an aquatic environment (water and bottom sediments) is not a reliable indicator of a potential threat of toxic compounds to living organisms. The amounts of elements accumulated in living organisms depend primarily on forms in which the elements occur in the environment. The measurement of the rate of uptake of trace elements by living organisms is known as a bioaccumulation coefficient. Copper is one of the most toxic metals to water organisms. In respect of its toxicity, it is the third (after mercury and silver) most toxic element among metals commonly present in the environment. The paper aimed to determine the copper accumulation in individual links of a food chain in a water ecosystem of a pond used for extensive carp aquaculture. Based on the copper concentration in the carps, the value of the copper bioaccumulation coefficient in the water ecosystem was calculated and the degree of contamination of the pond was estimated.

The study comprised a breeding fish pond situated in Mydlniki, fed with water from the Rudawa River. The following were sampled: pond water, sediment from the pond's bottom, benthos organisms represented by larvae of *Diptera* family *Chironomidae* and carps (*Ciprinus carpio* L.). Organs from the fish (gills, muscles, livers and gonads) most strongly connected with the metabolism of metals were excised and prepared. Copper concentrations were determined in all samples by means of atomic emission spectrometry on a JY 238 Ultrace apparatus (Jobin Yvon Emission) after wet mineralization in a closed system using microwave energy. The results enabled us to conclude that there was no hazard of copper contamination in the examined ecosystem, although the copper content in the pond's sediments and water suggest some enrichment from anthropogenic sources. A lower value of the copper enrichment factor was determined in the sediment than in water. The bioaccumulation coefficient for copper in benthos and fish muscles assumed higher values, being much lower in fish gills in comparison with the literature data. The values of the copper bioaccumulation coefficient in the livers of the examined carps in relation to its content in water and bottom sediments were 1,532 and 0.623, respectively. Literature references

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dr inż. Marcin Niemiec, Chair of Agricultural and Environmental Chemistry, University of Agriculture in Krakow, Al. Mickiewicza 21, 31-120 Krakow, Poland, e-mail: niemiecm@tlen.pl

indicate that – regardless of the water pollution degree – the bioacummulation coefficient in this organ assumes a value approximately the same as obtained in the authors' own investigations. The copper concentration in the liver is a reliable indicator of the environmental pollution with copper compounds. The highest amounts of copper were found in the liver; less copper was in gills and gonads, and the lowest concentration of copper was detected in the carps' muscles.

Key words: copper, bioaccumulation, food chain, aquatic ecosystem.

AKUMULACJA MIEDZI W WYBRANYCH ELEMENTACH ŁAŃCUCHA POKARMOWEGO EKOSYSTEMU STAWOWEGO

Abstrakt

Zawartość pierwiastków w środowisku wodnym (w wodzie i osadach dennych) nie jest miarodajnym wskaźnikiem zagrożenia dla organizmów żywych. Ilość pierwiastków akumulowanych w organizmach żywych zależy przede wszystkim od form, w jakich występują w środowisku. Miarą intensywności pobierania pierwiastków śladowych przez organizmy żywe jest współczynnik bioakumulacji. Miedź jest jednym z najbardziej toksycznych metali dla organizmów wodnych. Pod względem toksyczności zajmuje 3 miejsce (po rtęci i srebrze) wśród metali powszechnie występujących w środowisku. Celem pracy było określenie akumulacji miedzi w poszczególnych ogniwach łańcucha pokarmowego ekosystemu wodnego w warunkach ekstensywnej hodowli karpi. Na podstawie zawartości miedzi w tych organizmach obliczono wartość współczynnika bioakumulacji miedzi w ekosystemie wodnym i oszacowano stopień zanieczyszczenia stawów.

Badania wykonano w stawie hodowlanym, położonym w Mydlnikach, zasilanym wodą z rzeki Rudawa. Z badanego stawu pobrano: wodę, osad z dna stawu, organizmy bentosu reprezentowane przez larwy muchówek (*Diptera*) z rodziny ochotkowatych (*Chironomidae*) oraz karpi (*Cyprinus carpio* L.). Z organizmów karpi wypreparowano narządy (skrzela, gonady, wątrobę i mięśnie) w największym stopniu związane z metabolizmem metali. We wszystkich próbkach oznaczono zawartość miedzi metodą emisyjnej spektrometrii atomowej w aparacie JY 238 Ultrace Jobin Yvon Emission, po uprzedniej mineralizacji metodą na mokro w systemie zamkniętym z wykorzystaniem energii mikrofalowej.

Stwierdzono, że w badanym ekosystemie nie ma zagrożenia zatrucia miedzią, chociaż zawartość miedzi w osadach i wodzie wskazuje na ich antropogeniczne wzbogacenie. Stwierdzono małą wartość współczynnika wzbogacenia osadów w miedź w stosunku do jej zawartości w wodzie. Współczynnik bioakumulacji tego pierwiastka w bentosie oraz w mięśniach ryb przyjmował większe wartości, natomiast w skrzelach znacznie mniejsze w porównaniu z danymi literaturowymi. Wartość współczynnika bioakumulacji miedzi w wątrobie badanych karpi w stosunku do jej zawartości w wodzie i osadach dennych wynosiła odpowiednio 1532 i 0,623. Dane literaturowe wskazują, że niezależnie od stopnia zanieczyszczenia wody, współczynnik bioakumulacji w tym organie przyjmuje wartość zbliżoną do uzyskanych w badaniach własnych. Zawartość miedzi w wątrobie jest najbardziej miarodajnym wskaźnikiem zanieczyszczenia środowiska związkami miedzi. Najwięcej miedzi stwierdzono w wątrobie, następnie w skrzelach, gonadach, a najmniej w mięśniach karpi.

Słowa kluczowe: miedź, bioakumulacja, łańcuch pokarmowy, ekosystem wodny.

INTRODUCTION

Complex determination of the threat to water ecosystems caused by trace elements is very difficult due to high temporal and spatial changeability of the chemical composition of water and bottom sediments in any water reservoir. Amounts of elements accumulated in living organisms above all depend on the form in which elements occur in the environment, which determines their bioavailability. Depending on the species of an aquatic organism species, which determines the type of food, the way of it is ingested, life activity and metabolic specificity, both amounts of accumulated copper and sources from which it is absorbed are different. Pelagic organisms take up mainly elements contained in water and in the seston. For benthic or benthic-foraging organisms, including carp, the basic source of heavy metals are bottom sediments (LIANG et al. 1999, ALAM et al. 2002, SCHJOLDEN et al. 2007). The best method of estimating environmental hazards posed by trace elements is by determining the bioaccumulation coefficient through an assessment of their accumulation in organisms at the subsequent trophic levels in an ecosystem. Determination of the environmental pollution with bioindicators is preceded by a selection of appropriate animal and plant species, which are distinguished by high capacity to accumulate a given xenobiotic. In the case of aquatic macrofauna, it is also necessary to choose an organ which accumulates the highest amounts of the analysed element.

Copper is one of the most toxic metals to aquatic organisms. In fact, it is the third (after mercury and silver) most toxic substance among metals commonly present in the environment (KHANGAROT 1991). The element may negatively affect fish even if present in small amounts in the environment (YUVANATEMIYA, BOYD 2006).

The aim of the paper was to determine the copper accumulation in individual links of of a food chain in an ecosystem in a pond ecosystem used for extensive carp (*Ciprinus carpio* L.) breeding. On the basis of conducted analyses the value of copper bioaccumulation coefficient in aquatic ecosystem was computed and the degree of pond pollution was estimated.

MATERIAL AND METHODS

In 2008, research on copper cycling was conducted in a fish pond, owned by the Experimental Station of the Department of Ichtyobiology and Fisheries, University of Agriculture in Krakow, situated in Mydlniki and fed with water from the Rudawa River. It was a commercial pond covering an area of about 4 ha. The analyses comprised copper assessments in water, bottom sediments, benthos and carp (*Cyprinus carpio* L.) organs.

Copper concentrations in water were assessed three times during the fish rearing season. The samples were collected at the beginning of the feeding season (in May), during the most intensive fish feeding (in July) and in September, by the end of the fattening period. Water was sampled from six points in the pond. Bottom sediments were collected from the topmost layer of the pond's bottom (0-5 cm) after emptying the pond. The pond was divided into 8 sections, and samples were taken from each one (two samples in the vicinity of the inlet box, 4 samples from the middle part of the pond and two close to the outlet box). The bottom sediments were dried, sifted through a sieve with 1mm mesh and ground in a mortar. Samples of benthic organisms (larvae of *Diptera* family *Chironomidae*) were collected in the same sites. The copper content in carp (*Cyprinus carpio* L.) was assessed in 25 randomly chosen fish reared for human consumption. The fish gender (by inspection), their age (fish rearing logbooks) and mass of the analysed carps (gravimetric method) were determined. The fish came from a three-year rearing period, and their weight ranged from 1,500 to 2,200 g. Carps were sacrificed by decapitation and their individual organs (gills, muscles, livers and gonads) were prepared. The laboratory samples were wet mineralized in a closed system using microwave energy. A weighted portion for analyses was *ca* 0.5 g in conversion to dry weight. Biological material was dissolved in a mixture of HNO_3 and H_2O_2 (5:1, v/v), whereas bottom sediments were dissolved in *aqua regia* in the quantitative ratio of sediment/ reagent equal 1:10. Water samples for analyses were condensed tenfold. The copper concentration in the solutions was determined using atomic emission spectrometry in inductively coupled plasma, in a JY 238 apparatus (Ultrace Jobin Yvon Emission) at the wavelength of 327.393 nm. The limit of detection in the applied method was 0.0097 mg Cu dm^{-3}. The measurement uncertainty of the applied method was ±6 %. The limit of detection of the analyses was 0.291 µg g^{-1} of the biological material dry mass and 0.47 µg g^{-1} of the sediment dry mass. The correctness of copper analyses was verified by means of the certified reference material CRM 16-050. Moreover, the following properties of sediments were assessed: pH in water suspension (potentiometrically), grain-size composition (areometric method) and organic carbon content (oxidation method using acidified dichromate ($K_2Cr_2O_7$--H_2SO_4) solution) (Ostrowska et al. 1991).

RESULTS AND DISCUSSION

The average copper concentration in water of the analysed pond was 5.62 µg Cu dm^{-3} (Table 1). No statistically significant differences in this metal concentration were assessed in water collected on various dates. Water collected in July contained slightly more copper. Less copper was as-

Table 1

Statistical parameters of copper content in studied elements of ecosystem

Statistical parameter	Water	Sediment	Bentos	Organ of *Ciprinus carpio* L.			
				gills	gonads	muscle	liver
	(µg dm^{-3})	(mg kg^{-1} d.m.)					
Minimum	4.080	6.763	13.55	1.739	1.340	0.870	3.953
Maximum	8.080	21.38	16.48	4.492	9.369	3.701	29.32
Mean	5.618	13.83	14.66	2.880	2.888	1.763	8.612
Median	5.440	12.97	14.48	2.638	2.474	1.447	6.886
Standard deviation	1.064	4.649	1.096	0.667	1.641	0.843	5.174
Relative standard deviation (%)	18.94	33.61	7.476	23.14	56.81	47.84	60.08

sessed in samples collected from water flowing into fish ponds (ŁUSZCZEK-TROJNAR et al. 2011), which evidences slight enrichment of the pond water. WIŚNIOWSKA-KIELIAN and NIEMIEC (2004) recorded a similar content of this metal in a section of the Dunajec River flowing through agricultural and recreational land, with a moderate level of anthropopressure. In a section of the same river passing through an area where some municipal and industrial sewage was discharged, the copper concentration was higher than found in the present research. High water temperature causes higher copper toxicity, which is a result of the depressed oxygen content in water due to an increased concentration of dissolved forms of copper in water; it also limits the ability of living organisms for detoxication (ALLEN, BRISBI 1980, RUSSEL et al. 2009). NIEMIEC and WIŚNIOWSKA-KIELIAN (2011) state that mean copper concentrations in reservoirs holding water running off a road were 15.20 µg Cu dm^{-3}. In polluted reservoirs, a copper concentration reaching 35 µg Cu dm^{-3} may prove harmful to biological life. KAHLE and ZAUKE (2003) found copper concentrations from 0.1 to 0.2 µg Cu dm^{-3} in water from the Arctic Sea. In fresh waters from unpolluted areas, the copper content is usually below 1 µg Cu dm^{-3} (KABATA-PENDIAS, PENDIAS 1999).

Copper contained in water has a strong affinity for organic matter. The biodiversity of this element depends firstly on the content of its water-soluble forms, which is connected not only with this element's amount but also with physical, chemical and physicochemical properties of water (ALLEN, BRISBIN 1980).

Bottom sediments from the analysed pond revealed a small amount of organic matter, which depending on the sampling place ranged from 1.38 to 5.05%. In intensively utilized ponds, this parameter often exceeds 10% (YUVANATEMIYA, BOYD 2006). The small quantity of organic matter detected in the uppermost layer of the pond sediments is caused by a low amount of indigenous sediments due to the extensive fishery management and proper agrotechnical measures applied to the pond bottom. The grain-size composition of the analysed sediments revealed a considerable percentage of the silt fraction. Three of the analysed sediment samples were classified as ordinary silt, three belonged to the silt loam group and two corresponded to heavy silt loams. The content of floatable particles fluctuated between 25-54%. The grain-size composition of the analysed sediments is typical for water sediments. The pH value in the analysed sediments measured in water suspension ranged from 7.8 to 8.3.

The average copper content in bottom sediments from the analysed pond ecosystem was 13.83 mg Cu kg^{-1} (Table 1). WIŚNIOWSKA-KIELIAN and NIEMIEC (2005ab) reported similar copper content in bottom sediments of the Dunajec River and its tributaries. Similar data were presented by SKORBIŁOWICZ and SKORBIŁOWICZ (2009) for the Supraśl River and its tributaries. Differences in the content of this metal between individual samples reached 33%. According to the classification of BOJAKOWSKA and SOKOŁOWSKA (1998), the examined sediments presented the 1st degree of copper pollution. In the research of YUVANATEMIYA and BOYD (2006), bottom sediments from unpolluted fish ponds contained 7 mg Cu kg^{-1}. In the pond ecosystem analysed herein, a slight accumulation of copper in bottom sediments was noted in relation to its quantity in water. VINOT and PIHAN (2005) reported almost 20-fold higher copper concentrations in bottom sediments from Mirgenbach Lake in northern France than in its water. In our investigations, the factor representing the copper enrichment in sediments was about 2.5 thousand. It may have been caused by a low content of organic matter in pond sediments. Organic matter is the most important substance binding copper in bottom sediments and its amount is positively correlated with the value of the sediment copper enrichment coefficient (ALLEN, BRISBIN 1980, YUVANATEMIYA, BOYD 2006, VINOT, PIHAN 2005). The authors' own research revealed a statistically significant (at $p<0.01$) correlation coefficient between the organic matter content and copper concentration in sediments. The copper content in the analysed sediments was not high, being typical of bottom sediments from unpolluted ecosystems.

Copper bioaccumulation in living organisms is determined by two processes. One limits the absorption of the element from the environment by a given organism and the other one determines the intensity of copper excretion from the organism. Depending on the strategy adopted by an organism, one of these processes prevails, thus protecting it against poisoning (RUSSEL et al. 2009).

The copper content in benthic organisms of the examined ecosystem revealed the least changeability, ranging from 13.55 to 16.48 mg Cu kg^{-1} d.m., on average 14.66 mg Cu kg^{-1} d.m. (Table 1). The copper content in scuds (*Gammarus* sp.) from Mirgenbach Lake was 134 mg Cu kg^{-1} d.m., whilst the water Cu concentration was 35 µg dm^{-3} and the copper concentration in sediments was 744 mg Cu kg^{-1} (VINOT, PIHAN 2005). The copper bioaccumulation coefficient in larvae of *Diptera* family *Chironomidae* analysed in the present research was high, reaching 2,610 and 1.06 of the amounts in the water and the bottom sediments, respectively. The value of the bioaccumulation coefficient in polluted ecosystems is much lower. VINOT and PIHAN (2005) reported that this coefficient's value in relation to the copper concentration in water and sediments for scuds (*Gammarus* sp.) from polluted Mirgenbach Lake was about 3,800 and 0.18, respectively. Copper bioaccumulation coefficient for snails (*Gastropoda*) from this lake in relation to bottom sediments was about 1.0. LIANG et al. (1999) conducted research on copper bioacummulation in ponds to which sewage was disposed. Value of copper bioaccumulation coefficient in benthos from the ponds in relation to its content in the sediments was about 0.5, however this metal content was approximate to its amount assessed in the studied pond. Generally, metal bioacummulation coefficient in benthos of water ecosystems is adversely proportional to their concentration in the environment (DEFOREST et al. 2007).

The use of fish for bioindication of water environment pollution with heavy metals provides an answer concerning the element content in water ecosystem, but first of all allows estimate the element toxicological effect in the environment. Carp is the fish well suitable for an assessment of the environment pollution with copper due to high correlation between copper amount in the environment and this element content in its organism, in the first place in liver (DE BOECK et al. 2003).

Gills are an important organ of ionic exchange in fish organism. Fish take up metals from the water and remove them from the organism. Copper is absorbed by gills as free ions and most probably hydroxyl complexes of this element. Other water soluble compounds are not taken up by gills. On basis of an analysis of the forms in which copper occurs in water it is possible to predict its toxicity for living organisms (TAO et al. 2002). Excessive amount of copper causes an injury and oedema of gill lobes leading to impairment of mucus production. Healthiness of gills and their copper content are good indicators of the environment pollution with this element (REYNDERS et al. 2008, KUNWAR et al. 2009). Copper content in the analysed fish gills revealed little diversification and ranged from 1.739 to 4.492 mg kg^{-1} d.m., with an average of 2 880 mg kg^{-1} d.m. (Table 1). Copper content in the gills of carps living in unpolluted waters is about 1.0 mg Cu kg^{-1} f.m. (SCHJOLDEN et al. 2007), which in conversion to dry matter gives the contents similar to obtained in the presented investigations. The copper bioaccumulation coefficient in the analysed organs was 512 in relation to the water concentrations, and 0.208 in relation to the content in the sediments. The concentration of this element concentration in carp gills was fivefold smaller than in the benthos (Table 2). REDDY et al. (2006) stated that the copper bioaccumulation coefficient in gills of fish living in water containing 39 µg

Table 2

Bioaccumulation coefficients of copper in benthos and individual organs of *Ciprinus carpio* L. in studied ecosystem

Element of ecosystem	Benthos	Gills	Gonads	Muscle	Liver
Water	2610	512.7	514.1	313.8	1532
Sediments	1.060	0.208	0.209	0.127	0.623
Benthos	-	0.196	0.197	0.120	0.587

Cu dm^{-3} as copper sulphate reached the value of over 3,500. Such a high value of this coefficient is connected with the form in which this element occurs. In water with high calcium and magnesium concentrations, the copper bioaccumulation coefficient is lower, which may explain its low value reported for the analysed fish.

The copper content in carp muscles was highly variable, fluctuating from 0.870 to 3.701 mg Cu kg^{-1} (Table 1), and the average was 1.763 mg Cu kg^{-1}. LIANG et al. (1999) obtained similar values for muscles of carps living in ponds with approximately similar copper accumulation in sediments. The copper bioaccumulation coefficient in these organs of the fish tested in our experiment, in relation to this metal's concentrations in water, bottom sediments and benthos was 313.8; 0.127 and 0.120, respectively (Table 2). ALAM et al. (2002) reported copper contents in muscles of carps kept in ponds of 0.249 mg Cu kg^{-1} in f.m. in the case of a low concentration of this element in water (2.2 μg Cu dm^{-3}). Under these conditions, the copper bioaccumulation coefficient in relation to its concentration in water was 450 and fell down to 1.0 in relation to the content in bottom sediments. VINOT and PIHAN (2005) found similar copper contents in muscles of breams (*Abramis brama*) from the pond whose water contained over 7-fold more copper than the water of the pond analysed in our study, whereas the bottom sediments had 60-fold more copper than herein. A much lower value of the bioaccumulation coefficient for breams is connected with this species's biology (bream is a pelagic fish) and lower water hardness. LIANG et al. (1999) assessed a high copper content (about 4 mg Cu kg^{-1}) in mosquitofish (*Gambusia affinis*) in an ecosystem with copper concentrations approximately same as found in the investigated pond. These authors point to a high copper bioaccumulation coefficient in phytoplankton and zooplankton in relation to the copper concentration in water. When the binding of copper with sediments is limited, more copper is absorbed by phyto- and zooplankton and by pelagic fish. In ecosystems with large amounts of organic matter or with bicarbonate of calcium and magnesium, the copper uptake from water is lower owing to its fast binding in sediments by organic matter or precipitation as carbonates. The Rudawa River catchment, which feeds the analysed pond, is abundant in limestone.

The liver is the main site of copper accumulation in fish organisms. The concentration of copper in this organ is a sensitive indicator of the environmental pollution and degree of fish poisoning with copper. The content of copper accumulated in livers of the analysed carps revealed the highest changeability, fluctuating between 3.953 to 29.32 mg Cu kg^{-1} d.m. (Table 1), at an average content of 8.612 mg Cu kg^{-1} d.m. This element's bioaccumulation coefficient in relation to its amount in water was 1,532, whereas in relation to bottom sediments equalled 0.623. The copper content determined in carps' livers was almost half the amount observed in the benthos (Table 2). Alam et al. (2002) reported values of the copper bioaccumulation coefficient in livers of wild carps in an unpolluted environment approximately same as presented in this paper (in relation to the concentration in water and sediments, the cited coefficients were about 2,000 and 0.288, respectively). Copper bioaccumulation coefficients in organisms of carps from aquacultures assumed lower values. Vinot and Pihan (2005) obtained similar values of the copper bioaccumulation coefficient in livers of various fish species living in copper-polluted water.

The copper concentration in the liver of bream (*Abramis brama*) was 70 mg kg^{-1} d.m., of roach (*Rutilus rutilus*) – 50 mg kg^{-1} d.m. and of perch (*Perca fluviatilis*) – 30 mg kg^{-1} d.m., with the content of this element in water being 35 µg dm^{-3}, and in the sediments – 744 mg kg^{-1}. The value of copper bioaccumulation from sediments was much lower than in the presented investigations. Mersch et al. (1993) reported the copper concentrations in bream's and roach's livers equal 56 and 36 mg Cu kg^{-1} d.m., respectively, with the concentration of this element in water amounting to 20.5 µg Cu dm^{-3}. The coefficient of copper bioaccumulation in lives of these fish species reached 2,700 and 1,750, respectively. The bioaccumulation coefficient of copper in fish livers from the ecosystem analysed in our experiment in relation to its water concentrations reached a similar level to the data quoted in literature. Irrespective of the degree of water pollution, it assumes values from 1,400 to 3,000. In the case of the bioaccumulation coefficient in the carp's liver computed in relation to the copper content in sediments, its value is high and characteristic for ecosystems unpolluted with copper. Thee results obtained by the authors imply a higher copper uptake from water than from bottom sediments.

Gills are organs which do not accumulate copper. The content of this element in gills was highly varied, fluctuating from 1.340 to 9.369 mg Cu kg^{-1} d.m., with the mean content of 2.89 mg Cu kg^{-1} d.m. (Table 1). No statistically significant differences in concentrations of this element were found in testicles or ovaries. Vinot and Pihan (2003) reported similar quantities of copper, as observed in the authors' own studies, in gonads of carps caught from a lake unpolluted with this element, i.e. 1.264 mg Cu kg^{-1} f.m. in gonads of wild carps and 0.607 mg kg^{-1} f.m. in bred ones. The cited researchers determined that the copper bioaccumulation coefficient in gonads in relation to its content in water was 559, which is a value approximately same as obtained in the authors' own research. The values of the bioaccumulation coefficient in relation to bottom sediments found herein are characteristic for ecosystems in slightly polluted environments.

Considering all the studied organs, the highest amounts of copper were found in livers, then in gonads and gills, whereas the smallest quantities of Cu occurred in muscles of the analysed carps (Figure 1). The quantitative ratio of the copper content in these organs was 1:0.34, 34:0.19, respectively. No statistically significant differences were determined between the copper content in male and female organs. Quantitative ratios of trace elements in individual fish organs depend on availability of the said substances in the environment. In ecosystems polluted with copper, its higher concentrations appear in the liver and gills, whereas in the environments poor in copper, the biggest quantities of Cu may accumulate in gonads, which is connected with the physiological functions performed by copper in these organs (MOISEENKO et al. 2008, ALAM et al. 2002, REDDY et al. 2006, REYNDERS et al. 2008).

In soft waters and waters with a low content of organic matter, copper is taken up mainly from water, both by plankton and fish. In ecosystems with a high content of organic matter, organisms in a food chain have limited availability of copper from water, and the metal is taken up mainly from sediments.

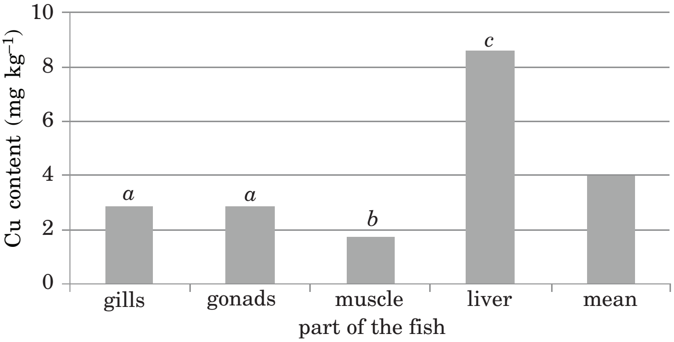

Fig. 1. Mean content of copper in studied organs of *Ciprinus carpio* L.

CONCLUSIONS

1. Both water and bottom sediments from the pond had copper concentrations normally observed in areas with a low degree of anthropopressure.

2. Copper concentrations in individual fish organs can be put in order from the highest: liver > gonads > gills > muscles. The copper content in the carp's liver is the most reliable indicator of copper hazard to the environment.

3. Copper concentrations in all the analysed organs were on a level observed in fish from unpolluted environments.

4. The coefficient of copper enrichment in the sediments was not high and characteristic for ecosystems poor in organic matter.

5. The calculated copper bioaccumulation coefficients in the benthos and fish muscles were high in relation to the content in water and sediments. Similar values of these coefficients are noted in unpolluted ecosystems.

6. Low values of the copper bioaccumulation coefficients were recorded in gills, which is connected with the limited availability of this element from water.

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ARYLSULPHATASE ACTIVITY AND THE CONTENT OF TOTAL SULPHUR AND ITS FORMS UNDER THE INFLUENCE OF FERTILISATION WITH NITROGEN AND OTHER MACROELEMENTS

Anetta Siwik-Ziomek, Joanna Lemanowicz, Jan Koper

Department of Biochemistry
University of Technology and Life Sciences in Bydgoszcz

Abstract

The aim of this research was to determine the effect of mineral fertilisation on the activity of arylsulphatase (EC 3.1.6.1.) and the content of total sulphur and its fraction in soil under winter wheat. The soil was collected from a field experiment set up at the Agricultural Experimental Station in Grabów nad Wisłą by the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy. The sampling was carried out twice during the vegetative period of maize in 2008. The experiment involved exclusive mineral fertilisation composed of increasing rates of ammonium nitrate (0, 50, 100, 150, 200 kg N ha^{-1}), and fertilisation with P – 80 kg P_2O_5 ha^{-1}, K – 140 kg K_2O ha^{-1}, Ca – 200 kg CaO ha^{-1}, Mg – 70 kg MgO ha^{-1} and S in fertilisation forms with other elements. The activity of arylsulphatase was determined following the Tabatabai and Bremner method, and sulphate sulphur was assayed compliant with the Bardsley–Lancaster method. The fertilisation with ammonium nitrate determined the content of total sulphur and its fraction as well as the activity of arylsulphatase in Luvisol. The optimum nitrogen rate leading to the highest arylsulphatase activity in soil was the rate of 100 kg N ha^{-1}. The analysed Luvisol demonstrated low sulphur abundance and should be enriched with this nutrient to ensure that the plants would produce yields adequate in terms of both quality and quantity.

Key words: arylsulphatase activity, sulphur, soil, macroelements.

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dr inż. Anetta Siwk-Ziomek, Department of Biochemistry, University of Technology and Life Sciences in Bydgoszcz, Bernardyńska 6 street, 85-029 Bydgoszcz, Poland, tel. +48 (052) 374-95-55, e-mail: ziomek@utp.edu.pl

AKTYWNOŚĆ ARYLOSULFATAZY ORAZ ZAWARTOŚĆ SIARKI OGÓŁEM I JEJ FORM POD WPŁYWEM NAWOŻENIA AZOTEM I POZOSTAŁYMI MAKROELEMENTAMI

Abstrakt

Celem pracy było określenie wpływu mineralnego nawożenia na aktywność arylosulfatazy (EC 3.1.6.1.) oraz zawartość siarki ogółem i jej frakcji w glebie spod uprawy kukurydzy. Próbki gleby pobrano z doświadczenia polowego, założonego na terenie RZD w Grabowie nad Wisłą przez IUNG w Puławach, dwukrotnie w sezonie wegetacyjnym kukurydzy w 2008 roku. W doświadczeniu zastosowano tylko nawożenie mineralne: wzrastające dawki saletry amonowej (0, 50, 100, 150, 200 kg N ha^{-1}) oraz nawożenie: P – 80 kg P_2O_5 ha^{-1}, K – 140 kg K_2O ha^{-1}, Ca – 200 kg CaO ha^{-1}, Mg – 70 kg MgO ha^{-1} i S w formach nawozowych z innymi pierwiastkami. Aktywność arylosulfatazy oznaczono wg metody Tabatabai i Bremnera, a siarkę ogółem i siarczanową zgodnie z metodą Bardsleya-Lancastera. Nawożenie saletrą amonową wpływało na koncentrację siarki organicznej i siarczanowej oraz aktywność arylosulfatazy w glebie płowej. Zawartość siarki ogółem wynosiła średnio 0,053 g kg^{-1} i nie zależała od zastosowanego nawożenia mineralnego. Optymalną dawką azotu, po której zastosowaniu stwierdzono w glebie największą aktywność arylosulfatazy, było 100 kg N ha^{-1}. Na podstawie zawartości siarki siarczanowej (średnio 11.0 mg kg^{-1}) zakwalifikowano badaną glebę do gleb o średniej zasobności w siarkę, dlatego uprawa kukurydzy wymaga uzupełnienia zawartości tego pierwiastka, by umożliwić roślinom wydanie plonu o odpowiedniej jakości i wielkości.

Słowa kluczowe: arylosulfataza, siarka, gleba, makroskładniki.

INTRODUCTION

Over the last twenty years, sulphur deficiency has been recognized as a constraint on crop production all over the word (ERIKSEN et al. 2004, MASCAGNI et al. 2008, SCHERER 2009). The main reasons are the reduction of sulphur emission from various industrial sources, increasing use of high-analysis low-S-containing fertilizers, and the decreasing use of S-containing fungicides and pesticides (SCHRERER 2001, ERIKSEN et al. 2004.). Sulphur in soil occurs in inorganic and organic forms and it is cycled between these forms by mobilization, mineralization, immobilization, oxidation and reduction processes. Organic sulphur occurs mainly in two primary forms: as ester sulphates and carbon bonded (C-S). While organic sulphur compounds are largely immobile, inorganic sulphur is more mobile and sulphate (SO_4^{2-}) is the most mobile form (SCHERER 2001). Arylsulphatases are enzymes that catalyse the hydrolysis of aromatic sulphate esters ($C\text{-}O\text{-}SO_3^{-}$) to phenols (R-OH) and sulphate (SO_4^{2-}) In soil, sulphate esters represent a large fraction (25.3-93.1%) of the total S and therefore, arylsulphatases may be important for mobilisation of inorganic SO_4^{2-} for plant nutrition (FITZGERALD 1976). Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (SINSABAUGH 1994). Mineral fertilisation stimulates biochemical reactions in soils, however, it is also a supplier of anions an excessive concentration of which can lead to the inhibition of enzymatic reactions in soil.

The aim of the paper was to determine the effect of mineral fertilisation on the activity of arylsulphatase and the content of total sulphur and its fraction in soil under maize cultivation.

MATERIAL AND METHODS

The soil was collected from a field experiment set up in the Agricultural Experimental Station in Grabów nad Wisłą, conducted by the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy. The soil sampling was performed twice in the vegetative period of maize in 2008. The soil was a very good rye complex soil. The experiment involved only mineral fertilisation consisting of increasing rates of ammonium nitrate (0, 40, 80, 120, 160 kg N ha^{-1}), and fertilisation with P – 80 kg P_2O_5 ha^{-1}, K – 140 kg K_2O ha^{-1}, Ca – 200 kg CaO ha^{-1}, Mg – 70 kg MgO ha^{-1} and S in fertilisation forms with other elements. The experiment was performed as a split-plot design, the first factor involved increasing rates of nitrogen fertiliser, and the second one corresponded to fertilisation with the other macroelements. There were six treatments of the second factor. The first level involved a fertiliser combination of all the minerals, and then one element was absent in the following treatments; hence, the following six treatments:

1. P, K, Ca, Mg, S;
2. – , K, Ca, Mg, S;
3. P, – , Ca, Mg, S;
4. P, K, – , Mg, S;
5. P, K, Ca, – , S;
6. P, K, Ca, Mg, – .

In the treatments with S, single superphosphate and potassium sulphate were used, while the treatments without S consisted of triple superphosphate and high-percentage potassium salt.

The activity of arylsulphatase was determined following the TABATABAI and BREMNER method (1970), and the sulphate sulphur activity was analyzed according to the BARDSLEY-LANCASTER method (1960). Organic sulphur was calculated from the difference between the content of total sulphur and $S\text{-}SO_4^{2-}$. The results of the sulphur content and the activity of the examined enzyme were processed using an analysis of variance with Tukey'a semi-intervals of a confidence test ($p = 0.05$).

RESULTS AND DISCUSSION

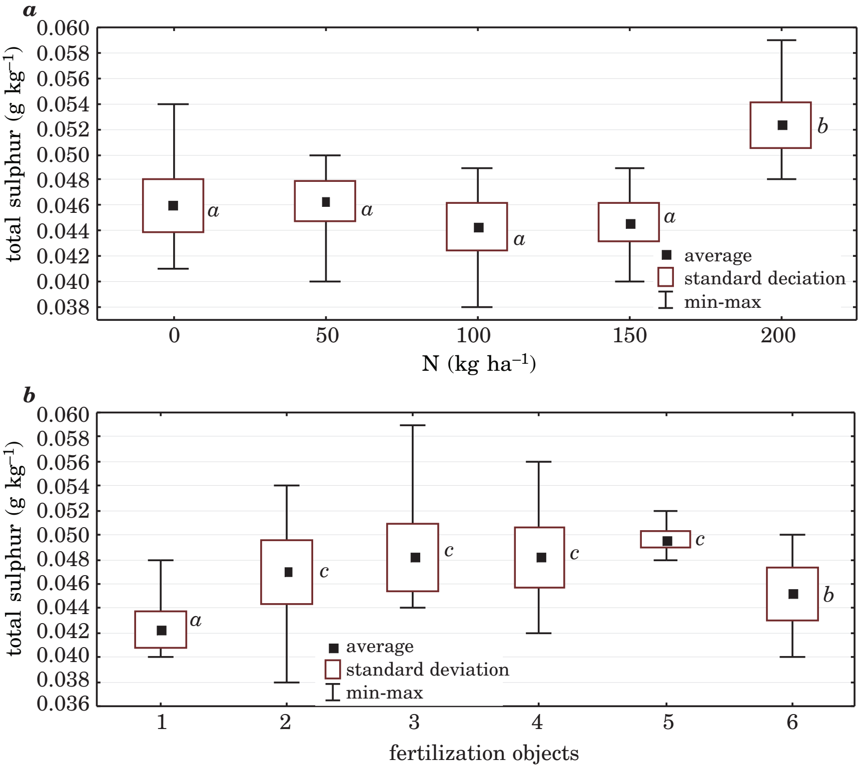
The reaction of the soil collected from the field in Grabów nad Wisłą ranged from 6.18 to 6.81 pH (Table 1). The reaction of the analysed soil ranged from slightly acid to neutral. Changes in the soil reaction were caused by neither the increasing rates of ammonium nitrate nor the various combinations of P, K, Ca, Mg and S fertilisation. CZEKAŁA et al. (2002) found that changes in the soil reaction were to a greater extent caused by the crops grown than nitrogen fertilisation. The content of sulphur available to plants depends on the soil reaction; when exposed to a higher soil pH, more sulphur is released owing to a higher rate of organic matter decomposition; when the soil pH is lower, an increased adsorption of sulphates on hydrate iron and aluminium oxides as well as kaolinite occurs (JOHNSON 1984). Similarly, the activity of soil enzymes depends on the concentration of hydrogen ions in soil. Arylsulphatase has a broad pH optimum from 5.8-8.2 (KLOSE et al. 1999).

Table 1

Reaction (pH in 1 mol KCl dm^{-3}) of soil in the year of the experiment

	Nitrogen fertilization (kg ha^{-1})				
Treatments	0	50	100	150	200
1. P, K, Ca, Mg, S	6.28	6.59	6.54	6.76	6.56
2. – , K, Ca, Mg, S	6.51	6.36	6.49	6.59	6.25
3. P, – , Ca, Mg, S	6.23	6.51	6.72	6.18	6.37
4. P, K, – , Mg, S	6.72	6.45	6.63	6.56	6.64
5. P, K, Ca, – , S	6.67	6.33	6.58	6.38	6.58
6. P, K, Ca, Mg, –	6.81	6.51	6.75	6.36	6.61

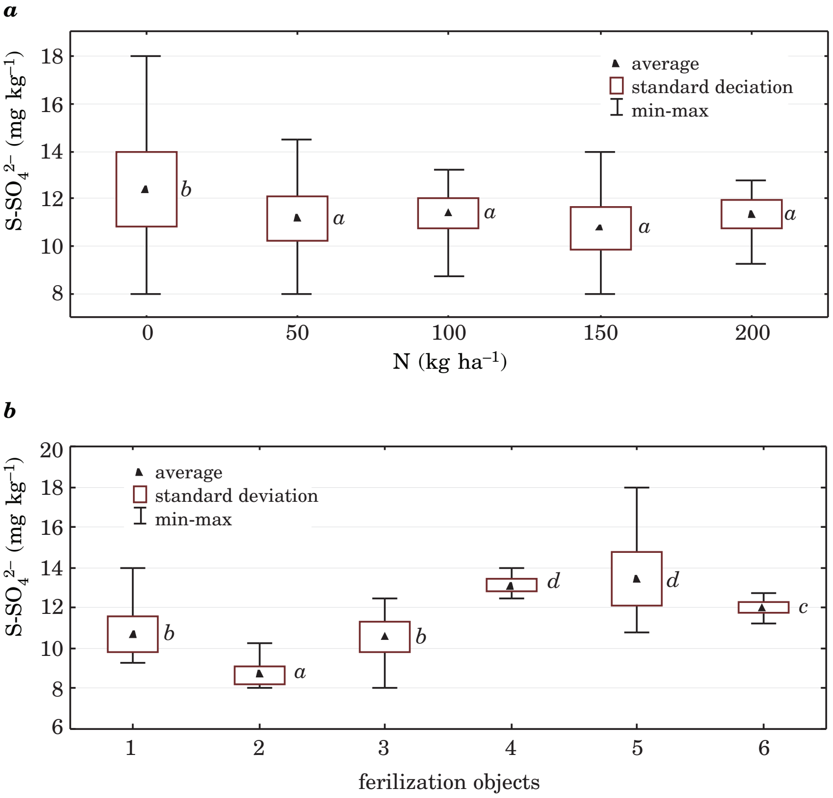
The content of total sulphur in soil ranged from 0.038 g kg^{-1} to 0.059 g kg^{-1}. MOTOWICKA-TERELAK and TERELAK (1998) indicated that the content of S_{og} in mineral soils under agricultural use ranged from 0.07 to 1.07 g kg^{-1}. In the examined soil, the content of total sulphur was found below the range characteristic for mineral soils in Poland's agricultural areas. According to YANG et al. (2007) or KOTKOVA et al. (2008), the total soil sulphur depends on the type of fertilisers applied (mineral or organic). In our experiment, both types of fertilisation, with nitrogen and with the other macroelements, affected the content of sulphur in the soil. In the plots receiving either NK or NPKS fertilizers for more than 80 years, no significant accumulation of total sulphur, as compared with the control, could be detected (YANG et al. 2007). The highest total sulphur content was observed in the soil collected from the treatments with the highest rate of ammonium nitrate (200 kg ha^{-1}) – Figure 1. The other nitrogen fertiliser rates did not show any significant effect on the content of this macroelement in soil. However, fertilisa-



1. P, K, Ca, Mg, S; 2. –, K, Ca, Mg, S; 3. P, –,Ca, Mg, S; 4. P, K, –, Mg, S;
5. P, K, Ca, –, S; 6. P, K, Ca, Mg, –
a, b, c – homogenous groups are marked the same letter

Fig. 1. Content of total sulphur in soil as dependent on fertilization

tion with the other macroelements resulted in a significant effect of the type of macroelements on the content of total sulphur. Its highest rate was determined in soil fertilised with P, K, Ca, –, S (treatment 5) – Figure 2. The total sulphur content in soil from that treatment was 8% higher than in the control treatment without sulphur fertilisation and 14% higher than in the treatment including fertilisation with all the macroelements. The present research did not identify the effect of the soil sampling date on the content of this sulphur fraction. Total sulphur includes the total content of organic and mineral compounds of this element which occurs in soil and so the application of fertilisers containing sulphur results in an increase in the content of the nutrient in soil, whereas the immobilization and mineralization processes depend on the physicochemical soil properties, climate and the vegetation (McGrath et al. 2003) and occur at varies intensity in the vegetation period (Wielebski 2000).



1. P, K, Ca, Mg, S; 2. –, K, Ca, Mg, S; 3. P, –, Ca, Mg, S; 4. P, K, –, Mg, S;
5. P, K, Ca, –, S; 6. P, K, Ca, Mg, –
a, b, c, d – homogenous groups are marked the same letter

Fig. 2. Content of sulphate sulphur in soil as dependent on fertilization

Inorganic S is generally much less abundant than organically bound S in most of the agricultural soils (Bohn et al., 1986). Sulphate, the most common form of inorganic S, is divisible into SO_4^{2-} in soil solution, adsorbed SO_4^{2-} and mineral sulphur (Scherer 2001). Sulphate sulphur in the analysed soil ranged from 8 to18 mg kg^{-1}, depending on the fertilisation with nitrogen and the other nutrients (Figure 2). In most agriculturally used soils in Poland, the content of sulphate sulphur does not exceed 25 mg kg^{-1} of soil. In most soils, i.e. 70% of the total arable land, the content of this sulphur fraction ranges from 5.0 to 20.0 mg kg^{-1} (Lipiński et al. 2003). The content of sulphate sulphur determined in our study was, on average, 11 mg kg^{-1}. This level of soil richness in available sulphur is considered a medium one, hence maize needs supplementary sulphur fertilisation (Lipiński et al. 2003). The content of sulphate sulphur depended significantly on the rates of ammonium nitrate. The highest content of this sulphur form, 8% higher, was reported in the soil sampled from the control treatments with no nitrogen fertilisation (Figure 2a). As for the fertilisation with macroelements, the highest content of sulphates occurred in the soil collected from the treatments fertilised with P, K, Mg, S (4) and P, K, Ca, –, S (5) – Figure 2b.

In Polish soils, organic sulphur is a dominant form of the element, with its content falling within a wide range of 6 to 688 mg kg^{-1}. The share of this sulphur fraction in total sulphur varies from 50 to 80% in the humus horizons of mineral soils (MOTOWICKA-TERELAK, TERELAK 1998). In the soil sampled from the Grabów experiment, the share of organic sulphur in total sulphur depended on the fertilisation variant, and ranged from 61 to 80% (Figure 3). The content of sulphur in organic compounds found in the soil was within the range from 26 to 46 mg kg^{-1} (Figure 4) and did not undergo significant changes during the maize growing season, although it depended on the applied fertilisation. The highest amount of this sulphur fraction was noted in the treatments fertilised with the highest rate of ammonium nitrate and fertilisers containing –, K, Ca, Mg, S (treatment 2) and P, –, Ca, Mg, S (treatment 3) – Figure 5. KNIGHTS et al. (2001) found that a 153-year-long application of FYM in the experiment in Broadbalk resulted in an increase in the content of organic sulphur in soil, whereas soil fertilised with mineral fertilisers only showed a low level of organic sulphur in all plots. The authors also observed an increase in the level of mineralization in the soil sampled from the plots fertilised with FYM, as compared with those treated with mineral fertilisation and so they claim that long-term FYM fertilisation definitely supplies plants with sulphur.

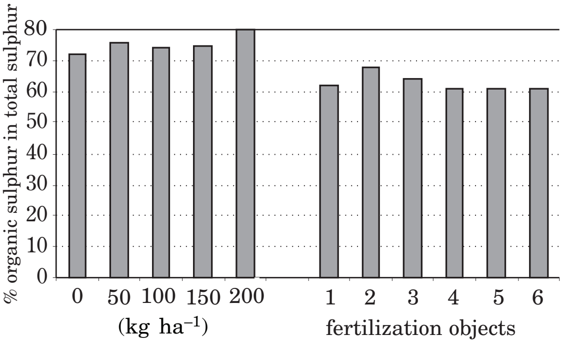
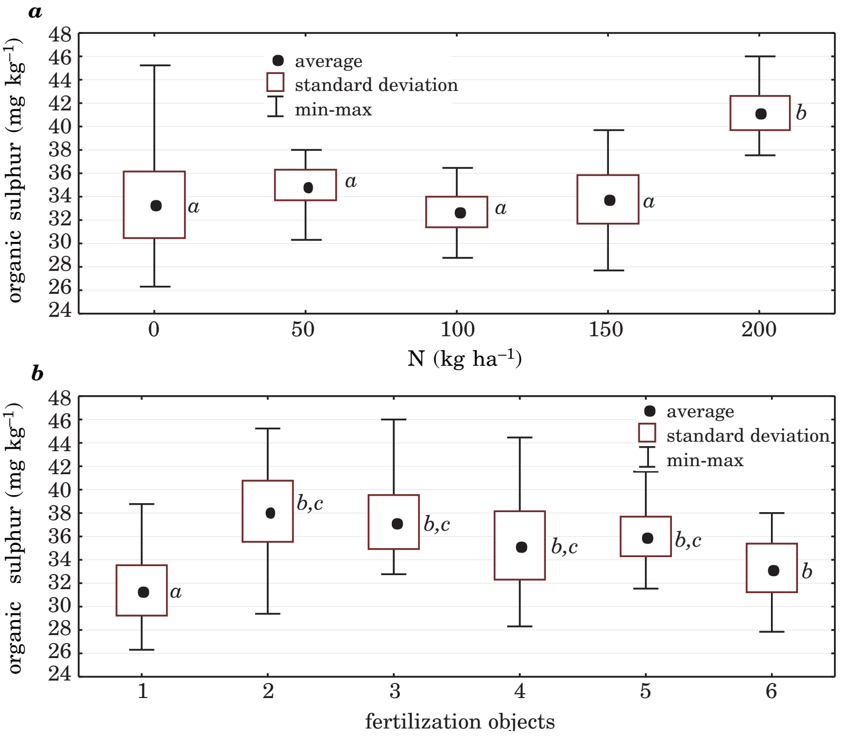


Fig. 3. Percentages of organics sulphur in total sulphur in soil as dependent on fertilization

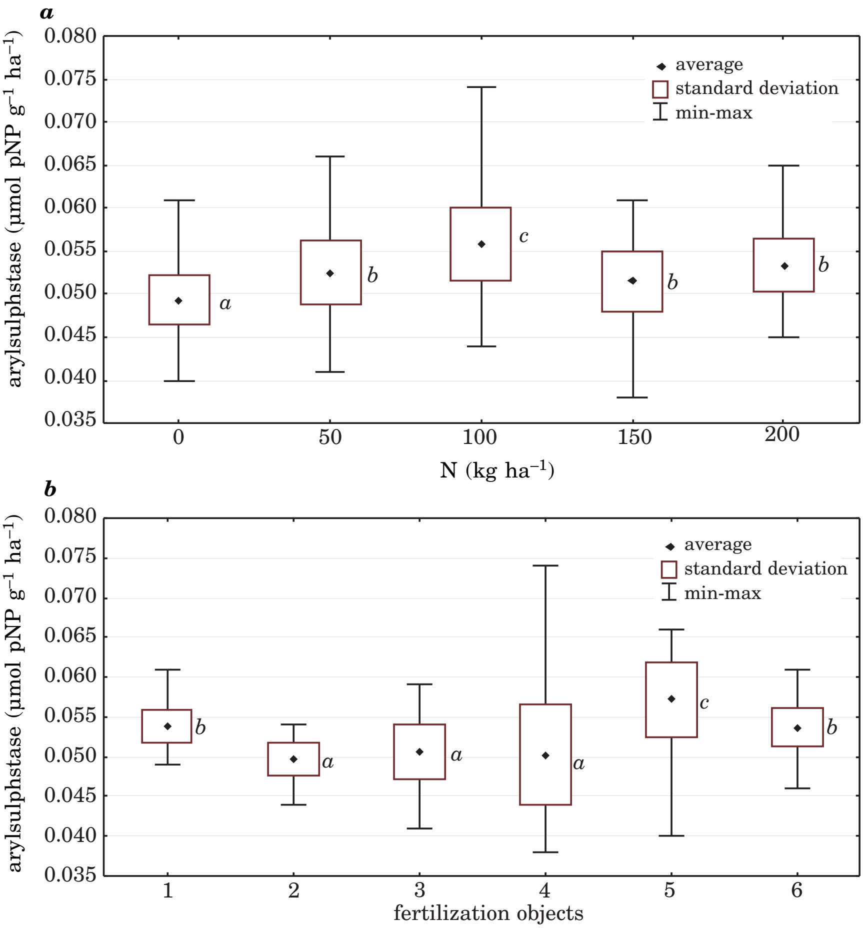
Soil enzymes play a biochemical function in the overall process of organic matter decomposition in the soil system (BURNS 1983). They are engaged in catalysing several important reactions necessary for the life processes of microorganisms in soils and the stabilisation of soil structure, the decomposition of organic waste, formation of organic matter and nutrient cycling (DICK et al. 1994). All soils contain enzymes that determine soil met-



1. P, K, Ca, Mg, S; 2. –, K, Ca, Mg, S; 3. P, –, Ca, Mg, S; 4. P, K, –, Mg, S;
5. P, K, Ca, –, S; 6. P, K, Ca, Mg, –
a, b, c – homogenous groups are marked the same letter

Fig. 4. Content of organic sulphur in soil as dependent on fertilization

abolic processes, which in turn depend on its physical, chemical, microbiological and biochemical properties (Makoi, Ndakidemi 2008). The activity of arylsulphatase in the analysed Luvisol soil depended on the fertilisation, ranging from 0.038 to 0.066 μmol pNP g^{-1} h^{-1}. The optimal rate of ammonium nitrate which coincided with the highest activity of the hydrolase was 100 kg N ha^{-1}. In an experiment analysing soil from a field under potato fertilized with different doses of farmyard manure (0, 20, 40, 60 and 80 t ha^{-1}) and mineral nitrogen (0, 45, 90, 135 kg N ha^{-1}), the highest activity of arylsulphatase (an average 0.018 μmol pNP g^{-1} h^{-1}) was determined in the samples fertilised with nitrogen at the amount of 45 kg ha^{-1} (Siwik-Ziomek, Koper 2010). Fertilisation with the other macronutrients affected the activity of arylsulphatase, which was the highest in samples of the soils fertilised with P, K, Ca, –, S (treatment 5) and all the macroelements applied in the experiment (treatment 1) and P, K, Ca, Mg, –, (treatment 6) – Figure 5. Literature reports (Ganeshamurthy, Nielsen 1990, Germida et al. 1992) discuss a negative effect of ions (NO_3^-, NO_2^-, PO_4^{3-}, SO_4^{2-}, Cl^-) on the soil enzy-



1. P, K, Ca, Mg, S; 2. –, K, Ca, Mg, S; 3. P, –, Ca, Mg, S; 4. P, K, –, Mg, S;
5. P, K, Ca, –, S; 6. P, K, Ca, Mg, –
a, b, c – homogenous groups are marked the same letter

Fig. 5. Arylsulphatase activity in soil as dependent on fertilization

matic activity. The experiments made in Grabów on Haplic Luvisol did not reveal any inhibiting effect of mineral fertilisation on the activity of this enzyme. WYSZKOWSKA, KUCHARSKI, KUCHARSKI (2010) showed in a pot experiment that β-glucosidase, arylsulfatase and phosphatases were less resistant to copper contamination in cropped than in uncropped soil. The soil enzymatic activity undergoes complex biochemical processes, e.g. integrated and organic synthesis, immobilisation and stability of enzymes (KHAZIYEV, GULKE 1991).

In the current experiment, no significant coefficients of correlation were found between the content of total sulphur and its fractions versus the activity of arylsulphatase.

CONCLUSIONS

1. The effect of fertilisation with nitrogen and the other macroelements (P, K, Ca, Mg, S) on the content of total sulphur and its fraction and the activity of arylsulphatase was identified.

2. In respect of the sulphate sulphur content (an average of 11.0 mg kg^{-1}) the soil was classified as having an average sulphur content, and therefore must be supplemented for maize to achieve the right quality and quantity of yields.

3. The optimal rate of ammonium nitrate for which the highest activity of arylsulphatase was reported was 100 kg N ha^{-1}.

4. Low concentrations of total and organic sulphur in the soil fertilised with mineral fertilisers only would suggest that natural and organic fertiliser should be applied.

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EFFECT OF BORON FOLIAR APPLICATION AT CRITICAL GROWTH STAGES ON SUNFLOWER (*HELIANTHUS ANNUUS* L.) YIELD AND QUALITY

Petr Škarpa

Department of Agrochemistry, Soil Science
Microbiology and Plant Nutrition
Mendel University in Brno

Abstract

The objective of the reported vegetation experiment, established in 2008-2011, was to explore the effect of the date of foliar boron application on the yield and quality of sunflower. Four treatments were established in the experiment: 1) control – not fertilised with boron; 2) application of 300 g B ha^{-1} in the growing stage of 4 developed leaves (V-4); 3) application of 300 g B ha^{-1} at the beginning of elongation growth (R-1); and 4) split rate of boron application of 150 g B ha^{-1} at stage V-4 (4 developed leaves) and 150 g B ha^{-1} at stage R-1 (terminal bud forms).

Foliar nutrition with boron increased the content of this element in leaves and stems, and raised the biomass production of sunflower plants. The increased uptake of nutrients by plants was essential for achene production. Application of boron fertilizer at stage V-4 increased the N content in the plant. Boron had a considerable effect on achene production. The highest increase in yields was related to the foliar boron application at stage V-4 (by 8.3% relatively). The relative increase in the oil content in response to boron application, which ranged within 1-2%, was not significant. The higher yields and a constant oil content increased oil production, most markedly after the boron foliar application at stage V-4 (by 10.2%). It was only the content of stearic acid that was increased significantly by foliar application of B at stage V-4 and split application (by 8.8% and 9.4%, respectively).

Key words: sunflower, boron, foliar application, yield, oil content.

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ing. Petr Škarpa, Ph.D., Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic, e-mail: petr.skarpa@mendelu.cz

WPŁYW NAWOŻENIA DOLISTNEGO BOREM W TRAKCIE KRYTYCZNYCH FAZ WZROSTU NA PLON I JAKOŚĆ PLONU SŁONECZNIKA (*HELIANTHUS ANNUUS* L.)

Celem doświadczenia wegetacyjnego, przeprowadzonego w latach 2008-2011, było zbadanie wpływu terminu nawożenia dolistnego borem na plon i jakość plonu słonecznika. Doświadczenie składało się z czterech obiektów: 1) kontrolnego – nie nawożonego borem; 2) z zastosowaniem 300 g B ha^{-1} w fazie wzrostu 4 rozwiniętych liści (V-4); 3) z zastosowaniem 300 g B ha^{-1} na początku fazy strzelania w łodygę (R-1); 4) z zastosowaniem podzielonej dawki boru: 150 g B ha^{-1} w fazie V-4 (4 rozwinięte liście) i 150 g B ha^{-1} w fazie R-1 (tworzenie pąków).

Nawożenie dolistne borem zwiększało zawartość tego pierwiastka w liściach i łodygach słonecznika oraz ilość wytworzonej biomasy. Zwiększony pobór składników odżywczych przez rośliny jest fundamentalny dla ich owocowania. Zastosowanie nawozu w postaci boru w fazie V-4 zwiększyło zawartość azotu w roślinach. Bor miał znaczny wpływ na plon słonecznika. Największy wzrost plonu odnotowano w przypadku stosowania boru w fazie V-4 (wzrost o 8,3%). Stosunkowy wzrost zawartości oleju pod wpływem zastosowania boru, który wahał się od 1 do 2%, nie był istotny. Wyższe plony i stałą zawartość oleju uzyskano najbardziej wyraźnie po zastosowaniu boru w fazie V-4 (wzrost o 10,2%). Jedynie zawartość kwasu stearynowego zwiększyła się istotnie pod wpływem dolistnej aplikacji boru w fazie V-4 (wzrost o 8,8%) lub dawki podzielonej na dwie części (wzrost o 9,4%).

Słowa kluczowe: słonecznik, bor, nawożenie dolistne, plon, zawartość oleju.

INTRODUCTION

As a certain problem in growing sunflower, the second most important oil plant in the Czech Republic, we see the stagnation of yields. From 1996 to 2011, the yields were 1.99 t/ha on average, while the genetic potential of sunflower is to produce as much as 5 tons per hectare. Implementation of new and more efficient varieties, as well as optimization of sunflower nutrition by means of rational application of fertilisers could stimulate the yields.

Sunflower is a plant with high demand for boron (B) and, depending on specific soil conditions, for other trace elements. To some extent, the uptake of nutrients by plant roots depends on soil properties (particularly soil pH) and supply of micronutrients in the soil. The critical content of boron at the time of sunflower emergence is 20 mg kg^{-1} of soil (Asad 2002). That is the reason why farmers prefer foliar nutrition when applying micronutrients. Some authors (Asad et al. 2003, Zerrari, Moustaoui 2005) have described the effects of foliar application of boron on the growth and development of sunflower. Zerrari and Moustaoui (2005) stated that the boron content corresponding to its deficit is 32.5 mg B kg^{-1} of dry matter. The demand of sunflower for boron is varied, depending on the stage of plant growth. The critical content of boron in sunflower at the age of 4 weeks is 46.0-63.0 mg B kg^{-1} of dry matter, while 8-week old plants need just 36.0 mg B kg^{-1} (Rashid, Rafique 2005). Sunflower plants with the tissue content of boron ranging between 16.5 and 23.0 mg kg^{-1} of dry matter require 300 g B ha^{-1}, and for plants containing between 23.0 and 32.5 mg B kg^{-1} of dry matter, the rate of 150 g B ha^{-1} is sufficient Boron application significantly increases not only its content in the plant, but also the dry matter production and achene yields (Sharma et al. 1999, Rashid, Rafique 2005, Zerrari, Moustaoui 2005). Boron is an important element, which affects yields of sunflower, cotton (Dordas 2006a), rape (Asad et al. 2002), alfalfa (Dordas 2006b) and maize (Hosseini et al. 2007).

The objective of this study was to determine the critical stage of boron foliar applications in sunflower, assuming its positive effects on the biomass production, content of nutrients in dry matter, achene yield, oil content, oil production and content of fatty acid in sunflower oil.

MATERIAL AND METHODS

In 2008-2011, a field trial with sunflower (*Helianthus annuus*) was established at the School Farm of the Mendel University in Brno in Žabčice (49°00'45"N, 16°37'50"E) and the Ivaň Farm Cooperative in the district of Vranovice (48°57'26"N, 16°36'18"E). In the experiment, we explored the effect of the date of boron application on sunflower production and achene quality.

The content of nutrients in the soil analysed prior to the establishment of the experiment (Table 1) was on an adequate to very high level. The soil reaction (pH/$CaCl_2$) was slightly acid (2010, 2011) to neutral (2008, 2009). The content of available B in soil (Berger and Truog) is given in Table 1.

In all the years of the experiment, we used the hybrid Orasole (early hybrid with high achene yields, high oil content and a higher proportion of oleic acid, i.e. a high oleic plant). Prior to sowing, the plot was fertilized to a rate of 100 kg N ha^{-1} (this rate included the content of N_{min} determined before sowing – Table 1). On sowing, the inter-row distance was 75 cm, the seeds in the row were spaced 20 cm apart and planted to a depth of 4-6 cm. After sowing, the plot was compacted and a pre-emergence application of herbicides followed.

After the emergence of the plants, a micro-plot experiment was established (4.5 m x 15 m plot area; 450 plants on plot). Application of B was performed in the form of foliar nutrition during the developmental stages V-4 (4 developed leaves and stage) and R-1 (terminal bud forms a miniature floral head rather than a cluster of leaves), in combinations and rates given in Table 2. Boron was applied in the form of ethanolamine as sodium pentaborate. Each treatment was repeated 4 times.

The content of dry matter and the levels of nutrients (N, P, K, Ca, Mg and B) were determined in plant mass in developmental stages V-4, R-1 and

Table 1

Agrochemical characteristics of the soil

Year	pH/CaCl$_2$	Nmin (kg ha^{-1})	Content of nutrients in (mg kg^{-1} DM) soil (Mehlich 3)				Available B (mg kg^{-1})
			P	K	Ca	Mg	
2008	6.2	15.0	91	254	2672	244	0.83
2009	6.2	5.0	66	179	4477	313	0.80
2010	6.7	8.6	63	111	2321	164	0.71
2011	6.8	12.6	78	206	2864	262	0.75

Table 2

Treatments of the experiment

Treatments of fertilization	Dose of B (g ha^{-1})	Time of application*
Control	0	–
Boron 1	300	V-4
Boron 2	300	R-1
Boron 3	150	V-4
	150	R-1

* Stages of sunflower development (SCHNEITER, MILLER 1981)

R-2 (immature bud elongates 0.5 to 2.0 cm above the nearest leaf attached to the stem). The samples of plant mass were dried at a temperature of 60°C, then crushed in a grinder and homogenized. The resultant crushed plant mass was mineralized using a mixture of H_2SO_4 and H_2O_2 (ZBÍRAL 2005). The amount of N in the mineralized sample was determined using the Kjeldahl's method. The content of P in the extract was determined colorimetrically on an ATI Unicam 8625 UV/VIS spectrophotometer. The levels of K, Ca, and Mg were determined in mineralized samples using Atomic Absorption Spectrophotometry (AAS) with a Carl Zeiss Jena AAS-30 instrument. The content of B was determined by ICP-AES.

Sunflower was harvested when it reached physiological ripeness. The yield of achenes, oil content, oil production and levels of fatty acids (palmitic, stearic, oleic and linoleic) in the achenes were evaluated after harvest. The oil content was determined using the Soxhlet method based on the extraction of sunflower achenes in a continuous flow extractor. The levels of fatty acids (FA) were determined as methyl esters using Gas Chromatography (HOUGEN, BODO 1973).

The Statistica 9 programme was used for the determination of overall characteristics. Arithmetic means were calculated when evaluating the results of experiments from 2008-2011. In order to elaborate the significance of differences among the arithmetic means of each characteristic, we used one- and two-factor analyses of variance followed by testing at 95% ($p < 0.05$), 99% ($p < 0.01$) and 99.9% ($p < 0.001$) levels of significance using the Fischer's LSD test.

RESULTS AND DISCUSSION

According to Cerkal et al. (2011), the nutrient content determined in stage V-4 (Table 3) showed that sunflower was well nourished. However, the soil had a low content of B and was therefore unable to provide the plant with an amount sufficient according to the plant material analyses (the optimal B content ranges between 35 and 100 mg kg^{-1}).

Table 3

Dry weight (g per plant) and nutrients concentration (% DM, mg kg^{-1} DM) of plant in the V-4 stage of sunflower development

Dry weight of plant (g per plant)	Content of nutrients					
	(% DM)					(mg kg^{-1} DM)
	N	P	K	Ca	Mg	B
3.01±0.33	4.27±0.33	0.40±0.04	5.04±0.30	1.94±0.43	0.77±0.11	35.60±3.07

Values show means of experiments in 2008-2011± SEM

Foliar application of boron in stage V-4 increased the boron content in the plant biomass; in plant leaves the increase was significant ($p < 0.05$). Asad et al. (2003) also discovered that foliar application of boron increased the concentration of B in all parts of the sunflower plant. The rate of 150 g B ha^{-1} (Boron 3) increased the concentration of B in leaves comparably to the treatment with 300 g B ha^{-1}. The relative increase in the boron content thus ranged between 37.6 and 52.6 mg kg^{-1}. Foliar nutrition did not significantly affect its content in the stem. Boron application increased the uptake of macro biogenic nutrients (especially N), as can be seen in analyses of plants taken in stage R-1 (Table 4). Foliar nutrition with boron at V-4 also increased the dry matter yield of the plants (Table 4), both in leaves (by 21.0-22.5%) and in stems (by 18.6-36.0%). Boron deficiency in sunflower inhibits plant growth and reduces dry matter production (Bonacin et al. 2008).

The positive effect of boron fertilization in stage R-1 on dry matter production (Boron 2) is obvious from the results of analyses of plants in stage R-2 (Table 5). The dry matter yield of leaves and stems increased by 21.1%

Table 4

Dry weight (g per plant) and concentration of nutrients concentration (% DM, mg kg^{-1} DM) of plants in the R-1 stage of sunflower development

Treatments of fertilization	Part of plant	Dry weight of plant (g per plant)	Content of nutrients					
			(% DM)					(mg kg^{-1} DM)
			N	P	K	Ca	Mg	B
Control	leaves	8.68±1.51	4.13±0.42	0.40±0.04	4.23±0.39	2.05±0.63	0.89±0.20	66.44 ^{a}±1.58
Boron 1	leaves	10.63±1.62	4.51±0.14	0.47±0.02	4.05±0.44	2.07±0.64	0.85±0.20	101.38 ^{b}±2.17
Boron 3	leaves	10.50±1.84	4.56±0.22	0.45±0.03	4.24±0.44	2.07±0.70	0.83±018	91.45 ^{b}±1.58
Control	stems	3.77±1.41	2.09±0.38	0.28±0.02	5.87±1.05	1.18±0.22	0.81±0.17	49.07 ^{a}±1.85
Boron 1	stems	4.47±0.86	2.39±0.18	0.36±0.05	5.83±0.85	1.23±0.30	0.91±0.25	44.96 ^{a}±1.39
Boron 3	stems	5.13±1.36	2.18±0.10	0.34±0.05	5.96±0.71	1.19±0.31	0.86±0.24	43.75 ^{a}±1.30

$P < 0.05$ – statistical significance at a 95% level of significance.

Variants with identical letters express statistically insignificant differences. Values show means of experiments in 2008-2011 ± SEM

Table 5

Dry weight (g per plant) and nutrients concentration (% DM, mg kg^{-1} DM) of plant in the R-2 stage of sunflower development

Treatments of fertilization	Part of plant	Dry weight of plant (g per plant)	Content of nutrients					
			(% DM)					(mg kg^{-1} DM)
			N	P	K	Ca	Mg	B
Control	leaves	27.55±4.20	3.02±0.28	0.36±0.02	3.35±0.65	1.74±0.53	0.83±0.16	61.99 ^{a}±1.51
Boron 1	leaves	28.92±1.01	3.05±0.38	0.38±0.03	3.52±0.50	1.63±0.45	0.82±0.16	72.23 ^{b}±2.12
Boron 2	leaves	33.34±3.87	3.17±0.22	0.38±0.02	3.65±0.39	1.63±0.47	0.73±0.17	76.47 ^{b}±2.31
Boron 3	leaves	28.39±4.09	3.06±0.33	0.40±0.03	3.53±0.51	1.63±0.49	0.75±0.16	78.41 ^{b}±2.34
Control	stems	26.15±2.46	1.10±0.38	0.25±0.02	4.49±1.07	0.83±0.20	0.71±0.19	24.02 ^{a}±1.82
Boron 1	stems	29.56±3.23	1.11±0.28	0.23±0.01	4.02±0.75	0.68±0.13	0.68±0.24	23.68 ^{a}±0.67
Boron 2	stems	30.87±4.85	1.16±0.37	0.24±0.01	4.42±0.84	0.73±0.18	0.64±0.25	23.35 ^{a}±2.21
Boron 3	stems	27.10±3.49	1.08±0.40	0.26±0.02	4.59±1.33	0.77±0.15	0.70±0.20	26.43 ^{b}±0.12

$p < 0.05$ – statistical significance at a 95% level of significance. Variants with identical letters express statistically insignificant differences. Values show means of experiments in 2008-2011 ± SEM.

and 18.0%, respectively. Foliar boron application increased the boron content in the sunflower plant biomass statistically significantly ($p < 0.05$) versus the unfertilised treatment (in leaves by 23.4%). Likewise, in the treatment where a split rate of boron was applied, the biomass yield increased and its content in the plant increased significantly ($p < 0.05$).

Boron applications had a positive effect on achene production in all fertilisation treatments (Figure 1). Blamey et al. (1997), Zerrari and Moustaoui (2005) and Sumathi et al. (2005) also reported elevated achene yields after fertilisation with boron. Achene yields increased statistically significantly ($p < 0.05$) after boron application at V-4 (Boron 1) by 8.3%. Souza et al. (2004) and Martin et al. (2010) reported 15 to 40% reduction in yields due to boron deficiency. Additional B nutrition in treatments Boron 2 and Boron 3 increased yields, although not statistically significantly ($p < 0.05$).

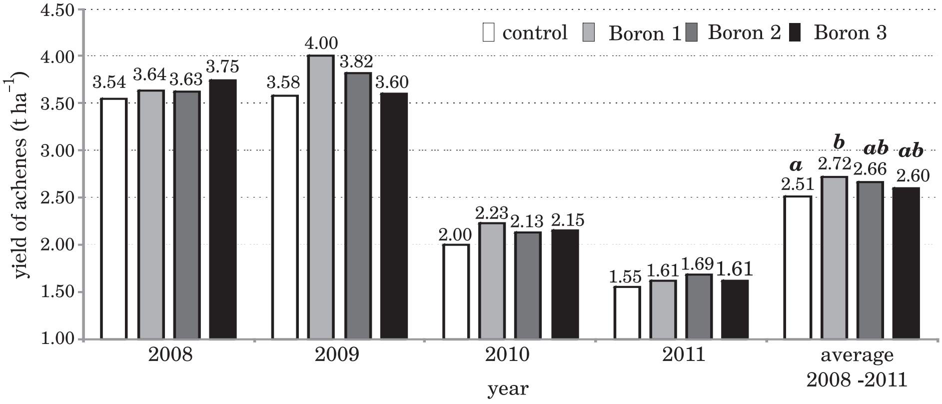

Fig. 1. Effect of different foliar boron application on yield of achenes
Means followed by different letters are significantly different (yield of achenes – $P < 0.05$).
Values show means of experiments in 2008-2011

Foliar boron nutrition increased the oil content in treatments with single boron application (Boron 1 and Boron 2), with an increase ranging between 1.2 and 1.8% (Table 6), which was not statistically significant ($p < 0.05$). In all the treatments, the oil content ranged between 44.47 and 45.65%. Also Ceyhan et al. (2008) reported lower oil content due to B application.

Owing to higher yields and a stabile oil content, the resulting effect of fertiliser application was higher oil production (in t ha^{-1}); the fertilisation combinations statistically significantly ($p < 0.05$) exceeded the control treatment, most highly the one with boron application at the beginning of vegetation (Boron 1; an increase by 10.2%).

Table 6

Effect of different foliar boron fertilization on oil content (%) and oil production (t ha^{-1})

Treatments of fertilization	Oil content (%)	Oil production (t ha^{-1})
Control	44.83 ^{a} ± 0.61	1.191 ^{a} ± 0.083
Boron 1	45.65 ^{a} ± 0.56	1.313 ^{b} ± 0.026
Boron 2	45.37 ^{a} ± 0.50	1.258 ^{ab} ± 0.024
Boron 3	44.47 ^{a} ± 0.77	1.258 ^{ab} ± 0.025

Means followed by the different letters are significantly different ($p < 0.05$).
Values show means of experiments in 2008-2011 ± SEM

In terms of the qualitative parameters of sunflower yield, we evaluated the share of fatty acids in sunflower oil; their composition is given in Table 7. Oleic acid is claimed to be among the most important fatty acids (the proportion of oleic acid in sunflower of the high oleic type should be at least 82%). The results of our analysis showed that the content of oleic acid ranged between 86.51 and 88.42%, and foliar nutrition with boron did not have a significant ($p < 0.05$) effect on its amount. Application of B increased significantly ($p < 0.05$) only the content of stearic acid, which ranged between 1.5 and 9.4%, and was the highest in the treatment with split rates of boron.

Table 7

The content of fatty acid (%) in sunflower oil

Treatments of fertilization	C 16:0	C 18:0	C 18:1	C 18:2
Control	3.39 ^{a}±0.10	3.20 ^{a}±0.08	87.01 ^{a}±1.24	6.28 ^{a}±1.26
Boron 1	3.41 ^{a}±0.08	3.48 ^{b}±0.09	86.51 ^{a}±0.95	6.50 ^{a}±0.96
Boron 2	3.45 ^{a}±0.17	3.25 ^{ab}±0.10	88.06 ^{a}±0.86	5.13 ^{a}±0.82
Boron 3	3.24 ^{a}±0.08	3.50 ^{b}±0.11	88.42 ^{a}±0.64	4.72 ^{a}±0.65

Palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2).
Means followed by the different letters are significantly different ($p < 0.05$). Values show means of experiments in 2008-2011 ± SEM.

CONCLUSIONS

Foliar application of boron increased the biomass production of sunflower plants and raised its content in leaf and stem dry matter. Early application of boron (stage V-4) also increased the uptake of macro biogenic nutrients (particularly N). Similarly to the year of cultivation, the application of boron had a statistically significant effect on sunflower yields. The achene yield increased significantly (by 8.3%) after the application of 300 g B ha^{-1} at the beginning of vegetation (stage V-4). Owing to the increased yields and stabile oil content, the oil production increased and was the highest after the application of boron fertilisation at the beginning of vegetation (by 10.2% relatively).

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CONTENT OF SELECTED ELEMENTS IN THE MUSCLE TISSUE OF PLAICE *(PLEURONECTES PLATESSA)* AND GARFISH *(BELONE BELONE)* FROM THE BALTIC SEA

Agnieszka Staszowska, Piotr Skałecki, Mariusz Florek, Anna Litwińczuk

Department of Commodity Science
and Processing of Raw Animal Materials
University of Life Sciences in Lublin

Abstract

Fish are an important component of a balanced human diet. Marine fish are more popular than freshwater fish among Polish consumers. All fish are an excellent source of minerals, which the human body cannot synthesize. Hence, they should be provided with food in right amounts and proportions. However, fish can be contaminated by heavy metals. For this reason, it is very important to determine the levels of selected trace elements in fish.

The aim of this study was to compare the content of mineral compounds in the muscle tissue of two species of fish from the Baltic Sea, i.e. plaice *(Pleuronectes platessa)* and garfish *(Belone belone)*. Wet-digested fish muscle samples were used to analyze the content of macro- (K, Na, Ca, Mg), and micronutrients (Zn, Fe, Mn, Cu) determined with flame atomic absorption spectrometry (AAS), and the levels of heavy metals (Cd, Pb) using flameless AAS.

No significant differences between the species in the concentrations of K, Mg, Ca, Cu and heavy metals appeared, while the Na, Mn, Zn and Fe concentrations were significantly different between the two fish. Higher levels of Na, Mg, Mn and heavy metals were determined in muscle tissue of European plaice, while concentrations of the other macro- and micronutrients were higher in muscles of garfish. The muscles of both species showed equal levels of Cd and Pb, below the maximum allowable limits.

Key words: garfish, plaice, muscle tissue, micronutrients, macronutrients, heavy metals.

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mgr inż. Agnieszka Staszowska, Department of Commodity Science and Processing of Raw Animal Materials, University of Life Sciences in Lublin, ul. Akademicka 13, 20-950 Lublin, Poland, e-mail: agnieszka.staszowska@up.lublin.pl

ZAWARTOŚĆ WYBRANYCH PIERWIASTKÓW W TKANCW MIĘŚNIOWEJ GŁADZICY *(PLEURONECTES PLATESSA)* I BELONY *(BELONE BELONE)* Z MORZA BAŁTYCKIEGO

Abstrakt

Ryby są istotnym elementem zbilansowanej diety człowieka. Polacy częściej spożywają ryby morskie niż słodkowodne. Wszystkie jednak są doskonałym źródłem związków mineralnych, które muszą być dostarczane do organizmu człowieka w odpowiedniej ilości i w odpowiednich proporcjach. Jakkolwiek ryby mogą być skażone metalami ciężkimi. Dlatego też bardzo istotne jest monitorowanie zawartości metali ciężkich.

Celem badań było porównanie zawartości związków mineralnych w mięsie dwóch gatunków ryb z Morza Bałtyckiego. Materiał badawczy stanowiła tkanka mięśniowa gładzicy *(Pleuronectes platessa)* i belony *(Belone belone)*. Pobrane próby poddano mineralizacji na mokro, a następnie oznaczono w nich zawartość makro- (Mg, Ca, Na, K) i mikroelementów (Zn, Cu, Mn, Fe) oraz metali ciężkich (Pb, Cd). Analizę mikro- i makroelementów przeprowadzono techniką płomieniowej atomowej spektrometrii absorpcyjnej (AAS), natomiast stężenie metali ciężkich oznaczono metodą bezpłomieniowej atomowej spektrometrii absorpcyjnej.

Nie wykazono istotnych różnic między badanymi gatunkami w przypadku K, Mg, Ca, Cu oraz metali ciężkich, natomiast statystycznie istotne różnice wykazano w przypadku zawartości Na, Mn, Zn oraz Fe. Wyższe stężenie Na, Mg, Mn oraz metali ciężkich stwierdzono w mięsie gładzicy, poziom pozostałych makro- i mikroelementów był wyższy w mięsie belony. Zawartość metali ciężkich w mięsie badanych gatunków ryb nie przekroczyła dopuszczalnych limitów.

Słowa kluczowe: belona, gładzica, tkanka mięśniowa, mikroelementy, makroelementy, metale ciężkie.

INTRODUCTION

People today are interested in nutrition as a way to ensure good health. Many chronic diseases, such as cardiovascular disorders, cancer or type 2 diabetes, are blamed to an unbalanced diet and lack of physical activity (ŁUCZYŃSKA et al. 2006, HONKANEN 2010). Minerals are a very important component of a diet, because they cannot be synthesized by the human body and therefore should be provided with food in the right amounts and proportions. Minerals play many crucial physiological functions and their proportions in tissues and cells condition the health of the body. Fish are an excellent source of elements, for example with respect to the mineral content some fish are superior to other types of food (POLAK-JUSZCZAK 2007). BRUCKA-JASTRZĘBSKA et al. (2010) revealed that a culture site and culture conditions exerted significant influence on the levels of macro- and micronutrients in freshwater fish. Moreover, the content of trace elements in the muscle tissue of fish can vary due to biological factors, such as the species, gender, age, feeding, living environment, or technical conditions, e.g. the method of fishing as well as transport (RYWOTYCKI 2005, ŁUCZYŃSKA et al. 2006, BRUCKA-JASTRZĘBSKA et al. 2009).

The European plaice (*Pleuronectes platessa*) is an important commercial flatfish, one of the most popular table fish in the Baltic Sea region. It is usually found on muddy seabeds from the low shore to depths exceeding 50 m. Juveniles forage on plankton and insect larvae, and adults feedn on polychaetes, molluscs, crustaceans and small fish. Similarly, the garfish (*Belone belone*) living in the pelagic zone of the Baltic Sea is fished for its tasty meat. This needlefish is a typical predator, flying in flocks near the surface and feeding on small fish and crustaceans.

Sea fish are more popular than freshwater fish in Poland, for example in 2008 the sea fish consumption was 9.4 kg per person, compared to just 3.4 kg of freshwater fish consumed per person (ŁUCZYŃSKA et al. 2011). However, low consumption of fish can be associated with an individual perception of benefits and risk associated with fish eating (VAN DIJK et al. 2011).

The pollution of seafood is both a hygienic and toxicological problem. It is extremely important to determine the levels of selected trace elements in fish. The Baltic Sea countries have monitored the content of heavy metals in fish since the 1980's (POLAK-JUSZCZAK 2009). The content of heavy metals in fish muscle tissue cannot be higher than 0.050 mg kg^{-1} for cadmium and 0.30 mg kg^{-1} for lead (European Commission 2006).

The aim of this study was to compare the concentration of selected elements in muscles of two fish species with different species-specific characteristics (coastal water vs. pelagic fish), both fished from the Baltic Sea.

MATERIAL AND METHODS

The study included two fish species from the Baltic Sea: plaice *(Pleuronectes platessa)* and garfish *(Belone belone)*. Twenty fish were analyzed, i.e. ten fish from each species, caught in the summer of 2010 from the Bay of Gdańsk, which lies in the southeastern part of the Baltic Sea (FAO Major Fishing Area 27, subarea III, division IIId, subdivision 26). Samples of the dorsal muscles of fish were wet digested in a mixture of HNO_3 and $HClO_4$ according to AOAC 986.15 standard (AOAC 2000).

The mineralized samples were used to analyze the concentration of macronutrients (potassium, sodium, calcium, magnesium), micronutrients (zinc, iron, manganese, copper), and heavy metals (cadmium and lead). Micro- and macronutrients were determined with the use of flame atomic absorption spectrometry (SOLAR 939 Unicam Spectrometer). The content of heavy metals was detected using flameless atomic absorption spectrometry (SpectrAA 880Z Varian).

Solution of lanthanum was added to all the samples and standards when determining calcium in order to eliminate the influence of phosphorus. The detection limits were 0.01 mg kg^{-1} for Na, 0.04 mg kg^{-1} for K, 0.47 mg kg^{-1}

for Mg, 0.22 mg kg^{-1} for Ca, 0.01 mg kg^{-1} for Mn, 0.01 mg kg^{-1} for Cu, 0.01 mg kg^{-1} for Zn, 0.09 mg kg^{-1} for Fe, 0.02 µg kg^{-1} for Cd, 0.24 µg kg^{-1} for Pb. The accuracy of the method was verified by means of trace metals determined in DORM-3 standard reference material. The achieved results were in good agreement with the certified values. The content of macro- and micronutrients as well as heavy metals in the muscle tissue of fish was expressed in mg kg^{-1} wet mass.

Statistical analysis was carried out using Statistica ver. 6.0 software (Statsoft 2003). The data were subjected to one-way Anova and Tukey's test was applied for comparisons of the means, considering $p < 0.05$ as significant.

RESULTS AND DISCUSSION

The concentrations of ten elements including macro-, micronutrients and heavy metals in the muscles of European plaice and garfish from the Baltic Sea are shown in Table 1. While analyzing the macronutrients, a significantly ($P \leq 0.05$) higher concentration of sodium (689.19 mg kg^{-1}) was found

Table 1

Concentration of macro- and micronutrients and heavy metals in muscle tissue of European plaice and garfish (mg kg^{-1} wet mass)

Specification	Plaice (*Pleuronectes platessa*)		Garfish (*Belone belone*)	
	$\overline{x}$	s	$\overline{x}$	s
Macronutrients (mg kg^{-1}wet mass)				
Na	689.19^{b}	176.61	480.56^{a}	53.09
K	1960.92	439.76	2014.16	335.54
Mg	333.79	53.83	258.93	37.59
Ca	1023.71	223.20	1225.55	109.05
Micronutrients (mg kg^{-1}wet mass)				
Mn	1.42^{b}	0.39	0.37^{a}	0.08
Cu	1.22	0.31	1.60	0.35
Zn	10.79^{a}	3.85	17.29^{b}	2.97
Fe	6.81a	1.58	9.34^{b}	0.97
Heavy metals (mg kg^{-1} wet mass)				
Cd	0.003	0.001	0.002	0.001
Pb	0.096	0.018	0.093	0.015

Means with different letters *a,b* differ significantly at $P \leq 0.05$

in the muscles of European plaice as compared with garfish (480.56 mg kg^{-1}). Nevertheless, the concentrations of this element found herein are within the range given by SIKORSKI (2004). Moreover, a similar content of sodium in muscles of European plaice was stated earlier by KWOCZEK (2006), who examined European flounder (692±70.5 mg kg^{-1}).

The concentrations of other macronutrients (K, Mg, and Ca) did not differ significantly between the compared species. Considerably lower concentrations of these elements in muscles of European flounder were reported by KWOCZEK (2006): K – 340, Mg – 88.5, and Ca – 413 mg kg^{-1}, respectively, in comparison with the muscles of plaice analzyed in the present study. The differences may stem from the fact that the cited study investigated European flounder, while the present results concern muscles of European plaice. However, both species belong to the same family (*Pleuronectidae*) living in the Baltic Sea.

While analyzing micronutrients, a significantly ($P \leq 0.05$) higher concentration of manganese (1.42 mg kg^{-1}) was found in muscles of European plaice than in garfish (0.37 mg kg^{-1}). However, the mean concentration of copper in muscles of the compared species did not differ significantly, and ranged from 1.22 mg kg^{-1} in plaice to 1.60 mg kg^{-1} in garfish.

Regional differences in Mn and Cu concentrations in muscles of garfish from Turkish waters were examined by TÜRKMEN et al. (2009). The authors obtained concentrations ranging from 0.25 to 1.33 mg kg^{-1} for manganese, and from 0.21 to 5.89 mg kg^{-1} for copper. However, considerably lower concentrations of these metals were reported by KWOCZEK (2006), i.e. 0.1 Mn and 0.3 mg Cu kg^{-1} for manganese and copper.

The species correlated significantly ($P \leq 0.05$) to the concentrations of zinc and iron in muscles of the examined fish. Higher levels of Zn and Fe were determined in the muscles of garfish (17.29 and 9.34 mg kg^{-1} for Zn and Fe, respectively) than in plaice (10.79 and 6.81 mg kg^{-1} for Zn and Fe, respectively).

TÜRKMEN et al. (2009) determined lower concentrations of zinc in the muscles of garfish, that is 8.1 to 15.0 mg kg^{-1}. Also, KWOCZEK (2006) reported a lower content of zinc (averaged 5.7 mg kg^{-1}) in the muscles of flounder than found in plaice in the present study. The above differences may be caused to the different living environments (the coastal waters of Turkey vs. the coastal waters of Poland). With respect to plaice, KWOCZEK (2006) analzyed fish of another species which belonged to the same family.

Our values of iron concentrations were in agreement with literature (SIKORSKI 2004). Also, a similar Fe content (averaged 9.99 mg kg^{-1}) was found by TÜRKMEN et al. (2009) in the meat of garfish from the Sea of Marmara. However, the same authors reported a higher Fe level for garfish meat from the Black Sea, ranging from 16.5 to 43.3 mg kg^{-1}. According to KWOCZEK (2006), the concentration of iron in flounder muscles averaged 5.4 mg kg^{-1}, and was lower than in plaice muscles analyzed in the present study.

Numerous factors, both biological (species and feeding type) and environmental ones, affect the degree of contamination of fish muscle tissue with heavy metals, mainly lead, mercury and cadmium. Fish absorb heavy metal through the intestinal tract from ingested food, and through the gills and skin from ambient waters (POLAK-JUSZCZAK 2009). Trace element levels are known to vary in fish depending on various factors such as the habitat, feeding behavior and migration, even in the same area (TÜRKMEN et al. 2009).

No differences between the investigated species in terms of the heavy metal concentration were found in the present study. It is noteworthy that the Pb and Cd concentrations in all the samples were found to be lower than the threshold proposed for human consumption by the European Commission (2006). However, a considerably higher concentration of both elements were determined by TÜRKMEN et al. (2009) in garfish muscles from Turkish waters. The concentrations of the heavy metals from 0.01 to 0.07 mg kg^{-1} for cadmium and from 0.19 to 0.81 mg kg^{-1} for lead.

Finally, POLAK-JUSZCZAK (2009), who analyzed temporal trends in the bioaccumulation of trace metals in fish from the southern Baltic Sea in 1994--2003, indicates that the most likely cause of decreasing trends in concentrations of cadmium, lead and mercury in the herring, sprats and cod is the lowering accumulation of these metals in the Baltic waters.

CONCLUSION

Out of the two species from the Baltic Sea analyzed in this study, the European plaice was a coastal water fish and garfish was a pelagic fish. These species-specific characteristics may be responsible for differences in the accumulation of individual elements accumulation between the two fish speices from the Baltic waters.

No significant differences between the species in the concentrations of potassium, magnesium, calcium, copper and heavy metals were determined. However, significant differences were observed in the sodium, manganese, zinc and iron concentration. Higher levels of sodium, magnesium, manganese and heavy metals were determined in muscles of European plaice, while the concentrations of the other macro- and micronutrients were higher in muscles of garfish. The muscles of both species showed equal levels of cadmium and lead.

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THE EFFECT OF SOWING TIME/METHOD ON YIELD AND UPTAKE OF MACROELEMENTS IN AUTUMN REGROWTH OF GENOTYPES OF RED FESCUE

Małgorzata Szczepanek, Edward Wilczewski, Zbigniew Skinder

Chair of Agrotechnology
University of Technology and Life Sciences in Bydgoszcz

Abstract

The yield of autumn regrowth of grasses and the content of macronutrients are affected by environmental and agronomic factors as well as genetic traits of species and varieties. Biomass yield and the concentration of mineral elements determine their uptake by plants. The aim of this study was to evaluate dry matter yield as well as the content and uptake of N, P, K, Ca and Mg in the autumn regrowth of three varieties of red fescue in the first two production years depending on different sowing time and methods. This study was based on a strict field experiment conducted in 2004-2007, located in the Province of Kujawy and Pomorze (*województwo kujawsko-pomorskie*) (53°09′N, 17°35′E). The first factor was the time/method of sowing (spring: pure or with spring barley, and summer: in mid- and late July and in mid-August), while the second factor was the variety (chewings, slender creeping and strong creeping fescue cultivars). An average of 3.5 t ha^{-1} and 3.9 t ha^{-1} of regrowth of dry matter was obtained in the first and second production years, respectively. A delay of the sowing date until summer caused an increase in yield during the first production year in comparison with the spring sowing. In both years, the creeping variety gave the highest yield. The yield of the chewings variety was higher than that of the slender creeping one in the second production year. The concentration of N in the strong creeping variety was lower than that of the chewings one in the first year, and also in relation to the slender creeping variety in the second production year. The chewings and slender creeping varieties were characterized by a higher concentration of P than the strong creeping cultivar. The K and N uptake in the average regrowth yield from the first and second production years was the highest (106.3 kg ha^{-1} K and 62.7 kg N);

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dr inż. Małgorzata Szczepanek, Chair of Agrotechnology, University of Technology and Life Sciences in Bydgoszcz, Ks. A. Kordeckiego 20C, 85-225 Bydgoszcz, Poland, e-mail: malgorzata.szczepanek@utp.edu.pl

the P and Ca uptake was low (12.5 and 11.3 kg ha^{-1}) and that of Mg was the lowest (6.6 kg ha^{-1}). A rise in the regrowth yield caused an increase in the N, P, K, Ca and Mg uptake in summer sowing crops compared with spring ones in the first year and a high uptake of these elements by the strong creeping variety in both production years. In the first year, owing to the high N and P concentration, the uptake of these elements by the chewings and strong creeping fescue was similar. In the second year, the uptake of N, P, K, Ca by the slender creeping variety was the smallest due to a strong reduction in yield. In the first year, with no differences in the regrowth yield and K, Ca and Mg content, the uptake of these nutrients by the chewings and slender creeping varieties was similar.

Key words: macroelements, autumn regrowth, red fescue, sowing time/method, variety.

WPŁYW TERMINU/SPOSOBU SIEWU NA PLON ORAZ POBRANIE MAKROSKŁADNIKÓW W ODROŚCIE JESIENNYM GENOTYPÓW KOSTRZEWY CZERWONEJ

Abstrakt

Na wielkość plonu biomasy odrostu jesiennego traw oraz zawartość makroskładników mają wpływ czynniki siedliskowe, agrotechniczne oraz genetyczne właściwości gatunków i odmian. Plon biomasy oraz koncentracja składników mineralnych określają wielkość ich pobrania z plonem. Celem badań była ocena wielkości plonu suchej masy, zawartości i pobrania N, P, K, Ca i Mg w odroście jesiennym trzech odmian kostrzewy czerwonej w dwóch latach pełnego użytkowania, w warunkach różnych terminów i sposobów zakładania plantacji nasiennych. Podstawą badań było ścisłe doświadczenie polowe prowadzone w latach 2004-2007, w rejonie kujawsko-pomorskim (53°09′N; 17°35′E). Pierwszym czynnikiem był termin/sposób siewu (wiosenny: czysty lub z jęczmieniem jarym oraz letni: połowa i koniec lipca oraz połowa sierpnia), drugim – odmiana (kępowa, półrozłogowa oraz rozłogowa). Uzyskano 3,5 t ha^{-1} suchej masy odrostu w pierwszym roku pełnego użytkowania i 3,9 t ha^{-1} w drugim. Opóźnienie zasiewów letnich spowodowało zwiększenie plonu w pierwszym roku w stosunku do zasiewów wiosennych. W obu latach odmiana rozłogowa dała największy plon. W drugim roku plon odmiany kępowej był większy niż półrozłogowej. Koncentracja N w przypadku odmiany rozłogowej była mniejsza niż w przypadku kępowej w pierwszym roku użytkowania i najmniejsza w drugim. U odmiany kępowej i półrozłogowej stwierdzono większą koncentrację P niż u rozłogowej. Pobranie K i N z plonem odrostu, średnio z dwóch lat użytkowania, było największe (106,3 kg ha^{-1} K i 62,7 kg N), P i Ca niewielkie (12,5 i 11,3 kg ha^{-1}), a Mg najmniejsze (6,6 kg ha^{-1}). Zwiększona produkcja suchej masy spowodowała wzrost pobrania N, P, K, Ca i Mg w zasiewach letnich w porównaniu z wiosennymi w pierwszym roku, a także wysokie pobranie tych składników u odmiany rozłogowej w obu latach użytkowania. W pierwszym roku, wskutek wysokiej koncentracji N i P, pobranie tych składników u odmiany kępowej było podobne jak u rozłogowej. Z silnej redukcji plonu wynikało najmniejsze pobranie N, P, K, Ca u odmiany półrozłogowej w drugim roku użytkowania. W pierwszym roku, przy braku różnic w plonie odrostu oraz zawartości K, Ca i Mg, pobranie tych składników u odmiany kępowej i półrozłogowej było podobne.

Słowa kluczowe: makroskładniki, odrost jesienny, kostrzewa czerwona, termin/sposób siewu, odmiana.

INTRODUCTION

Seed plantations of grasses can be a valuable source of bulky feeds in the form of autumn regrowth (Rogalski, Łyduch 1981). Additional sources of cheap fodder must be found if an area of permanent grassland is insufficient to sustain a given cattle stock (Wilczewski et al. 2008). The size of the autumn regrowth yield of grasses and its phytochemical composition are affected by the species and even cultivars of plants (Kozłowski, Goliński 1997, Rogalski, Łyduch 1981, Golinski et al. 2004). Yield and the content of mineral elements in grass green forage are also determined by fertilization, particularly with nitrogen (Kozłowski, Goliński 1997, Goliński 2003, Kolczarek et al. 2008, Kaczor, Brodowska 2009, Skinder et al. 2011), and by row spacing as well as the sowing time and method (Szczepanek, Skinder 2006). The content of mineral elements in the biomass determines its usefulness in animal feeding (Rogalski, Łyduch 1983, Radkowski et al. 2005). Together with the yield, it also determines the uptake of minerals, the fact which should be taken into consideration when making a fertilization balance.

The aim of this study was to assess the size of dry weight yield as well as the content and uptake of N, P, K, Ca and Mg in the autumn regrowth of three different genotypes of red fescue in the first and second year of production under conditions of different season and methods of establishing seed plantations.

MATERIAL AND METHODS

The study was based on a strict field experiment, located in the region of Kujawy and Pomorze (53°09’ N; 17°35’ E), on lessive soil developed from heavy loamy silty sands deposited on loose sands. The soil had a very high content of phosphorus (104 mg P kg^{-1}) and potassium (212 mg K kg^{-1}). Its reaction was neutral (pH in KCl 6.75).

The experiment was conducted in two series, each including one establishment year (2004 in series I and 2005 in series II) and two successive years of production (2005 and 2006 in series I, 2006 and 2007 in series II). The quantity and chemical composition of autumn regrowth of red fescue harvested in each production year on the last ten days of September (three months after seed harvesting). A split plot design with four replications was used. The area of a plot was 15 m^2. The first factor was the time/method of sowing and the second one was the cultivar. Fescue was sown in spring (the middle of April) in a pure stand or as a crop undersown into spring barley. The grass was also sown in summer: in the middle and end of July and in the middle of September. Spring barley was sown prior to sowing red fescue, at a row spacing of 12 cm, depth of 3 cm and amount of seeds equal 125 kg ha^{-1}. Red fescue was sown at a row spacing of 24 cm, depth of 1 cm an amount of 8 kg ha^{-1}. Three lawn cultivars of red fescue (*Festuca rubra* L.) were investigated: chewings (ssp. *commutata* Gaud.) cv. Mirena, slender creeping (ssp. *trichophylla* Gaud.) cv. Womira and strong creeping (ssp. *rubra* = *genuina* Hack.) cv. Nista.

The rates of fertilizing nutrients were N 40, P 26 and K 66 kg ha^{-1}. They were applied in the establishment year in August, after harvesting barley from mixed sowing plots, and on fescue green forage plots from the spring pure sowing as well as presowing on summer sown fescue plots. Identical rates of these elements were applied immediately after harvesting fescue seeds (the beginning of July) in the years of production. Before the plants began to grow, at the end of March and at the beginning of April in the first and second year of production, the rate of mineral nitrogen reached 60 kg ha^{-1}.

At harvest, the fresh weight yield of the autumn regrowth from each plot was weighed and 1 kg samples were collected for determination of dry weight content and performance of chemical analyses. Dry weight was determined with the drying method at 105°C. Chemical analyses of plant material involved determination of the content of total N (with Kjeldahl method), P (vanadium–molybdenum method), K and Ca (flame photometry method) and Mg (colorimetrically with titan yellow). The uptake of macroelements was calculated as a product of their content and the size of dry weight yield. The results were subjected to an analysis of variance for multi-factorial experiments and a synthesis of the data was made according to the model of combined inaccuracies for the first and second year of production. Significance of differences between means was verified with Tukey's test. With no directional interactions, the results are presented as means for the tested factors and years.

RESULTS AND DISCUSSION

Red fescue can be established as a catch crop in a cover crop or in pure sowing from spring till late summer. The sowing time/method determines the amount of green biomass produced in the first half of the next growing season (the first production year) (Boelt 1997a, b, Fairey, Lefkovich 2001). In the present study, it was proven that the sowing time/method had a significant effect on the amount of vegetative biomass produced (autumn regrowth) in the second half of the growing season (Table 1). In the first production year, the dry matter yield of regrowth was significantly higher from swards sown in summer than in spring. Red fescue sown in spring produced 22% more of threshed straw biomass in the first half of growth than if sown in

Table 1

The dry matter yield of autumn regrowth of red fescue depending on time/method of sowing in the first and second production year (t ha^{-1})

Production year	Time/method of sowing					LSD (p=0.05)
	spring		summer			
	pure	with barley	middle of July	end of July	middle of August	
First, mean 2005-2006	2.695	3.107	3.800	4.140	3.999	0.642
Second, mean 2006-2007	3.743	3.771	4.093	3.954	3.902	n.s.

July and August (data not presented). This could have been caused by drier soil and lower quantities of available nutrients in soil later on in the plant growing season, particularly in the early regrowth of the sward. Hence, a decrease in the production of autumn regrowth biomass from spring sowing of grass. The availability of nutrients and water is a major determinant of grass yield (GOLIŃSKI 2003, KACZOR, BRODOWSKA 2009, SKINDER et al. 2011).

No effect of the time/method of sowing on the content of macroelements in the autumn regrowth of red fescue was found, either in the first or in the second production year (Table 2). According to MCCARTNEY et al. (2004), the chemical composition of red fescue green forage is dependent on the duration of its regrowing period. New sward (at 3-4 cuts in the growing

Table 2

Content of macroelements in autumn regrowth of red fescue depending on time/method of sowing in the first and second production year (%)

Production year	Element	Time/method of sowing					LSD (p=0.05)
		spring		summer			
		pure	with barley	middle of July	end of July	middle of August	
First, mean 2005-2006	N	1.753	1.793	1.837	1.825	1.898	n.s.
	P	0.303	0.308	0.296	0.291	0.290	n.s.
	K	2.423	2.603	2.622	2.617	2.773	n.s.
	Ca	0.313	0.323	0.321	0.341	0.332	n.s.
	Mg	0.182	0.191	0.190	0.191	0.189	n.s.
Second, mean 2006-2007	N	1.605	1.721	1.517	1.575	1.634	n.s.
	P	0.330	0.350	0.332	0.376	0.348	n.s.
	K	2.739	2.883	2.750	2.844	2.955	n.s.
	Ca	0.274	0.314	0.281	0.294	0.289	n.s.
	Mg	0.175	0.175	0.162	0.176	0.175	n.s.

season) contained more N than older one (cut twice in the season). In the present study, the duration of sward regrowing in all the variants of fescue sowing times/methods was identical, hence a similar chemical composition of the regrowth. The K content in the autumn fescue regrowth determined in the present study can be claimed to almost reach the highest amount in this grass species, as implied by the literature data (GOLIŃSKI et al. 2004, RADKOWSKI et al. 2005, SKINDER, SZCZEPANEK 2011). The high content of this element in soil and regular application of mineral fertilizers favoured high accumulation of K in the regrowth. The optimal concentrations of nutrients for dairy cows are claimed to be 0.4-0.5% P, 0.6-0.9% Ca, 0.2-0.3% Mg (KRZYWIECKI 1985), hence the concentrations of these elements, particularly Ca, in the regrowth of the tested cultivars seem deficient. Similar conclusions are presented also in other studies concerning the fodder value of the autumn regrowth of grasses (ROGALSKI, ŁYDUCH 1981, KOZŁOWSKI, GOLIŃSKI 1997, GOLIŃSKI et al. 2004, SKINDER et al. 2011).

The uptake of macroelements by the red fescue regrowth in the first and second year of production was similar (Table 3). The grass took up most K and N: on average, 106.3 kg K and 62.7 kg N ha^{-1} in the two-year utilization period. The P and Ca uptake was considerably lower and similar for both macroelements (12.5 and 11.3 kg ha^{-1}). The smallest uptake was found in the case of Mg (6.6 kg ha^{-1}).

Table 3

Uptake of macroelements with autumn re growth of red fescue depending on time/method of sowing in the first and second production year (kg ha^{-1})

Production year	Element	Time/method of sowing					LSD (p=0.05)
		spring		summer			
		pure	with barley	middle of July	end of July	middle of August	
First, mean 2005-2006	N	44.93	52.23	67.24	75.44	74.70	11.58
	P	8.860	10.31	12.34	13.11	12.54	2.031
	K	71.47	88.55	108.83	119.28	121.57	18.37
	Ca	8.346	9.846	11.72	13.71	12.98	2.097
	Mg	4.891	5.731	7.166	7.609	7.400	1.207
Second, mean 2006-2007	N	59.78	64.94	62.07	61.87	63.58	n.s.
	P	12.32	13.10	13.59	14.96	13.53	1.884
	K	103.1	108.0	113.7	113.0	115.5	n.s.
	Ca	10.27	11.91	11.57	11.56	11.25	1.603
	Mg	6.542	6.595	6.627	6.894	6.801	n.s.

A significant effect of the time/method of sowing on the uptake of N, P, K, Ca and Mg by the autumn regrowth occurred. In the first year of production, the uptake of macronutrients was larger in summer sowings than in spring ones (the said difference in the uptake of Ca by the grass undersown into barley was not significant). Having confirmed no differences in concentrations of these elements between the compared times/methods of sowing (Table 2), it was concluded that the uptake corresponded most closely to the volume of dry weight yield (Table 1). In the second year of production, a smaller uptake of P and Ca in the spring pure sowing was observed. Regarding P, the difference was significant only in comparison with the sowing at the end of July. With respect of Ca, it was significant in relation to all the other times/methods of sowing. The limited uptake of those elements grasses from the spring pure sowing resulted from both a reduction in yield and a lower content of these elements in biomass (Tables 1 and 2).

Red fescue cultivars (*Festuca rubra* L.*)* from three subspecies (ssp. *commutata* – chewings , *trichophylla* – slender creeping and *rubra* – strong creeping) show different physiologicaland morphological traits as well as different commercial value (DELEURAN, BOELT 1997, HUYLENBROECK, BOCKSTAELE 1999, MARTINIELLO, D'ANDREA 2006, SZCZEPANEK, OLSZEWSKI 2009, CHASTAIN et al. 2011). In the present study, differences in dry matter yields of autumn regrowths of red fescue cultivars/subspecies were found both in the first and second production year (Table 4). The strong creeping cultivar Nista was characterized by the highest yield in both years. In the first production year, the yields of the chewings cultivar Mirena and slender creeping Womira were similar, while in the second the chewings cultivar gave better yields. Similarly, GOLIŃSKI et al. (2004) showed that strong creeping cultivars of red fescue yielded better than chewings and slender creeping ones.

Table 4

The dry matter yield of autumn regrowth of red fescue depending on variety in the first and second production year (t ha^{-1})

Production year	Variety			LSD (p=0.05)
	chewings cv. Mirena	slender creeping cv. Womira	strong creeping cv. Nista	
First, mean 2005-2006	3.400	3.376	3.869	0.164
Second, mean 2006-2007	3.832	3.421	4.425	0.156

Significant variability of the chemical composition of the studied genotypes was observed (Table 5). The strong creeper Nista usually contained less N and P than the chewings and slender creeping varieties. Similarly, GOLIŃSKI et al. (2004) proved a significant difference between cultivars in the content of nitrogen (total protein) and phosphorus in the red fescue regrowth.

Table 5

Content of macroelements in autumn regrowth of red fescue depending on variety in the first and second production year (%)

Production year	Element	Variety			LSD (p=0.05)
		chewings cv. Mirena	slender creeping cv. Womira	strong creeping cv. Nista	
First, mean 2005-2006	N	1.926	1.812	1.7269	0.155
	P	0.314	0.303	0.276	0.016
	K	2.611	2.578	2.634	n.s.
	Ca	0.321	0.319	0.337	n.s.
	Mg	0.182	0.185	0.199	n.s.
Second, mean 2006-2007	N	1.645	1.645	1.540	0.101
	P	0.361	0.352	0.328	0.018
	K	2.862	2.749	2.892	n.s.
	Ca	0.286	0.291	0.294	n.s.
	Mg	0.161	0.187	0.169	n.s.

The authors reported that the protein content in strong creeping cultivars was usually smaller than in chewings and slender creeping ones. Kozłowski and Goliński (1997) reported that ecotypes of red fescue rich in protein can accumulate twice as much of this element as compared ones with little protein.

The uptake of nutrients by the autumn regrowth was different between the cultivars, both in the first and second year of production (Table 6). In the first year, the chewings cv. Mirena and the strong creeping cv. Nista took up more N than the slender creeping cv. Womira. In the chewings cultivar, an increased uptake of N resulted from a high concentration of this element in the biomass, and in the strong creeping cultivar the same effect was achieved owing to a high dry weight yield of the regrowth (Tables 4 and 5). Also in the case of P, its increased concentration in the cultivar Mirena resulted from a higher uptake of this element than by the cultivar Womira.

The rate of K, Ca and Mg uptake, in the absence of differences in the concentration of these elements in the biomass, was most strongly determined by the volume of yield, and was higher in the strong creeping cultivar Nista than in the other cultivars.

In the second year of production, the uptake of N, P K and Ca was the highest in the strong creeping cultivar, significantly smaller in the chewings one, and the smallest in the slender creeping one. An increase in the dry weight yield of the autumn regrowth of the strong creeping cultivar Nista

Table 6

Uptake of macroelements with autumn regrowth of red fescue depending on variety in the first and second production year (kg ha^{-1})

Production year	Element	Variety			LSD (p=0.05)
		chewings cv. Mirena	slender creeping cv. Womira	strong creeping cv. Nista	
First, mean 2005-2006	N	63.31	60.21	65.21	2.194
	P	11.61	11.12	11.57	0.462
	K	98.03	96.45	111.34	4.561
	Ca	10.65	10.65	12.66	0.418
	Mg	6.116	6.174	7.388	0.253
Second, mean 2006-2007	N	63.01	56.26	68.08	2.533
	P	13.88	12.07	14.56	0.550
	K	109.9	94.0	128.0	4.481
	Ca	10.98	9.955	13.00	0.457
	Mg	6.175	6.413	7.487	0.281

(by 15.5% and 29.3% versus the cultivars Mirena and Womira) (Table 4) indicates its crucial effect on the values of uptake in the second year. Concerning Mg, its uptake was also the largest in the strong creeping cultivar, but an increased concentration in the slender creeping cultivar resulted in an increase in the uptake of this element to the level found in the chewings cultivar.

CONCLUSIONS

1. In the first production year of red fescue, a significant increase in the autumn regrowth yield from summer sowings (from the middle and end of July and from the middle of August) in comparison with spring sowings (pure and with barley) was noticed (by 1.08 t ha^{-1} dry weight), This in turn resulted in an increase in the N, P, K, Ca and Mg uptake by 23.9; 3.1; 36.6; 3.7; 2.1 kg ha^{-1}, respectively.

2. The N and P content in the autumn regrowth of the strong creeping cultivar was less than that of the chewings and slender creeping ones, but the uptake of these elements along with the highest dry weight yield was as high as in the chewings cultivar in the first year of production and higher than both compared cultivars in the second production season.

3. The K, Ca and Mg uptake by the autumn regrowth biomass of red fescue, in the absence of differences between the cultivars in the concentration of these nutrients, was determined mainly by the amount of dry weight yield; it was the largest in the creepings cultivar, both in the first and second production years.

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EFFECT OF NITROGEN RATE AND STUBBLE CATCH CROPS ON CONCENTRATION OF MACROELEMENTS IN SPRING WHEAT GRAIN

Edward Wilczewski[1] , Małgorzata Szczepanek[1],
Anna Piotrowska-Długosz[2], Anna Wenda-Piesik[3]

[1]Chair of Agrotechnology
[2] Department of Biochemistry
[3]Chair of Plant Growing Basis and Experimental Methods
University of Technology and Life Science in Bydgoszcz

Abstract

The aim of this study was to determine the effect of different rates of nitrogen fertilizer and stubble catch crops on the content of nitrogen, phosphorus, potassium and magnesium in the grain of spring wheat. Field experiment was carried out in 2005-2008 at Mochełek near Bydgoszcz (53° 13' N, 17° 51' E) on the Alfisols soil classified as a very good rye complex and IIIb quality class. Nitrogen fertilization was applied at the rates of (kg N ha^{-1} $year^{-1}$): 0; 40 (before sowing), 80 (40 kg before sowing and 40 kg in BBCH 31-32 stage), 120 (60 kg before sowing and 60 kg in the BBCH 31 – 32 stage) and 160 (60 kg before sowing and 60 kg in BBCH 31-32 stage and 40 kg in BBCH 45-47 stage). Stubble catch crops (field pea and oilseed radish) were sown in the period from 3 to 11 of August in 2005 to 2007. After 70-77 days of plant growing, the whole biomass was plowed in as green manure. Spring wheat cv. Tybalt was sown following the catch crop,between March 30 to April 11 in 2006 to 2008. Nitrogen fertilization affected the concentrations of N, P and Mg in grain of spring wheat. Every 40 kg increment of nitrogen fertilization per ha resulted in a significant increase in the N content in wheat grain. The concentration of Mg in spring wheat grain was significantly higher when nitrogen fertilization was dosed at 120 or 160 kg per ha than at lower doses. The phosphorus content in grain of wheat grown after field pea catch crop was not related to nitrogen fertilization rates, while after the radish and in the control (without catch crop), it was significantly higher at 120 and 160 kg N per ha than at 40 kg N per ha. No effect of nitrogen fertilization on the K

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dr inż. Edward Wilczewski, Chair of Agrotechnology, University of Technology and Life Sciences in Bydgoszcz, Ks. A. Kordeckiego 20, 85-225 Bydgoszcz, Poland, e-mail: Edward.Wilczewski@utp.edu.pl

concentration in grain of spring wheat was found. Mean values from the three years of the N and Mg content in the grain of spring wheat were significantly influenced by the stubble catch crops. The effect of this factor on the content of macroelements in spring wheat grain was detected only in 2008, which had very low rainfalls during the growth of plants. Under these conditions, the stubble catch crops caused an increase in the content of N and a decrease of P and Mg in grain.

Key words: spring wheat, macroelements, nitrogen, stubble catch crops.

WPŁYW DAWKI AZOTU I MIĘDZYPLONÓW ŚCIERNISKOWYCH NA ZAWARTOŚĆ MAKROELEMENTÓW W ZIARNIE PSZENICY JAREJ

Abstrakt

Celem badań było określenie wpływu dawki azotu i międzyplonów ścierniskowych na zawartość azotu, fosforu, potasu i magnezu w ziarnie pszenicy jarej. Badania polowe wykonano w latach 2005-2008 na glebie płowej, kompleksu żytniego bardzo dobrego, w Mochełku k. Bydgoszczy (53° 13' N, 17° 51' E). Nawożenie azotem stosowano w dawkach (kg ha^{-1}): 0; 40; 80; 120 i 160. Międzyplony ścierniskowe (groch siewny i rzodkiew oleistą) wysiewano w terminie 3-11 sierpnia w latach 2005-2007, i po 70-77 dniach wegetacji przyorywano całą biomasę jako zielony nawóz. Pszenicę jarą cv. Tybalt wysiewano w stanowisku z przyoraną biomasą międzyplonów w latach 2006-2008. Nawożenie azotem wpływało na koncentrację azotu, fosforu i magnezu w ziarnie pszenicy jarej. Każde zwiększenie dawki o 40 kg N ha^{-1} powodowało istotne statystycznie zwiększenie zawartości N. Koncentracja P i Mg w ziarnie pszenicy jarej nawożonej azotem w dawkach 120 lub 160 kg ha^{-1} była istotnie wyższa niż po zastosowaniu niższych dawek. Zawartość P w ziarnie pszenicy uprawianej po rzodkwi oraz bez międzyplonu (obiekt kontrolny) po zastosowaniu 120 i 160 kg N ha^{-1} była wyższa niż w pszenicy nawożonej w ilości 40 kg ha^{-1}. Nie stwierdzono wpływu nawożenia azotem na koncentrację K w ziarnie pszenicy jarej. Międzyplony ścierniskowe powodowały zwiększenie średniej z lat zawartości N oraz zmniejszenie koncentracji Mg w ziarnie. Wpływ międzyplonów ścierniskowych na zawartość makroskładników w ziarnie pszenicy jarej był istotny tylko w 2008 r., charakteryzującym się bardzo małą sumą opadów w okresie wzrostu roślin. W tych warunkach przyorana biomasa międzyplonu ścierniskowego powodowała zwiększenie zawartości N oraz zmniejszenie zawartości Mg i P w ziarnie. Stwierdzono dodatnią korelację między zawartością Mg a koncentracją N i P w ziarnie pszenicy jarej. Zależności te były silniejsze w obiektach kontrolnych niż po międzyplonach ścierniskowych.

Słowa kluczowe: pszenica jara, makroelementy, azot, międzyplony ścierniskowe.

INTRODUCTION

Regular supply of soil organic matter, which sustains the right level of biological activity in soil, is necessary to ensure suitable conditions for cultivated plants. In Poland, the total livestock has been over the last thirty years, which has led to a substantial reduction of the soil fertilization with farmyard manure. The average total livestock density in Poland during the last twelve years has been about 45 livestock units (LUs) per 100 ha of agricultural land (Central Statistical Office 2011).

The use of rape and cereal straw for heating purposes is an another unfavorable factor, which deprives farmland from an essential source of organic carbon. Under such conditions, the only meaningful supply of plant nutrients comes from fertilizers. Unfortunately, high doses of mineral nutrients lead to excessive mineralization of soil organic matter. Among fertilizers, nitrogen supplied intensively could be an especially disturbing factor regarding the soil organic matter content (CORRÉ et al. 2000).

One of the management practices aimed at limiting the soil organic matter depletion is the use of stubble catch crops and their biomass as green manure. Some earlier results concerning this solution have suggested that catch crops can significantly affect physical (ZIMNY et al. 2005) and biological (PIOTROWSKA, WILCZEWSKI 2012) soil properties. Additionally, they can stimulate higher yields and better quality of spring cereal grain (WOJCIECHOWSKI 2009). Stubble catch crops improve nitrogen nutrition of plants grown afterwards. The amount of nitrogen taken up by spring cereals during the shooting, earing and grain formation can be increased by 35-50% after plowing into the soil of the biomass of catch crops (THORUP-KRISTENSEN 1994, BERNTSEN et al. 2006). Mineral compounds released from the mineralized biomass are accessible during the whole growing period of cereals, making it possible to improve all important crop features, so that their concentrations in grain can be higher (THORUP-KRISTENSEN 1994, SKINDER, WILCZEWSKI 2004). The fertilizing significance of catch crop biomass depends on the availability of nutrients from the soil resources and on application of mineral fertilizers. Therefore, it is particularly important for crops on the soils less rich in nutrients and humus, or on fields with a low input of fertilizers.

The aim of the research was to assess:

- the influence of stubble catch crops on the nitrogen, phosphorus, potassium and magnesium content in spring wheat grain as dependent on the dosage of nitrogen fertilization applied;
- interrelations among the concentrations of specific components in wheat grain.

Based on previous findings concerning the chemical, physical and biological properties of soil improved by catch crop plowing, a working hypothesis in this study was that the biomass mineralization in spring ensured a better nutrient supply for spring wheat grain.

MATERIAL AND METHODS

A two-factorial, field experiment was carried out between 2005-2008 in order to evaluate the effects of nitrogen fertilization and catch crops on the concentration of macroelements in spring wheat grain. A split-plot experimental design was used. The field experiment was carried out at the

Mochełek (53° 13' N, 17° 51' E), Experimental Station of the University of Technology and Life Sciences in Bydgoszcz, on *Alfisols* formed from sandy loam (Soil Survey Staff 2010). The concentrations of available phosphorus and potassium in soil were very high (111 mg P kg^{-1} and 367 mg K kg^{-1}). The content of total nitrogen and organic carbon was 0.67 and 7.17 g kg^{-1} of dry soil, respectively.

The first experimental factor was nitrogen fertilization applied before and during spring wheat growing period: 0; 40 kg of N (before sowing); 80 kg of N (40 kg before sowing and 40 kg at the BBCH 31-32 stage (LANCASHIRE et al. 1991)); 120 kg N (60 kg before sowing and 60 kg at the BBCH 31-32 stage); 160 kg N (60 kg before sowing, 60 kg at the BBCH 31-32 stage and 40 kg at the BBCH 45-47 stage). The second experimental factor was stubble catch crop treatments: field pea, oilseed radish, and the control without a catch crop. The catch crops were sown after spring barley harvest, from 3 to 11 August in 2005, 2006, 2007, on a plot area of 30 m^2.

Before seeds of catch crops were sown, the soil was ploughed and tilled using a cultivator with a roller. The catch crops were grown without fertilization.

The catch crops were harvested and ploughed between 20 of October and 10 of November. In the spring of the next year, spring wheat (*Triticum aestivum* L.) cv. Tybalt was sown from 30 of March to 1 of April in the years 2006-2008. Mineral fertilization was applied in spring, before soil preparation. Phosphorus and potassium were applied at the doses of 28.4 kg P ha^{-1} and 74.7 kg K ha^{-1}.

Chemical analyses were made after the mineralization of shredded spring wheat grains (wet combustion with perhydrol and sulphuric acid) using the following methods: phosphorus (P) content by the vanadium-molybdenum method, potassium (K) content by the flame photometry method, magnesium (Mg) content by the colorimetric method with titan yellow. The total nitrogen content in grains was determined using the Kjeldahl method (BREMNER, MULVANEY 1982).

The working hypotheses concerning the effect of various nitrogen doses, the stubble catch crops effect as well as the interactions between two experimental factors on the Mg, N, P and K content in wheat grain were verified by analyses of variance in a split-plot mixed model and tests of significance at $P<0.05$. For significant effects from the Anova, means were separated using Tukey's HSD test ($P < 0.05$). Simple correlations between the macronutrient content, grain yields and doses of N fertilizer per ha were calculated using Pearson's coefficient with significance at $P<0.05$. Significant relations between the macronutrient content in wheat grain and a dose of N fertilizer were explained using linear regression equations.

RESULTS AND DISCUSSION

Individual years of the study were highly varied in the total rainfall during the growing time of spring wheat. In 2006, the amount of rainfall was very high from wheat emergence to stem elongation (Table 1). During the further stages, substantial rainfall deficit was observed. In the following year (2007), the total rainfall and its distribution corresponded very well to spring wheat's needs. The worst supply of precipitation at a critical time in the water demand occurred in May and June 2008.

Table 1

Total precipitation and mean air temperature at the experimental station

Month	Year			Mean for 1949-2008
	2006	2007	2008	
Monthly precipitation (mm)				
April	77.0	17.6	38.7	28.0
May	59.9	73.1	11.5	41.7
June	21.8	105.5	15.5	53.5
July	24.2	104.7	58.7	70.8
Total III – VII	182.9	300.7	124.4	194.0
Mean monthly air temperature (^{o}C)				
April	7.1	8.5	7.6	7.3
May	12.5	13.8	13.2	12.8
June	16.8	18.2	17.6	16.2
July	22.4	18.0	19.2	18.0
Mean III – VII	14.7	14.6	14.4	13.6

The least favourable air temperature was in 2006, due to cool March and early April, which caused a delay in the sowing time of spring wheat. Moreover, in the same year, the grain formation was very poor due to high temperatures in July. The air temperatures were relatively high from April to June 2007 and throughout the growing period of spring wheat in 2008.

The investigated factors significantly influenced the concentrations of macronutrients in the grain of spring wheat. Especially the contents of N and Mg were greatly differentiated. In the three-year experiment (2006-2008), the total N content in spring wheat grain was influenced significantly by the rate of N fertilization (Table 2). No interaction between the rate of N fertilization and the years of investigation with reference to this variable wasa detected. Spring wheat, grown both after catch crops and in the con-

Table 2

Content of nitrogen (N) in grain of spring wheat (g kg^{-1})

Year	Nitrogen fertilization (kg ha^{-1})	Catch crop			
		field pea	oilseed radish	control	mean
2006-2008	0	19.37^{Ad}	18.49^{Bd}	17.80^{Cd}	18.55^{e}
	40	20.14^{Acd}	19.66^{Bc}	19.16^{Cc}	19.65^{d}
	80	21.70^{Bc}	22.20^{Ab}	21.49^{Bb}	21.80^{c}
	120	22.85^{Ab}	22.96^{Aab}	22.29^{Bb}	22.70^{b}
	160	24.06^{Aa}	23.84^{Ba}	23.92^{ABa}	23.94^{a}
2006	mean	21.48^{A}	21.61^{A}	21.60^{A}	21.56
2007		21.11^{A}	20.61^{AB}	19.90^{B}	20.54
2008		22.28^{A}	22.07^{A}	21.29^{B}	21.88
Mean		21.62^{A}	21.43^{B}	20.93^{C}	21.33

A, B, C – means marked with the same capital letter constitute a homogenous group across rows
a, b, c – means marked with the same small letter constitute a homogenous group across columns

trol plots, revealed significant increase in the total N content along with an increasing N fertilization rate. The highest concentration of N in grain was obtained when 160 kg N ha^{-1} had ben applied, while a significantly lower N concentration was found in wheat fertilized with 120 kg N ha^{-1}. The lowest N content was observed in grain of wheat unfertilized with N. Based on the linear regression, it was found that an average increase in N content in wheat grain is 0.030, 0.035 and 0.038 g kg^{-1} of dry matter per each kg of this ingredient in the fertilizer applied after field pea and oilseed radish stubble catch crops and in the control, respectively (Figure 1a). Stubble catch crops cultivated for green manure caused a significant increase in the total N content in grain of spring wheat. The effect of field pea on this variable was significantly stronger than oilseed radish. As for this variable, an interaction between the kind of plant species cultivated as stubble catch crops and the years of the investigation was shown. In 2008, both plants cultivated as catch crops influenced the total N content significantly, while in 2007 the N content in the grain of wheat harvested from the control plots was significantly lower than in the field pea treatment. In 2006, no significant impact of catch crops on this variable was noted.

The concentration of total N in wheat grain was relatively high, especially when the grain yield was low and the supply of nitrogen was good supply. Nitrogen fertilization strongly influenced the content of N in grain also in the years with rainfall deficit during the spring wheat vegetation. This significant effect of N fertilization rates on the total N concentration in barley grain is in agreement with numerous research results (GAUER et al. 1992, WALLEY et al. 2001, KARAMANOS et al. 2005, DENYS et al. 2006). The said impact was especially evident in our study, which resulted from a relatively

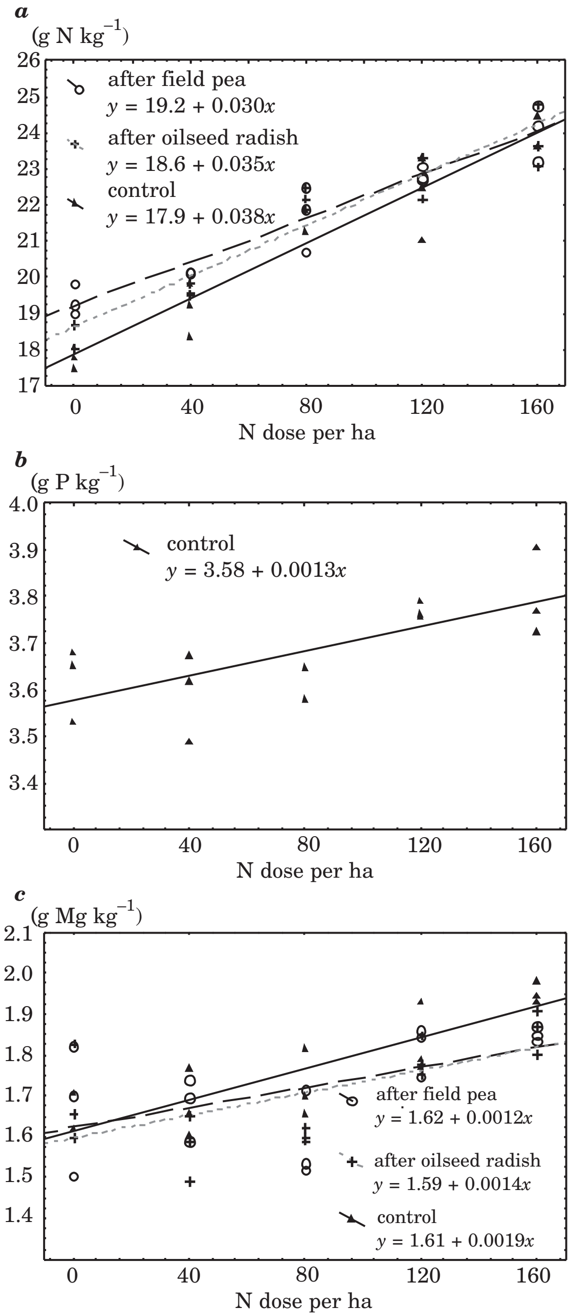

Fig. 1. The dependence of concentration of macroelements in spring wheat grain on a dose of N fertilizer per ha

low influence of the N rate on the grain yield (data not shown) and, consequently, from a higher availability of this nutrient in soil during the wheat maturation period alongside an increasing N fertilization rate. The uptake of nitrogen with wheat grain and straw crop was low and ranged from 52.2 kg ha^{-1} in the control plots to 87.3 kg ha^{-1} in wheat fertilized with the highest rate of N (data not shown). In some other studies, no significant influence of an N fertilization rate on the content of this nutrient in grain was observed, which was due to a strong influence of N on the grain yield achieved on good soil and with a good water supply (WOJCIECHOWSKI 2009), or under the shortage of rainfall during the wheat ripening period (SOUZA et al. 2004).

The influence of an N fertilization rate on the P concentration in grain of spring wheat depended on stubble catch crops ploughed in entirely in November of the year prior to the sowing time (Table 3). No significant effects of N fertilization rates on the P concentration in grain were found in spring wheat grown after the field pea treatment. After the oilseed radish treatment, a significantly lower P content in wheat grain was noted after the application of 40 kg N ha^{-1} compared to 120 kg N ha^{-1} and/or 160 kg N ha^{-1}. The grain of spring wheat harvested from the control plots fertilized with the two lowest rates of N and not fertilized with N had significantly less P than after the application of 160 kg N ha^{-1}. The increasing dosage of N fertilizer in the control caused a higher concentration of P in grain by 0.0013 g kg^{-1} dry matter per each kg of N applied, as demonstrated by the linear regression (Figure 1b).

Table 3

Content of phosphorus (P) in grain of spring wheat (g kg^{-1})

Year	Nitrogen fertilization (kg ha^{-1})	Catch crop			
		field pea	oilseed radish	control	mean
2006-2008	0	3.687 ^{Aa}	3.650 ^{Aab}	3.622 ^{Abc}	3.653 ^{ab}
	40	3.574 ^{Aa}	3.550 ^{Ab}	3.594 ^{Ac}	3.573 ^{b}
	80	3.600 ^{Aa}	3.599 ^{Aab}	3.625 ^{Abc}	3.608 ^{b}
	120	3.726 ^{Aa}	3.752 ^{Aa}	3.771 ^{Aab}	3.750 ^{a}
	160	3.733 ^{Aa}	3.722 ^{Aa}	3.801 ^{Aa}	3.752 ^{a}
2006	mean	4.022 ^{A}	4.014 ^{A}	3.955 ^{A}	3.997
2007		4.267 ^{A}	4.356 ^{A}	4.284 ^{A}	4.302
2008		2.703 ^{B}	2.594 ^{C}	2.810 ^{A}	2.702
Mean		3.664 ^{A}	3.655 ^{A}	3.683 ^{A}	3.667

A, B, C – means marked with the same capital letter constitute a homogenous group across rows
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The stubble catch crops influenced the P concentration in spring wheat grain only in 2008, the year characterized by the water supply unfavourable to plants. In that year, a negative impact of the catch crops, especially oilseed radish, on this variable was noted. In a study by Kraska (2011), the effect of catch crops on the said trait was dependent on the species of plants used for this purpose. The biomass of tansy phacelia negatively affected P levels, while white mustard had no significant effect on P concentrations in wheat grain.

Nitrogen fertilization did not influence significantly the concentration of K in spring wheat grain (Table 4). The stubble catch crop treatments did not affect the content of this nutrient in wheat grain either, although an interaction between the catch crops and years of investigation was demonstrated. In 2006 and 2007, the catch crops did not influence the K concentration in wheat grain, but in 2008 the K content in the grain of wheat grown after oilseed radish was significantly lower than in the control.

Table 4

Content of potassium (K) in grain of spring wheat (g kg^{-1})

Year	Nitrogen fertilization (kg ha^{-1})	Catch crop			
		field pea	oilseed radish	control	mean
2006-2008	0	3.302 ^{Aa}	3.210 ^{Aa}	3.340 ^{Aa}	3.284 ^{a}
	40	3.137 ^{Aa}	3.099 ^{Aa}	3.154 ^{Aa}	3.130 ^{a}
	80	3.210 ^{Aa}	3.136 ^{Aa}	3.209 ^{Aa}	3.185 ^{a}
	120	3.320 ^{Aa}	3.134 ^{Aa}	3.227 ^{Aa}	3.227 ^{a}
	160	3.209 ^{Aa}	3.191 ^{Aa}	3.292 ^{Aa}	3.231 ^{a}
2006	mean	3.198 ^{A}	3.108 ^{A}	3.129 ^{A}	3.145
2007		3.796 ^{A}	3.731 ^{A}	3.731 ^{A}	3.753
2008		2.713 ^{AB}	2.623 ^{B}	2.871 ^{A}	2.736
Mean		3.236 ^{A}	3.154 ^{A}	3.244 ^{A}	3.211

A, B, C – means marked with the same capital letter constitute a homogenous group across rows
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Following the fertilization of spring wheat with nitrogen at the rates of 120 and 160 kg ha^{-1}, as compared to the lower rates of nitrogen fertilization, the concentration of Mg in the grain was significantly higher (Table 5). This effect occurred both in the treatments with the catch crops and on the control plots. Having ananlyzed the effect of N doses on the content of Mg in wheat grain using linear regression, it was found that the average increase was 0.0012, 0.0014 and 0.0019 g kg^{-1} dry matter per each kg of N fertilizer used, respectively after field pea, oilseed radish and in the control (Figure 1c). The stubble catch crops had an adverse effect on the concentration of Mg in grain of spring wheat cultivated afterwards as the main

Table 5

Content of magnesium (Mg) in grain of spring wheat (g kg^{-1})

Year	Nitrogen fertilization (kg ha^{-1})	Catch crop			
		field pea	oilseed radish	control	mean
2006-2008	0	1.676^{Ab}	1.695^{Abc}	1.650^{Ac}	1.674^{b}
	40	1.675^{Ab}	1.579^{Bd}	1.677^{Ac}	1.644^{b}
	80	1.590^{Bb}	1.605^{Bcd}	1.725^{Ac}	1.640^{b}
	120	1.817^{Aa}	1.795^{Ab}	1.831^{Ab}	1.814^{a}
	160	1.851^{Ba}	1.861^{Ba}	1.953^{Aa}	1.889^{a}
2006	mean	1.650^{A}	1.627^{A}	1.627^{A}	1.634
2007		1.963^{A}	1.936^{A}	1.959^{A}	1.953
2008		1.553^{B}	1.558^{B}	1.715^{A}	1.609
Mean		1.722^{B}	1.707^{B}	1.767^{A}	1.732

A, B, C – means marked with the same capital letter constitute a homogenous group across rows
a, b, c – means marked with the same small letter constitute a homogenous group across columns

crop only in 2008, while in 2006 and 2007 this factor had no influence on the Mg content in wheat grain. This differentiated impact of catch crops in specific years was probably caused by the variable water supply in the spring period during the growth of wheat. The low total rainfall in May and June of 2008, compared to 2006 and 2007, might have reduced the soil enzymatic activity, thus depressing the decomposition of catch crops and the accessability of macrocompounds accumulated in the biomass. In fact, the enzymatic activities involved in the C (β-glucosidase), N (urease) and P cycling (alkaline phosphatase) studied in the experiment were significantly lower in that year compared to 2006 and 2007 (Piotrowska, Wilczewski 2012). Some other results confirmed that soil enzymatic activity is strongly moisture-dependent. Thus, higher soil moisture owing to rainfall enhanced the activity of soil enzymes, specially phosphatase and dehydrogenase, as reported by Akmal et al. (2012). Similar results have been found by Sardans, Penuclas (2005), who observed a strong positive relationship between the activity of soil phosphatases and soil water availability. Frey et al. (1999) reported that moisture influenced soil enzymatic activity indirectly, by enhancing the microbial growth and substrate availability.

The influence of the catch crops on the Mg content in spring wheat grain depended on the N fertilization rate. No significant influence of the ploughed catch crop biomass on this variable was shown in the control plots (without N fertilization) and after application of 120 kg N ha^{-1}. The concentration of Mg in the grain of spring wheat fertilized with N at the rates of 80 kg N ha^{-1} and 160 kg N ha^{-1} was significantly lower in the case of plots with preceding catch crops than in control. Earlier studies showed a beneficial impact of ploughed biomass of oilseed radish and common sunflower, grown as stubble catch crops, on the Mg content in wheat grain and a negative influence of sunflower or phacelia biomass on the Mg concentration in wheat straw (Wilczewski, Skinder 2011).

Significant Pearson's correlation coefficients between the content of nutrients and the spring wheat yield are presented in Table 6. In 2006 and 2007, the N concentration was positively correlated with the grain yield, while in 2008 this relationship was negative. The Mg concentration was correlated with the yield of spring wheat positively in 2007 and negatively in 2008. In 2006, no significant relationship between these variables appeared. Generally positive correlations between concentrations of macronutrients and yield were detected in 2007, while in 2006 and 2008 this tendency was opposite. Such evident differences in the relationships between the analyzed features of spring wheat were attributed to highly varied weather conditions, which affected the yield of wheat grain in individuals years of the investigations.

Table 6

Simple correlation coefficients for the relation between yield of grain of spring wheat and macronutrient content (n=45)

Year	Nutrient			
	N	P	K	Mg
2006	0.67*	-0.03	-0.46*	-0.26
2007	0.83*	0.16	0.07	0.33*
2008	-0.53*	-0.58*	-0.27	-0.74*

* correlation significant at $\alpha = 0.05$

Based on the linear correlation coefficients between the content of macroelements (N, P, K and Mg) in spring wheat grain, the N dose per ha and wheat grain yield after two catch crops, it was found that the above relationships could appear with different strength on plots with previous stubble catch crops as compared with the control. Significant, positive correlations were found between the Mg and N or P concentrations in wheat grain, stronger in the control treatment than after stubble catch crops (Table 7). There were also the same values of correlations, $r = 0.67$, $P < 0.01$, between the P and K content in wheat grain from plants sown after field pea or oilseed radish. The potassium content did not explain the concentration of Mg in wheat grain and *vice versa*. Meanwhile, the content of N in wheat grain was positively correlated with the concentration of P ($r = 0.73$, $P < 0.01$) but only in the control treatment.

Table 7

Linear correlation coefficients between the content of macroelements in spring wheat grain, N dose per ha and wheat grain yield for each catch crop (n=15)

Correlates	Catch crop treatment	N dose	Grain yield	N	P	K	Mg
N dose	control	-	**	***	**	ns	***
	field pea	-	ns	***	ns	ns	*
	oilseed radish	-	*	***	ns	ns	*
Grain yield	control	0.71	-	***	ns	ns	*
	field pea	0.47	-	ns	ns	ns	ns
	oilseed radish	0.57	-	**	ns	ns	ns
N	control	0.96	0.76	-	**	ns	***
	field pea	0.96	0.47	-	ns	ns	*
	oilseed radish	0.96	0.67	-	ns	ns	*
P	control	0.73	0.26	0.65	-	ns	***
	field pea	0.32	-0.21	0.35	-	**	**
	oilseed radish	0.43	0.03	0.43	-	**	*
K	control	-0.03	-0.41	-0.14	0.04	-	ns
	field pea	-0.003	-0.41	-0.02	0.67	-	ns
	oilseed radish	-0.002	-0.11	-0.01	0.67	-	ns
Mg	control	0.86	0.54	0.83	0.83	-0.04	-
	field pea	0.55	-0.12	0.52	0.67	0.21	-
	oilseed radish	0.63	0.05	0.54	0.65	0.15	-

CONCLUSIONS

1. Nitrogen fertilizer rates influenced the N, P and Mg concentrations but did not affect the content of K in the grain of spring wheat. The content of N was highest at 160 kg N ha^{-1}, while the concentrations of P and Mg were biggest at 120 or 160 kg N ha^{-1}.

2. Overall, the stubble catch crops caused a decrease in the Mg content and an increase in N concentration in spring wheat grain.

3. A linear relationship was found between increasing doses of nitrogen fertilizer and the content of N, P and Mg. These correlations were especially strong with regard to the N content. The relationships between N doses and the concentration of P and Mg were relatively high in control crops, which corresponded to an interaction between N fertilization and stubble catch crops.

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THE ANALYSIS OF SELECTED MICROELEMENTS IN NEONATAL UMBILICAL CORD BLOOD

Barbara Zych[1], **Małgorzata Sztanke**[2], **Bożena Kulesza-Brończyk**[3], **Bogumił Lewandowski**[1], **Krzysztof Sztanke**[2]
Kazimierz Pasternak[2]

[1]Institute of Obstetrics and Emergency Medicine, University of Rzeszow
[2]Department of Medical Chemistry, Medical University of Lublin
[3]Department of Obstetrics, Gynecology and Obstetrics/Gynecological Care
Medical University of Bialystok

Abstract

Microelements and trace elements play a vital role in the body. Low quantities of these elements are essential to ensure proper metabolic processes.

The aim of the thesis was to determine levels of selected microelements (zinc, copper, iron, manganese, chromium and aluminium) in cord blood plasma, and to find out if the gestational age, gender and birth weight of a newborn affect these concentrations.

The research was conducted on the cord blood of 71 newborns. Regarding the gestational age, three groups were distinguished: I – neonates born after 37 week of pregnancy (n=7), II – neonates born between 38 and 41 week (n=59), and III – neonates born after 42 week of pregnancy (n=5). Subsequently, another division concerned the gender: male (n=35) and female (n=36) neonates, as well as their weight: neonates with regular birth weight – 2.500-3.500 g (n=61), and neonates with high birth weight – over 3.500 g (n=10). The concentrations of zinc, copper, iron, manganese, chromium, and aluminium in cord blood plasma were determined on the basis of inductively coupled plasma atomic emission spectroscopy method. The results were subjected to statistical analysis using Statistica 10.0 software, with the assumption that the level of significance was $p<0.05$.

The average concentrations of analysed elements in umbilical cord blood were the following: 18.67±3.05 μmol Zn dm^{-3}, 16.60±2.64 μmol Cu dm^{-3}, 23.32±3.29 μmol Fe dm^{-3}, 0.96±0.21 μmol Mn dm^{-3}, 2.81±0.14 μmol Cr dm^{-3} and 0.041±0.028 μmol Al dm^{-3}, which is within the standard ranges, suggesting efficient regulating mechanisms of the developing foetuses. The research proved that the neonate's gestational age, gender and birth weight

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dr n. med. Barbara Zych, Institute of Obstetrics and Emergency Medicine, University of Rzeszow, Wesoła 5, 35-322 Rzeszów, Poland, e-mail: ba.zyc@wp.pl

had no substantial impact on concentrations of these elements in the cord blood, except the concentration of iron, which was statistically significantly correlated with the gender of neonates.

Key words: microelements, neonate, cord blood.

ANALIZA STĘŻEŃ WYBRANYCH MIKROPIERWIASTKÓW W KRWI PĘPOWINOWEJ NOWORODKA

Abstrakt

Dużą grupę pierwiastków wchodzących w skład organizmu ludzkiego stanowią pierwiastki śladowe. Wśród pierwiastków niezbędnych do prawidłowego funkcjonowania organizmu znajdują się również te, które określa się mianem toksycznych (mangan, chrom i glin). Niewielkie ich ilości zapewniają prawidłowe funkcjonowanie enzymów, syntezę hormonów oraz budowę związków o ważnej roli biologicznej, budulcowej i regulacyjnej organizmu ludzkiego.

Celem pracy było oznaczenie stężenia wybranych mikropierwiastków (cynku, miedzi, żelaza, manganu, chromu i glinu) w osoczu krwi pępowinowej noworodków oraz ustalenie, czy termin porodu, płeć i urodzeniowa masa ciała noworodka mają wpływ na stężenie tych mikropierwiastków.

Badano krew pępowinową 71 noworodków urodzonych w Szpitalu Wojewódzkim Nr 2 w Rzeszowie. Noworodki pochodziły od zdrowych matek w wieku od 16 do 44 lat. Porody odbywały się siłami natury lub przez cesarskie cięcie. Ze względu na tydzień ukończenia ciąży wyodrębniono 3 grupy: pierwszą stanowiły noworodki urodzone do 37. tyg. ciąży (n=7), drugą – noworodki urodzone między 38. a 41. tyg. ciąży (n=59), trzecią – noworodki urodzone w 42. i późniejszych tygodniach ciąży (n=5). Ponadto dokonano podziału noworodków ze względu na płeć: noworodki płci męskiej (n=35) i noworodki płci żeńskiej (n=36), a także ze względu na urodzeniową masę ciała: noworodki z normalną masą urodzeniową: 2500-3500 g (n=61) i noworodki z dużą masą urodzeniową: powyżej 3500 g (n=10). W osoczu oznaczono stężenia cynku, miedzi, żelaza, manganu chromu i glinu metodą emisyjnej spektrometrii atomowej z plazmą indukcyjnie wzbudzoną (ICP-AES – Inductively Coupled Plasma – Atomic Emission Spektrometry) z użyciem spektrometru Liberty 2 AX firmy Varian. Wyniki poddano analizie statystycznej, stosując oprogramowanie Statistica 10.0 (test Anowa Kruskala-Wallisa, test Manna-Whitneya), przyjmując za istotne statystycznie różnice przy p<0,05.

Wykazano, że średnie stężenia cynku, miedzi, żelaza, manganu, chromu i glinu we krwi pępowinowej noworodków mieściły się w zakresie wartości uznawanych za prawidłowe (Cu: 16,60±2,64 μmol dm^{-3}; Zn: 18,67±3,05 μmol dm^{-3}; Fe: 23,32±3,29 μmol dm^{-3}; Mn: 0,96±0,21 μmol dm^{-3}; Cr: 2,81±0,14 μmol dm^{-3}; Al: 0,041±0,028 μmol dm^{-3}), co może świadczyć o sprawnie funkcjonujących mechanizmach regulacyjnych w organizmie rozwijającego się płodu. Zarówno termin porodu, jak i płeć oraz masa urodzeniowa noworodka nie wpłynęły w istotny sposób na stężenia badanych pierwiastków we krwi pępowinowej noworodków. Jedynie znamienne statystycznie różnice w stężeniach żelaza zanotowano w zależności od płci noworodków.

Słowa kluczowe: mikropierwiastki, noworodek, krew pępowinowa.

INTRODUCTION

Microelements and trace elements play an important role in the body. Low quantities of these elements are essential for the proper functioning of numerous compounds which play some crucial biological, building and regulating roles, e.g. enzymes or hormones.

One of such essential microelements is zinc. It is a cofactor of over 200 enzymes; it participates in the metabolism of hormones and in the synthesis of proteins; it also acts as a regulator of transcription and metabolism of vitamin A. As a component of copper-zinc superoxide dismutase (CuZnSOD), a vital antioxidative enzyme, zinc is involved in the antyoxidative defence of the body (Merialdi et al. 2004, Cantin et al. 2007, Ozdemir et al. 2007). Zinc deficiency in the foetal period may lead to an innate immune system failure, neural tube defects, and even teratogenic disorders (Merialdi et al. 2004, Cantin et al. 2007). Zinc is also involved in interactions with other elements, e.g. copper and iron (Ozdemir et al. 2007, Shirvani et al. 2010).

Copper occurs in serum in combination with the ceruloplasmin, albumins and histidine, while inside a cell it is bound with metallothioneins, which prevents its cytotoxicity and plays an important part in protecting the body against free radicals (Komaszyńska, Maćkiewicz 2007). Copper is indispensable in the synthesis of haemoglobin in a developing organism, and, as a cofactor of many enzymes, it forms copper-zinc (CuZnSOD) and extracellular superoxide dismutase (ecSOD), eliminating reactive oxygen species (ROS) (Yien-Ming et al. 2006, Formigari et al. 2007). Copper deficiency interferes with the synthesis of catalase and manganese-dependent superoxide dismutase (MnSOD), reduces the concentration of ceruloplasmin in serum, and, in the prenatal period, can contribute to foetal growth disorders, cause anaemia, neutropenia and bone demineralization (Schulpis et al. 2004). Due to a high copper concentration in the body, lipids, proteins and DNA are prone to oxidative damage, which leads to disorders of the nervous system, the development of neurodegenerative diseases and cancer (Yien-Ming et al. 2006, Formigari et al. 2007, Zeyrek et al. 2009).

Iron is involved in many biochemical processes, hence it is necessary for the proper growth and functioning of the body. It is an essential component of haemoglobin, myoglobin and other proteins. Cooperating with low molecular weight antioxidants, iron ions stabilize the structure of cell membranes, which protects the body from oxidative processes (Cantin et al. 2007, Formigari et al. 2007). Iron plays an important role in the process of erythropoiesis, being simultaneously a substantial component of the DNA and RNA synthesis, and an element required in the process of building cellular immunity and in the synthesis of myelin during the foetal brain development (Lieu et al. 2001, Gurzau et al. 2003, Shirvani et al. 2010). Health consequences of iron deficiency in pregnant women are associated with miscarriages, premature births with low birth weight, increased risk of neonatal deaths, and psychomotor disorders and hearing impairment later in children's life (O'BRIEN et al. 2003, ANDERSEN et al. 2006, YOUNG et al. 2010).

The proper functioning of the human body is also dependent on trace amounts of manganese, chromium and aluminium (TAKSER et al. 2004, ASCHNER et al. 2005, BRONISZ, PASTERNAK 2008). A major function of manganese is related to antioxidant processes because, as a constituent of superoxide dismutase (MnSOD), it helps to scavenge harmful free radicals generated in metabolic transformations of a cell. Manganese is essential in the processes of cellular respiration, prevents the formation of atherosclerosis and participates in the process of hematopoiesis (ASCHNER et al. 2005, SOLDIN, ASCHNER 2007, VIGEH et al. 2008, RUIZ-SANZ et al. 2011). Low levels of manganese result in an impaired psychomotor development of the organism, growth inhibition and anaemia. On the other hand, prolonged exposure to high doses of manganese during the foetal period causes nervous system disorders, diseases of the skeletal system and intrauterine growth restriction (IUGR) (TAKSER et al. 2004, ASCHNER et al. 2005, SOLDIN, ASCHNER 2007, VIGEH et al. 2008).

Chromium appears to be an essential component of a diet owing to its effects on the glucose metabolism (ANDERSON et al. 2006, BRONISZ et al. 2007). Chromium deficiency among pregnant women is a factor in the development of metabolic syndrome. On the other hand, excess of chromium, apart from the prooxidative influence, can cause iron metabolism disorders facilitating the development of anaemia (GOMES et al. 2005, ANDERSEN et al. 2006, RUIZ-SANZ et al. 2011).

Aluminium in the human body is an inhibitor of enzymes participating in the carbohydrate metabolism, processes of phosphorylation, dephosphorylation, and proteolytic enzymes connected with the cell membrane (SANZ-MEDEL et al. 2002, BRONISZ, PASTERNAK 2008). High levels of aluminium help to reduce the activity of antioxidant enzymes (superoxide dismutase and glutathione peroxidase), disturb iron and zinc distribution and increase lipid peroxidation (SANZ-MEDEL et al. 2002, GURZAU et al. 2003, ALEXANDROV et al. 2005). Exposure to high levels of aluminium in the intrauterine life leads to progressive neurodegeneration and death (ALEXANDROV et al. 2005, KAWAHARA 2005).

The aim of the thesis was to determine the levels of selected microelements (zinc, copper, iron, manganese, chromium and aluminium) in cord blood plasma, and to find out if the gestational age, gender and birth weight of a newborn affect these concentrations.

MATERIAL AND METHODS

The research was conducted on the cord blood of 71 neonates born in the Provincial Hospital No 2 in Rzeszów. The children were born by healthy mothers aged 16 to 44. These were both natural labours and caesarian sections.

Regarding the gestational age, three groups were distinguished: the first group comprised neonates born up to the 37th week of pregnancy (n=7), the second one consisted of neonates born between the 38th and 41st week of pregnancy (n=59), and the third one was composed of neonates born in the 42nd week and later (n=5). Two other divisions distinguished between the genders: male (n=35) and female (n=36) neonates, newborns' weight: neonates with regular birth weight – 2500-3500 g (n=61), and neonates with high birth weight – over 3500 g (n=10).

Immediately after the delivery, a fragment of the umbilical cord was clamped and the blood was taken out from the placental vein to heparinized test tubes. The plasma was obtained by spinning a tube of fresh blood for 15 minutes at 3000 rpm and was stored until the analysis at the temperature of -20°C.

Trace elements such as zinc, copper, iron, manganese, chromium and aluminium were determined in the blood plasma by the inductively coupled plasma atomic emission spectroscopy (ICP-AES), using a Varian Liberty 2 AX spectrometer.

The results underwent statistical analysis using Statistica 10.0 software (Anova Kruskal-Wallis test, Mann-Whitney test), with the level of significance set at $p<0.05$.

RESULTS AND DISCUSSION

The concentrations of zinc, copper, iron, manganese, chromium and aluminium in the umbilical cord blood and the standard values for these microelements are presented in Table 1.

Average concentrations of the analysed microelements in particular subdivisions relating to the gestational age, gender and birth weight of a newborn are presented in Table 2.

According to the research, the average zinc concentration in the cord blood of the examined neonates was 18.67±3.05 μmol dm^{-3} and stayed within the standard limits (Table 1). The level of this element was on the increase in relation to the gestational age. A little higher zinc concentration was observed in female neonates, but the results were similar in all the sub-groups distinguished according to the newborns' birth weight (Table 2).

Table 1

Concentrations of zinc, copper, iron, manganese, chromium, aluminum in neonatal umbilical cord blood

Micro-element	Microelement concentration (µmol dm^{-3}) in umbilical cord blood						Range of norm (µmol dm^{-3})
	$\overline{x}$	SD	range of variation	bottom quartile	median	upper quartile	
Zn	18.67	3.05	11.84-24.48	16.30	19.16	21.23	12.2-21.3
Cu	16.60	2.64	12.99-26.02	14.59	15.96	17.87	12.6-25.2
Fe	23.32	3.29	14.28-27.52	20.38	23.94	26.42	12.5-26.9
Mn	0.96	0.21	0.14-1.54	0.86	0.93	1.05	0.72-3.64
Cr	2.81	0.14	2.48-3.16	2.69	2.79	2.90	2.80-2.88
Al	0.041	0.028	0.014-0.142	0.021	0.035	0.051	> 0.37

$\overline{x}$ – mean, SD – standard deviation

Similar observations were made by ELIZABETH et al. (2008), who examined relationships between the zinc level, gestational age and birth weight. They proved differences in the concentration of zinc depending on the gestational age and birth weight. OZDEMIR et al. (2007) analysed the zinc level in neonates in respect of the birth weight. Their research showed that the zinc concentration in neonates was related to the birth weight.

Despite differences in levels of the examined elements, the research of ELIZABETH et al. (2008), OZDEMIR et al. (2007), as well as authors' own study revealed that the zinc concentration in the cord blood stayed within the range of reference values.

The copper concentration in the cord blood in the examined neonates equalled 16.60±2.64 µmol dm^{-3}, which falls into the standard range (Table 1). Regarding the date of delivery, the copper level results showed a downward trend depending on the gestational age. The highest concentration of that microelement was observed for neonates born before 37th week of pregnancy, while the lowest one was determined for those born after the 42nd week of pregnancy. Yet, the differences between these groups were not statistically significant. Similarly, the gender and birth weight had no substantial impact on the copper concentration, although slightly higher results were observed for male neonates and newborns with regular birth weight (Table 2).

OZDEMIR et al. (2007) proved that the copper level in the cord blood of neonates born between 38th and 42nd week of pregnancy varied depending on the birth weight of these newborns. They noted that the highest concentration of Cu was characteristic for newborns with low birth weight (up to 2,500 g), and the lowest one – for those with birth weight over 4,000 g. The copper level in the cord blood of neonates with high birth weight

Table 2

Concentrations of zinc, copper, iron, manganese, chromium and aluminum in the subgroups in dependence of the week of delivery, gender and birth weight

Microelement	Microelement concentration (µmol dm^{-3}) in umbilical cord blood in dependence of:						Statistical analysis
	week of delivery						p*
	37th week and below		38-41th week		42th week and above		
	$\overline{x}$	SD	$\overline{x}$	SD	$\overline{x}$	SD	
Zn	17.78	2.22	18.63	3.22	20.32	0.72	0.474
Cu	16.76	4.82	16.65	2.43	15.75	0.30	0.507
Fe	21.71	2.86	23.48	3.23	23.73	4.54	0.160
Mn	1.06	0.26	0.95	0.21	0.87	0.12	0.293
Cr	2.82	0.20	2.80	0.13	2.88	0.19	0.730
Al	0.045	0.043	0.041	0.027	0.035	0.008	0.938
	gender of a neonate						p**
	male			female			
	$\overline{x}$	SD		$\overline{x}$	SD		
Zn	18.50	3.10		18.83	3.03		0.645
Cu	16.70	2.66		16.49	2.65		0.683
Fe	22.49	3.55		24.13	2.83		0.046
Mn	0.98	0.25		0.94	0.17		0.447
Cr	2.81	0.15		2.81	0.14		0.861
Al	0.047	0.033		0.035	0.021		0.095
	birth weight of a newborn						p**
	normal birth weight			high birth weight			
	$\overline{x}$	SD		$\overline{x}$	SD		
Zn	18.67	3.07		18.68	3.10		0.716
Cu	16.60	2.70		16.57	2.33		0.849
Fe	23.31	3.22		23.40	3.87		0.791
Mn	0.96	0.22		0.92	0.15		0.524
Cr	2.80	0.15		2.83	0.12		0.336
Al	0.040	0.026		0.049	0.039		0.804

$\overline{x}$ – mean, SD – standard deviation, p – level of the probability,
* ANOVA Kruskal-Wallis test, ** Mann-Whitney test

was significantly lower than of those with low or regular birth weight. Jones et al. (Jones et al. 2010) proved that the copper concentration in neonates of Afro-American origins born by mothers with pre-eclampsia was lower than in the case of those born by healthy mothers. Moreover, they noticed that neonates with low birth weight (<2,500 g) born before 37th week of pregnancy are characterized by a higher copper concentration in comparison to neonates with the birth weight over 2,500 g born on the expected date of delivery or later.

Contrary to the research by Jones et al. (2010) that substantiated the relation between the copper level and birth weight, the authors' own research did not prove any statistically significant differences between copper concentrations versus the gestational age, gender and birth weights.

The average concentration of iron in the cord blood of examined neonates reached to 23.32±3.29 µmol dm^{-3}, which was within the standard range (Table 1). During the last four weeks before due date, most iron bound to maternal serum with transferrin passes to the placenta, and from there, by the placental transferrin receptor, goes to the foetus (O'brien et al. 2003). This is confirmed by the authors' own research, which indicated that iron concentrations in the examined neonates showed an upward trend in relation to the gestational age. The level of this element in the cord blood depending on the gender was significantly higher in case of female neonates, and a little higher for the newborns with high birth weight. The research demonstrated that a statistically significant difference in the iron level was related to a neonate's gender (Table 2).

Elizabeth et al. (2008) showed that neonates with low birth weight born prematurely had a lower iron level than the control group. Both this research and the authors' own investigations indicate that higher iron concentrations are positively correlated with the gestational age. Young et al. (2010), however, showed different results, having observed as lower iron levels in neonates with birth weight ranging from 2,150 to 4,705 g and born around the due date (40.0±1.2 week of pregnancy). The researchers claimed there was a relationship between the concentration of iron in neonates and their mothers, and the placental TfR protein. They stated that the demand for iron during the foetal period is related to an increase in the mother's needs and the developing pregnancy, Besides, the expression of the placental TfR protein seems to be a vital factor for the pregnancy.

Trace amounts of manganese are essential for the proper functioning of the body because this element participates in antioxidative processes. It is also required to keep a healthy bone structure in a developing organism (Aschner et al. 2005, Soldin, Aschner 2007).

The average manganese concentration in the examined sample was 0.96±0.21 µmol dm^{-3}, falling into the range of values regarded as standard (Table 1). The concentrations of this element showed a downward trend in relation to the gestational age, with the highest values for neonates born before 37th week of pregnancy and the lowest ones for those born after 42nd week of pregnancy. Additionally, higher manganese levels were observed in male neonates and newborns with regular birth weight (Table 2).

Similar research was conducted by Jones et al. (2010) who determined manganese concentrations in the cord blood depending on the gestational age and birth weight. These researchers claimed that the level of manganese was higher in neonates with low birth weight (<2,500 g) born before 37th week of pregnancy than in those with birth weight over 2,500 g and born on due date. However, the differences were not statistically significant, similarly to the results obtained by the authors. Vigeh et al. (2008) compared the manganese levels in the maternal whole blood and cord blood of healthy neonates and IUGR neonates, depending on a neonate's gestational age and gender. They proved that the manganese concentration in the maternal whole blood of mothers having IUGR newborns was significantly lower than in mothers with healthy newborns. At the same time, the concentration of manganese in the cord blood was higher for IUGR newborns than for healthy ones.

It seems that trace amounts of chromium in pregnant women's diets are essential since its deficiency is a factor of the metabolic syndrome. Pregnancy increases the excretion of and the demand for trivalent chromium, simultaneously boosting the insulin resistance and the concentrations of hormones acting antagonistically to insulin (Anderson 2007).

According to the authors' own research, the average chromium concentration in the cord blood of the examined neonates was 2.81±0,14 µmol dm^{-3}, which is within the standard limits (Table 1). The level of this element changed in relation to the gestational age, as the highest values were observed in neonates born after 42nd week of pregnancy and the lowest ones - for those born between 38th and 41st week of pregnancy. The average chromium concentrations depending on the gender and birth weight were comparable in both groups. Similarly, the research by Jones et al. (2010) on chromium concentrations in relation to the gestational age and birth weight did not show any statistically significant differences. The chromium levels in the cord blood they detected were comparable to the results reported herein.

Aluminium is one of the most common metals in nature. According to the authors' own research, the average aluminium concentration in the cord blood reached 0.041±0.028 µmol dm^{-3}, which was in the range of standard values (Table 1). During pregnancy, aluminium toxicity appears to be negatively correlated to the gestational age. The physiological immaturity of preterm infants' kidneys favours certain accumulation of aluminium as a result of the substitution of calcium ions, a process which weakens bone mineralization processes (Reinke 2003, Gura, Puder 2006). However, the research did not show any statistically significant relationships between the gestational age and aluminium concentrations. It was demonstrated that the level of this microelement shows a downward trend in relation to the gestational age. Also the gender and birth weight had no substantial impact on the aluminium concentration in the cord blood (Table 2).

The role of this element appears to be vital during pregnancy and lactation, as it induces an increase of newborns' body weight rate (YOKEL 1984). On the other hand, there is a wealth of research (REINKE et al. 2003, CHU et al. 2006, GURA et al. 2006, MIU et al. 2006) indicating that it is necessary to limit contact with this element due to its possible interference with the normal development of the organism. The aluminium is excreted from the body by kidneys, and preterm newborns are especially prone to the effect of this element. Aluminium toxicity seems to be negatively correlated with the gestational age because the physiological immaturity of preterm infants' kidneys favours some accumulation of aluminium as a result of the substitution of calcium ions, which weakens bone mineralization processes (REINKE et al. 2003, GURA et al. 2006).

CONCLUSIONS

1. The average concentrations of zinc, copper, iron, manganese, chromium and aluminium in cord blood stayed within the ranges recognized as standard, which indicates efficient regulating mechanisms of the developing foetuses.

2. The neonate's gestational age, gender and birth weight had no substantial impact on concentrations of the examined elements in the cord blood, except iron, whose concentration was noticed to be statistically significantly correlated to the neonates' gender.

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RESPONSE OF MAIZE (*ZEA MAYS* L.) TO SOIL CONTAMINATION WITH COPPER DEPENDING ON APPLIED CONTAMINATION NEUTRALIZING SUBSTANCES

Andrzej C. Żołnowski, Marta K. Busse, Paweł K. Zając

Chair of Environmental Chemistry
University of Warmia and Mazury in Olsztyn

Abstract

Copper is an essential trace element in living organisms, but its excess interferes with metabolic transformations in plant, animal and human cells. The uptake of copper from contaminated soils can be regulated, for example, by soil liming or application of other substances which enhance soil adsorption capacity and Cu binding in the substrate. The purpose of this study has been to assess the response of maize to soil contamination with copper depending on the applied neutralizing substances. The tested factors were increasing concentrations of Cu in soil: 0, 200, 400 and 600 mg Cu kg^{-1} dm, and soil application of mineral (lime, loam and zeolite) and organic (manure, peat and bark) neutralizing substances.

Soil contamination with copper within the range of 200 to 600 mg kg^{-1} of soil caused reduction in maize yields. Positive influence such as alleviation of the harmful effect of copper contamination was demonstrated by lime and manure, which enhanced yields of maize, especially in the series polluted with 200 and 400 mg Cu kg^{-1}. The other soil amending substances, especially peat added in amounts of 400 and 600 mg kg^{-1} of soil, caused a considerable depression in maize yields. A linear dependence has been demonstrated between the concentration of Cu in maize plants and the content of Cu in the substrate, with the root content of Cu being on average six-fold higher than in aerial organs. Mineral soil amendments significantly decreased the BTC index in maize compared to organic substances, and lime as well as pine bark decreased the BCF index to 2.33 and 1.67 versus the value of 4.21 found in the control treatment without any neutralizing substances. The uptake of copper depended on the volume of yield and – to a lesser degree – on the concentration of Cu in plants. The uptake of copper by plants was the highest in treatments

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dr inż. Andrzej Żołnowski, Chair of Environmental Chemistry, University of Warmia and Mazury in Olsztyn, Plac Łódzki 4, 10-727 Olsztyn, Poland, e-mail: anzol@uwm.edu.pl

contaminated with a rate of 200 mg Cu kg^{-1}, which was the consequence of higher yields from that treatment than from the plots polluted with 400 or 600 mg Cu kg^{-1} of soil.

Key words: copper, BTC, BCF, BAC, phytotoxicity, neutralizing amendments.

REAKCJA KUKURYDZY (*ZEA MAYS* L.) NA ZANIECZYSZCZENIE GLEBY MIEDZIĄ W ZALEŻNOŚCI OD ZASTOSOWANYCH DODATKÓW NEUTRALIZUJĄCYCH

Abstrakt

Miedź to niezbędny w funkcjonowaniu organizmów żywych pierwiastek śladowy, jednak jej nadmiar objawia się zakłóceniem metabolizmu roślin, zwierząt oraz ludzi. Pobranie miedzi z gleb zanieczyszczonych można regulować m.in. stosując zabieg wapnowania oraz inne substancje wpływające na zwiększenie sorpcji i wiązanie Cu w glebie. Celem badań była ocena reakcji kukurydzy na zanieczyszczenie gleby miedzią oraz zastosowane dodatki neutralizujące. Czynnikami były rosnące stężenia Cu w glebie: 0, 200, 400 i 600 mg Cu kg^{-1} s.m. oraz doglebowe dodatki neutralizujące: mineralne (wapno, ił i zeolit) oraz organiczne (obornik, torf i kora).

Zanieczyszczenie gleby Cu w ilości od 200 do 600 mg kg^{-1} gleby powodowało spadek plonowania kukurydzy. Pozytywny wpływ na łagodzenie skutków szkodliwego działania miedzi wykazały wapno oraz obornik, poprawiając plonowanie roślin, głównie w odniesieniu do gleby zanieczyszczonej 200 i 400 mg Cu kg^{-1}, pozostałe dodatki, szczególnie torf stosowany w glebie zanieczyszczonej 400 i 600 mg Cu kg^{-1}, przyczyniły się do znacznego obniżenia plonu. Wykazano liniową zależność między zawartością Cu w roślinach kukurydzy a zawartością Cu w podłożu, przy czym zawartość Cu w korzeniach była średnio ponad 6 razy wyższa niż w części nadziemnej. Dodatki mineralne istotnie wpłynęły na wskaźnik BTC w kukurydzy w stosunku do zastosowanych dodatków organicznych. Wapno i kora sosnowa wpłynęły na obniżenie wskaźnika BCF do wartości 2,33 i 1,67 w stosunku do wartości 4,21 w obiekcie kontrolnym – bez dodatków. Pobranie miedzi zależało od wielkości plonu, w mniejszym stopniu od zawartości Cu w roślinach. Na obiektach zanieczyszczonych 200 mg Cu kg^{-1} – wyżej plonujących – średnia wielkość pobrania była istotnie wyższa niż na obiektach o bardzo dużej zawartości miedzi – 400 i 600 mg Cu, ale dużo niżej plonujących.

Słowa kluczowe: miedź, BTC, BCF, BAC, fitotoksyczność, dodatki neutralizujące.

INTRODUCTION

Progress in our civilization is invariably associated with environmental pollution. The contamination of the natural environment is mainly caused by emission of metal-bearing dusts, which are eventually deposited on the surface of earth. Other sources of trace metals are industrial waste, municipal sewage and wastewater, fertilizers and plant protection chemicals (BOWSZYS et. al 2009, WOŁOSZYK et al. 2009). In Poland, soil contamination with heavy metals is detectable locally, mainly in industrialized areas, although in some locations near sources of emission, soils can be heavily polluted (ROSADA 2007, DOPIERAŁA 2009, SZULC, KOBIERSKI 2010). The content of Cu in Polish soils ranges from 0.2 to 725.0 mg Cu kg^{-1} d.m. and the average is 6.50 mg Cu kg^{-1}. Near metal smelting plants, e.g. the Głogów Copper Smelter (Poland), the concentration of Cu is 1440 mg Cu kg^{-1} on average (Karczewska et al. 2012), and contamination rates detected near metal smelters in other parts of the world range from 510-9700 mg Cu kg^{-1} – Sudbury (Canada), 1400--3700 mg Cu kg^{-1} – Coniston (Canada) (CCME 1997), 11 600-14 200 mg Cu kg^{-1} – Lubumbashi (DR-Congo) (Narendrula et al. 2012). Soil contamination with copper can also result from inadequate and repetitive application of plant protection chemical preparations. Copper-based fungicides used in viniculture until the end of the 19th century (the Bordeaux mixture $CuSO_4 + Ca(OH)_2$) as well as the ongoing application of copper compounds such as $Cu(OH)_2$ $CuCl_2$, $CuSO_4$ $3Cu(OH)_2$, Cu_2O, $Cu(OH)_2$ have led to serious accumulation of Cu in soil of grapevine plantations in France, Brazil, Croatia and Spain. In these countries, average concentrations of Cu in the 0-20 cm soil layer are 332, 665, 700 and 560 mg Cu kg^{-1} respectively (Komarek et al. 2010). Copper-based fungicides are also applied for protection of other crops (Schramel et al. 2000, Li et al. 2005, Komarek et al. 2009). Trace elements are not biodegradable (Antoniadis et al. 2007) and in soil can be dissolved, chelated, adsorbed by humus, bound by soil minerals, uptaken by plants or titrated with other compounds. Persistence of copper bonds depends on the soil pH, quantity and type of other mineral components, redox potential, soil adsorption capacity and content of organic matter (Dube et al. 2001, USEPA 2002). One possible method which alleviates the harmful effect of heavy metals consists in soil amendment with neutralizing substances (Nwachukwu, Pulford 2008) that bind copper into insoluble metal and mineral or metal and organic forms, which – under favourable conditions – may remain in soil for a long time as harmless compounds (Zolnowski et al. 2009). Such soil amending substances include zeolites, loam, lime, ground tree bark, manure and peat (Nwachukwu, Pulford 2008, Komarek et al. 2009, Najmowicz et al. 2010). Methods for immobilization of metals in soil remain the only available and economically viable way to rehabilitate contaminated soils, which enable restoration of biological life and plant cover on degraded land (Karczewska, Kabała 2010). High concentrations of heavy metals in soil may lead to their plant concentrations higher than normal, which in turn can cause their increased transfer to subsequent links in the trophic chain, where they can accumulate and produce lasting harmful effects.

The aim of this study has been to assess the response of maize to soil contamination with copper and to determine the effectiveness of copper immobilization in soil by using mineral (lime, loam and zeolite) and organic (manure, peat and tree bark) neutralizing soil amendments.

MATERIAL AND METHODS

A pot experiment was set up in 2011, in a greenhouse at the University of Warmia and Mazury in Olsztyn. The experiment was designed according to the method of randomly selected blocks with four replicates. There were two groups of factors: 1) incremental contamination of soil with copper: 0, 200, 400 and 600 mg Cu kg^{-1} of soil, which was simulated by adding to soil aqueous solution of copper (II) sulphate ($CuSO_4$ $5H_2O$; produced at the POCh in Gliwice), and 2) neutralizing substances: lime in an amount equivalent to 1 Hh, i.e. 8,48 g $CaCO_3$ pot^{-1}, loam, natural zeolite ∅ 1-2.5 mm (SUBIO EKO Polska, Sp. z o.o.), cattle manure (P.P.H.U. CDN Ireneusz Cal), peat (Athena Bio-Produkty Sp. z o.o.) and pine bark (fraction ∅ 2-5 mm) (Hollas Sp. z o.o.) 3% of soil mass per pot, i.e. 240 g pot^{-1}. NPK fertilization was: 2.17 g N as ammonia, $CO(NH_2)_2$, 0.6 g P as KH_2PO_4, 1.25 g K as KH_2PO_4 (0.75 g K) and K_2SO_4 (0.5 g K), 0.18 g Mg as $MgSO_4$ and 0.25 mg B as H_3BO_3 pot^{-1}. Typical brown soil developed from poor loamy sand class IVb and collected from the Ap horizon was used in the experiment. In the Polish soil valuation system, the soil belongs to good rye complex. In the FAO/WRB (World Reference Base for Soil Resources) taxonomy (FAO 2006), the soil was classified as Cambisol – Brown Soils. The basic properties of soil are specified in Table 1.

Table 1

Some physicochemical properties of soil used in experiment

Soil type	pH KCl	pH H_2O	Hydro-lytic acidity (cmol kg^{-1})	Alka-line cations (cmol kg^{-1})	Cation exchange capacity CEC (cmol kg^{-1})	Total base saturation (%)	Salinity (µS cm^{-1})	N total (g kg^{-1})	C org. (g kg^{-1})	C:N ratio
Cambisols – brown soil	4.50	5.70	2.84	5.47	8.31	65.83	71.60	0.76	5.55	7.30

The experiment was set up in Kick-Brauckmann pots, by filling each pot with 8 kg of soil passed through a ∅ 1 cm mesh sieve. During the vegetative growth of plants, the soil moisture was maintained at the level of 60% full water capacity. Maize (*Zea mays* L.) cv. San (Hodowla Roślin Smolice Sp. z o.o.) was grown for green matter and harvested 100 days after sowing. After harvest, the yield of aerial parts and roots (after washing off the soil residue and drying) was determined. Samples were dried at 65°C, ground and wet digested according to the EPA Method 3052 (Microwave Assisted Acid Digestion of Siliceous and Originally Based Matrices) (EPA 1996), using microwave heating with an appropriate microwave system (MARS-5, CEM Corporation USA). Concentration of Cu was determined by flame atomic absorption spectroscopy (FLAAS) (VARIAN SpectrAA – FS240, Varian Inc. Australia). The content of copper was calculated per absolutely dry matter, determined at 105°C. The biological transfer coefficient for copper BTC – 1, biological concentration factor BCF – 2, and biological accumulation coefficient BAC – 3, were determined (TUKURA et al. 2012).

$$\mathrm{BTC} = \frac{\text{metal concentration (mg kg}^{-1}\text{ DM) abveground part of plant}}{\text{metal concentration (mg kg}^{-1}\text{ DM) in roots}} \tag{1}$$

$$\mathrm{BCF} = \frac{\text{metal concentration (mg kg}^{-1}\text{ DM) in roots}}{\text{metal concentration (mg kg}^{-1}\text{ DM) in soil}} \tag{2}$$

$$\mathrm{RAC} = \frac{\text{metal concentration (mg kg}^{-1}\text{ DM) aboveground part of plant}}{\text{metal concentration (mg kg}^{-1}\text{ DM) in soil}} \tag{3}$$

The uptake of copper by plants was expressed in mg of copper taken up from 1 kg of soil. The results were processed statistically with Anova at the level of significance of α = 0.05, using a Statistica v. 9.0 software package (StatSoft 2009). The correlation between the analyzed factors was established using a simple linear correlation model, with the Microsoft Excel programme (Microsoft 2002).

RESULTS AND DISCUSSION

The tested soil contamination with copper caused a significant, linear decrease in the mass of roots and aerial matter yield produced by maize, except the rate of 200 mg Cu kg^{-1} of soil, which stimulated the roots yield, raising it by 13.80 g DM pot^{-1} relative to the control and by 28.8 g versus the manure-amended pots (Table 2). No such effect was observed in respect of the aerial mass yield in the control treatment without soil amending substances. Out of the tested amendments, lime and manure are noteworthy as they raised the yield of root dry matter from pots contaminated with 200 mg Cu kg^{-1} of soil by 16.50 and 15.63 g DM pot^{-1}. In this experiment, copper contamination was observed to have inhibited the growth of maize roots, which became thicker and shorter. A similar response of plants to Cu contamination of soil has been described by ROSSI et al. (2004), SZULC, KOBIERSKI (2010) and SINGH et al. (2007). Crops can be improved when soil is amended with substances which can arrest copper. Such substances include composts made from plant waste, coir and tree bark or, less often used, bone meal and peat (NWACHUKWU, PULFORD 2008). However, organic soil amendments do not always produce same effects on chosen heavy metals. In a study completed by CIEĆKO et al. (2001), application of charcoal or compost with lime or in a series without liming did not improve yields of triticale.

Table 2

Effects of soil pollution with copper on the dry matter weight of roots and aboveground part of maize

Soil pollution with copper (mg Cu kg^{-1})	Objects							Average
	without amendments	lime	clay	zeolite	FYM	peat	pine bark	
Weight of roots (DM g pot^{-1})								
0	46.95	98.63	60.10	51.53	47.58	72.08	58.28	62.16
200	60.75	77.25	48.25	26.25	76.38	68.35	42.25	57.07
400	4.25	5.78	5.18	3.70	5.88	2.20	4.13	4.45
600	1.60	1.80	1.80	1.93	1.28	1.93	2.53	1.84
$\bar{x}$	28.39	45.87	28.83	20.85	32.78	36.14	26.80	31.38
R_2	0.64*	0.80**	0.84**	0.83**	0.54*	0.79**	0.81**	0.75**
LSD $_{\alpha=0.05}$ for Cu soil pollution = 5.31; for amendments = 7.03; for interaction = 14.06								
Yield of aerial part of maize (DM g pot^{-1})								
0	168.15	166.46	152.09	156.60	175.48	160.98	163.85	163.37
200	145.84	147.61	107.98	74.86	149.53	83.34	101.85	115.86
400	18.92	18.96	20.85	15.32	21.19	3.97	18.23	16.78
600	3.40	3.30	3.88	2.69	2.62	3.04	3.42	3.19
$\bar{x}$	84.08	84.08	71.20	62.37	87.21	62.83	71.84	74.80
R_2	0.83**	0.87**	0.89**	0.89**	0.84**	0.83**	0.66*	0.83**
LSD $_{\alpha=0.05}$ for increasing Cu soil pollution = 13.21; for amendments = 17.47; for interaction = n.s.								

* determination coefficient R_2 significant for $\alpha \leq 0.05$;
** determination coefficient R_2 significant for $\alpha \leq 0.01$; n.s. – not significant; n=8

Manure introduced to soil may have a stimulating effect on mobility of metals, possibly because it contains nitric compounds, which are often physiologically acidic (Sienkiewicz et al. 2009). The concentration of copper in maize plants was varied and depended on the analyzed part of the plant and the degree of soil contamination with copper (Table 3). Roots contained on average 1661.38 mg Cu kg^{-1} DM in the series without neutralizing additives and the aerial organs had 299.02 mg Cu kg^{-1} DM.

Application of lime depressed the concentration of copper in plant roots by an average of 771.37, zeolite by 225.83 and loam by 113.12 mg Cu kg^{-1} DM of roots. Among the tested organic soil amendments, pine bark caused a significant decrease of the concentration of Cu in roots by 1141.68 mg kg^{-1} DM, while manure decreased it by 461.91 mg Cu kg^{-1} DM compared to the average concentration found in the non-amended series.

Table 3

Effects of soil pollution with copper on the copper content in roots and aboveground part of maize

Soil pollution with copper (mg Cu kg^{-1})	Objects							Average
	without amendments	lime	clay	zeolite	FYM	peat	pine bark	
Copper content in roots (mg kg^{-1} DM)								
0	19.18	17.93	31.00	53.50	11.00	12.20	14.75	22.79
200	176.28	117.85	276.20	266.20	143.78	171.38	156.53	186.89
400	1292.65	525.08	1242.35	898.75	1006.25	2542.40	643.18	1164.38
600	5157.40	2899.17	4643.50	4523.75	3636.85	6080.00	1264.35	4029.29
$\overline{x}$	1661.38	890.01	1548.26	1435.55	1199.47	2201.49	519.70	1350.84
R_2	0.79**	0.74**	0.80**	0.75**	0.81**	0.88**	0.93**	0.81**
LSD $_{\alpha=0.05}$ for Cu soil pollution = 60.58; for amendments = 80.14; for interaction = 160.28								
Copper content in aerial part of maize (mg kg^{-1} DM)								
0	15.30	6.05	10.08	8.30	8.50	5.38	8.00	8.80
200	10.80	10.93	9.30	11.35	23.03	52.50	9.85	18.25
400	64.33	20.83	32.58	27.78	49.38	510.20	118.00	117.59
600	1105.65	483.60	735.20	571.43	330.05	1398.40	280.13	700.64
$\overline{x}$	299.02	130.35	196.79	154.71	102.74	491.62	103.99	211.32
R_2	0.63*	0.62*	0.62*	0.63*	0.70**	0.86**	0.86**	0.70**
LSD $_{\alpha=0.05}$ for increasing Cu soil pollution = 10.26; for amendments = 13.57; for interaction = 27.15								

* determination coefficient R_2 significant for $\alpha \leq 0.05$;
** determination coefficient R_2 significant for $\alpha \leq 0.01$; n.s. -not significant; n=8

The concentration of Cu in roots was the highest in maize plants grown on soil amended with peat: 2201.49 mg Cu kg^{-1} DM on average and the maximum concentration of 6080.00 mg Cu kg^{-1} DM. on soil polluted with 600 mg Cu kg^{-1}. Significant reduction in the concentration of Cu in aerial parts of maize was achieved through enrichment of soil with lime as well as manure or pine bark, where it decreased by 168.67, 196.28 and 195.03 mg Cu kg^{-1} DM., respectively. In contrast, the application of peat caused an increase in the content of Cu in aerial parts of maize by 192.6 mg Cu kg^{-1} DM. versus the control. Rossi et al. (2004) also demonstrated that the roots of *Brassica napus* accumulated several-fold more copper than the aerial organs, which is in accord with the present results.

The coefficients of biological transfer (BTC), biological accumulation (BAC) and biological concentration factor (BCF) determined for copper in maize plants depended on the applied neutralizing substances (Table 4).

Table 4

Effects of soil pollution with copper on the biological transfer coefficient (BTC), biological concentration factor (BCF), and biological accumulation coefficient (BAC)

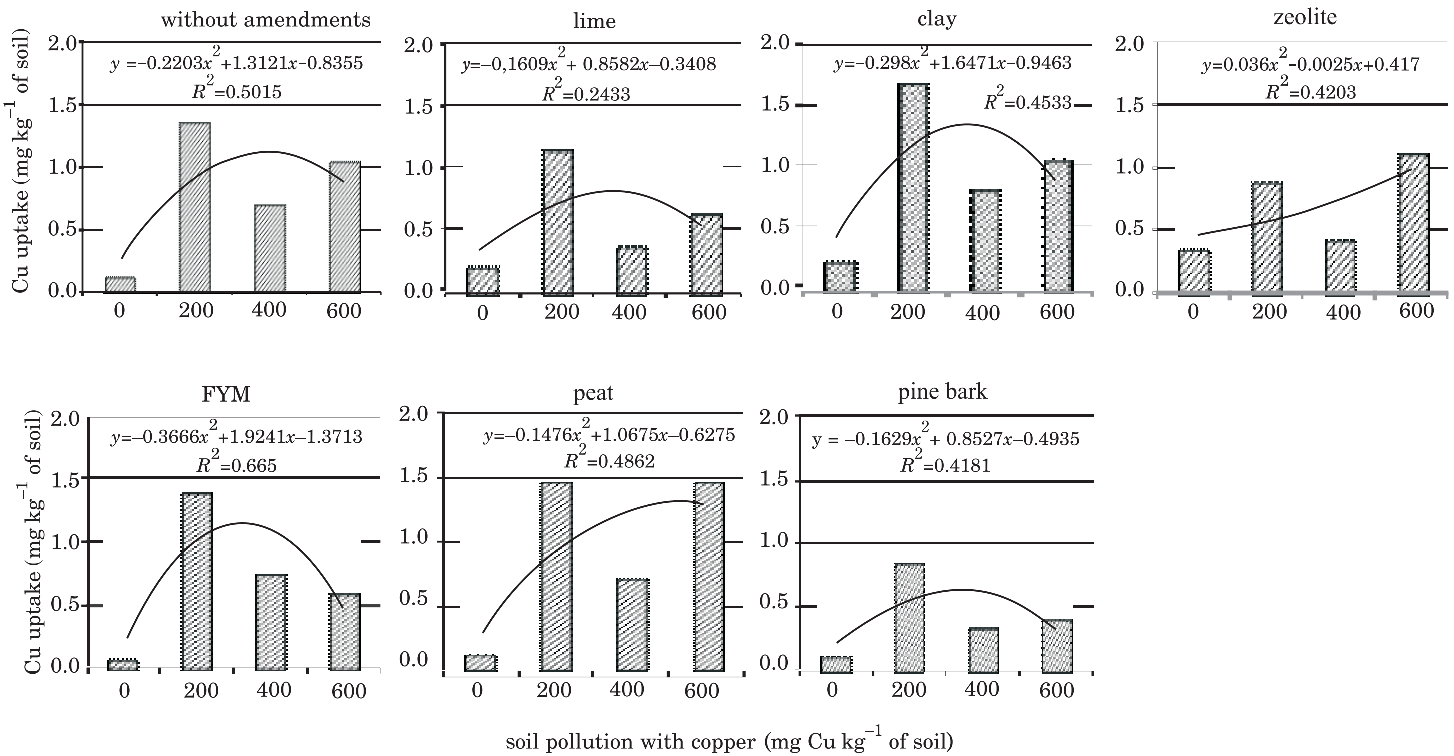
Soil pollution with copper (mg Cu kg^{-1})	Objects							Average
	without amendments	lime	clay	zeolite	FYM	peat	pine bark	
Biological transfer coefficient (BTC)								
0	0.82	0.34	0.32	0.15	0.80	0.44	0.69	0.51
200	0.06	0.09	0.03	0.04	0.18	0.31	0.06	0.11
400	0.05	0.04	0.03	0.03	0.05	0.20	0.18	0.08
600	0.21	0.17	0.16	0.13	0.09	0.23	0.22	0.17
$\overline{x}$	0.29	0.16	0.14	0.09	0.28	0.29	0.29	0.22
R_2	n.s.	n.s.	n.s.	n.s.	0.64**	0.68**	n.s.	n.s.
LSD $_{\alpha=0.05}$ for Cu soil pollution = 0.10; for amendments = 0.13; for interaction = 0.26								
Biological concentration factor (BCF)								
0	2.85	2.06	4.00	5.91	1.81	3.16	0.32	2.87
200	0.95	0.63	1.56	1.41	1.45	0.97	1.11	1.15
400	3.77	1.34	3.57	2.37	2.63	7.37	2.26	3.33
600	9.29	5.28	8.34	7.71	7.15	10.64	2.98	7.34
$\overline{x}$	4.21	2.33	4.37	4.35	3.26	5.54	1.67	3.68
R_2	0.64**	n.s.	n.s.	n.s.	n.s.	0.74**	0.96**	0.65**
LSD $_{\alpha=0.05}$ for Cu soil pollution = 0.23; for amendments = 0.30; for interaction = 0.61								
Biological accumulation coefficient (BAC)								
0	2.34	0.70	1.30	0.91	1.38	1.40	0.19	1.17
200	0.06	0.06	0.05	0.06	0.23	0.30	0.07	0.12
400	0.19	0.05	0.09	0.07	0.13	1.48	0.41	0.35
600	1.99	0.88	1.32	0.97	0.65	2.45	0.66	1.27
$\overline{x}$	1.14	0.42	0.69	0.50	0.60	1.41	0.33	0.73
R_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.74**	n.s.
LSD $_{\alpha=0.05}$ for Cu soil pollution = 0.19; for amendments = 0.25; for interaction = 0.51								

* determination coefficient R_2 significant for $\alpha \leq 0.05$;
** determination coefficient R_2 significant for $\alpha \leq 0.01$; n.s. -not significant; n=8

Maize was characterized by a relatively low BTC, which means that quite a large portion of absorbed copper stayed in roots. As a rule, plants with BTC >1.0 are said to be hyperaccumulators and can be used for phytoextraction and phytostabilization (TUKURA et al. 2012). Lower BTC values than in other series were found in the treatment with zeolite, where the mean BTC was 0.09. This means that peat had a significant effect on arresting metals in roots. A high BTC value was determined for the series without any neutralizers (0.29), and the applied organic soil amendments did not affect the transfer of copper from roots to the upper parts of maize plants. The BCF >1.0 confirms extensive binding of metals, which is a desirable characteristic of plants used for phytoremediation of polluted soils. High BCF informs about the presence of detoxication mechanisms, which lead to sequestration of ions of trace metals, and this in turn prevents translocation of harmful metals of aerial parts of plants (GHOSH, SINGH 2005). The tested maize was characterized by a relatively high BCF, which suggests a considerably high uptake of copper and its arrest in roots. Out of the mineral soil amendments, lime caused a decrease in the concentration of Cu in maize (BCF=2.33); organic substances produced diverse effects. Pine bark depressed the biological concentration factor down to 1.67, while peat induced a very high increase in its value – to 5.54 on average, with the highest values of 7.37 and 10.64 in treatments polluted with 400 i 600 mg Cu kg^{-1} of soil, respectively.

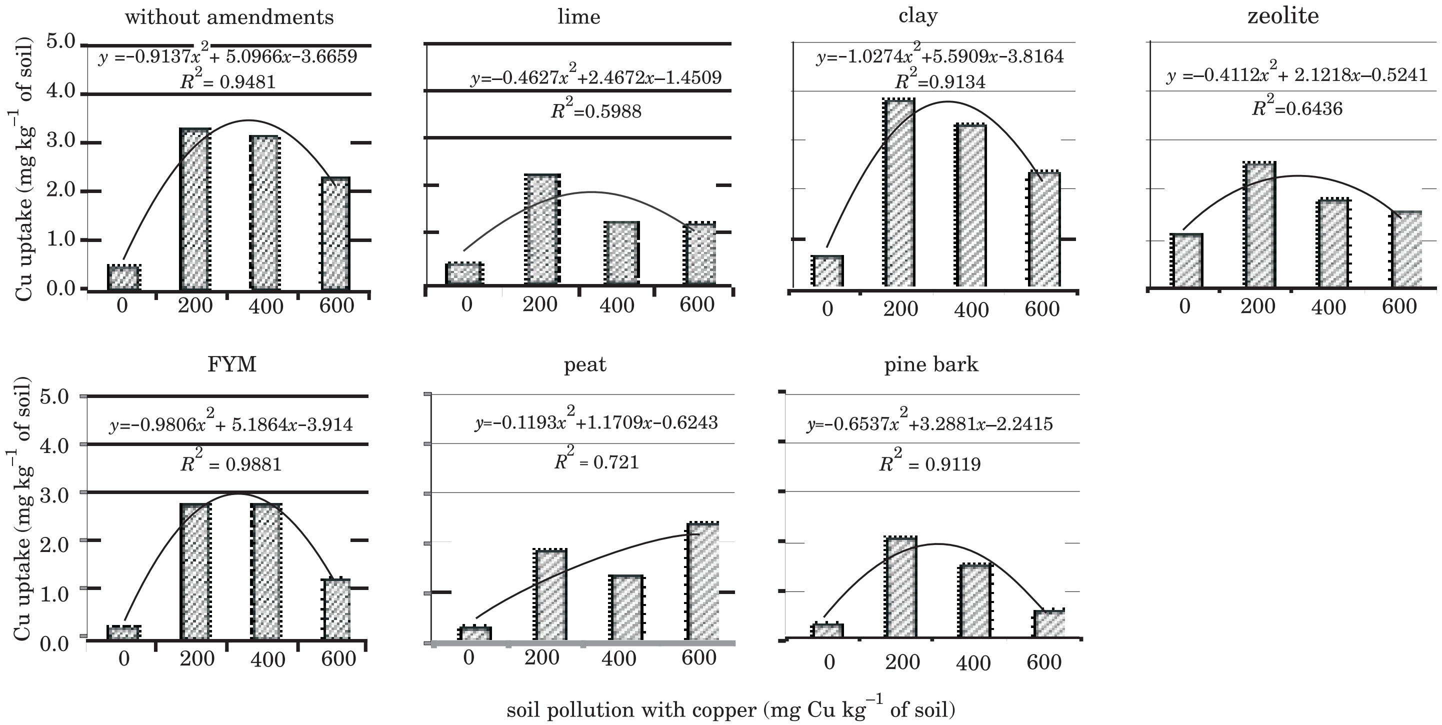
A relatively low BAC indicated high efficiency of lime and pine bark added to soil (0.42 and 0.33, respectively). These neutralizing substances inhibited the transfer of copper from soil to stems and leaves of maize. The highest accumulation coefficient was determined for maize grown on soil with peat (1.41) and without any neutralizers (1.14). The uptake of copper by maize plants (Figures 1, 2) to a large extent depended on the degree of soil contamination with this metal. The highest uptake occurred in the treatments polluted with 200 mg Cu kg^{-1} of soil. In maize grown on soil with higher rates of copper, despite an increase in copper concentration in plant tissues, a significant decrease in the uptake of the metal was observed as a result of the strong depression of maize yield due to the toxicity of copper. Lime, zeolite and bark distinctly depressed the uptake of copper by maize roots compared to the treatments without soil amending substances and with added loam, manure and peat.

In respect of the aerial parts of maize, lime and zeolite as well as peat and pine bark limited the uptake of copper. The amounts of copper removed with harvested parts of maize depended above all on the properties of soil neutralizing substances. Lime was most effective as it enhanced yields, generated a low biological concentration factor and resulted in the lowest uptake of copper. As reported by SIENKIEWICZ et al. (2009), application of organic substances to soil may be followed by an increase in the uptake of copper, an effect induced by nitric compounds found in such substances which acidify the soil and increase the solubility of metals, thus facilitating their translocation into plants, the event that was also demonstrated in this study.



LSD $_{\alpha=0.05}$ for increasing Cu soil pollution = 0.09; for amendments = 0.13; for interaction = 0.25 correlation coefficient between soil Cu pollution and Cu uptake r=0.36, ns – not significant correlation

Fig 1. Copper uptake by roots of maize from 1 kg of soil polluted with copper



LSD $_{\alpha=0.05}$ for increasing Cu soil pollution = 0.15; for amendments = 0.20; for interaction = 0.40 correlation coefficient between soil Cu pollution and Cu uptake r=0.24, ns – not significant correlation

Fig 2. Cu uptake by aboveground parts of maize from 1 kg of soil polluted with copper

CONCLUSIONS

1. Soil contamination with copper had a significant effect on maize yields. The rate of 200 mg Cu kg^{-1} of soil stimulated the growth of maize roots, while the doses of 400 and 600 mg Cu kg^{-1} led to reduced root yields. Soil contamination with copper had a significantly negative effect on yields of aerial parts of maize at all the tested rates of contamination.

2. Copper contamination significantly raised the concentration of Cu in plants. In roots, it was on average over 6-fold higher than in aerial organs, which indicates that there are certain mechanisms involved limiting the uptake of toxic amounts of copper by maize.

3. Mineral contamination neutralizing substances depressed the biological transfer coefficient (BTC) for Cu in maize compared to plants grown on soil with organic soil amendments. The biological concentration factor (BCF) fell down to 2.33 and 1.67 in response to application of lime and, exceptionally, pine bark compared to 4.21 in the control. The BAC in the same treatments decreased down to 0.42 and 0.33 versus 1.14 found in the control treatment, without any neutralizers. The substances introduced to soil evidently inhibited the translocation of copper from soil to maize stems and leaves.

4. The uptake of copper by maize depended primarily on the volume of yields and, to a lesser degree, on the concentration of Cu in maize plants. Plants growing on the treatments polluted with 200 mg Cu kg^{-1} absorbed on average more copper than the ones from pots with soil polluted with 400 and 600 mg Cu kg^{-1}, despite a very high concentration of Cu in plant tissues in the latter case.

5. The smallest average unit uptake of copper by maize, expressed in mg Cu per kg of soil, was demonstrated by plants grown on soil enriched with lime and pine bark, which confirms the claim that these soil amendments belong to substances that most strongly inhibit the uptake of heavy metals by plants.

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CONTENT OF MACRO- AND MICROELEMENTS IN THE YIELD OF GARLIC CHIVES (*ALLIUM TUBEROSUM* ROTTLER EX SPRENG.) ACCORDING TO THE PLANT AGE

Agnieszka Żurawik, Dorota Jadczak, Piotr Żurawik

Chair of Horticulture
West Pomeranian University of Technology in Szczecin

Abstract

The experiments were carried out in 2002-2005, at the Chair of Vegetable Cultivation of the Agricultural University in Szczecin (currently Chair of Horticulture, West Pomeranian University of Technology in Szczecin). The aim of the study was to estimate the effect of age of grown plants on the content of macro- and microelements in leaves of garlic chives. Seeds of chives were sown on 10th April, directly to the soil, at a density of 30×20 cm, 12 seeds per point. The content of macro- and microelements was assessed in the harvested yield. After mineralization of plant dry matter in a mixture of H_2SO_4 and H_2O, the content of total nitrogen, phosphorus, potassium, sodium, calcium, magnesium and sulphur was determined. The content of copper, zinc, manganese and iron was assessed after mineralization of plant dry matter in a mixture of HNO_3 and $HClO_4$. The research material consisted of one-, two- and three-year-old plants.

A significant effect of the age of garlic chives on the content of macro- and microelements in the yield was found. One- and three-year-old garlic chives were characterized by a higher content of nitrogen while two-year-old plants contained less nitrogen. The highest content of phosphorus was determined in three-year-old garlic chives. However, the least amount of this element was found in one-year-old plants. The highest content of sodium was noted in yield of three-year-old plants, while the least Na was in two-year-old plants. Moreover, the highest content of calcium and magnesium was determined in the yield of two-year-old plants, and the lowest – in three-year-old ones.

Three-year-old garlic chives contained less potassium and sulphur. In contrast, the highest content of these elements was determined in one-year-old plants. The highest content of copper was assessed in one-year-old garlic chives, and the lowest – in three-year-old plants. The yield of one-year-old plants contained significantly more zinc in comparison

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dr inż. Agnieszka Żurawik, Chair of Horticulture, West Pomeranian University of Technology in Szczecin, P. Pawła VI 1, 71-459 Szczecin, Poland, e-mail: azurawik@zut.edu.pl

with two- and three-year-old ones. However, the highest content of manganese was noted in three-year-old plant leaves and the lowest – in leaves of two-year-old plants. Three-year-old garlic chives were characterized by a significantly higher content of iron in comparison with one- and two-year-old plants.

Key words: garlic chives, dry matter, macroelements, microelements.

ZAWARTOŚĆ MAKRO- I MIKROSKŁADNIKÓW W PLONIE SZCZYPIORKU CZOSNKOWEGO (*ALLIUM TUBEROSUM* ROTTLER EX SPRENG.) W ZALEŻNOŚCI OD WIEKU ROŚLIN

Abstrakt

Doświadczenia przeprowadzono w latach 2002-2005 w Katedrze Warzywnictwa Akademii Rolniczej w Szczecinie (obecnie Katedra Ogrodnictwa, ZUT w Szczecinie). Celem było określenie wpływu wieku uprawianych roślin na zawartość makro- i mikroskładników w liściach szczypiorku czosnkowego. Nasiona szczypiorku wysiewano 10. kwietnia, bezpośrednio na pole, w rozstawie 30x20 cm, gniazdowo po 12 nasion. W plonie określono zawartość makro- i mikroskładników. Po zmineralizowaniu suchej masy w mieszaninie H_2SO_4 i H_2O oznaczono zawartość: azotu ogólnego, fosforu, potasu, sodu, wapnia, magnezu i siarki. Zawartość miedzi, cynku, manganu i żelaza oznaczono po zmineralizowaniu suchej masy w mieszaninie HNO_3 i $HClO_4$. Ocenie poddano rośliny jednoroczne, dwu- i trzyletnie.

Wykazano istotny wpływ wieku roślin szczypiorku czosnkowego na zawartość w plonie analizowanych makro- i mikroskładników. Więcej azotu zawierał jednoroczny i trzyletni szczypiorek czosnkowy, a mniej dwuletni. Istotnie najwięcej fosforu zawierał trzyletni szczypiorek czosnkowy, najmniej tego pierwiastka oznaczono w plonie zebranym z roślin jednorocznych, a sodu – z dwuletnich. Najwięcej wapnia i magnezu zawierał plon z roślin dwuletnich, a najmniej – trzyletnich. W trzyletnim szczypiorku czosnkowym oznaczono także najmniej potasu i siarki, najwięcej tych pierwiastków zawierał plon zebrany z roślin jednorocznych. Najwięcej miedzi zawierał jednoroczny szczypiorek czosnkowy, a najmniej trzyletni. Plon zebrany z roślin jednorocznych, w porównaniu z dwu- i trzyletnimi, zawierał istotnie więcej cynku. Natomiast najwięcej manganu zawierały liście roślin trzyletnich, a najmniej – dwuletnich. W trzyletnim szczypiorku czosnkowym oznaczono istotnie więcej żelaza, w porównaniu z roślinami jednorocznymi i dwuletnimi.

Słowa kluczowe: szczypiorek czosnkowy, sucha masa, makroskładniki, mikroskładniki.

INTRODUCTION

Garlic chives are a valuable vegetable plant, which offer many health and taste benefits, like chives and garlic. Nowadays, it is cultivated mainly in Japan, China, Korea and countries of South Asia. This species is also popular in Great Britain, where it is sold in bunches. Unfortunately, despite its high nutritional value, simple and low-cost cultivation, resistance to diseases and insects and ornametal value, garlic chives are still unknown in Poland.

Alium tuberosum is a perennial plant. Owing to its resistance to onion vegetable diseases and insects, it does not require the use of chemical substances for plant protection. In the climatic conditions of Poland, it overwinters in open field without use of covers. The whole plant of garlic chives is edible: bulbs with leaves and young flowering stems. It is very often used in the kitchen as an excellent spice in many dishes. Moreover, it can be lyophilized and used in the food processing industry as an additive to meat and dairy products. For consumption as a fresh product, garlic chives are grown and sold in containers or, after leaf cutting, in bunches. It is a very decorative plant, forming a large number of white, beautiful smelling, umbrella-like flower heads, blooming the whole summer.

In national and international literature, there is little information about garlic chives, such as data concerning the macro- and microelement content. The content of micro- and macroelements in plants is affected by the plant species and cultivar, but also by fertilization (Markiewicz, Golcz 2010), use of biostimulators (Majkowska-Gadomska, Wróblewska-Wierzbicka 2013) and harvest date (Kołota et al. 2012). However, no information is available about the influence of the age of plants. According to Żurawik and Jadczak (2008, 2009), garlic chives are a valuable source of sugars and vitamin C. According to Kotlińska et al. (2005), and Tendaj and Mysiak (2006), bulbs and leaves of onion vegetables are rich in many minerals, including calcium and potassium, which are able to neutralize mild metabolic acidosis. In view of the above, we decided to conduct an experiment in order to assess the content of macro- and microelements in the yield of garlic chives depending on the year of its cultivation.

MATERIAL AND METHODS

A field experiment was carried out in 2002-2005, at the Vegetable Experimental Station in Dołuje, near Szczecin. In the experiment, the effect of plant age on the content of macro- and microelements in garlic chives leaves was tested. The experimental material consisted of one-, two- and three-year-old plants.

The experiment was set up in a split-block design with four replications. The harvest plot area was 1.44 m^2 (1.2×1.2 m). Seeds of chives were sown directly on a field on 10th April, at a density of 30×20 cm (12 seeds per point), and in seedbeds in rows spaced by 30 cm. The seedlings produced in seedbeds were transplanted into an open field when they had formed 2-3 leaves. The plants were analysed after one, two and three years of cultivation. Directly after each harvest, the dry matter content was assessed. Moreover, the content of macro- and microelements determined in the plants. For the chemical analyses, aggregated samples were taken from all of the treatments, separately for one-, two- and three-year-old plants.

During the vegetative season, chemical plant protection from diseases and insects of onion vegetables was provided according to the Vegetable Protection Program of the Research Institute of Vegetable Crops in Skierniewice.

The content of dry matter in fresh yield was assessed by the method of drying at 105°C to constant weight. After mineralization of plant dry matter in a mixture of H_2SO_4 and H_2O, the content of total nitrogen (by the Kjeldahl's method), phosphorus (by the colorimetric method), potassium, sodium, and calcium (by flame photometry), magnesium (by the method of atomic absorption spectrophotometry ASA) and sulphur (by the turbidimetric method) was determined.

The content of copper, zinc, manganese, iron (by atomic absorption spectrophotometry ASA) was assessed after mineralization of plant dry matter in a mixture of HNO_3 and $HClO_4$ (Krełowska-Kułas 1993). The analyses of macro- and microelements were carried out at the Regional Chemical-Agricultural Station in Szczecin.

The results concerning yield quality were subjected to an analysis of variance. The means were separated by Tukey's test at p=0.05.

RESULTS AND DISCUSSION

In the opinion of Kotlińska et al. (2005), leaves of Japanese bunching onion contain 5-13% of dry matter. Similar results were obtained for leaves of garlic chives in our study, which had on average 13.1% of dry matter. However, there were differences in the dry matter content in garlic chive plants correlated to the plants' age. In the first year of the study, a significantly higher content of dry matter was determined in one- and two-year-old plants in comparison with three-year-old ones. However, on the basis of the results in the subsequent year of cultivation and the statistical analysis of means of both years of the study, it was demonstrated that one-year old plants were characterized by the highest amount of dry matter while three-year old plants had the lowest dry matter content (Table 1).

Bulbs and leaves of onion vegetables are a rich source of many minerals, for example calcium and potassium, which are able to neutralize mild metabolic acidosis (Kotlińska et al. 2005, Tendaj, Mysiak 2006). In our experiment, in the plant material (leaves) of garlic chives, the average content of Ca was on average 10.7 g kg^{-1} d.m. and that of K was 47.7 g kg^{-1} d.m. Moreover, there were statistical differences in the content of macroelements between younger and older plants (Table 2). In the first year of chemical analyses, a significantly higher content of nitrogen and sodium was determined in three-year-old plants than in one- and two-year-old ones. The highest content of potassium was in two-year-old plants and the lowest – in one-

Table 1

Content of dry matter in leaves of garlic chives

Garlic chives	Dry matter (%)		
	experiment I	experiment II	mean
One-year-old	14.7	14.0	14.3
Two-year-old	15.0	12.0	13.5
Thrre-year-old	12.6	10.4	11.5
Mean	14.1	12.1	-
LSD α=0.05	1.3	1.3	0.5

Table 2

Macroelements in edible parts of one-, two- and three-year-old plants of garlic chives

Macro-elements	Years of study	Garlic chives (g kg^{-1} d.m.)			Mean	LSD α=0.05
		one-year -old	two-year -old	thrre-year -old		
N	experiment I	33.4	25.9	44.6	34.6	10.6
	experiment II	41.0	29.2	32.4	34.2	7.5
	mean	37.2	27.5	38.5	-	3.6
K	experiment I	41.6	56.5	47.4	48.5	3.5
	experiment II	60.2	40.3	40.3	46.9	0.6
	mean	50.9	48.4	43.8	-	1.0
Ca	experiment I	13.2	7.8	11.4	10.8	1.7
	experiment II	7.2	18.1	6.6	10.6	0.5
	mean	10.2	12.9	9.0	-	0.5
Na	experiment I	0.2	0.1	0.5	0.3	0.2
	experiment II	0.3	0.2	0.2	0.2	n.s.
	mean	0.3	0.2	0.4	-	0.04
P	experiment I	3.3	3.6	4.4	3.8	n.s.
	experiment II	2.6	3.6	5.9	4.0	0.2
	mean	2.9	3.6	5.1	-	0.5
Mg	experiment I	2.7	3.4	1.2	2.4	0.4
	experiment II	1.8	1.9	1.8	1.8	n.s.
	mean	2.2	2.6	1.5	-	0.3
S	experiment I	9.5	10.0	7.9	9.1	1.0
	experiment II	13.7	8.3	7.3	9.8	1.9
	mean	11.6	9.1	7.6	-	0.6

year-old ones. However, one- and two-year-old plants were characterized by a significantly higher content of magnesium and sulphur than three-year-old plants of garlic chives. One-year-old plants contained the highest amount of calcium, while the lowest Ca content was in two-year-old ones.

In the following year of the study, a higher content of nitrogen, potassium and sulphur was found in one-year old plants in comparison with two- and three-year-old ones. The highest content of calcium (18.1 g kg^{-1} d.m.) was determined in two-year-old plants while the least Ca (6.6 g kg^{-1} d.m.) was found in three-year-old ones. However, the highest content of phosphorus was assessed in three-year-old plants, while the lowest was in one-year-old garlic chives. On the basis of the statistical analysis of two-year means, it was proven that three-year-old plants were characterized by the highest content of phosphorus while the least of this element was noted in one-year-old plants. The highest content of sodium was determined in the yield of three-year-old plants, while the lowest was found in two-year-old plants. One- and three-year-old plants contained higher amounts of nitrogen than with two-year-old ones. Moreover, the highest content of magnesium and calcium (respectively 2.6 and 12.9 g kg^{-1} d.m.) was determined in two-year-old plants while the lowest concentrations of the two elements were determined in three-year-old garlic chives. The three-year-old plants were also characterized by the smallest concentrations of potassium and sulphur, while the highest content of these compounds was detected in one-year-old plants.

In the first year of the study, there was a significant effect of the plant age on the microelement content (Table 3). A significantly higher content of zinc was determined in one- and two-year-old plants than in three-year-old ones. However, the highest concentration of copper was found in one-year-old plants (20.5 mg kg^{-1} d.m.) and the lowest – in three-year-old ones (10.4 mg kg^{-1} d.m.). The highest content of manganese and iron was assayed in the yield collected from three-year-old plants. Two-year-old plants were characterized by the smallest content of manganese, and one-year-old plants had the lowest of content of iron. In the following year of the study, one-year-old plants were characterized by a higher content of iron in comparison with two- and three-year-old plants. The highest content of copper (61.8 mg kg^{-1} d.m.) was assessed in one-year-old plants, while the least copper (19.4 mg kg^{-1} d.m.) was in three-year-old garlic chives. Significantly higher amounts of manganese were determined in two-year-old plants in comparison with one- and three-year-old ones. However, one-year-old plants were characterized by the highest content of zinc (89.7 mg kg^{-1} d.m.). The least of this compound (60.3 mg kg^{-1} d.m.) was determined in two-year-old plants. On the basis of two-year means, it was evidenced that one-year-old plants contained the highest amount of copper, while the least copper was in three-year-old plants. Moreover, one-year-old plants of garlic chives were characterized by a higher amount of zinc than two- and three-year-old plants. However, three-year-old plants were characterized by the highest content

Table 3

Microelements in edible parts of one-, two- and three-year-old plants of garlic chives

Macroelements	Years of study	Garlic chives (mg kg^{-1} d.m.)			Mean	LSD α=0.05
		one-year old	two-year old	thrre-year old		
Mn	experiment I	31.3	15.9	34.6	27.3	1.7
	experiment II	15.5	19.0	16.0	16.8	2.4
	mean	23.4	17.4	25.3	-	0.9
Cu	experiment I	20.5	14.2	10.4	15.0	1.9
	experiment II	61.8	24.8	19.4	35.3	1.4
	mean	41.1	19.5	14.9	-	0.7
Zn	experiment I	49.8	51.4	43.5	48.2	4.6
	experiment II	89.7	60.3	65.7	71.9	1.7
	mean	69.7	55.8	54.6	-	1.5
Fe	experiment I	100.0	150.5	197.0	149.2	5.4
	experiment II	148.0	122.0	119.0	130.0	8.7
	mean	124.0	136.2	158.0	-	3.1

of manganese (25.3 mg kg^{-1} d.m.) while two-year-old plants had the least of this element (17.4 mg kg^{-1} d.m.). Moreover, three-year-old plants contained more iron than one- and two-year-old plants.

CONCLUSIONS

1. The plant age had a significant effect on the content of dry matter. The highest amount of dry matter was assessed in leaves of one-year-old plants, while the least dry matter was contained in three-year-old plants.

2. It has been demonstrated that the plant age has a significant effect on the content of macro- and microelements. Leaves of one-year-old plants contained the highest amounts of potassium, sulphur, copper and zinc. Two-year-old plants were characterized by a high content of magnesium and calcium, while three-year-old plants contained the highest amounts of phosphorus and manganese.

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REVIEWER PAPER

CALCIUM AND PHOSPHORUS IN MEDICINE AND TREATMENT

Agata Maziarka, Kazimierz Pasternak

Chair and Department of Medical Chemistry
Medical University of Lublin

Abstract

Calcium and phosphorus are essential for skeletal mineralization and perform a wide array of other biological functions. Calcium is a prime intracellular signalling molecule. It is also involved in muscle contractions (including the myocardium), digestion and blood coagulation. (Theobald 2005) Phosphorus is an intrinsic constituent of many organic substances such as nucleotides, nucleosides, phosphoamino acids and proteins, phospholipids, phosphoglycans and others (Huang, Moe 2011). In addition, it plays a critical role in cellular signalling through phosphorylation of proteins and other substances (Shaikh et al. 2008).

The present paper focuses on the role of these two elements in skeletal mineralization, and their use in treatment and medicine. First, it briefly discusses the calcium and phosphorus homeostasis, which occurs in three major organs: kidneys, intestines and bones, and involves an integrated hormonal system which maintains their normal serum levels. Moreover, disorders relevant to their abnormally high levels, hypercalcaemia and hyperphosphataemia, and excessively low levels, hypocalcaemia and hypophosphataemia, are described. Additionally, the physiology and pathology of bone as a prime store of both calcium and phosphorus are presented. Adequate intakes of these nutrients are essential for achieving the peak bone mass (PBM). A negative balance results from age-related bone loss, hence possible osteoporosis. This skeletal disorder is characterized by compromised bone strength, thus predisposing to an increased risk of fractures. One of the pharmacological interventions used in the treatment of osteoporosis is the administration of bisphosphonates. They inhibit the action of osteoclasts, prevent further bone losses and improve it strength (Ferrone, Geraci 2007). In the final part of the article, calcium phosphate based-ceramics, such as coralline hydroxyapatite, calcium hydroxyapatite (HAp), tri-calcium phosphate (TCP), bioglass, calcium phosphate cements (CPC) and their clinical applications are described.

Agata Maziarka, MSc., Chair and Department of Medical Chemistry, Medical University of Lublin, Chodźki 4a, 20-093 Lublin, Poland, e-mail: agatamaziarka@gmail.com

Key words: calcium, phosphorus, hypercalcaemia, hypocalcaemia, hyperphosphataemia, hypophosphataemia, osteoporosis, bisphosphonates, ceramics.

WAPŃ I FOSFOR W MEDYCYNIE I LECZNICTWIE

Abstrakt

Wapń i fosfor są niezbędne nie tylko dla mineralizacji kośćca, ale również w wielu procesach biologicznych. Wapń jest podstawowym przekaźnikiem informacji wewnątrzkomórkowej. Zaangażowany jest on również w skurcze mięśni (w tym mięśnia sercowego), trawienie oraz krzepnięcie krwi. Fosfor jest nieodłącznym składnikiem wielu substancji organicznych, takich jak: nukleotydy, nukleozydy, kwasy fosfoaminowe oraz białka, fosfolipidy, fosfoglikany itp. Ponadto wskutek fosforylacji białek i innych substancji odgrywa kluczową rolę w sygnalizacji komórkowej.

Praca skupia się na ich roli budulcowej oraz zastosowaniu w lecznictwie i medycynie. Na wstępie krótko przedstawiono homeostazę wapnia i fosforu, która dotyczy trzech głównych narządów: nerki, jelita i kości oraz obejmuje zintegrowany system hormonalny utrzymujący ich prawidłowe stężenie w osoczu. Ponadto opisano zaburzenia związane z ich nieprawidłowo wysokim – hiperkalcemia, hiperfosfatemia, oraz niskim – hipokalcemia, hipofosfatemia poziomem. Dodatkowo przedstawiono fizjologię i patologię kości, jako głównego magazynu zarówno wapnia, jak i fosforu. Odpowiednie spożycie tych substancji odżywczych jest niezbędne do osiągnięcia szczytowej masy kostnej (PBM). Ujemny bilans kostny wynika ze związanej z wiekiem utraty tkanki kostnej i może prowadzić do powstania osteoporozy. To zaburzenie funkcjonowania układu kostnego charakteryzuje się zmniejszoną wytrzymałością kości, co jest związane ze zwiększoną skłonnością do złamań. Bisfosfoniany są jedną z możliwości interwencji farmakologicznych stosowanych w leczeniu osteoporozy. Hamują one działanie osteoklastów, a tym samym zapobiegają dalszej utracie tkanki kostnej i wzmacniają jej wytrzymałość. Na zakończenie opisano wraz z klinicznym zastosowaniem ceramiki fosforanowo-wapniowe: hydroksyapatyt koralowy, hydroksyapatyt wapnia (HAp), fosforan trójwapniowy (TPC), bioszkło oraz cementy fosforanowo- wapniowe (CPC).

Słowa kluczowe: wapń, fosfor, hiperkalcemia, hipokalcemia, hiperfosfatemia, hipofosfatemia, osteoporoza

INTRODUCTION

Calcium is the fifth and phosphorus the sixth most abundant element in the human body (HUANG, MOE 2011). An adult human body contains approximately 1200 g of calcium (THEOBALD 2005) and approximately 700 g of phosphorus in total (MOE 2008). Most of these elements (~99% of calcium and 80-90% of phosphorus) are found in bones, with smaller amounts stored in teeth. These two elements form an inorganic crystalline structure – hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. The remaining ~1% of calcium and 20-20% of phosphorus (10- 20%) are present in soft tissues and body fluids (THEOBALD 2005, SHAIKH et al. 2008).

The recommended dietary intake of calcium is from 1,000 to 1,500 mg d^{-1} by healthy individuals, depending on the age. The key sources of dietary calcium are milk and dairy products. In contrast to calcium, dietary phosphorus is present in abundance in most foods. It parallels dietary protein and is absorbed virtually twice as efficiently as dietary calcium. (BONJOUR 2009)

NORMAL PHYSIOLOGY

Calcium homeostasis

Calcium is metabolised mainly in kidneys, intestines and bones. It is regulated by the parathyroid hormone (PTH), its receptor PTHR, (POTTS, GARDELLA 2007), calcitriol (1,25 $(OH)_2$ vitamin D) and the vitamin D receptor (VDR). (JURUTKA et al. 2001)Extracellular ionized calcium levels are also maintained within the physiologic range owing to the calcium sensing receptor (CaR), which controls the PTH secretion. (THEMAN, COLLINS 2009) The reduction in plasma calcium inactivates CaR in the parathyroid cells, causing an increased PTH secretion, which acts on the PTHR. This results in an elevated calcium reabsorption in the kidney and net bone resorption. An increased secretion of calcitriol, which is also caused by the increased PTH, stimulates calcium absorption in the intestine through the activation of the VDR, decreased PTH secretion in the parathyroid glands and increased resorption in bones. An increase in serum calcium has contrary effects. (MOE 2008) This integrated hormonal response ensures the maintenance of normal serum calcium levels. It is strictly controlled within a narrow range, usually 8.5-10.5 mg dL^{-1} (2.12-2.55 mmol L^{-1}). (MAEDA et al. 2006)

Phosphorus homeostasis

Phosphorus metabolism is regulated by the fibroblast growth factor 23 (FGF-23) and the FGF receptor/Klotho complex (HUANG, MOE 2011) and, similarly to calcium homeostasis, by the PTH and PTHR. An increase in serum phosphorus causes FGF-23 secretion from the bone. The fibroblast growth factor 23 acts on the Na/Pi II co-transporters in renal cells, causing a decrease in phosphorus reabsorption. The FGF-23 also induces a decrease in the renal secretion of calcitriol, which is responsible for reducing the intestinal phosphorus absorption. The PTH has an opposite effect on 1,25 $(OH)_2$ vitamin D; its increase stimulates the secretion of calcitriol. A higher level of the PTH also decreases the renal phosphate reabsorption by acting on the renal Na/Pi II co-transporters. A decrease in serum phosphorus evokes contrary effects, leading to the restoration of normal levels of serum phosphorus (SHAIKH et al. 2008). Normal homeostasis maintains a serum phosphorus concentration between 2.5 to 4.5 mg dl^{-1} (0.81 to 1.45 mmol L^{-1}) (MOE 2008).

DISORDERS OF MINERAL METABOLISM

Hypercalcaemia

Hypercalcaemia occurs when the concentration of serum ionized calcium, which represents the biologically active fraction of total calcium, is abnormally increased. The most common symptoms caused by high serum calcium levels are gastrointestinal disorders, e.g. nausea, vomiting, constipation and abdominal pain. Hypertension and the shortening of the QT interval on an electrocardiogram are the cardiovascular effects. Hypercalcaemia can cause nephrogenic diabetes insipidus and lead to acute renal failure. In most cases, such disorders in calcium concentrations are caused by malignancy and hyperparathyroidism (Renkema et al. 2008). One of the pharmacological options used in the therapy of malignancy associated with hypercalcaemia is the intravenous administration of bisphosphonates. There are two such agents which have been approved in the United States, pamidronate and zoledronate. They induce osteoclast apoptosis. preventing bone resorption and therefore reducing the level of serum calcium (Drake et al. 2008).

Hypocalcaemia

Hypocalcemia results from a low ionized calcium concentration. Mild hypocalcaemia is asymptomatic. The periodontal numbness and carpopedal spasms are typical of large or abrupt changes in ionized calcium. In some cases, tetany might develop. Abnormally low calcium levels may be caused by precipitation into the extraskeletal tissue as well as some malignancies with blastic bone metastases. The hungry bone syndrome, which follows parathyroidectomy, induces a reduction in both ionized calcium and phosphorus concentration due to a sudden decrease in the PTH. Ionized calcium is also bound by phosphorus in acute hyperphosphataemia. There are two forms of intravenous calcium infusions which are recommended in symptomatic hypocalcaemia, calcium gluconate (10 ml vial = 94 mg elemental calcium) and calcium chloride (10 ml vial = 273 mg elemental calcium). Due to the risk of precipitation, this kind of treatment is not indicated for severe hyperphosphataemia. (Moe 2008) In asymptomatic hypocalcaemia, oral supplementation of calcium carbonate (which has 40% of calcium per tablet) is recommended (Nieves 2005).

Hyperphosphataemia

Hyperphosphataemia can be induced by increased intestinal absorption, cellular release or sudden intracellular to extracellular dislocations and reduced renal excretion. Persistent hyperphosphataemia is solely caused by acute or chronic kidney disease. High oral intake of phosphate-containing laxatives or enemas and vitamin D overdose induce an increase in the intestinal absorption. An acute tumour lysis syndrome, leukaemia as well as hyperthermia, haemolysis, rhabdomyolysis and profound catabolic stress increase the tissue release of phosphorus. Acute hyperphosphataemia typically does not cause symptoms, although the precipitation of phosphorus with calcium induces the symptoms of hypocalcaemia (Moe 2008). The treatment of hyperphosphataemia involves dietary phosphate limitation and oral phosphate binders such as calcium carbonate or calcium acetate (Rees, Shroff 2010).

Hypophosphataemia

Hypophosphataemia generally results from reduced intestinal absorption or increased gastrointestinal losses as well as excess renal wasting from renal tubular defects, extracellular to intracellular displacements and hyperparathyroidism. It is often classified as mild (<3.5 mg dl^{-1}), moderate (<2.5 mg dl^{-1}) and severe (<1.0 mg dl^{-1}). The haemolysis, rhabdomyolysis, impaired platelet and WBC function, muscle weakness and rarely neurologic disorders are the characteristic symptoms of moderate and severe hypophosphataemia. The most common causes of hypophosphataemia are antacid abuse, chronic diarrhoea, vitamin D deficiency, alcoholism, extracellular fluid volume expansion and primary hyperparathyroidism. In the treatment of moderate and severe hypophosphataemia, oral supplementation is more appropriate than intravenous phosphate preparations. After intravenous administration, phosphorus can complex calcium and induce extraskeletal calcification. Neutraphosph K capsules® or Neutraphosph® solution are the pharmacological oral intake options. (Moe 2008)

BONE PHYSIOLOGY AND PATHOLOGY

The skeleton is a dynamic organ which consists of long bones, vertebrae and the skull. The prime store for both calcium and phosphorus is bone. It contains a number of specialized cells and collagen fibres encrusted with crystalline material. Osteoblasts are responsible for the production of bone collagen fibres and other organic constituents of the matrix. These forms of fibroblast are engaged in the process of bone mineralisation and partially in the regulation of bone resorption. Osteocytes, which are mature osteoblasts, cannot create new bone because they are encased in mineralized bone. The third kind of bone cells are osteoclasts, i.e. macrophages derived from stem cells of bone marrow. They are situated on the surface of bone and induce bone resorption.

Each bone consists of two forms of bone tissue: compact and trabecular. The exterior parts of bones, especially the shafts of long bones, are built of compact bone, which is thick and dense. It plays the structural role and forms cortices. The interior parts of long bones are made up of a network of trabeculae, which have a metabolic function. This spongy trabecular bone tissue occurs primarily in vertebrae of the spine and in the pelvis. Compact bone constitutes about 80% of bone; the remaining 20% is trabecular (THEOBALD 2005).

All bones in the human body are continually remoulded throughout the lifespan. The bone remodelling process repairs the bones, hence strengthens the skeleton. This bone turnover consists of the balanced activity of osteoclasts, which induce bone resorption, and osteoblasts, which result in bone formation. Approximately 4% of cortical bone and 28% of trabecular bone undergo modification each year (FERRONE, GERACI 2007). Healthy skeletal growth of children ensures positive bone balance (formation > resorption). The peak bone mass (PBM) reached by healthy adults indicates neutral bone balance (formation = resorption). Negative bone balance (formation < resorption) is characteristic of elderly individuals and results in aged-related bone loss.

Osteoporosis

Osteoporosis is described as a skeletal disorder characterized by low bone mass and compromised bone strength, causing an increase in bone fragility and susceptibility (HONIG 2010), The diagnostic criteria for osteoporosis are based on the bone mineral content (BMC) or bone mineral density (BMD). BMD within 1 standard deviation (SD) of a young adult is considered normal, between -1 and -2.5 SD is diagnosed as osteopenia and more than -2.5 SD is considered as osteoporosis or established osteoporosis if, at the same mass definition, an osteoporosis-related fracture occurs. Fragility fractures, which are specific to osteoporosis, involve the vertebral body, distal forearm and proximal femur, although they can occur in various parts of the skeleton (CASHMAN 2007). The fractures resulting from osteoporosis induce an increase in morbidity and mortality. After the age of 50 years, the risk of such fracture in women is 40% and in men 15% (EASTELL 2005).

Adequate nutrition plays a crucial role in prevention and treatment of osteoporosis. Calcium, often in combination with vitamin D, and phosphorus are the nutrients of greatest significance.

Calcium in osteoporosis

A number of studies have demonstrated that higher calcium intake at every stage of life is associated with higher bone mineral density. Increased calcium intake can maximize the peak bone mass and optimize the rate of bone loss associated with aging (North American Menopause Society 2006, TANG et al. 2007, HUNCHAREK 2008).

An inadequate intake of calcium results in an increase in the PTH levels, which in turn causes an increase in bone remodelling, leading to a significant loss of bone and increased fracture risk. Elevations in the serum PTH and larger bone losses are usually associated with lower levels of vitamin D. Calcium given in sufficiently large doses may reduce elevated PTH levels and lower the rate of bone remodelling. Thus, when adequate quantities of dietary calcium cannot be provided, calcium supplementation is required. Calcium carbonate and calcium citrate should be used, optimally in doses <500 mg a day, because calcium absorption decreases with larger loads. Calcium is also better absorbed with food, hence most supplements should be taken with meals (Nieves 2005). Calcium in a dose of 1,000 mg day^{-1} in combination with vitamin D in a dose of 500 IU day^{-1} has been demonstrated to prevent hip fractures in elderly housebound patients (Eastell 2005). Moreover, calcium supplementation also delays bone losses in postmenopausal women. This effect on bone mass may be altered by factors such as the age, number of years since the menopause, baseline calcium intake before supplementation and physical activity. The benefits of taking calcium supplements may be greater in elderly and late postmenopausal women as well as in women with low baseline calcium intakes. Hormonal replacement therapy (HRT), which is one of the ways to prevent osteoporosis, appears to be more effective with calcium supplementation (Nieves 2005, Cashman 2007).

Phosphorus in osteoporosis

An adequate phosphorus intake is crucial for bone health. Hence, a decrease in serum phosphorus will restrict bone formation and mineralization. (Heaney 2004) On the other hand, an excessive intake of phosphorus, especially when combined with a low calcium intake, may be deleterious. In such cases, it may also lead to secondary hyperparathyroidism and bone loss. Phosphorus deficiency resulting from a low phosphorus intake or negative phosphorus balance due to food phosphorus bound to supplemental calcium can restrict the activity of osteoblasts and increase the bone resorption. Higher bone density is relevant to a diet adequate in calcium, moderate protein and sufficient phosphorus, hence the ratio of phosphorus to calcium is more significant than the intake of phosphorus alone. (Heaney, Nordin 2002)

Bisphosphonates

Phosphorus is a basic component of primary pharmacologic agents currently used against bone loss due to osteoporosis. Bisphosphonates are synthetic analogues of natural pyrophosphate, in which oxygen occupies the place of a carbon atom making the compounds resistant to hydrolysis. Their crucial feature is a very high affinity for bone mineral, especially at sites of active bone remodelling and consequent deposition. Bisphosphonates inhibit bone resorption by decreasing the recruitment and function of osteoclasts and increasing apoptosis (Gass, Dawson-Hughes 2006), Their anti-resorption activity peaks within approximately 3 months of a course of oral bisphosphonate therapy and remains fairly constant with the continuation of treatment. Intravenous (IV) bisphosphonate administration causes a more rapid decreasein bone resorption than oral bisphosphonate therapy. The duration of the inhibition mostly depends potency of bisphosphonates for binding hydroxyapatite crystals. All bisphosphonates are poorly absorbed in the gastrointestinal tract, which is due to their hydrophilic properties. Additionally, about 50% of the uptaken quantity is retained in the skeleton and the remaining amount is cleared from the circulation by renal excretion. Importantly, when oral bisphosphonate is administered, patients should remain upright for 30 minutes and avoid eating for 2 hours before and at least 30 minutes after taking a pill. Maintenaning an adequate calcium and vitamin D supply is also a key issue in the bisphosphonate therapy.

Possible pharmacological options include alendronate and risedronate, which can be administered once a week, and ibandronate and risedronate taken once a month. Less frequent dosing requires intravenous preparations such as pamidronate, ibandronate and zoledronic acid (Drake et al. 2008). Recently, bisphosphonates have become the major therapeutic solution, commonly chosen to treat not only osteoporosis but also Paget's disease (Reid et al. 2005), osteogenesis imperfecta (OI) (Akcay et al. 2008), hypercalcaemia and malignancy metastatic to bone (Clemons et al. 2006). However, the latest reports indicate that the use of bisphosphonate therapy is implied in pathologic conditions, including osteonecrosis of the jaw (Kramer, Fantasia 2011).

Calcium phosphate as ceramics

Calcium and phosphorus in a form of calcium phosphate-based ceramics have been used in dentistry and in orthopaedics since 1980s. These synthetic scaffolds have been proven to induce a biologic response similar to that of bone. Alone, the ceramics show minimal immediate structural support and do not have any osteogenic or osteoinductive properties. Once attached to healthy bone, the osteoid is produced directly onto the surfaces of the ceramic, thus it mineralizes and causes new bone remodelling.

Coralline hydroxyapatite

The naturally occurring porous structure made by calcium phosphate (coralline), which is similar to human cancellous bone, was found in certain coral species. These sources of hydroxyapatite of natural origin are transformed, yet maintaining their macroporous and interconnected structure. Hence, they provide an in-growth of host tissue upon implantation and diffusion of a nutrient throughout the graft material. These coralline-derived ceramics are known as Pro Osteon ® (Interpose Cross International, USA) and Biocoral ® (Biocoral, USA) (Kretlow et al. 2009).

Calcium hydroxyapatite (HAp)

Synthetically obtained hydroxyapatite (HA) is typically sintered at temperatures above 1,000°C and is a highly crystalline form of calcium phosphate. It has the formula $Ca_{10}(PO_4)_6(OH)_2$ with a calcium-to-phosphate atomic ratio of 1.67. Its chemical similarity to the mineralized phase of bone explains the osteoconductive and biocompatible properties. Synthetic hydroxyapatite is typically used as a coating for dental and orthopaedic implants (Nandi et al. 2010). Plasma spray HA has been applied to a metallic femoral stem and cup. It prevents complications caused by the use of the poly(methyl methacrylate -PMMA (Moroni et al. 2005). Hydroxyapatite-coated pins improve pin attachment regardless of the type of bone and loading conditions. They also depress the rate of infection and losses during external fixation (Nguyen et al. 2004).

Tri-calcium phosphate (TCP)

This synthetic material, $Ca_3(PO_4)_2$, is similar in chemical composition and crystallinity to the mineral phase of bone. Similarly to hydroxyapatite, the bioabsorbility and biocompatibility are also characteristic of TCP. There are two forms of tri-calcium phosphate, α and β-crystalline. In contrast to hydroxyapatite, which is non-resorbable under physiological conditions, β-TPC has been demonstrated to degrade within 6 weeks after implantation (Kretlow et al. 2009). Tri-calcium phosphate implants have been used for two decades in dental applications and in orthopaedics as synthetic bone void fillers (Shigaku, Katsuyuki 2005).

Bioglass

Bioactive glass ceramics are composed primarily of silica, sodium oxide, calcium oxide and phosphates. They bind to bone without an intervening fibrous connective tissue interface. This material is also biocompatible and osteoconductive (Dorozhkin 2009). Bioglasses have been used in clinical applications as filling material in benign tumour surgery, in obliteration of frontal sinuses, for tympanoplastic reconstruction, repair of orbital floor fractures, treatment of periodontal bone defects, lumbar fusion, reconstruction of facial bone defects as well as for reconstruction of iliac crest defects after bone graft harvesting (Nandi et al. 2010).

Calcium phosphate cements (CPC)

Generally, calcium phosphate cements are formed by combining dry powder (CaP) and a liquid component in a reaction which occurs under physiologic pH and temperatures. Dicalcium phosphate, dicalcium phosphate dihydrate, calcium-deficient hydroxyapatite and amorphous calcium phosphate have been used as the solid phase. As a liquid phase, an inorganic or organic acid, or else sodium phosphate solutions can be used. The injectability and ability to mould for variable periods before hardening are specific to calcium phosphate cements. At least three formulations of CPC have been approved by the Federal Drug Administration for clinical use: Norian ® (Synthes Craniomaxillofacial, USA), Mimix ® (Walter Lorenz Surgical, USA) and BoneSource ® (Stryker Leibinger, Germany) (Kretlow et al. 2009) They have been successfully used for clinical applications such as cranial defect repair (Ji, Ahn 2010).

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