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ORIGINAL PAPERS

EFFECT OF CULTURE CONDITIONS ON MAGNESIUM AND ZINC CONCENTRATIONS IN MUSCLES OF FRESHWATER FISH*

Ewa Brucka-Jastrzębska¹, Dorota Kawczuga¹, Mikołaj Protasowicki², Monika Rajkowska²

¹Chair of Physiology, University of Szczecin
²Chair of Toxicology
West Pomeranian University of Technology of Szczecin

Abstract

The aim of the study was to estimate the effect of culture conditions and culture site on magnesium (Mg) and zinc (Zn) concentrations in freshwater fish. The study encompassed dorsal muscles in five fish species: common carp (Cyprinus carpio L.), rainbow trout (Oncorhynchus mykiss Walbaum) and Siberian sturgeon (Acipenser baeri Brandt), northern pike (Esox lucius L.) and grass carp (Ctenopharyngodon idella Valenciennes).

A total of 125 fish comprised 25 individuals of each species, aged from 6, 9, and 12 months. The fish were cultured in privately owned fish breeding ponds (Western Pomerania, Poland). For chemical and biochemical assays, samples of dorsal muscles were taken from each fish. Tissue samples were wet mineralised in concentrated HNO₃ in a CEM MDS 2000 microwave oven. Magnesium and zinc concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP-MS) in a Jobin Yvon type JY-24 apparatus. The pursuit of the research we had an approval of the Polish Local Ethics Committee nr 9/05. The magnesium concentration in the dorsal muscles ranged from 95.3⁻347.6 mg kg⁻¹ w.w. The highest Mg concentration was found in rainbow trout.
Zinc concentration varied from 6.7±98.8 mg kg⁻¹ w.w. The highest was found in the muscles of Siberian sturgeon (98.8±0.4 mg kg⁻¹ w.w.), and the lowest in rainbow trout (6.7±0.7 mg kg⁻¹ w.w.). It was found that the breeding place significantly affected the Zn and Mg concentrations in the muscle tissue among individual freshwater fish species. The magnesium and zinc concentrations were also significantly affected by the type of feed.

Key words: common carp, rainbow trout, Siberian sturgeon, northern, grass carp, zinc, magnesium.

INTRODUCTION

Breeding conditions, including feeding regime, have a significant effect on the development of fish. In the growth and development of both terrestrial and aquatic animals, culture conditions play one of the keys roles. In the natural environment, there are many various chemicals but most of them, despite being in direct contact with live organisms do not penetrate inside them in significant amounts. Due to human actions, we can observe...
an increasing man-made impact on the environment. It disturbs the homeostasis of aquatic environments, which may lead to some disturbance of the balance between fish.

During the evolution, countless relationships have been developed between organisms and their environment. When those relationships are disrupted by altered environmental conditions, diseases or even death of an organism may occur. Elements of the environment such as water, air and food provide organisms with essential components, but at the same time they may be sources of xenobiotics and harmful substances which are capable of causing impairment of life functions in organisms. Human activity exerts increasing pressure on the environment, which results in elevated pollution levels in aquatic and terrestrial ecosystems. By living in aquatic environment, fish are particularly exposed to anthropogenic pressure.

Monitoring micro and macro-elements levels in fish is a significant diagnostic research as it shows the physiological condition of organisms. Concentration of some mineral elements in fish depends mainly on the culture and water type in which the fish are bred, as well as the season of the year and the pastures the fish are fed. All the elements affect the homeostatic behavior of fish, which may vary due to some excess or deficiency of any of the factors. Excessive or deficient value of a factor may lead to serious disorders in biochemical processes, which may result in many diseases.

The aim of this study was to evaluate the effect of culture conditions and culture site on levels of a microelement (Zn) and macroelement (Mg) in five species of freshwater fish: rainbow trout (*Oncorhynchus mykiss* Walbaum), common carp (*Cyprinus carpio* L.), Siberian sturgeon (*Acipenser baeri* Brandt), northern pike (*Esox lucius* L.) and grass carp (*Ctenopharyngodon idella* Valenciennes). The study encompassed dorsal muscles in fish.

**MATERIAL AND METHODS**

The study involved 125 individuals of freshwater fish reared in commercial fish farms in West Pomeranian Province, Poland. The fish were represented by 25 individuals of each of the five species: rainbow trout, common carp, Siberian sturgeon, northern pike and grass carp. The research had been approved by the Polish Local Ethics Committee nr 9/05.

The fish aged from 6 to 12 months, weighed from 176.5 to 615.4 g and measured from 20.2 to 35.7 cm. The fish were collected three times: in December, April and August.

The fish were fed Aller Aqua pelleted feeds (Table 1), each species with an appropriate feed type, and a mixture of oats and rape. All the fish feed products are produced by extrusion. Fish feed must cover the basic metabo-
lism of fish and ensure healthy growth. In order to meet these requirements, the fish feed composition must meet all needs for nutrients, vitamins (A, E and D3) and minerals. The daily food ration was 3.4±0.8 g per fish. The fish were fed twice a day.

Table 2 presents chemical and biochemical parameters of water in which the fish were kept.

Table 1

<table>
<thead>
<tr>
<th>Researched parameters of feed</th>
<th>Examined fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>common carp</td>
</tr>
<tr>
<td>Name of feed</td>
<td>Aller classic</td>
</tr>
<tr>
<td>Largeness of feed (mm)</td>
<td>5.0</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>35.0</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>9.0</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>43.0</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>7.0</td>
</tr>
<tr>
<td>Fiber (%)</td>
<td>5.0</td>
</tr>
<tr>
<td>All-out energy (Gross energy) (Kcal/MJ)</td>
<td>4325/18.1</td>
</tr>
<tr>
<td>Energy digestible (Kcal/MJ)</td>
<td>3353/14.0</td>
</tr>
<tr>
<td>Nitrogen (d.m. %)</td>
<td>5.2</td>
</tr>
<tr>
<td>Phosphorus (d.m. %)</td>
<td>1.3</td>
</tr>
<tr>
<td>Energy in dry matter (Kcal/MJ)</td>
<td>4701/19.6</td>
</tr>
</tbody>
</table>

The fish behaviour and appearance were recorded. Intravital examination involved observation of fish behaviour, assessment of rearing conditions, as well as evaluation of the quality and general appearance of fish skin, fins, eyes and gills. Post mortem examination involved autopsy to verify if there were any anatomical or pathological changes in internal organs.

For chemical analysis, samples of dorsal muscles were collected from each fish. The collected material was stored at -20 °C. Prior to analysis, 1-g subsamples of organs, weighed to the nearest 0.001 g, were wet mineralized in 3 cm³ HNO₃ in a CEM MDS 2000 microwave oven. The solutions were quantitatively transferred to polyethylene vials and brought up to 25 g with deionised water. Magnesium (Mg) and zinc (Zn) were determined with inductively coupled plasma atomic emission spectrometry (ICP-AES) in a JY-24 Jobin Yvon apparatus. Tissue concentrations of metals were reported as mg kg⁻¹ wet weight (mg kg⁻¹ w.w.).
The results were subjected to statistical treatment with the Statistica 6.0 software. Analyses of variance (ANOVA) were performed at the significance level of $P = 0.05$.

**RESULTS AND DISCUSSION**

Increasing industrial and agricultural production has resulted in a rising number of systems affected by contaminants present in discharged wastewater. For example, heavy metals (e.g. Cu, Zn) are known to accumulate in organs of fish (Balint et al. 1997). These metals pollute aquatic and terrestrial ecosystems, adversely affecting the environment and inhabiting organisms. High concentration of metals in fish tissues can lead to redox reac-
tion, generating free radicals, especially reactive oxygen species (DAUTREME-EPUITS et al. 2002). These highly reactive compounds may induce tissue alterations and change some physiological responses of fish (PARIS-PALACIOS et al. 2000, VARANKA et al. 2001). Aquatic organisms are more sensitive to exposure and toxicity compared to terrestrial organisms, including mammals, and in this respect they may provide experimental data for evaluation of subtle effects of oxidative stress, mutagenicity and other adverse effects of pollutants (VALAVANIDIS et al. 2006). The influence of high temperature on aquatic biocenoses manifests in an increase in the biological production rate and also in the shortening of lifecycles of aquatic organisms, which die in large numbers due to lack of synchronization with climate rhythms. This results in accumulation of organic matter and increased biological oxygen demand, along with a decrease in oxygen solubility and availability.

Nutritional studies have shown that minerals may play a crucial role in preventing oxidative stress. Increases or decreases in their concentrations may disrupt internal homeostasis and produce various pathological conditions. Toxic effects of metals on different tissues and organs involve structural damage and functional disorders, which may be reflected in changes in blood composition and levels of ions, proteins, hormones or glucose and its metabolites, as well as in changed enzyme activities.

Intravital and post mortem examination showed no changes in fish behaviour, as well as in their external and internal appearance. Comparison of water parameters (Table 2) revealed only slight differences among the five fish culture sites.

The research has shown that Mg and Zn concentrations have changed during the growth of fish. An average magnesium content ranged from 95.3 to 347.6 mg kg\(^{-1}\) w.w. (Table 3). The highest magnesium levels were detect-

<table>
<thead>
<tr>
<th>Fish research</th>
<th>Mg (mg kg(^{-1}) w.w.)</th>
<th>Month of fish life</th>
<th>Statistically significant differences P ≤ 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Common carp</td>
<td>103.6 ± 13.6</td>
<td>168.6 ± 35.7</td>
<td>147.6 ± 18.2</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>287.6 ± 42.5</td>
<td>321.6 ± 28.9</td>
<td>347.6 ± 32.2</td>
</tr>
<tr>
<td>Siberian sturgeon</td>
<td>127.6 ± 27.2</td>
<td>134.6 ± 16.8</td>
<td>143.8 ± 21.2</td>
</tr>
<tr>
<td>Northern pike</td>
<td>95.3 ± 11.3</td>
<td>98.7 ± 5.6</td>
<td>103.3 ± 7.3</td>
</tr>
<tr>
<td>Grass carp</td>
<td>95.3 ± 11.3</td>
<td>96.7 ± 5.6</td>
<td>98.3 ± 13.3</td>
</tr>
</tbody>
</table>

\(^a\)w.w. – wet weight, SD – standard deviation; A – statistically significant differences P ≤ 0.05; ns – no significant differences
ed in dorsal muscles of rainbow trout (347.6±32.2 mg kg\(^{-1}\) w.w.). The lowest magnesium levels were found in dorsal muscles of grass carp (95.3±11.3 mg kg\(^{-1}\) w.w.). An average zinc content ranged from 6.7÷98.8 mg kg\(^{-1}\) w.w. (Table 4). The highest zinc levels were detected in dorsal muscles of Siberian sturgeon (98.8±0.4 mg kg\(^{-1}\) w.w.). The lowest zinc levels were found in dorsal muscles of rainbow trout (6.7±0.7 mg kg\(^{-1}\) w.w.). It was found that the breeding site significantly affected the Zn and Mg concentrations in muscle tissue among individual freshwater fish species. The magnesium and zinc concentrations were also significantly influenced by the type of feed. It was noticed that in the fish (common carp, Siberian sturgeon and rainbow trout) fed Aller Aqua pellet pasture, the Zn and Mg concentrations were higher compared to the fish (northern pike and grass carp) fed oat and oilseed rape blend. We have also found that the Mg concentration in all the examined fish was higher than the Zn concentration. The results allow us to state that the breeding site, breeding conditions and the feeding type have a significant effect on the Mg and Zn levels in muscle tissue of the examined fish.

Magnesium was distributed in fish bodies according to the following pattern of decreasing concentrations: grass carp > northern pike > common carp > Siberian sturgeon > rainbow trout. Zinc was distributed in fish bodies according to the following pattern of decreasing concentrations: rainbow trout > grass carp > common carp > northern pike > Siberian sturgeon. Many authors have reported considerably higher levels of these elements in muscles of freshwater and marine fish (PuJin et al. 1990, Kargin 1996, Grosseva et al. 2000, Jurkiewicz-Karnakowska 2001).

### Table 4

<table>
<thead>
<tr>
<th>Fish research</th>
<th>Zn (mg kg(^{-1}) w.w.)</th>
<th>Statistically significant differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>month of fish life</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Common carp</td>
<td>mean ± SD</td>
<td>mean ± SD</td>
</tr>
<tr>
<td></td>
<td>25.3 ± 5.6</td>
<td>26.7 ± 3.9</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>6.7 ± 0.7</td>
<td>19.7 ± 5.3</td>
</tr>
<tr>
<td>Siberian sturgeon</td>
<td>93.7 ± 5.6</td>
<td>98.8 ± 0.4</td>
</tr>
<tr>
<td>Northern pike</td>
<td>37.1 ± 5.6</td>
<td>41.2 ± 5.6</td>
</tr>
<tr>
<td>Grass carp</td>
<td>20.7 ± 3.9</td>
<td>20.1 ± 2.6</td>
</tr>
</tbody>
</table>

*W.w. – wet weight, SD – standard deviation; A – statistically significant differences P≤0.05; ns – no significant differences
Zinc is weakly accumulated in fish tissues, as it is retained in the gills, where the metal is deposited in large amounts (Witeska 2003). This may be explained by the fact that it penetrates into blood less easily than other metals (cadmium, nickel). In turn, changes in zinc levels in the examined tissues resulted from its affinity to erythrocyte membranes (Barron, Adelman 1984) and serum proteins (Bettger et al. 1987) that participate in its transport. Zinc is transported mainly as zinc-albumin and zinc-macroglobulin complexes, and is excreted mainly in the faeces (70-80%). Zinc displays low toxicity to freshwater fish. Its adverse influence is mainly connected with secondary deficit of copper and does not produce any specific symptoms. Zinc absorption by animals is influenced by food quality and interactions among zinc and other elements. A metabolically significant antagonism occurs between zinc and cadmium, as well as between Zn and Cu. Additionally, calcium and magnesium may reduce zinc absorption.

Levels of some bioelements in fish bodies depend on culture methods, water chemistry, and season of the year and feed quality. All these factors together influence the physiological condition of fish, which can be disturbed by excess or deficiency of minerals. Excess or deficiency of minerals may seriously disturb biochemical processes and upset internal homeostasis, leading, in consequence, to various diseases. Tacon (1992) reported that disorders occurred in organisms of various fish species due to deficiency or excess of micro- and macroelements which were caused by improper nutrition,avitaminosis or poisoning. It is therefore important to monitor levels of macro- and microelements in fish organisms.

Among the examined freshwater fish species, statistically significant differences in the levels of macro- and microelements were observed. The analyzed bioelements (Zn, Mg), which are regarded as some of the most important macro- and microelements, were reported to accumulate in excess in disease conditions caused by bacterial and viral infections, as well as during an increased activity of hepatocytes (Pouramahad, O'Brien 2000, Lushchak et al. 2005). Levels of microelements recorded in this study were not high (Tables 3, 4) and remained within the normal range for salmonids (Salmonidae) and cyprinids (Cyprinidae). For the sturgeon family (Acipenseridae), an accurate normal range of macro- and microelements has not been determined.

Magnesium is the 11th most abundant element by mass in the vertebrate body. Its ions are essential to all living cells, where they play a major role in manipulating important biological polyphosphate compounds like ATP, DNA, and RNA. Over 300 enzymes require the presence of magnesium ions for their catalytic action, including all enzymes utilizing or synthesizing ATP, or those which use other nucleotides to synthesize DNA and RNA. Normally, ATP exists in cells as a chelate of ATP and a magnesium ion. Magnesium plays a regulatory role in prooxidant and antioxidant processes (Lopez-Tores et al. 1993, Ozmen et al. 2004). In our research, we have observed that
magnesium concentration in fish muscles increased with the age of fish. We have also found statistically significant differences between dorsal muscles during the growth of rainbow trout and Siberian sturgeon. Oikari et al. (1985) have shown that infusion of magnesium salt into the body cavity of a freshwater-adapted fish (rainbow trout) affects the magnesium concentration in the plasma. Magnesium could either be reabsorbed or secreted in control freshwater-adapted trout, apparently as a function of nutritional status. Fish could switch from reabsorption to secretion in response to magnesium loading. It is suggested that freshwater fish eliminate excess dietary magnesium renally (Oikari et al. 1985). Variability of metal concentrations in freshwater fish must be seen in the wide perspective of other variables such as habitat, seasonal variations, age of fish, Fulton’s condition factor and individual ability for metal uptake (Allen 1993, Canli, Atli 2003, Lopez-Tores et al. 1993, Martinez-Alvarez et al. 2005, Ritola et al. 2002, Svobodova et al. 1997).

Zinc has both catalytic and structural roles in enzymes and its antioxidant properties have been widely recognized (Powell 2000). However, zinc deficiency was reported not to induce hepatic degeneration in trout liver (Ogino, Yang 1978). Anyway, Hidalgo et al. (2002) have demonstrated that dietary Zn deficiency induced oxidative stress in rainbow trout liver, with changes in the SOD band pattern. In the fish examined hereby, zinc levels remained within the physiological reference range reported by Protasowicki and Chodyniecki (1988). Zinc is accumulated in fish tissues to a small extent only, as most of the element is absorbed by gills, where it accumulates in considerable amounts. Zinc levels similar to the ones recorded in this study were reported by Yoshitomi et al. (1998) and Areechon and Plumb (1990). Statistically significant differences were found in the zinc concentration during the growth of northern pike, common carp and rainbow trout.

Fish are characterized by species and seasonal changeability of micro- and macroelements. Sopinska (1985) and Stosik and Deptula (2000) found changes in zinc and magnesium concentrations due to the season of the year and the change of lymphocytes level in the examined fish. According to these authors, such changes resulted form a close relationship of the season and the sun exposure. In many other studies, it has been shown that Mg and Zn levels were different depending on the temperature, season, sex, feeding type and culture type. (Blaxhall 1972, Thomas et al. 1999).

We have found that feeding common carp, Siberian sturgeon and rainbow trout Aller Aqua pellet pasture affected concentration of the analyzed elements.

The results have shown that the content of the analyzed elements was within the physiological reference ranges for fish. The differences found in the levels of the bio-elements resulted from individual and seasonal variability typical of the fish. Owing to their environmental requirements, fish may be regarded as indicators that supply information on the degree of pollution of aquatic environments.
CONCLUSIONS

1. The culture site was found to have statistically significant influence on the magnesium and zinc concentrations in muscle tissue in the examined freshwater fish species.

2. We have found that feeding common carp, Siberian sturgeon and rainbow trout Aller Aqua pellet pasture affected concentrations of the analyzed elements.

3. The differences in concentrations of the elements are a result of individual differences between species.

4. Zn and Mg concentrations in muscle tissue of the examined freshwater fish were significantly influenced by the experimental factors.

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EFFECT OF FERTILIZATION ON YIELD AND QUALITY OF CULTIVAR KENT STRAWBERRY FRUIT

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Abstract

In an experiment carried out in 2006-2007, influence of different fertilizers on yield and quality of cv. Kent strawberry fruit was determined. Two combinations were tested, each consisting of 3 types of fertilizers. The control plants remained unfertilized. In both combinations, two types of multi-component fertilizers were used (T – 5% N, 5% P₂O₅, 15% K₂O and O – 10% N, 5% P₂O₅, 10% K₂O) as well as one rate of ammonium nitrate to provide 50 kg N ha⁻¹ in the first combination and 70 kg N ha⁻¹ in the second one. The usage of multi-component fertilizers, especially O type resulted in an increase of cv. Kent strawberry yield. The fruit collected from the control plants and the ones fertilized with ammonium nitrate weighed less than berries obtained from plants fertilized with multi-component fertilizers. The treatment with multi-component fertilizers enhanced firmness as well as calcium, phosphorus and potassium content in fruit. The berries fertilized with higher rate of T fertilizer contained more soluble solids, total sugar and reducing sugar. Neither the type of fertilizers, nor their rate affected acidity, vitamin C, total phenol and magnesium content in berries. Higher antioxidant activity towards DPPH radicals was observed in fruit obtained from plants fertilized with lower and higher rate of ammonium nitrate. The lowest nitrogen content was observed for control berries. Practically, the differences regarding nitrogen content between the fruits of the first (50 kg N ha⁻¹) and second combination (70 kg N ha⁻¹) were negligible.

Key words: strawberry, multi-component fertilizers, fruit quality, chemical composition.
Wpływ nawożenia na wysokość i jakość plonu truskawek odmiany Kent

Abstrakt

W doświadczeniu przeprowadzonym w latach 2006-2007 określano wpływ nawozów o zróżnicowanym składzie chemicznym na plonowanie oraz jakość owoców truskawki odmiany Kent. Na tle kontroli badano 2 warianty nawożenia mineralnego, z których każdy obejmował 3 kombinacje nawozowe. W obu wariantach zastosowano posypowo dwa rodzaje nawozów wieloskładnikowych (T – 5% N, 5% P₂O₅, 15% K₂O oraz O – 10% N, 5% P₂O₅, 10% K₂O) oraz jedną dawkę saltry amonowej w taki sposób, aby dostarczyć w 1. wariantie doświadczenia 50 kg N ha⁻¹, a w 2. wariantie 70 kg N ha⁻¹. Stosowanie nawozów wieloskładnikowych, zwłaszcza typu O, spowodowało wzrost plonowania truskawki odmiany Kent. Owoce z roślin kontrolnych oraz nawożonych saltryą miały mniejszą masę niż uzyskane z zastosowaniem nawozów wieloskładnikowych. Nawozy wieloskładnikowe wpłynęły na zwiększenie jądrości i zwiększenie koncentracji wapnia, fosforu i potasu w owocach. Owoce z roślin nawożonych wyższą dawką nawozu T odnosiły się większą zawartością ekstraktu, cukrów ogółem i cukrów redukujących. Nie stwierdzono istotnego wpływu zastosowanego nawożenia na kwasowość owoców, zawartość witaminy C, polifenoli ogółem i magnezu. Wyższą aktywnością przeciwutleniającą wobec rodników DPPH odnosiły się owoce pochodzące z roślin nawożonych niższą i wyższą dawką saltry amonowej. Najmniejszą zawartość azotu miały owoce kontrolne, natomiast różnice w zawartości azotu między owocami z wariantu 1. (50 kg N ha⁻¹) i wariantu 2. (70 kg N ha⁻¹) z praktycznego punktu widzenia nie były istotne.

Słowa kluczowe: truskawka, nawozy wieloskładnikowe, jakość owoców, skład chemiczny.

INTRODUCTION

Mineral fertilization as well as cultivar, weather conditions, agronomic practice and water supply affect directly the quality of strawberry fruit (NESTBY et al. 2004). Optimal fertilization is conducive to obtaining high yield of good quality and high biological value (TREDER 2001). It is recommended to adjust fertilization to soil type, planting age and vegetation period. In spring time, strawberry plants show an increased demand mainly for nitrogen and potassium, which continues to rise during the blossom and fruit-setting phases (CIEŚLIŃSKI 2005). During the harvest, the demand for these elements declines, only to grow again afterwards (SZCZYGIEL, PIERZGA 2004).

The aim of the study was to estimate the influence of multi-component fertilizers on yield and quality of cv. Kent strawberry fruit.

MATERIAL AND METHODS

The experiment was carried out in 2006-2007 at the Pomological Experimental Station in Rajkowo, near Szczecin. Frigo seedlings of cv. Kent were
planted in spring 2006 in a raised-bed system at 20x80 cm spacing, in three replicates, with 20 plants per plot in a split-block design. The plants were irrigated using a T-Tap dripping line. The multi-component fertilizers and ammonium nitrate were applied so as to supply a suitable amount of nitrogen to soil: 1 – 50 kg N ha\(^{-1}\), 2 – 70 kg N ha\(^{-1}\), and K\(_0\) – control plots without fertilization.

Combination 1

K\(_1\) – N-50 kg ha\(^{-1}\) (ammonium nitrate);
T\(_1\) – 150 kg ha\(^{-1}\) (5% N, 5% P\(_2\)O\(_5\), 15% K\(_2\)O) + 42.5 kg N ha\(^{-1}\) (ammonium nitrate);
O\(_1\) – 200 kg ha\(^{-1}\) (10% N, 5% P\(_2\)O\(_5\), 10% K\(_2\)O) + 30 kg N ha\(^{-1}\) (ammonium nitrate);

Combination 2

K\(_2\) – 70 kg ha\(^{-1}\) (ammonium nitrate)
T\(_2\) – 300 kg ha\(^{-1}\) (5% N, 5% P\(_2\)O\(_5\), 15% K\(_2\)O) + 55 kg N ha\(^{-1}\) (ammonium nitrate);
O\(_2\) – 400 kg ha\(^{-1}\) (10% N, 5% P\(_2\)O\(_5\), 10% K\(_2\)O) + 30 kg N ha\(^{-1}\) (ammonium nitrate).

For the all plants under experiment, the yield per plot was determined each year. Moreover, one-fruit weight (0.01 g accuracy) and fruit firmness were measured with a FirmTech 2 apparatus (BioWorks, USA). The firmness of 100 randomly selected berries from each replicate was expressed as a gram-force causing fruit surface to bend 1 mm. Further, titratable acidity was determined by titration of a water extract of fruit homogenate with 0.1 N NaOH to an end point of pH 8.1 (measured with an Orion 720 A pH meter, USA). Soluble solids content was determined with an Abbé refractometer. L-ascorbic acid content was measured with the iodometric method (SAMOTUS et al. 1982) and expressed as mg per 100 g fruit. Total sugar and reducing sugar content was measured by Luff–Schoorl method and saccharose content was calculated (DRZAZGA 1997). The DPPH radical scavenging activity was measured spectrophotometrically at 517 nm (YEN, CHEN 1995), and DPPH percent inhibition was calculated according to ROSSI et al. (2003). Total phenol content was determined by the spectrophotometric metod at 760 nm, using Folin-Ciocalteu reagent and gallic acid as a standard.

The fruits were collected from each harvest and deep-frozen. After the harvest, all the samples were aggregated. Chemical analyses of macronutrients (N, P, K, Ca, Mg) were performed according to the Polish Standards.

The results were subjected to one-way analysis of variance for each year of the experiment. The means were separated by Duncan’s test at significance level \(P = 0.05\).
RESULTS AND DISCUSSION

Yield is one of the basic factors which determine profitability of production, although the quality of yield is also important. The quality of strawberry yield is a product of fruit size, firmness and chemical composition (PELAYO et al. 2003). The experiment showed that the best yields were obtained after fertilizer type O had been used. The total yield per plant expressed as a sum for 2006 and 2007 exceeded 0.4 kg (Table 1). However, in the experiment carried out by MAKOWSKA et al. (2005) cv. Kent strawberries yielded 25% better. The treatment with multi-component fertilizers resulted in obtaining fruit of the highest 100-fruit weight (Table 1). The firmness is a feature directly affecting fruit quality while calcium is a essential element for the development of hardness (TREDER 2004). In turn, potassium affects water balance in plants (CIEŚLIŃSKI 2003). In this study, the highest firmness was found for fruit collected from the plots fertilized with multi-component fertilizers (171-193 G mm⁻¹) – Table 1. These berries were found to contain increased potassium and calcium levels compared to the berries from the plots fertilized only with ammonium nitrate.

The two-year-long observation showed that an average soluble solids content in cv. Kent strawberries varied from 8.15% (control) to 8.85% (T₂) – Table 2. ZHENG et al. (2003) determined similar content of soluble solids for cv. Allstar cultivar (8.1%). In our experiment, more total sugar and reducing sugar were found in the fruit collected from the plots fertilized with the highest rates of multi-component fertilizers (T₂ and O₂), less in the control plants (K₀) or the ones fertilized with 50 kg of ammonium nitrate per ha (K₁) – Table 2. The total sugar content in 5 cultivars of ripe strawberries studied by CORDENUNSI et al. (2003) (37.3-58.8 mg g⁻¹), especially in the range of higher values, was similar to the data observed in this experiment (5.96-6.62 g 100 g⁻¹). The mean saccharose content in cv. Kent berries obtained from the plants fertilized with T₁ (1.46 g 100 g⁻¹) was higher than that of K₁ (1.14 g 100 g⁻¹), although neither differed significantly versus the berries from the other fertilization regimes. In the literature, there are reports that much greater differentiation in saccharose content in ripe fruit may

<table>
<thead>
<tr>
<th>Fertilization</th>
<th>K₀</th>
<th>K₁</th>
<th>T₁</th>
<th>O₁</th>
<th>K₂</th>
<th>T₂</th>
<th>O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total yield per plant (g)</td>
<td>366 ab</td>
<td>358 ab</td>
<td>375 ab</td>
<td>417 b</td>
<td>331 a</td>
<td>313 a</td>
<td>439 b</td>
</tr>
<tr>
<td>Mean weight of 100 fruits (g)</td>
<td>528 a</td>
<td>525 a</td>
<td>608 bc</td>
<td>591 abc</td>
<td>536 a</td>
<td>548 ab</td>
<td>645 c</td>
</tr>
<tr>
<td>Fruit firmness (G mm⁻¹)</td>
<td>163 ab</td>
<td>151 a</td>
<td>193 b</td>
<td>171 ab</td>
<td>148 a</td>
<td>186 b</td>
<td>179 b</td>
</tr>
</tbody>
</table>
occurred between the genotypes 0.59-22.75 g kg⁻¹ (KAFKAS et al., 2007). In this study, the fertilization did not exert an influence on acidity, vitamin C, and total polyphenol content in Kent berries (Table 2). On the other hand, the plants fertilized with both lower and higher rate of ammonium nitrate yielded berries of better ability to scavenge DPPH radicals compared to other treatments, except for control fruits (Table 2). In 3-year experiment SKUPIEN (2003) determined for Kent strawberries obtained from plantation of conventional growing procedure 8.7% of mean soluble solids content, 0.66 g malic acid 100 g⁻¹, 6.87 g 100 g⁻¹ total sugar, 5.99 g 100 g⁻¹ reducing sugar, 0.84 g 100 g⁻¹ saccharose, 41.1 mg 100 g⁻¹ vitamin C, and 218.3 mg 100 g⁻¹ total polyphenol.

### Table 2

<table>
<thead>
<tr>
<th>Item*</th>
<th>Fertilization</th>
<th>K₀</th>
<th>K₁</th>
<th>T₁</th>
<th>O₁</th>
<th>K₂</th>
<th>T₂</th>
<th>O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble solids (%)</td>
<td>8.15 a</td>
<td>8.60 ab</td>
<td>8.50 ab</td>
<td>8.25 a</td>
<td>8.55 ab</td>
<td>8.85 b</td>
<td>8.25 a</td>
<td></td>
</tr>
<tr>
<td>Total sugar (g 100 g⁻¹)</td>
<td>5.96 a</td>
<td>6.00 a</td>
<td>6.45 ab</td>
<td>6.23 ab</td>
<td>6.43 ab</td>
<td>6.62 b</td>
<td>6.61 b</td>
<td></td>
</tr>
<tr>
<td>Reducing sugar (g 100 g⁻¹)</td>
<td>4.63 a</td>
<td>4.80 a</td>
<td>4.91 ab</td>
<td>4.89 ab</td>
<td>5.04 ab</td>
<td>5.26 b</td>
<td>5.24 b</td>
<td></td>
</tr>
<tr>
<td>Saccharose (g 100 g⁻¹)</td>
<td>1.27 ab</td>
<td>1.14 a</td>
<td>1.46 b</td>
<td>1.27 ab</td>
<td>1.33 ab</td>
<td>1.30 ab</td>
<td>1.30 ab</td>
<td></td>
</tr>
<tr>
<td>Titratable acidity (g citric acid 100 g⁻¹)</td>
<td>1.11 a</td>
<td>1.15 a</td>
<td>1.11 a</td>
<td>1.09 a</td>
<td>1.13 a</td>
<td>1.13 a</td>
<td>1.11 a</td>
<td></td>
</tr>
<tr>
<td>Vitamin C (mg 100 g⁻¹)</td>
<td>47.1 a</td>
<td>48.3 a</td>
<td>46.5 a</td>
<td>41.8 a</td>
<td>44.9 a</td>
<td>44.7 a</td>
<td>42.2 a</td>
<td></td>
</tr>
<tr>
<td>Polyphenols (mg 100 g⁻¹)</td>
<td>405 a</td>
<td>452 a</td>
<td>424 a</td>
<td>415 a</td>
<td>440 a</td>
<td>432 a</td>
<td>437 a</td>
<td></td>
</tr>
<tr>
<td>DPPH % inhibition</td>
<td>24.0 bc</td>
<td>26.5 c</td>
<td>20.9 b</td>
<td>16.8 a</td>
<td>25.3 c</td>
<td>19.7 ab</td>
<td>20.7 ab</td>
<td></td>
</tr>
</tbody>
</table>

* The data is expressed in terms of fresh fruit.

### Table 3

<table>
<thead>
<tr>
<th>Fertilizer</th>
<th>Dry matter (%)</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₀</td>
<td>10.45 a</td>
<td>10.12 a</td>
<td>2.52 a</td>
<td>16.29 a</td>
<td>2.96 a</td>
<td>1.26 a</td>
</tr>
<tr>
<td>K₁</td>
<td>9.43 a</td>
<td>10.92 b</td>
<td>2.98 ab</td>
<td>16.58 ab</td>
<td>3.22 a</td>
<td>1.45 a</td>
</tr>
<tr>
<td>T₁</td>
<td>10.51 a</td>
<td>10.95 bc</td>
<td>3.21 ab</td>
<td>16.54 a</td>
<td>3.30 ab</td>
<td>1.28 a</td>
</tr>
<tr>
<td>O₁</td>
<td>9.86 a</td>
<td>11.64 c</td>
<td>3.26 b</td>
<td>17.14 bc</td>
<td>3.41 b</td>
<td>1.25 a</td>
</tr>
<tr>
<td>K₂</td>
<td>10.10 a</td>
<td>11.14 bc</td>
<td>2.84 a</td>
<td>16.04 a</td>
<td>3.17 a</td>
<td>1.39 a</td>
</tr>
<tr>
<td>T₂</td>
<td>10.45 a</td>
<td>11.56 bc</td>
<td>3.25 b</td>
<td>17.47 c</td>
<td>3.46 b</td>
<td>1.52 a</td>
</tr>
<tr>
<td>O₂</td>
<td>10.50 a</td>
<td>11.60 bc</td>
<td>3.40 b</td>
<td>16.93 bc</td>
<td>3.35 ab</td>
<td>1.31 a</td>
</tr>
</tbody>
</table>
The results presented in Table 3 show that fertilization did not affect dry matter (9.43-10.51%) and magnesium content (1.25-1.52 g kg\(^{-1}\)) in cv. Kent berries. OCHMIAN et al. (2008) determined a much higher magnesium content in cv. Senga Sengana strawberries exceeding 2.5 g kg\(^{-1}\). The use of multi-component fertilizers, especially O\(_1\) and O\(_2\) resulted in higher nitrogen (11.6 g kg\(^{-1}\)) and potassium (16.93-17.47 g kg\(^{-1}\)) content in fruit. The cultivar Senga Sengana berries were characterized by a higher nitrogen content, up to 28 g kg\(^{-1}\), but the potassium content was lower, 11-14 g kg\(^{-1}\) (OCHMIAN et al. 2008). Application of O and T type fertilizers enhanced the phosphorus content in comparison to the control berries, containing less than 3 g P per kg d. m. The multi-component fertilizers enhanced the calcium content in fruit. HAKALA et al. (2003) observed similar values of K and Mg but slightly lower for Ca (on fresh weight basis) for 12 cultivars of strawberries grown under traditional and organic conditions (1.64-2.53 g kg\(^{-1}\), 112-223 mg kg\(^{-1}\), and 171-223 mg kg\(^{-1}\), respectively).

**CONCLUSIONS**

1. Application of multi-component fertilizers, especially O type, caused an increase in the yield of cv. Kent strawberry.

2. The multi-component fertilizers improved firmness and increased the calcium content in berries.

3. Practically, the fertilization used in this experiment did not cause any considerable enhancement in the content of soluble solids, total sugar and reducing sugars in cv. Kent strawberries.

4. The fertilization applied did not affect acidity, vitamin C and total polyphenol content in the berries.

5. The fruit collected from the control plots and the plants fertilized only with ammonium nitrate showed higher ability of scavenging DPPH radicals than strawberries from the plants fertilized with multi-component fertilizers.

6. The use of multi-component fertilizers resulted in higher phosphorus and potassium concentration in fruit. The lowest nitrogen content was observed in the control fruit, while the differences between the berries from the first (50 kg N ha\(^{-1}\)) and second combination (70 kg N ha\(^{-1}\)) were not essential in practice.
REFERENCES


CONTENT OF MINERAL ELEMENTS
IN MILK AND HAIR OF COWS
FROM ORGANIC FARMS

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Abstract

The value of the routine analyses, applied until present, of whole blood, serum and urine for bioelements is limited. The blood mineral level does not often correspond to the content of minerals in the whole body because the composition of plasma results from supplementation of deficiencies by different homeostatic mechanisms. Moreover, the blood concentration of bioelements is relatively low and depends on a current diet, therefore the diagnostic value of such analytical results may be fairly small. Studies have shown that the analysis of hair and nails are an appropriate alternative for blood and urine tests or for biopsy. Chemical treatments in agriculture, animal production and food processing introduce many food contaminants into the food chain. Organic methods in agriculture are safer and therefore very important. Nutrition based on organically produced foods and anthroposophic lifestyle can play an important role in health prophylaxis. The objective of this study was to determine correlations between concentrations of 29 major and trace elements in cow's milk and hair. The experimental material consisted of 33 cows of Polish Holstein-Fresian (HF) breed from three dairy organic farms. All the farms were located in one climatic zone and under similar soil conditions. The cows were kept in traditional tied-up barns. The feeding was traditional, with ration components given separately. The cows were grazed from May to October. Depending on pasture yield and availability of other feeds, the feeding ration was supplemented with hay, straw, silage and cereals. Samples of milk and hair for analyses of minerals were collected in September, i.e., during the pasture feeding. The hair was taken from the poll. The concentration of Ca, K, Mg, Na, P, S, B, Ba, Co, Cr, Cu, Fe, Ge, I, Li, Mn, Mo, Ni, Se, Si, Sn, Sr, V, Zn, Al, As, Cd, Hg, Pb was determined. The content of toxic elements in milk was low and below the admissible level. The statistically significant positive correlations between concentration in milk and in hair were detected for such elements as Ba, Ge, Mo and Pb. In the case of major elements K and Mg and trace elements Al, As, Co, Fe, Hg, Se, Sr, positive correlations were observed but they were not statistically significant. Negative correlations occurred for such elements.
as Cr, Cu, I, Li, Ni, S, Si, Sn, V and Zn. It was only for V and Zn that they were statistically significant. Very low values (near zero) of coefficient r were observed for Ca, Cd, Li, Mn, Na, Ni, P, S and Sn. It seems that broader investigations of mineral composition of cow’s hair could be useful for establishing reference values for some elements and would make a contribution to better animals’ welfare.

Key words: cow, hair, milk, macro- and microelements, organic farms.

ZAWARTOŚĆ SKŁADNIKÓW MINERALNYCH W MLEKU I WŁOSACH KRÓW Z GOSPODARSTW EKOLOGICZNYCH

Abstrakt

Badanie zawartości biopierwiastków w pełnej krwi, surowicy i moczu ma ograniczoną wartość. Poziom składników mineralnych we krwi często nie odpowiada ich zawartości w całym organizmie, ponieważ skład osocza jest wynikiem kompensowania deficytów przez różne mechanizmy homeostazy. Poza tym zawartość biopierwiastków we krwi jest relatywnie niska i zależy od stosowanej diety. Wobec tego wartość diagnostyczna jej wyników, w dłuższym okresie prowadzenia obserwacji, może być ograniczona. Badania pokazują, że analizy zawartości biopierwiastków we włosach i paznokciach mogą być alternatywne dla analiz zawartości we krwi, moczu lub biopsji. Chemizacja rolnictwa, produkcji zwierzęcej i przetwórstwa żywności prowadzi wiele zanieczyszczeń w łańcuchu produkcji żywności. Ekologiczne (organiczne) metody w rolnictwie są bezpieczniejsze. Żywanie oparte na żywności produkowanej w gospodarstwach ekologicznych i proekologicznych styl życia mogą odkrywać ważną rolę w profilaktyce zdrowia ludzi. Celem badań było obliczenie korelacji między zawartością 29 makro- i mikroelementów we włosach a ich zawartością w mleku krów. Do badań wybrano 33 krowy rasy polskiej holsztyńsko-fryzyjskiej (hf) z trzech gospodarstw ekologicznych. Wszystkie gospodarstwa były położone w tej samej strefie klimatycznej i miały podobne warunki glebowe. Krowy korzystały z pastwiska od maja do października. W zależności od wydajności pasywnej i potrzeb krów dawka pokarmowa była uzupełniana o siano, słomę, kiszonkę i zboże. Próbki mleka i włosów do oznaczenia składników mineralnych pobrano we wrześniu, w trakcie żywienia pastwiskowego. Włosy pobrano z pasa międzyrożnego. W mleku i włosach oznaczono zawartość Cu, K, Mg, Na, P, S, B, Ba, Co, Cr, Cu, Fe, Ge, I, Li, Mm, Mo, Ni, Se, Si, Sn, Sr, V, Zn, Al, As, Cd, Hg i Pb. Poziomy elementów toksycznych w mleku okazały się niskie i leżały poniżej wartości dopuszczalnych. Korelacje między zawartością Ba, Ge, Mo, Pb we włosach i w mleku były dodatnie i statystycznie istotne. Korelacje między zawartością makroelementów K i Mg oraz mikroelementów Al, As, Co, Fe, Hg, Se, Sr były również dodatnie i wysokie, ale nieistotne statystycznie. Ujemne korelacje uzyskano między zawartościami Cr, Cu, I, Li, Ni, S, Si, Sn, V oraz Zn. Bardzo niskie wartości (bliskie zeru) współczynnika korelacji otrzymano dla zawartości Ca, Cd, Li, Mn, Na, Ni, P, S i Sn. Wydaje się, że dalsze badania składu mineralnego włosów u krów mogą być przydatne do oszacowania w przyszłości wartości referencyjnych dla niektórych pierwiastków i mogą się przyczynić do lepszego dobrostanu zwierząt.

Słowa kluczowe: krowy, włosy, mleko, makro- i mikroelementy, rolnictwo ekologiczne.
INTRODUCTION

Proper growth and functions of plants and animals require basic nutrients as well as mineral components. Out of more than a hundred elements occurring in nature, four organically bound elements (carbon, hydrogen, oxygen and nitrogen) make up 96% of the animal’s body weight. The principal cations and anions together account for 3.5% of the body weight, the remainder comprising additional elements. The progress in analytical methods has led to elucidation of the biological role of many elements occurring in plant, animal and human organisms. Mineral distribution within the body’s tissues is not uniform, since some tissues selectively concentrate specific elements. There is no disagreement concerning the essential nature of major and trace elements for livestock (McDowell 1992).

The value of the routine analyses, applied until today, of whole blood, serum and urine samples for bioelements is limited. The blood level of minerals does not often correspond to the content of minerals in the whole body because the composition of plasma results from supplementation of deficiencies by different homeostatic mechanisms. Moreover, the blood concentration of bioelements is relatively low and depends on a current diet, therefore the diagnostic value of such analytical results may be fairly small. Studies have shown that the analysis of hair and nails are an appropriate alternative for the analysis of blood and urine, and for biopsy. The diagnostic value of hair analysis is confirmed by many authors, who have demonstrated the presence of correlation between the levels of principal elements in hair and their content in the body, both at the physiological equilibrium and during pathological disturbances (Radomska et al. 1993, 2005). Milk is important for satisfying the nutritional demands of mammalian neonates. Data on minerals are limited to identifying the nutrients in milk required for optimal growth and health of mammalian species, including humans (Anderson 1992). Chemical treatments in agriculture, animal production and food processing introduce many food contaminants into the food chain. Organic methods in agriculture are safer and therefore very important. Nutrition based on organically produced foods and anthroposophic lifestyle can play an important role in health prophylaxis (Rembiłkowska 2003). Gabryszuk at al. (2008) suggested that the mineral composition of milk and hair depended on a production system (conventional vs. organic system). It seems that broader investigations of mineral composition of cow’s hair could be useful for establishing reference values for some elements.

The objective of this study was to determine correlations between concentrations of 29 major and trace elements in cow’s milk and hair.
MATERIAL AND METHODS

The experimental material consisted of 33 cows of Polish Holstein-Friesian (HF) breed from three dairy organic farms. All the farms were located in one climatic zone and under similar soil conditions. The cows were kept in traditional tied-up barns. The feeding was traditional, with ration components given separately. The cows were grazed from May to October. Depending on pasture yield and availability of other feeds, the feeding ration was supplemented with hay, straw, silage and cereals. All of the herds were under the official milk recording system, provided by the Polish Society of Cattle Breeders and Dairy Farmers.

Samples of milk and hair for analyses of minerals were collected in September, i.e. during pasture feeding. Hair was taken from the poll. It was washed with analytically pure acetone pure and rinsed 3 times with deionized water. The concentration of Ca, K, Mg, Na, P, S, B, Ba, Co, Cr, Cu, Fe, Ge, I, Li, Mn, Mo, Ni, Se, Si, Sn, Sr, V, Zn, Al, As, Cd, Hg, Pb was determined. Samples of hair (0.3 g) and milk (1 ml) were mineralised in a mixture of 4 ml HNO₃ and 1 ml H₂O₂ in hermetic high-pressure vessels by heating in a microwave oven. Content of mineral elements was determined by inductively coupled plasma atomic emission spectroscopy in a (ICP-AES) Optima 5300 DV, Perkin Elmer.

Preliminary statistical evaluation showed no significant effect of parity and milk yield on the content of minerals in cows’ hair and milk. Pearson’s correlation coefficients between the content of the same element in milk and in hair were computed from raw data, with no adjustments. The GLM procedure from the SAS package (1999) was used for computation.

RESULTS AND DISCUSSION

The mean concentrations and their standard deviations (SD) for macro- and micronutrients in milk and hair of cows are shown in Tables 1 and 2. It is quite interesting to notice that the concentration of Ca, Na, P was higher in milk than in hair. PULS (1994) reported that levels of macronutrients in hair do not correlate with their dietary intake. Maintaining plasma Ca constant during lactation presents a formidable challenge to a dairy cow. If dietary sources of Ca are consumed at only 38% availability and dietary P at 45% availability, the cow must consume, on average, 90 to 100 g Ca and 60 to 70 g P daily just to meet her needs for lactation. Additional 25 to 30 g Ca and 15 to 20 g P must be supplied for daily maintenance of the cow (HORST et al. 1997). During the early weeks of lactation, most cows remain under the negative Ca balance. To maintain normal plasma Ca, resorption of bone Ca stores and absorption of Ca from intestines counterbalance the
negative Ca balance. Bone Ca mobilisation is stimulated by a concerted effort of parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D \([1,25(OH)_{2}D]\). The adaptation process begins with a dramatic increase in the plasma concentrations of PTH and 1,25(OH)\(_{2}\)D at the onset of hypocalcemia (HORST et al. 1997). Polish soils are considered to be low or very low in Mg. This can explain the fact that grazed cows showed significantly lower concentration of Mg in milk compared with TMR-fed cows (GABRYSZUK et al. 2008). The levels of Ca, Mg and P in milk were generally lower compared to values reported by KUNACHOWICZ et al. (2005). Disorders of calcium, phosphorus and magnesium homeostasis in ruminants provide natural models for the study of the physiology and pathophysiology of these minerals. The knowledge that can be acquired by improving our understanding of the pathogenesis of these diseases could give useful clues for solving the puzzle of human osteoporosis (RIOND et al. 1995).

The content of trace elements in milk and hair is shown in Table 2. The levels of trace elements were within published ranges. Concentrations reported in the literature for cow’s milk are (mg dm\(^{-3}\)): 3.3 for Zn, 0.559 for I, 217.2 for Ba, 0.079 for Cu, 84.9 for Cr, 74.3 for Mn, 84.5 for V, 61.0 for Ni, 60.5 for Se, 28.2 for Ge, 11.5 for Mo, and 6.5 for Co (DOBRAŃSKI et al. 2005). Other authors reported the following trace element content in cow’s milk: 300-600 \(\mu\)g dm\(^{-3}\) Fe, 2-6 mg dm\(^{-3}\) Zn, 0.1-0.6 mg dm\(^{-3}\) Cu, 20-50 \(\mu\)g dm\(^{-3}\) Mn, 0.26 mg dm\(^{-3}\) I, 5-67 \(\mu\)g dm\(^{-3}\) Se, 0.5-1.3 \(\mu\)g dm\(^{-3}\) Co, 8-13 \(\mu\)g dm\(^{-3}\) Cr, 18-120 \(\mu\)g dm\(^{-3}\) Mo, 0-50 \(\mu\)g dm\(^{-3}\) Ni, 750-7000 \(\mu\)g dm\(^{-3}\) Si and 0-310 \(\mu\)g dm\(^{-3}\) V (GOFF 1995). Differences in the concentrations of major and trace elements in milk of cows depended on nutrition, breed, age, dairy period and performance, geographical location, location of an experiment, occupation, production system, mineral status of cows and animal welfare. HERMANSEN et al. (2005) observed that organically produced milk in Denmark, compared with conventionally produced milk, contained a significantly higher concentration of Mo and lower concentrations of Ba, Mn and Zn.

### Table 1

<table>
<thead>
<tr>
<th>Element</th>
<th>Milk (mg dm(^{-3}))</th>
<th>Hair (mg kg(^{-1}))</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
</tr>
<tr>
<td>Ca</td>
<td>637.4</td>
<td>138.4</td>
<td>586.9</td>
</tr>
<tr>
<td>K</td>
<td>894.6</td>
<td>179.5</td>
<td>1297</td>
</tr>
<tr>
<td>Mg</td>
<td>61.85</td>
<td>12.35</td>
<td>63.24</td>
</tr>
<tr>
<td>Na</td>
<td>421.0</td>
<td>108.0</td>
<td>368.3</td>
</tr>
<tr>
<td>P</td>
<td>456.3</td>
<td>105.0</td>
<td>38.32</td>
</tr>
<tr>
<td>S</td>
<td>14.22</td>
<td>3.091</td>
<td>3968</td>
</tr>
</tbody>
</table>
The reference concentrations of minerals in cow’s hair dry matter are, for example, 0.1-2.5% for Ca, 130-455 ppm for Mg, 0.2 ppm for Cr, 6.7-32 ppm for Cu, 59-200 ppm for Fe, 0.5-1.32 ppm for Mn, 0.5-1.32 ppm for Se and 100-150 ppm for Zn (PULS 1994). According to this author, determination of certain elements in hair may be useful for long-term monitoring of mineral status of animals (PULS 1994). Also, the mineral element status in the sheep’s flock determined by wool analysis can be a good method. The content of mineral elements in wool showed statistically significant differences between Booroola and Polish Merino ewes. The results of concentration
of the same minerals in the blood plasma of the same ewes were within the reference value, and no significant differences were observed between breeds (GABRYSZUK et al. 2001). The mineral content of wool depended also on the physiological status (parturition, gestation, mating) of sheep (GABRYSZUK et al. 2000).

Milk yield may affect the mineral status of cows. The main problem in pasture feeding is that the composition and digestibility of nutrients, including the content of mineral elements, are highly affected by the stage of plant growth and can vary significantly in relatively short periods. Eventually, it is difficult to maintain constantly high milk production based on pasture feeding, even when the quality of grass is high and soil and water conditions are suitable for grass production.

A change in the body condition is a common physiological phenomenon in dairy cows. Usually, the condition worsens after parturition and then is gradually regained, which is more evident in the later part of lactation and during the dry period. This can also be related to cows’ mineral supply. A clear difference between production systems was observed for cow’s herd lifespan. The period from the first calving to disposal (culling) was around 2.5 years in the intensive (conventional) herd, while in the extensive (organic) herds it was twice as long (GABRYSZUK et al. 2008).

The content of toxic elements in milk was low and below the admissible level (Ordinance of minister for health 2003). The content of heavy metals in milk and hair depends on feed, content of these metals in soil, environmental contamination as well as the antagonistic interaction between bioelements and heavy metals, which influence their absorption and metabolism. For these reasons, the content of toxic elements in milk from ecological farms was not lower than in milk from conventional herds.

The statistically significant positive correlations between concentration in milk and in hair were determined for such elements as Ba, Ge, Mo, Pb. In respect of major elements K and Mg and trace elements Al, As, Co, Fe, Hg, Se, Sr, positive correlations were observed but they were not statistically significant. The negative correlations concern such elements as Cr, Cu, I, Li, Ni, S, Si, Sn, V and Zn. It was only for V and Zn that they were statistically significant. Very low values (near zero) of coefficient r were observed for Ca, Cd, Li, Mn, Na, Ni, P, S and Sn. DOBRZEŃSKI et al. (2005) reported that positive significant correlations between concentration in milk and in blood concern such elements as Mn, Ga, Ni, Ge, Mo, while for Al and V negative correlation were observed.
CONCLUSIONS

This study has demonstrated that the hair levels of macroelements (Ca, Na, P) do not correlate with their levels in the milk. The determination of K, Mg, Al, As, Bo, Co, Fe, Ge, Hg, Pb, Se and Se in hair can be useful for long-term monitoring of the mineral status of cows. It seems that broader investigations of mineral composition of cow’s hair could be useful for establishing reference values for some elements and would make a contribution to better animals' welfare.

REFERENCES


THE EFFECT OF WEED CONTROL METHODS ON MAGNESIUM AND CALCIUM CONTENT IN EDIBLE PEA SEEDS (PISUM SATIVUM L.)

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Abstract

A field experiment was carried out in 2006-2008 at the Experimental Farm in Zawady, owned by the University of Podlasie. The experimental design was a split-plot arrangement of treatments with three replicates. The factors examined included: factor I – three sowing densities (75, 100 and 125 plants per 1 m²), and factor II – five weed control methods (control – mechanical weed control and four herbicide-based treatments). The objective of the study was to determine the effect of weed control methods as well as sowing density on magnesium and calcium content in the seeds of edible pea (Pisum sativum L.) of Merlin cultivar.

The highest seed yield was obtained in the plots where weeds were chemically controlled (Afolon Dyspersynj 450 SC was sprayed just after sowing and followed by an application of a mixture of Basagran 600 SL + Fusilade Forte 150 EC when plants were 5 cm high). The yield from this treatment was 4.84 t ha⁻¹, on average. The lowest yield was harvested in the plots where weeds were mechanically controlled (the control) – on average 2.92 t ha⁻¹.

Variance analysis showed significant influence of weed control methods and weather conditions on magnesium and calcium contents in pea seeds. The herbicides applied in the experiment increased concentrations of the above elements compared with the control. The highest magnesium content (1.389 g kg⁻¹) in pea seeds was found in the plots where Afolon Dyspersynj 450 SC was applied just after sowing at a dose of 1.5 dm³ ha⁻¹ and followed by a mixture of Basagran 600 SL at a dose of 2.0 dm³ ha⁻¹ + Fusilade Forte at a dose of 1.5 dm³ ha⁻¹ applied post-emergence. The highest calcium content was recorded for treatment 2, consisting of an application of Afolon Dyspersynj 450 SC at a dose of 1.5 dm³ ha⁻¹ just after sowing and followed by post-emergence spraying with Basagran 600 SL at a dose of 2.0 dm³ ha⁻¹ – on average 0.989 g kg⁻¹. In turn, sowing density had no influence on the discussed characteristics although a tendency was observed towards increasing magnesium and calcium content in edible pea seeds.
Abstrakt

Doświadczenia polowe przeprowadzono w latach 2006-2008 w Rolniczej Stacji Doświadczalnej Zawady należącej do Akademii Podlaskiej w Siedlcach. Doświadczenie założono w układzie split-plot w trzech powtórzeniach. Badanymi czynnikami były: I czynnik – 3 gęstości siewu (75, 100 i 125 roślin na 1 m²), II czynnik – 5 sposobów pielęgnacji (1 obiekt kontrolny – pielęgnacja mechaniczna i 4 obiekty, na których zastosowano herbicydy). Celem badań było określenie wpływu sposobów pielęgnacji oraz gęstości siewu na zawartość magnezu i wapnia w nasionach grochu siewnego jadalnego (*Pisum sativum* L.), odmiany Merlin

Analizując sposoby pielęgnacji lanu, największy plon nasion uzyskano na obiekcie, na którym zastosowano pielęgnację chemiczną (bezpośrednio po siewie opryskiwanie preparatem Afalon Dyspersyjny 450 SC i po osiągnięciu przez rośliny wysokości 5 cm opryskiwanie mieszaniną herbicydów Basagran 600 SL + Fusilade Forte 150 EC). Plon na tym obiekcie wyniósł średnio 4,84 t ha⁻¹, natomiast najniższy plon nasion stwierdzono na obiekcie kontrolnym, na którym zastosowano pielęgnację mechaniczną – średnio 2,92 t ha⁻¹

Analiza wariancji wykazała istotny wpływ sposobów pielęgnacji i warunków pogodowych na zawartość magnezu i wapnia w nasionach grochu. Zastosowane w doświadczeniu herbicydy spowodowały wzrost zawartości omawianych pierwiastków w porównaniu z obiektem kontrolnym. Największą zawartość magnezu (1,389 g kg⁻¹) w nasionach grochu uzyskano na obiekcie, na którym zastosowano bezpośrednio po siewie Afalon Dyspersyjny 450 SC w dawce 1,5 dm³ ha⁻¹ i po wschodach mieszaniną herbicydów Basagran 600 SL w dawce 2,0 dm³ ha⁻¹ + Fusilade Forte w dawce 1,5 dm³ ha⁻¹. Natomiast największą zawartość wapnia odnotowano na obiekcie 4. po zastosowaniu bezpośrednio po siewie preparatu Afalon Dyspersyjny 450 SC w dawce 1,5 dm³ ha⁻¹ i po wschodach preparatu Basagran 600 SL w dawce 2,0 dm³ ha⁻¹ – średnio 0,989 g kg⁻¹. Natomiast gęstość siewu nie miała wpływu na omawiane cechy, jednakże zaobserwowano tendencję do podwyższania zawartości magnezu i wapnia w nasionach grochu siewnego.

Słowa kluczowe: gęstość siewu, sposoby pielęgnacji, plon nasion grochu, zawartość magnezu, zawartość wapnia.

INTRODUCTION

Magnesium is one of the essential elements which are extremely important for the proper functioning of human body. Magnesium is a catalyst involved in around 300 metabolic pathways. The human body is not able to synthesize magnesium and, as a result, the Mg amount and proportion in a diet should be adequately adjusted (USTYMOWICZ-FARBISZEWSKA et al. 2000).

Appropriate nutrition is one of basic factors influencing the growth and development of organisms. Minerals play a significant role as they are both
building blocks and regulators conditioning the course of a number of metabolic processes (Stefanska et al. 2003).

Over the last years, a growing interest in vegetarian food has been observed, including seeds of leguminous plants (Korus 2002). Legume seeds are a valuable source of nutrients in the human diet as they supply minerals in addition to protein, carbohydrates and B-group vitamins. In many developed countries there is a tendency towards increased use of legume seeds in human nutrition. In Western Europe, an over threefold increase in legume seed consumption in the last years has been observed, reaching now about 3 kg per capita. In Poland, the level is much lower than recommended in the standards, i.e. 10-12 g per capita daily (Podlesny 2005).

The objective of the study was to determine the effect of weed control methods as well as sowing density on magnesium and calcium contents in seeds of edible pea (Pisum sativum L.).

**MATERIALS AND METHODS**

A field experiment was conducted in 2006–2008 at the Experimental Farm in Zawady, owned by the University of Podlasie in Siedlce. The experimental design was a split-plot arrangement of treatments with three replicates.

The following factors were examined:

- **factor I** – three sowing densities:
  1. 75 plants per 1 m²; 2. 100 plants per 1 m²; 3. 125 plants per 1 m²,
- **factor II** – five weed control methods:
  1. Control treatment – mechanical weed control (harrowing once between sowing and emergence, harrowing twice post-emergence and until the stage when plants were 5 cm high);
  2. Chemical weed control (spraying with Afalon Dyspersyjny 450 SC at a dose of 1.5 dm³ ha⁻¹ just after sowing);
  3. Mechanical and chemical weed control (harrowing once pre-emergence, harrowing twice until plants were 5 cm high, then spraying with Basagran 600 SL at a dose of 2.0 dm³ ha⁻¹);
  4. Chemical weed control (spraying with Afalon Dyspersyjny 450 SC at a dose of 1.5 dm³ ha⁻¹ just after sowing followed by spraying with Basagran 600 SL at a dose of 2.0 dm³ ha⁻¹ when plants reached the height of 5 cm);
  5. Chemical weed control (spraying with Afalon Dyspersyjny 450 SC at a dose of 1.5 dm³ ha⁻¹ just after sowing followed by spraying with a mixture of Basagran 600 SL at a dose of 2.0 dm³ ha⁻¹ + Fusilade Forte at a dose of 1.5 dm³ ha⁻¹ when plants reached the height of 5 cm).
The edible pea cultivar Merlin, registered in 2001, was grown in the experiment. It is suitable for cultivation to harvest seeds for cooking or as animal feed. The experimental plots lie on light to medium soil, whose composition is similar to loamy fine sand, quality classes IVa and IVb, very good ryne complex of soil.

Pea followed cereals (triticale, rye, triticale) in rotation. After harvesting the preceding crop, post-harvest cultivation treatments were performed and followed by winter ploughing in autumn. The first spring treatment was dragging, after which a cultivation unit was applied. Mineral fertilization with the complex fertilizer Polifoska 6 at a rate of 400 kg ha\(^{-1}\) was applied pre-plant.

Pea seeds were sown in the first decade of April at a row spacing of 25 cm. Harvest was performed when seeds reached the stage of full maturity. Seed yield was calculated on the basis of the weight of seeds harvested from an area of 20 m\(^{-2}\) and expressed in tones per 1 ha. After weighing, a representative seed sample was taken in order to determine thousand-seed weight and perform chemical analyses. Magnesium and calcium contents were determined by atomic absorption spectrophotometry AAS.

The results were statistically analysed by means of variance analysis. Significance of sources of variation was checked using the F (Fisher-Snedecor) test and significance of differences between mean values was determined using Tukey’s test at a significance level of \(P=0.05\) (Trętowski, Wójcik 1991).

Variable weather conditions prevailed in each growing season of the study period (Table 1). The highest precipitation of 337.7 mm was recorded in the growing season of 2006 but the distribution of rainfall was very uneven in the individual months. Large fluctuations were observed in the summer months, which are decisive for the growth, development and yield of edible pea. On the basis of the calculated hydrothermal coefficient (\(K=1.01\)), it was inferred that the growing season of 2007 was free from drought over the months of rapid growth and yield accumulation (June, July), with the respective values of hydrothermal coefficient equal 1.08 and 1.23. The growing season of 2008 was warm but the moisture conditions varied in individual months. The highest precipitation was recorded in May, July and August. The growing seasons of both 2007 and 2008 favoured the growth and yield accumulation of edible pea.

RESULTS AND DISCUSSION

The analysis of the results of the experimental factors demonstrated that the sowing density, weed control methods and weather conditions in the individual years had a significant impact on edible pea seed yield.
The highest average seed yield of 4.02 t ha\(^{-1}\) was obtained at the assumed sowing density of 125 plants per 1 m\(^2\). The lowest was the seed yield (on average 3.21 t ha\(^{-1}\)) at the assumed sowing density of 75 plants per 1 m\(^2\). Similar results were reported by Sawicki et al. (2000) as well as Kozak and Kotecki (2006).

According to some authors (Podleśny et al. 1993, Ksiežak 2007), it is possible to obtain high yields when complex weed control is applied, which was confirmed in the present work. Compared with the control, significantly higher seed yields were obtained in the remaining plots, where either single herbicides or their mixtures were applied. The highest yield (on average 4.84 t ha\(^{-1}\)) was recorded in treatment 5, where sowing was immediately followed by spraying with Afalon Dyspersyjny 450 SC at a dose of 1.5 dm\(^3\) ha\(^{-1}\) and then Basagran 600 SL at a dose of 2.0 dm\(^3\) ha\(^{-1}\) + Fusilade Forte 150 EC at a dose of 1.5 dm\(^3\) ha\(^{-1}\), the mixture being applied when the plants reached the height of 5 cm. The lowest average yield of 2.92 t ha\(^{-1}\) was recorded in the control treatment, where mechanical weed control was applied.

<table>
<thead>
<tr>
<th>Years</th>
<th>Rainfalls (mm)</th>
<th>Sum</th>
<th>Air temperature (°C)</th>
<th>Mean</th>
<th>Sielianinov’s hydrothermic coefficients</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>29.8</td>
<td>39.6</td>
<td>24.0</td>
<td>16.2</td>
<td>228.1</td>
<td>337.7</td>
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<td>2007</td>
<td>21.2</td>
<td>59.1</td>
<td>59.0</td>
<td>70.2</td>
<td>31.1</td>
<td>240.6</td>
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<td>2008</td>
<td>28.2</td>
<td>85.6</td>
<td>49.0</td>
<td>69.8</td>
<td>75.4</td>
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<td>Multiyear average (1987-2000)</td>
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<td>0.93</td>
<td>1.25</td>
<td>1.36</td>
<td>1.35</td>
</tr>
</tbody>
</table>

Table 1

Weather conditions during the pea vegetation period in 2006-2008
(Zawady Meteorological Station)
Weather conditions in each growing season influenced the yield of edible pea seeds (Table 2). The highest yields were recorded in the growing seasons of 2007 and 2008. They reached 3.90 and 3.98 t ha\(^{-1}\), respectively, and were significantly higher than the yield harvested in 2006 (3.09 t ha\(^{-1}\)). According to ALVINO and LEONE (1993), MICHALSKA (1995), FOUGEREUS and DORE (1997), SZWEJKOWSKA (2004) as well as BOROWCZAK and RĘBARZ (2007), weather conditions have great influence on the level of edible pea seed yield. Variance analysis indicated that there was a significant interaction between weed control methods and sowing density as well as study years and weed control methods.

<table>
<thead>
<tr>
<th>Weed control methods</th>
<th>Sowing density seeds per 1 m(^2)</th>
<th>Years</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75</td>
<td>100</td>
<td>125</td>
</tr>
<tr>
<td>Control – mechanical weed control – 3x</td>
<td>2.51</td>
<td>2.95</td>
<td>3.29</td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC</td>
<td>2.79</td>
<td>3.23</td>
<td>3.62</td>
</tr>
<tr>
<td>Harrowing 3x + Basagrán 600 SL</td>
<td>2.91</td>
<td>3.57</td>
<td>3.63</td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC + Basagrán 600 SL</td>
<td>3.64</td>
<td>3.98</td>
<td>4.25</td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC + Basagrán 600 SL+Fusilade Forte</td>
<td>4.19</td>
<td>5.03</td>
<td>5.29</td>
</tr>
<tr>
<td>Mean</td>
<td>3.21</td>
<td>3.75</td>
<td>4.02</td>
</tr>
</tbody>
</table>

LSD\(_{0.05}\) between: years – 0.12; sowing rate – 0.12; weed control methods – 0.25; Interaction: weed control methods x sowing rate – 0.32; weed control methods x years – 0.41

Thousand-seed weight (Table 3) was found to be significantly influenced by the sowing density, weed control methods and weather conditions. As for the sowing density, the highest thousand-seed weight was recorded for pea seeds harvested from the plots with the densities of 75 and 100 seeds per 1 m\(^2\) – the mean value was 262.8 g; the lowest thousand-seed weight (259.0 g) was characteristic of the plots with the highest sowing density – 125 seeds per 1 m\(^2\). The findings agree with the results obtained by KSIŻAK (1996) as well as KOZAK and KOTECKI (2006). In contrast, KULIGA et al. (1999) and SAWICKI et al. (2000) reported that plant density had no significant influence on the characteristic in question.

The weed control methods significantly influenced thousand-seed weight. The most favourable method, which affected the value of TSW, was the procedure based on Afalon Dyspersyjny 450 SC followed by a mixture
of Basagran 600 SL + Fusilade Forte 150 EC applied post-emergence. The average TSW was 265.0 g, and this result is confirmed by Szwejkowska (2006).

The weather conditions over the growing season influenced thousand-seed weight, too. The highest and lowest mean TSW values were recorded in the year 2008 (269.0 g) and 2009 (247.2 g), respectively. Szukała and Maciejewski (1995) as well as Kulig et al. (1999) reported that thousand-seed weight was significantly affected by both precipitation and air temperature.

Statistically significant interactions of years and sowing density, and of years and weed control methods were found. The interactions indicate an individual response of sowing density to weather conditions as well as herbicides applied in the particular growing seasons.

The analysis of an impact of sowing density on magnesium content indicated no significant differences between magnesium concentrations, with the mean values ranging between 1.345 and 1.350 g kg⁻¹. The results were confirmed by Kotecki and Grządowska (1997).

The variance analysis revealed that weed control methods significantly influenced magnesium content in pea seeds (Table 4). The herbicides involved in treatment 3 (Basagran 600 SL), 4 (Afalon Dyspersyjny 450 SC + Basagran 600 SL) and 5 (Afalon Dyspersyjny 450 SC + Basagran 600 SL + Fusilade Forte 150 EC) significantly increased magnesium content compared with the control. Reduced magnesium content was recorded in treatment 2, where only Afalon Dyspersyjny 450 SC had been applied. The highest magnesium content (1.389 g kg⁻¹) in pea seeds was recorded in the plots

Table 3

<table>
<thead>
<tr>
<th>Weed control methods</th>
<th>Sowing density seeds per 1 m²</th>
<th>Years</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75</td>
<td>100</td>
<td>125</td>
</tr>
<tr>
<td>Control – mechanical weed control – 3x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC</td>
<td>261.9</td>
<td>262.0</td>
<td>258.1</td>
</tr>
<tr>
<td>Harrowing 3x + Basagran 600 SL</td>
<td>261.6</td>
<td>262.1</td>
<td>257.9</td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC + Basagran 600 SL</td>
<td>262.0</td>
<td>262.4</td>
<td>258.8</td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC + Basagran 600 SL + Fusilade Forte</td>
<td>268.0</td>
<td>265.9</td>
<td>261.3</td>
</tr>
<tr>
<td>Mean</td>
<td>262.8</td>
<td>262.8</td>
<td>259.0</td>
</tr>
</tbody>
</table>

LSD₀.₀₅ between: years – 0.36; sowing rate – 0.36 ; weed control methods – 0.51; Interaction: weed control methods x sowing rate – n.s.; weed control methods x years – 1.03

n.s. – not significant
which, following sowing, were sprayed with Afalon Dyspersyjny 450 SC at a dose of 1.5 dm$^3$ ha$^{-1}$ and then a mixture of Basagran 600 SL at a dose of 2.0 dm$^3$ ha$^{-1}$ + Fusilade Forte at a dose of 1.5 dm$^3$ ha$^{-1}$, applied post-emergence. Similar conclusions were reached by Ciszewska (1977). In their studies, Zarzecka et al. (2002) found a significant response of potato tubers, in terms of increased magnesium and calcium content, to an application of herbicides and their mixtures. By contrast, Adamas and Piotrowicz-Cieślak (2007) found no significant influence of an application of herbicides on the magnesium level.

The weather conditions in every growing season significantly affected magnesium content in pea seeds (Table 4). The highest value was recorded in the growing season of 2007, which was characterised by the most favourable precipitation and temperature distributions. In turn, the lowest magnesium content was obtained in the growing season of 2006, when precipitation was unevenly distributed in individual months. Marked fluctuations were recorded in June and July – the key months for the development and yield accumulation of edible pea. Stępnia-Solyga and Wojtasik (2003) reported significant influence of moisture conditions on seed mineral composition. The variance analysis showed that there was an interaction of sowing density and weed control methods.

Calcium content in pea seeds varied depending on sowing density. The highest content was obtained at the sowing density of 125 plants per 1 m$^2$ – on average 0.928 g kg$^{-1}$. In turn, the lowest was the content associated

<table>
<thead>
<tr>
<th>Weed control methods</th>
<th>Sowing density seeds per 1 m$^2$</th>
<th>Years</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75</td>
<td>100</td>
<td>125</td>
</tr>
<tr>
<td>Control – mechanical weed control – 3x</td>
<td>1.328</td>
<td>1.316</td>
<td>1.303</td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC</td>
<td>1.317</td>
<td>1.298</td>
<td>1.308</td>
</tr>
<tr>
<td>Harrowing 3x + Basagran 600 SL</td>
<td>1.342</td>
<td>1.339</td>
<td>1.354</td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC + Basagran 600 SL</td>
<td>1.370</td>
<td>1.380</td>
<td>1.387</td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC + Basagran 600 SL+Fusilade Forte</td>
<td>1.376</td>
<td>1.393</td>
<td>1.398</td>
</tr>
<tr>
<td>Mean</td>
<td>1.346</td>
<td>1.345</td>
<td>1.350</td>
</tr>
</tbody>
</table>

LSD$_{0.05}$ between: years – 0.006; sowing rate – n.s.; weed control methods – 0.08; Interaction: weed control methods x sowing rate – 0.011; weed control methods x years – 0.013

n.s. – not significant
with the sowing density of 75 plants per 1 m² – on average 0.923 g kg⁻¹. However, the differences were not significant. The findings were confirmed in the studies by KOTECKI and GRZĄDKOWSA (1997).

The statistical calculations indicated that the herbicides applied in the experiment significantly increased the calcium content in pea seeds compared with the control (Table 5). The highest calcium concentration (0.989 g kg⁻¹) was obtained in the pea seeds harvested from the plots where Afalon Dyspersyjny 450 SC, applied just after sowing at a dose of 1.5 dm³ ha⁻¹, was followed by Basagran 600 SL at a dose of 2.0 dm³ ha⁻¹, applied when plants were 5 cm high.

The weather conditions influenced the calcium content in edible pea seeds. The highest content, on average 0.975 g kg⁻¹, was recorded in 2007, when both precipitation and temperature distributions were favourable. The lowest calcium content, i.e. 0.848 g kg⁻¹, was found in 2006, when precipitation was unevenly distributed. The differences in Mg and Ca content linked to the impact of weather conditions were confirmed in the study by JADCZAK et al. (2006). Significant influence of weather conditions on magnesium and calcium content was reported by ZARZECKA et al. (2002).

### Table 5

<table>
<thead>
<tr>
<th>Weed control methods</th>
<th>Sowing density seeds per 1 m²</th>
<th>Years</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75</td>
<td>100</td>
<td>125</td>
</tr>
<tr>
<td>Control – mechanical weed control – 3x</td>
<td>0.879</td>
<td>0.888</td>
<td>0.883</td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC</td>
<td>0.890</td>
<td>0.897</td>
<td>0.911</td>
</tr>
<tr>
<td>Harrowing 3x + Basagran 600 SL</td>
<td>0.933</td>
<td>0.931</td>
<td>0.912</td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC + Basagran 600 SL</td>
<td>1.016</td>
<td>0.970</td>
<td>0.980</td>
</tr>
<tr>
<td>Afalon Dyspersyjny 450 SC + Basagran 600 SL + Fusilade Forte</td>
<td>0.899</td>
<td>0.951</td>
<td>0.954</td>
</tr>
<tr>
<td>Mean</td>
<td>0.923</td>
<td>0.927</td>
<td>0.928</td>
</tr>
</tbody>
</table>

LSD₀.₀₅ between: years – 0.02; sowing rate – n.s.; weed control methods – 0.03; Interaction: weed control methods x sowing rate – n.s.; weed control methods x years – n.s.

n.s. – not significant
CONCLUSIONS

1. Application of herbicides in edible pea cultivation contributed to increased seed yields as well as seed robustness. The highest yield and thousand-seed weight were obtained in the plots of the treatment where weeds were controlled with Afalon Dyspersyjny 450 SC applied just after sowing and followed by a mixture of Basagran 600 SL + Fusilade Forte 150 EC applied post-emergence.

2. The herbicides and their mixtures examined in the experiment significantly increased magnesium and calcium content compared with the control. The highest Mg and Ca content was associated with an application of three herbicides, namely Afalon Dyspersyjny 450 SC and a mixture of Basagran 600 SL + Fusilade Forte 150 EC.

3. Magnesium and calcium concentrations in edible pea seeds were significantly affected by weather conditions in individual growing seasons. The highest concentrations of the macroelements in pea seeds were obtained in the growing seasons characterised by an even distribution of precipitation and temperature.

REFERENCES


STEPIEN-SOŁYGA P., WOJTASIK J. 2003. Zawartość składników pokarmowych i mineralnych w nasionach grochu (Pisum sativum), lędźwiowym (Lathyrus sativus), soczewicą (Lens culinaris) i soi (Glycine max) [Content of nutrients and minerals in seeds of pea (Pisum sativum), grass pea (Lathyrus sativus), lentil (Lens culinaris) and soybean (Glycine max)]. Ann. UMCS, Sect. EE, 76: 175-185. (in Polish)


EFFECT OF BOTTOM SEDIMENT ON CONTENT, BIOACCUMULATION AND TRANSLOCATION OF HEAVY METALS IN MAIZE BIOMASS

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¹Chair of Agricultural and Environmental Chemistry
²Chair of Hydraulic Engineering and Geotechnics
University of Agriculture in Krakow

Abstract

The research aimed to assess the effect of bottom sediment on the content, bioaccumulation and translocation of heavy metals in maize biomass. The investigations were conducted in 2006-2007 as a pot experiment on light soil of the granulometric composition of weakly-loamy sand. The experimental design comprised 3 treatments: without sediment (I), a 5% sediment admixture (II) and a 10% sediment admixture to the soil (III). Bottom sediment was added to the soil in the first year of the investigations. The content of Zn, Cu, Ni, Pb, Cd and Cr was determined using the ICP-EAS method in the plant material after its dry mineralization and ash solution in HNO₃. The uptake of the above-mentioned metals by maize was computed alongside their bioaccumulation and translocation coefficients. The effect of bottom sediment admixture on heavy metal concentrations in maize was determined to be varied, e.g. a 5% dose of sediment added to soil decreased the content of all the analyzed heavy metals in the biomass of maize aerial parts, whereas a 10% admixture increased the content of Cu, Ni, Pb and Cr. The values of bioaccumulation coefficients revealed that an admixture of both doses of bottom sediment led to a decreased accumulation of Zn, Cu, Cd, Cr and Ni (5% dose) in maize aerial biomass. Moreover, the plant more easily accumulated Zn, Cd and Cu than Cr, Ni or Pb. Permissible amounts of heavy metals in plants to be used as animal fodder were not exceeded in the maize biomass.

Key words: bottom sediment, heavy metals, maize.
WPŁYW OSADU DENNEGO NA ZAWARTOŚĆ, BIOAKUMULACJĘ I TRANSLOKACJĘ METALI CIĘŻKICH W BIOMASIE KUKURYDZY

Abstrakt

Celem badań była ocena wpływu osadu dennego na zawartość, bioakumulację i translokację metali ciężkich w biomasie kukurydzy. Badania prowadzono w latach 2006-2007, w warunkach doświadczalnego, na glebie lekkiej o składzie granulometrycznym piasku słabogliniastego. Schemat doświadczenia obejmował 3 obiekty: bez osadu (I), z dodatkiem 5% osadu (II) i dodatkiem 10% osadu do gleby (III). Osad dennny dodano do gleby w pierwszym roku badań. Zawartość Zn, Cu, Ni, Pb, Cd, Cr w materiale roślinnym oznaczono po suchej mineralizacji i roztworzeniu popiołu w HNO₃. Obliczono wynos ww. metali przez kukurydzę oraz ich współczynniki bioakumulacji i translokacji. Stwierdzono niejednoznaczny wpływ dodatku osadu dennego na zawartość metali ciężkich w kukurydzy. Osad dodany w ilości 5% do gleby wpłynął na zmniejszenie zawartości wszystkich analizowanych metali w nadziemnej biomasie kukurydzy, natomiast 10% dodatek osadu na zwiększenie ich zawartości (Cu, Ni, Pb, Cr). Wartości współczynników bioakumulacji świadczą, że dodatek osadu dennego w obu dawkach spowodował zmniejszenie akumulacji Zn, Cu, Cd, Cr oraz Ni (dawka 5%) w biomasie nadziemnej kukurydzy, ponadto rośliny łatwiej akumulowały Zn, Cd i Cu niż Cr, Ni i Pb. W biomasie kukurydzy nie stwierdzono przekroczenia dopuszczalnych zawartości metali ciężkich przyjętych do oceny roślin pod względem ich przydatności paszowej.

Słowa kluczowe: osad denny, metale ciężkie, kukurydza.

INTRODUCTION

Most of the toxic substances, including heavy metals, which reach open waters as a result of human economic activities are trapped in bottom sediments. Bottom deposits which accumulate these substances are therefore an important source of information about the degree of anthropopressure on water environment (BOJAKOWSKA 2001). Another crucial aspect of the pollution of bottom sediments is how to handle or dispose of them after their extraction from the bottom of rivers, dam reservoirs, ports, channels or ponds (FONSECA et al. 1998, MADEYSKI 2003, POPENDA et al. 2007). Many authors emphasize that utilization of bottom sediments free from chemical or biological pollution in agriculture may be of considerable environmental and ecological importance and may prove to be the most rational way of their management (FONSECA et al. 1998, 2003, PLECZAR et al. 1998, WISNIOWSKA-KIELIAN, NIEMIEC 2007ab). The research aimed to assess the effect of bottom sediment on the content, bioaccumulation and translocation of heavy metals in maize biomass.
MATERIAL AND METHODS

The investigations were conducted in 2006-2007 as a pot experiment. The experiment was conducted on light soil of the granulometric composition of weakly-loamy sand and pH_{KCl} 6.21. With respect to the threshold levels of heavy metals in soil, the investigated soil had natural content of Cu, Pb, Ni, Cd and elevated content of Zn (KABATA-PENDIAS et al. 1995). Bottom sediments originated from a small retention reservoir localized in the village of Zesławice, 8.7 km of the Dłubnia River (Province of Małopolska, województwo małopolskie) (JASIEWICZ, BARAN 2006). The bottom sediment was classified as ordinary silt deposit of pH_{KCl} 7.35. It had a low content of available phosphorus and potassium but a high content of magnesium (Table 1). The bottom sediment evaluation concerning heavy metal concentrations was conducted according to the Ordinance of the Minister of the Natural Environment on types and concentrations of substances which cause yield pollution (Dz.U. 2002 nr 55, poz. 498), whereas the way it was handled was determined with respect to the IUNG criteria (KABATA-PENDIAS et al. 1995) and the Ordinance of the Minister of the National Environment on soil quality standards and earth quality standards (Dz.U. 2002, nr 165, poz. 1359). According to the above regulations and the IUNG assessment, the levels of heavy metal in the analyzed sediment did not exceed the content admissible for yield or for the soil and land of group B, and was classified as natural (degree 0) – Table 1.

Air dry bottom sediment was added to the soil in the first year of the investigations. The experimental design comprised 3 treatments: without sediment (I), a 5% sediment admixture (II) and a 10% sediment admixture to the soil (III). The same NPK fertilization, i.e. 1.8 g N, 1.1 g P, and 2.2 g K per pot (8 kg soil d.m.), was applied to all the treatments. The mineral salts NH₄NO₃, KH₂PO₄ and KCl were added each time prior to the test plant sowing.

Table 1

<table>
<thead>
<tr>
<th>Share of Ø (mm) fraction</th>
<th>Org. matter</th>
<th>Total N</th>
<th>P₂O₅</th>
<th>K₂O</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-0.1</td>
<td>0.1-0.02</td>
<td>&lt;0.02</td>
<td>(g kg⁻¹ d.m.)</td>
<td>(mg kg⁻¹ d.m.)</td>
<td></td>
</tr>
<tr>
<td>8%</td>
<td>66%</td>
<td>26%</td>
<td>25.8</td>
<td>1.0</td>
<td>44.6</td>
</tr>
<tr>
<td>Heavy metals (mg kg⁻¹ d.m.)</td>
<td>Cr</td>
<td>Zn</td>
<td>Pb</td>
<td>Cu</td>
<td>Cd</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>76.31</td>
<td>12.85</td>
<td>12.23</td>
<td>0.35</td>
</tr>
<tr>
<td>Norm*</td>
<td>&lt;200</td>
<td>&lt;1000</td>
<td>&lt;200</td>
<td>&lt;150</td>
<td>&lt;7,5</td>
</tr>
<tr>
<td>Norm (grounds B)**</td>
<td>150</td>
<td>300</td>
<td>100</td>
<td>150</td>
<td>4</td>
</tr>
<tr>
<td>IUNG</td>
<td>-</td>
<td>&lt;100</td>
<td>&lt;70</td>
<td>&lt;40</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

After harvest, the plant material was dried in a dryer with forced air flow at 65°C, after which the plant material was comminuted in a laboratory mill and subjected to chemical analysis. The content of Zn, Cu, Ni, Pb, Cd and Cr in the plant material was determined using the ICP-EAS method after its dry mineralization and ash solution in HNO₃. The uptake of the above-mentioned metals by maize was computed, as well as their bioaccumulation and translocation coefficients. The results were verified statistically by means of one way ANOVA and Tukey test at significance level \( \alpha = 0.05 \), using Statistica 8.1. programme.

RESULTS AND DISCUSSION

Our analysis of zinc distribution in the plant revealed that its roots contained on average 34% more of this metal than the aerial parts (Table 2). The highest Zn concentrations both in the aerial parts and roots found among the experimental treatments were in the control plants (Table 2). Significantly smallest quantities of zinc were assessed in the plants from treatments with a 5% supplement of the sediment to the soil. In these treatments, maize had 29% less of Zn (aerial parts) and 27% (roots) in comparison with the object without the sediment. The admixture of both bottom deposit doses to the soil significantly diminished Zn content in maize roots (Table 2). It might have been connected with the bottom sediment effect on the soil pH, where an increase in pH value decreases zinc bioavailability.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoots (mg kg⁻¹ d.m.)</th>
<th>Roots (mg kg⁻¹ d.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zn         Cu         Ni   Pb      Cd      Cr     Zn         Cu         Ni   Pb     Cd      Cr</td>
<td></td>
</tr>
<tr>
<td>Soil without sediment</td>
<td>35.39       1.55       0.30  0.74   0.23     0.59</td>
<td>5.60        0.33       0.11  s.n.  0.09   s.n.</td>
</tr>
<tr>
<td>Soil + 5% sediment</td>
<td>25.07       1.20       0.26  0.73   0.13     0.54</td>
<td>34.20       1.61       0.40  0.85  0.22     0.61</td>
</tr>
<tr>
<td>Soil +10% sediment</td>
<td>34.20       1.61       0.40  0.85   0.22     0.61</td>
<td>5.60        0.33       0.11  s.n.  0.09   s.n.</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td>5.60        0.33       0.11  s.n.  0.09   s.n.</td>
<td>5.60        0.33       0.11  s.n.  0.09   s.n.</td>
</tr>
</tbody>
</table>

s.n. – statistically non-significant
whereas a decline in pH value favours zinc availability to plants. The bottom sediment had alkaline reaction (pH_{KCl} 7.35) and therefore caused worse zinc solubility in soil.

The present investigations revealed that maize roots accumulated between 1.8- and 4-fold more copper than the aerial parts (Table 2). An admixture of bottom sediment to the soil did not produce an identical effect on the copper concentrations in maize biomass. Significantly the highest concentrations of this metal were found in maize aerial parts from the treatment with a 10% supplement of bottom deposit, and in roots from the treatment with a 5% share of bottom sediment in the soil. The lowest copper content in the aerial biomass was assessed in the treatment with a 5% share of the sediment and in roots in the treatment where a 10% admixture was used to light soil. In both cases, the relationships were statistically significant (Table 2).

In our experiment, maize roots had on average 86% more nickel than aerial parts. In the aerial biomass, the highest and statistically significant nickel concentrations were registered on the variant with a 10% admixture of bottom deposit to the soil whereas the lowest ones appeared in the variant with a 5% share (Table 2). The aerial parts from these treatments contained 25% more (10% of sediment) and 13% less Ni (5% of sediment) in comparison with the control. An admixture of bottom deposit to light soil increased nickel concentrations in roots, but the differences were statistically non-significant. Wiśniowska-Kielian and Niemiec (2007a) obtained slightly different results, i.e. between 1 and 4% admixture of bottom sediment led to increased nickel accumulation in maize aerial biomass, whereas an over 6% sediment supplement diminished its content. These authors found the highest nickel concentrations in maize roots in treatment with a 10% admixture of bottom sediment.

Maize roots accumulated over 4.5- to 5-fold more lead than the aerial parts (Table 2). The results confirm the hypothesis that relatively big quantities of lead present on the root surface are a mechanism limiting this metal uptake from soil (Baranowska-Morek 2003). An admixture of bottom sediment to soil did not diversify statistically significantly the content of lead in the examined parts of maize. In the aerial parts, the highest Pb concentrations were determined in the treatment with a 10% bottom sediment admixture to the soil (Table 2). Wiśniowska-Kielian and Niemiec (2007a) reported different results, i.e. lower doses of bottom sediment, i.e. between 1 and 4% increased Pb content, whereas the doses higher than 6% led to a decrease in Pb concentrations in aerial biomass. In the present experiment, a bottom sediment admixture caused a decline in Pb root concentrations (Table 2). Wiśniowska-Kielian and Niemiec (2007a) also found that the lead content diminished in roots as a result of bottom deposit application but only at its highest, i.e. 16%, share. In another experiment, Wiśniowska-Kielian and Niemiec (2007b) demonstrated that lead accumulation in aerial
The maize roots contained 9% more cadmium than the aerial parts (Table 2). As for zinc, the applied bottom sediment caused a significant decrease in cadmium content in aerial parts and roots of maize in comparison with the treatment without the deposit. The lowest amounts of cadmium were found in the plants from the treatments with a 5% sediment supplement to the soil. A 5% sediment admixture lowered the cadmium level by 44% in maize aerial biomass and by 23% in roots in comparison with the object without the sediment (Table 2). Similar relationships were reported from other studies, in which application of bottom sediment to soil led to a decline in the Cd content in maize, oat and narrowleafed lupine biomass (Wieńrowska-Kiełian, Niemiec 2007ab).

The maize roots accumulated on average 69% more chromium in comparison with the aboveground biomass (Table 2). An admixture of bottom sediment to soil in 5 and 10% were not diversify statistically significantly the content of chromium in the aerial parts and roots of maize. The highest Cr concentrations in the aerial parts were found in the treatment with a 10% admixture of bottom sediment to the soil (Table 2). In the maize roots, a decrease in Cr content was noticed under the influence of both doses of bottom sediment added to the soil.

Considering the share of individual maize parts in the general element uptake, it was noticed that the highest amounts of elements were removed with the maize aerial biomass (Table 3), which absorbed 86% Zn, 77% Cu,

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoots (mg kg⁻¹ d.m.)</th>
<th>roots (mg kg⁻¹ d.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zn</td>
<td>Cu</td>
</tr>
<tr>
<td>Soil without sediment</td>
<td>10.41ᵇ</td>
<td>0.46</td>
</tr>
<tr>
<td>Soil + 5% sediment</td>
<td>8.03ᵃ</td>
<td>0.38</td>
</tr>
<tr>
<td>Soil +10% sediment</td>
<td>10.23ᵇ</td>
<td>0.48</td>
</tr>
<tr>
<td>LSD₀.₀⁵</td>
<td>1.86</td>
<td>s.n.</td>
</tr>
</tbody>
</table>

**Table 3**

s.n. – statistically non-significant
74% Cr, 66% Pb, 55% Ni and 51% Cd of the total heavy metal concentrations. On the other hand, the analysis of heavy metal uptake depending on the applied quantities of bottom sediment revealed a significantly lower uptake of Zn, Cd (roots), Cu (roots), and Pb (5% dose). Plants growing on soil with a 5% addition of bottom sediment were characterized by the lowest metal uptake, which resulted from the highest maize yields from this treatment. Additionally, the aerial biomass produced from this treatment revealed the lowest metal concentrations (Table 2). In this treatment, 39% Pb, 31% Cd, 24% Zn, 9% Cr and 8% Cu less were taken up with yield than in the treatment without the deposit (Table 3). The highest uptake of Zn, Cu, Cd and Cr was recorded for the control plant yield, while most Ni and Pb were found in the plant biomass from the treatment with a 10% admixture of bottom sediment.

The assessment of the degree and direction of the translocation of individual elements in plant distinguishes two coefficients of plant sensitivity to heavy metals, i.e. bioaccumulation and translocation coefficient. The value of bioaccumulation coefficient (BC) shows the ability of a plant to absorb components from soil and the size of metal translocation from the soil solution to plant aerial parts. Bioaccumulation coefficient is the ratio of plant heavy metal concentration to its quantity in soil (Grzebisz et al.1998). The computed values of bioaccumulation coefficient revealed that maize more easily accumulated Zn, Cd and Cd (the highest values of BC) than Cr, Ni or Pb (Table 4), which evidences considerable mobility of Zn and Cd in comparison with other metals and their relatively easy absorption by plants. Moderate accumulation levels were assessed for zinc, cadmium and copper (BC; 0.1-1), whereas the other metals (BC; 0.01-0.1) produced low BC. While estimating the effect of bottom sediment, it was determined that both its doses added to the soil led to a decrease in Zn, Cu, Cd, Cr and Ni accumulation (5% dose) in maize aerial biomass in comparison with the plants which absorbed the highest amounts (Table 4). It was only the level of lead that rose by 4% (5% of sediment) and 17% (10% sediment) in comparison with the control plants as a result of soil enrichment with bottom sediment. Diminished values of bioaccumulation coefficient in maize may be explained by the fact that bottom sediment admixture to the soil alkalized the soil environment (increasing its pH value), therefore decreasing metal availability to plants. Mobility of the metals in maize was determined using translocation coefficient (TC). This parameter was computed as a ratio of metal content in the aerial parts to its content in roots (Jasiewicz, Antonkiewicz 2000). In maize, the values of TC coefficient appeared in the following increasing order: Cd < Ni < Pb < Cr < Cu < Zn (Table 4). Our analysis of the TC coefficient values showed that maize roots absorbed the highest quantities of Cd, Ni and Pb. A 5% admixture of bottom sediment caused a decline in this parameter value by 1.5% for zinc, 11% for chromium, 30% for copper and cadmium and 35% for nickel, but 7% increase for lead versus the control
values. This coefficient reached its highest values for all the analyzed metals when a 10% supplement of bottom sediment was added to the soil, which points to an increased metal mobility in plants under the influence of the applied bottom deposit (Table 4). The value of the metal translocation coefficient in this treatment increased by 2% for cadmium, 11% for lead, 18% for chromium and nickel, 24% for zinc and by over 30% for copper in comparison with the control. WISNIOWSKA-KIELIAN and NIEMIEC (2007a) obtained slightly different results. These authors found that bottom deposit added to soil significantly lowered values of metal translocation coefficients in maize. Also ANTONKIEWICZ and LOSAK (2007) revealed a decrease in value of this coefficient in plants as a result of ash admixture to soil due to the substrate alkalization.

To sum up, extracted sediments which are either neutral or alkaline, have a high content of silt and clay fractions and low heavy metal concentrations may be utilized for improving the properties of light and acid soils (WISNIOWSKA-KIELIAN, NIEMIEC 2007ab). The sediment applied in the present research had a high share of silt and clay fractions, alkaline pH and low content of heavy metals, therefore it may be used as an admixture to the above-mentioned soils to improve their productivity. It is commonly know that pH has much influence on metal mobility; the lower pH value, the greater the solubility of individual metals. Another environmentally justifiable method of dredged sediment management is to use it use as structure and soil forming material for soilless systems and wastelands (POPENDA et al. 2007). In the present experiment, the assessment of heavy metal content in maize included the assumption that the maize biomass would be used for production of animal feeds, therefore using threshold limits of heavy metals in plants stated by various authors CURYLO et al. (1985), KABATA-PENDIAS et al.
(1993) and the Ordinance of the Minister of Agriculture and Rural Development on the permissible quantities of undesirable substances in feeds (Dz.U. 2007. Nr 20, poz. 119). The permissible amounts of heavy metals in feeds are as follows: \(<100 \text{ mg Zn}, <10 \text{ mg Cu}, <10 \text{ mg Cr}, <10 \text{ mg Ni}, <5 \text{ mg Cd} \) and \(<10 \text{ mg Pb} \text{ kg}^{-1} \text{ d.m.} \). The assessment of the maize biomass obtained using these threshold levels proved that it met the requirements set for good quality fodder with respect to the contents of all the heavy metals.

**CONCLUSIONS**

1. The sediment added to the soil as a 5% dose decreased the content of all the analyzed heavy metals in maize aerial biomass, whereas a 10% admixture increased their content (Cu, Ni, Pb and Cr).

2. The values of bioaccumulation coefficients revealed that an admixture of both doses of bottom sediment led to a decreased accumulation of Zn, Cu, Cd, Cr and Ni (5% dose) in maize aerial biomass. However, under these conditions maize more easily accumulated Zn, Cd and Cu than Cr, Ni or Pb.

3. The values of translocation coefficient showed increased metal mobility in roots to aerial parts under the influence of a 10% bottom sediment admixture to the soil.

4. No excess of the permissible content of heavy metals in plants used as animal forage were found in the maize biomass.

**REFERENCES**


USE OF SELECTED METAL IONS FOR THE SEPARATION OF PEPTIDES ISOLATED FROM THERMALLY PROCESSED STRING BEANS

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Abstract

Recent years have witnessed growing interest in research on the structure and properties of proteins and peptides as physiologically active dietary components. The above has spurred a new interest in the isolation of animal, plant and microbiological peptides and investigation of their biological activity. The isolation and separation of protein and peptide mixture is not an easy procedure. Immobilised Metal Ion Affinity Chromatography (IMAC) is increasingly often used in this process. Affinity chromatography relies on the specific interactions between amino acids, their reactive groups in peptides and metal ions. The objective of this study was to determine whether copper and nickel ions can be used for the separation of peptides isolated from string beans than had been blanched and heated in a microwave oven. In this study, peptides extracted with 1% trichloroacetic acid (TCA) from string beans that had been blanched and heated in a microwave oven, were separated by chromatography on columns with copper and nickel ions immobilised through iminodiacetic acid (IDA). Peptide concentrations of the separated fractions were determined. Peptides found in string beans had similar affinity for metal ions in the Cu > Ni sequence, with selectivity in the Ni > Cu sequence. Microwave heating of string beans decreases the peptide content of extracts isolated with 1% TCA. The resulting changes are dependent on the duration of the process and the type of heating medium. Affinity chromatography with the use of metal ions immobilized to iminodiacetic acid (IDA)-Sephadex G-25 may be successfully used for the separation of peptides isolated from string beans.

Key words: peptides, IMAC, metal ions, string beans.
Abstrakt

W ostatnich latach obserwuje się coraz większe zainteresowanie badaniem struktury oraz właściwości białek i peptydów jako fizjologicznie aktywnych składników diety. W związku z powyższym wzrosta zainteresowanie izolowaniem i badaniem biologicznie aktywnych peptydów pochodzenia zwierzęcego, roślinnego oraz mikrobiologicznego.

W skomplikowanym procesie rozdziału i izolowania mieszanin białek i peptydów coraz szersze zastosowanie znalazła chromatografia powinowactwa na unieruchomionych jonach metali – IMAC (Immobilized Metal Ion Affinity Chromatography). Chromatografia powinowactwa wykorzystuje specyficzne oddziaływania między aminokwasami oraz ich reaktywnymi ugrupowaniami w peptydach a jonami metali. Celem pracy było zbadanie przydatności jonów miedzi i niklu w procesie rozdziału peptydów izolowanych z fasoli szparagowej poddanej blanszowaniu i ogrzewaniu mikrofalami. Fasolę szparagową poddano odpowiedniej obróbce cieplnej (blanszowanie i ogrzewanie w kuchence mikrofalowej), a z uzyskanego surowca ekstrahowano peptydy 1% kwasem trójchlorooctowym (TCA). Następnie przeprowadzono rozdział chromatograficzny na kolumnach z unieruchomionymi jonami niklu i miedzi poprzez kwas iminodioctowy (IDA). W otrzymanych frakcjach oznaczono zawartość peptydów. Peptydy obecne w fasoli szparagowej charakteryzowały się zbliżonym powinowactwem do jonów metali, co przebiegało w kolejności Cu > Ni, natomiast selektyność układła się w kolejności Ni > Cu. Ogrzewanie fasoli szparagowej obniża poziom peptydów w ekstraktach izolowanych 1% TCA. Zmiany uzależnione są od czasu trwania procesu i rodzaju zastosowanego czynnika grzewczego. Metoda chromatografii powinowactwa z wykorzystaniem unieruchomionych jonów metali na schelatowanym kwasem iminodioctowym (IDA) żelu Sephadex G-25 może być z powodzeniem stosowana do rozdziału peptydów izolowanych z fasoli szparagowej.

Słowa kluczowe: peptydy, IMAC, jony metali, fasola szparagowa.

INTRODUCTION

Increasing interest in the properties of proteins and peptides as bioactive dietary components has prompted further investigations into the isolation and activity of peptides of animal, plant and microbiological origin. It is not easy to select a technique appropriate for the separation of physiologically active peptides and proteins from impurities, and to ensure optimum isolation conditions so as to obtain a product with the highest biological activity.

Immobilised Metal Ion Affinity Chromatography (IMAC) relies on specific interactions between amino acids, their reactive groups in proteins and peptides and „transitory” metal ions, in particular Cu$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Co$^{2+}$, Fe$^{3+}$ (CHAGA 2001). These ions are immobilised by a chelating compound on the bed, forming specific adsorbents which bind proteins and peptides.
Protein affinity for metal ions is determined by numerous factors, including protein structure, type of chelating compound, pH, type of solvent, presence of salt and competitive ligands or type of metal ions. The chelating compound on the bed (without immobilized metal ions) can be applied for removing metal ions, which are contained in edible parts of some fresh vegetables or aquatic plants (Bosiacki, Tykśniński 2009, Senze 2009).

The objective of this study was to determine whether copper and nickel ions can be used for the separation of peptides isolated from string beans that had been blanched and heated in a microwave oven.

MATERIALS AND METHODS

Frozen pods of dwarf, green-podded string bean cv. Fana were used in the study. String beans were blanched at 90°C and heated in a microwave oven (Philips/Whirlpool – model 606), used 3 power range 500, 750 and 900 Watts. The heating process was carried out for 2, 4, 6, 8 and 10 min.

Peptides were extracted from well-homogenized string bean pods with 1% trichloroacetic acid (TCA). Homogenate of 1 g sample of heated string beans was stirred with a magnetic stirrer with 10 ml of 1% TCA (pH 1,4) for 2 hours at room temperature. The solid fraction was separated by centrifugation at 4000 rpm for 15 min. The collected extracts were separated by immobilized copper and nickel ion affinity chromatography.

Gel preparation for peptide separation by IMAC

Sephadex G-25 medium was mixed with a solution containing 0.0375 g NaBH₄, 10 cm³ 2 M NaOH and 1 cm³ of epichlorohydrin. The suspension was mixed slowly for 2 hours at room temperature, and 10 cm³ of 2 M NaOH and 5 cm³ of epichlorohydrin solutions were gradually added during this time. The mixture was left overnight to complete the reaction. The gel was washed on a Büchner funnel and dried. The dried gel was mixed with 25 cm³ of a solution containing 5.3 g Na₂CO₃, 2.5 g iminodiacetate acid (IDA) and 0.03 g NaBH₄. The suspension was left overnight at 60°C, being stirred slowly from time to time. After 24 hours, the gel was washed with distilled water followed by diluted acetic acid and distilled water again, to neutralize the pH. 50 ml of a solution containing copper (nickel) ions at a concentration of 1 mg cm⁻³ was added to the gel. Next, the bed was transferred to a 1.5 cm diameter and 12 cm long column. The column was equilibrated with 40 cm³ of a 0.05 M solution of Tris-HCl buffer, pH 7.5. A 2 cm³ sample was applied to a glass column packed with gel, and fractions 1-36 were eluted with a pH gradient of Tris-HCl buffer: 7.5, 5.5, 4.5 and back to 7.5 (adjusted with EDTA-fraction 37-70). 4 cm³ fractions were collected at a flow rate of 40 cm³ hour⁻¹.
The peptide content was determined spectrophotometrically with trinitrobenzene sulfonic acid (TNBS), according to the Habeeb’s method (HABEEB 1966) modified by ADLER-NISSEN (1979). Leucylglycine were used as standards (340 nm).

RESULTS AND DISCUSSIONS

Non-ionizing electromagnetic radiation induces specific intermolecular interactions which during traditional cooking occur at a much slower rate, or are not observed at all. Recent research focuses on untypical changes in the structure of saccharide and protein molecules, activation of certain enzymes, the change in the configuration of amino acids from the naturally occurring left-handed to right-handed, and changes in the structure of fatty acid molecules induced by free radicals. These processes proceed much faster during microwave heating. Microwave irradiation causes rapid dipole reorientation and hydrogen bond breaking, and creates molecular friction (SURÓWKA 1994). The thermal processing of string beans resulted in a significant decrease in the content of peptides (extracted with 1% TCA) obtained by chromatographic separation, regardless of the type of metal ions immobilized on the column (Figures 1, 2). Additional fractions of peptides showing high affinity for both copper and nickel ions were obtained after two minutes of blanching. The fact that these additional fractions were obtained by separation using immobilized nickel ions after two and then after six minutes of blanching may be indicative of conformational changes accompanied by denaturation and partial hydrolysis of molecules. The lowest degree of denaturation was observed in string beans processed with microwaves for 2 minutes at 500 W. The reason for these changes could be the differences in dissociation of fraction 11S into subunits with exposure of hydrophobic sur-
faces of the $\beta$-conformation of polypeptide chains, which may polymerize or interact with other molecules. After two and four minutes of heating with 500 W microwaves, the peptide content of fractions 3-7, determined with TNBS, was substantially lower than in blanched samples. Microwaves considerably accelerate the hydrolysis of peptide bonds: two-hour microwave heating of a protein solution in hydrochloric acid produces a comparable quantity of amino acids as traditional 24-hour hydrolysis (KROLL et al. 1998).

Columns with immobilized Cu ions are often used in the process of protein and peptide separation. They have been applied to selectively purify synthetic peptides containing cysteine and histidine (HANSEN et al. 1996), to separate peptides obtained through casein hydrolysis (LIN et al. 2000), to purify antibodies and their proteolytic/recombinant fragments (ROGUE et al. 2007), and to isolate human immunoglobulin IgG (VANCAN et al. 2002). The process of affinity chromatography involving chelate-bound metal ions is determined by many factors (UEDA et al. 2003). Due to the above, the results obtained in this study are difficult to compare with the findings of other authors. The effectiveness of different metal ions used in a given process can be tested reliably only with the use of the same bed and the same material subjected to separation, under identical elution conditions.

As shown in the fraction images obtained after the separation of extracts from string beans heated in a microwave oven, the largest peak was observed for fractions 3-5 irrespective of the heating time, microwave power and the type of metal ions immobilized on the column (Figures 3-6). The application of 750 W and 900 W microwaves for more than six minutes led to the surface drying and browning of string beans, which lost their nutritional value. The levels of peptides were affected by all the tested factors. The highest peptide content (expressed as the leucyl-glycine content) was noticed after microwave heating at the lowest power (500 W) for the shortest time (2 minutes). Larger amounts of peptides were determined in fractions obtained by separation with the use of immobilized Cu ions (0.0132 mg cm$^{-3}$), compared with Ni ions (0.0093 mg cm$^{-3}$). Similar results were reported after microwave heating at 750 W (Figures 1, 2).
Fig. 3. Elution profiles of non-bound peptides from IMAC chromatography of extracts from string bean (blanching: a-2 min., b-6 min.) on Cu(II)-IDA-Sephadex and Ni(II)-IDA-Sephadex.

Fig. 4. Elution profiles of non-bound peptides from IMAC chromatography of extracts from string bean (microwave-heating 500 W: a-2 min., b-6 min.) on Cu(II)-IDA-Sephadex and Ni(II)-IDA-Sephadex.

Fig. 5. Elution profiles of non-bound peptides from IMAC chromatography of extracts from string bean (microwave-heating 750 W: a-2 min., b-6 min.) on Cu(II)-IDA-Sephadex and Ni(II)-IDA-Sephadex.
At higher microwave power (900 W), peptide content obtained by separation on columns with immobilized Ni ions was higher than that obtained with Cu ions (0.0054 mg cm\(^{-3}\) vs. 0.0042 mg cm\(^{-3}\)). The peptide content determined with TNBS in fractions obtained by separation with both Cu and Ni ions decreased as the time of blanching was prolonged. Regardless of the blanching time and the type of metal ions used, fractions 3-5 contained the largest amounts of peptides (expressed as leucyl-glycine content) – Figures 1, 2. In the extract of string beans blanched for two minutes, the peptide content was higher following the application of Ni ions (0.00758 mg cm\(^{-3}\)) than Cu ions (0.00592 mg cm\(^{-3}\)). Prolonged blanching (6 minutes) caused a 2.5-fold and a 1.2-fold decrease in the peptide content of these fractions after separation using immobilized Ni and Cu ions, respectively (Figure 3). Very small peaks with an insignificant peptide content were detected with respect to samples blanched for 8 and 10 minutes. Following separation with the use of immobilized Cu ions, a small peak was observed for fractions 38-39 in samples heated for 4, 6, 8 and 10 minutes, with a peptide content of 0.0025, 0.0037, 0.0033 and 0.0071 mg cm\(^{-3}\) respectively.

Liesiene et al. (1997) obtained a fraction image identical to that reported in this study after separation in columns with immobilised copper ions on a cellulose bed. The application of a 0.5 M solution of sodium chloride in a phosphate buffer at pH = 7.8 enabled us to achieve the maximum protein content (measured spectrophotometrically, A\(_{280}\)) already in the first fractions. Further elution with an acetate buffer at pH from 6.6 to 4.3 produced several small peaks with a maximum absorbance below 0.1.

In the process of isolating green fluorescent protein (GFPuv), the highest efficiency was reported for copper ions, followed by nickel ions, while the lowest efficiency was noted for zinc and cobalt ions (Li et al. 2001). Varlamov et al. (1995) recommended the use of IDA-Sepharose with immobilized Co ions at the final purification stage of beta-dopamine hydroxylase.
from beef bone marrow adrenaline, owing to more effective interactions with these ions, compared with copper, zinc and nickel ions. Cobalt ions also proved to be more selective than Cu, Ni, Zn and Cd ions during the purification of selenoprotein P from human plasma (SIDENIUS et al. 1999), despite stronger interactions between proteins and nickel ions.

Peptides found in string beans had similar affinity for metal ions in the Cu > Ni sequence, with selectivity in the Ni > Cu order (Figure 3-6).

When analysing the affinity of two synthetic ATPase N-terminal peptides from *Helicobacter pylori*. VOLZ et al. (1998) observed that nickel ions are more effective in binding peptides containing histidine residues, while copper ions are more likely to interact with peptides containing cysteine residues. ZACHARIOU and HEARN (2000) suggested mixed-type interactions of proteins and peptides with metal ions – in addition to the formation of coordinate bonds, which are largely responsible for the formation of permanent bonds, they are also responsible for electrostatic interactions whose stability is determined by the eluent’s pH and ionic strength. The effect of the latter factor should be excluded in this study, because the elution process was conducted with the use of the same buffer at an identical concentration.

PATWARDHAN and ATAAI (1997) postulated the existence of two types of copper ions inside the matrix, which are marked by variable availability for protein molecules diffused through the column. According to the above authors, at higher pH values, proteins are bound to easily available copper ions and are first to be eluted, while at lower pH values the eluted proteins are proteins bound to less available copper ions. According to JOHNSON et al. (1996), in an alkaline environment, histidine and lysine – amino acids coordinated via the amino groups - are not bound to metal ions but to the solid support, and the process resembles ion-exchange chromatography, while in an acidic environment these amino acids form strong coordinate bonds with metal ions, and the process is analogous to affinity chromatography.

**CONCLUSION**

1. Microwave heating of string beans decreases the peptide content of extracts isolated with 1% TCA. The resulting changes are dependent on the duration of the process and the type of heating medium.

2. Peptides found in string beans had similar affinity for metal ions in the Cu > Ni sequence, with selectivity in the Ni > Cu sequence.

3. Affinity chromatography with the use of metal ions immobilized to iminodiacetic acid (IDA)-Sephadex G-25 may be successfully used for the separation of peptides isolated from string beans.
REFERENCES


DIFFERENTIATED MICROELEMENT CONTENT IN ANTHURIUM (ANTHURIUM CULTORUM BIRDSEY) LEAVES*

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Abstract

The main objective of the study was to determine the nutritional status in terms of the content of microelements, such as iron, manganese, zinc, copper and boron in several standard cultivars of anthurium (Anthurium cultorum Birdsey) called Baron, Choco, Midori, Pistache, President and Tropical. The plants were grown in expanded clay (§ 8-18 mm) using drip fertigation with standard nutrient for anthurium grown in inert substrates with the following components: N-NH₄<1.0, N-NO₃ 7.5 P 1.0, K 4.5, Ca 1.5, Mg 1.0, S-SO₄ 1.5 (mmol dm⁻³), Fe 15, Mn 3.0, Zn 3.0, Cu 0.5, B 20.0, Mo 0.5 (µmol dm⁻³), pH 5.5-5.7, EC 1.5-1.8 mS cm⁻¹. Every two months, anthurium indicator parts were sampled for chemical analyses. The indicator parts included fully developed leaves from plants after freshly cut flowers. The average microelement content in the indicator parts showed the following values (in mg kg⁻¹ d.m.): Fe 47.6-58.0, Mn 36.9-45.1, Zn 60.3-67.6, Cu 5.01-6.43, B 63.5-89.0. It was found that a significant effect on the nutritional status with respect to micro-elements was produced by the plant cultivar type. The highest content of iron in the indicator parts was found in cv. Baron; manganese was most abundant in cv. Choco; cv. Midori was the richest in zinc and boron appeared in the highest level in cv. Pistache. Coefficients of variability (CV) of the analyzed microelements were determined. The smallest variability during 3 years of studies was shown by copper (CV 15.4-24.3%), a mean value was found in boron (CV 39.7-44.7%) and in iron (CV 25.1-31.4%), while the highest values were shown by zinc (CV 39.7-44.7%) and by manganese (CV 40.4-58.5%).

Key words: tropical, plant analysis, microelement, coefficient of variability, inert substrate.

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Głównym celem badań było określenie zróżnicowania stanu odżywienia mikroelementami: żelazem, manganem, cynkiem, miedzią i borem standardowych odmian anturium (Anthurium cultorum Birdsey): Baron, Choco, Midori, Pistache, President i Tropical. Rośliny uprawiano w keramyzie (r 8-18 mm) z zastosowaniem fertygacji kroplowej pożywką standardową dla uprawy anturium w podłożach inertnych o następującej zawartości składników: N-NH₄<1.0, N-NO₃ 7.5, P 1.0, K 4.5, Ca 1.5, Mg 1.0, S-SO₄ 1.5 (mmol dm⁻³), Fe 15, Mn 3.0, Zn 3.0, Cu 0.5, B 20.0, Mo 0.5 (µmol dm⁻³), pH 5.5-5.7, EC 1.5-1.8 mS cm⁻¹. Co 2 miesiące pobierano do analiz chemicznych części wskaźnikowej anturium, którymi były w pełni wyrośnięte liście z roślin po święto świętem kwiecie. Przeciętna zawartość mikroelementów w częściach wskaźnikowych była następująca (w mg kg⁻¹ s.m.): Fe 47.6-58.0, Mn 36.9-45.1, Zn 60.3-67.6, Cu 5.01-6.43, B 63.5-89.0. Stwierdzono istotny wpływ odmian na stan odżywienia roślin mikroelementami. Największą zawartość żelaza w częściach wskaźnikowych stwierdzono u odmiany Baron, manganu i miedzi u Choco, cynku u Midori, a boru u Pistache. Wyznaczono współczynniki zmienności (CV) zawartości badanych mikroelementów. Najmniejszą zmienność w trakcie 3 lat badań wykazywała miedź (CV 15.4-24.3%), średnią bor (CV 20.9-26.7%) i żelazo (CV 25.1-31.4%), a największą cynk (CV 39.7-44.7%) i mangan (CV 40.4-58.5%).

Słowa kluczowe: anturium, mikroelementy, analiza roślin, współczynniki zmienności, podłoga inertne.

INTRODUCTION

Owing to a widespread use of inert media [mainly expanded clay] and fertigation, Poland is one of the main anthurium producer in Europe. In modern horticulture, an important role is played by controlled fertilization. In many cases, it is not enough to determine the content of components in a substrate or in a nutrient. In order to diagnose the nutritional status of plants, it is necessary to perform their chemical analyses. Usually, it is done by sampling plant indicator parts, which reflect most faithfully the differences in the component content under the influence of increasing fertilization (De Kreij et al. 1990). Mills, Scoggins (1998) proved that young, fully developed leaves, before developing a layer of suberine, are the best indicator parts for anthurium. Older leaves are not adequate for this purpose because their nutritive components are translocated to flowers.

Each year, a wide range of new commercial cultivars of the flower appear on the market. Meanwhile, many of the studies carried out so far concern cultivars of anthurium which are less popular in Poland (Higaki et al. 1992, Sonneveld, Voogt 1993, Mills, Scoggins 1998, Dufour, Guérin 2005).

The main objective of our present study was to determine the nutritional status in terms of iron, manganese, zinc, copper and boron in the most popular anthurium cultivars grown in Poland (Anthurium cultorum Bird-
sey), such as Baron, Choco, Midori, Pistache, President and Tropical, grown in expanded clay, as well as to determine the coefficients of variability and regression equations describing the dynamics of microelement content in leaves. These data are essential for the interpretation of the results of plant assays and for diagnosing their the nutritional status.

MATERIAL AND METHODS

A vegetation experiment was carried out at two speciality farms in Wielkopolska. Venlo type greenhouses were equipped with modern systems of fertigation, climate control and recording system, air humidity checks and energy-saving curtains. Anthurium cultivars (Anthurium cultorium Birdseye), such as Choco, Midori, Pistache, President and Tropical (Anthura B.V., Holland), grown in expanded clay (Ø 8-18 mm), underwent analyses. Cuttings grown in pots of mineral wool (75 cm³) were planted into beds in the greenhouse between 8th and 11th August 2000. The analyses started on 15th of January 2002 (on 2-year-old plants) and were terminated on 30th November 2004 (4-year-old plants). One bed measuring 12 x 46 m covered 55.2 m². Fourteen plants were grown per 1 m² 14. Agronomic treatments were carried out according to the current recommendations for anthurium. During the whole experiment, plant yielding was optimal regarding both the quantitative and qualitative values (KLEIBER, KOMOSA 2007).

In the vegetation experiment, drip fertigation in a closed system was used without nutrient recirculation. The nutrient was distributed in beds through dripping lines with emitters spaced in 20cm intervals. The frequency and time of irrigation depended on the season of the year. In summer, fertigation was applied 6-8 times supplying 4-5 dm³ of nutrient per 1 m², while in winter, it was done 2-3 times applying 2-3 dm³. About 20% of nutrient exuded from the root zone. In order to provide an adequate air and substrate humidity, the culture was sprinkled with rain water using micro-sprinklers.

Before the preparation of nutrients, chemical analyses of water were carried out (KLEIBER, KOMOSA 2006, 2008). In the experiments, the standard nutrient was used in drip fertigation for anthurium grown in inert substrates: N-NH₄<1.0, N-NO₃ 7.5, P 1.0, K 4.5, Ca 1.5, Mg 1.0, S-SO₄ 1.5 (mmol dm⁻³), Fe 15, Mn 3.0, Zn 3.0, Cu 0.5, B 20.0, Mo 0.5 (µmol dm⁻³), pH 5.5-5.7, EC 1.5-1.8 mS cm⁻¹ (after KOMOSA 2000).

Samples of plant material were taken in the years 2002-2004 in two-month intervals, between 14th and 16th January, March, May, July, September and November. The indicator parts of anthurium consisted of fully developed leaves from plants after freshly cut flowers (DE KREIJ et al. 1990). Leaves were randomly sampled from the total area of beds from plants char-
acteristic of a given cultivar, healthy, well yielding and without any symptoms of damages. One sample of a given cultivar consisted of 15-20 leaves sampled in both experimental farms. The details of chemical analyses of plant material were given previously (Analytical methods... 1972, KLEIBER et al. 2009).

Statistical analysis was carried out, with the calculation of variability coefficients and regression equilibrium coefficients and a description of the microelement content dynamics in the indicator parts, which changed with the age of plants. Conclusions were drawn at the significance level of \( \alpha = 0.05 \).

RESULTS AND DISCUSSION

The results of the chemical analyses of anthurium indicator parts are shown in Tables 1 and 2 and the dynamics of elements – in Figure 1. The plant cultivar and age of plants significantly modified the nutritional status of plants in terms of microelements. The mean iron content in the indicator parts was 47.6-58.0 mg Fe kg\(^{-1}\) (Table 1). The least amount of Fe was found in the youngest plants (in 2002), while the 3-4-year-old plants were characterized by significantly higher values (in 2003-2004). The Fe content tended to increase with the aging of plants (Figure 1), which was proven by a regression equation in the form of

\[
y = 0.011x^3 - 0.2829x^2 + 2.4241x + 45.50,
\]

where: \( y \) – expected Fe content in a given term; \( x \) – the term of sampling (1-18; from Jan. 2002 to Nov. 2004; 3 years 6 terms = 18). For example, in the 4\(^{th}\) term of sampling (July 2002), the Fe content was

\[
y = 0.011 \times 4^3 - 0.2829 \times 4^2 + 2.4241 \times 4 + 45.50 = 50.74 \text{ mg Fe kg}^{-1}
\]

The cultivar Baron showed the highest Fe content in the indicator parts (53.9-67.2 mg Fe), while cv. Choco was characterized by the smallest Fe content (38.3-53.9 mg Fe). Some earlier studies by KLEIBER, KOMOSA (2004) showed that the year of experiments can produce a considerable effect on the iron content.

The mean content of manganese in plant indicator parts ranged from 36.9mg (4-year-old plants) to 45.1 mg Mn kg\(^{-1}\) d.m. (3-year-old plants). Differences between the successive years have not been statistically proven. KLEIBER and KOMOSA (2004) confirmed significant differentiation of Mn in indicator parts in the particular years of studies. The cultivar Choco showed the highest Mn content (41.5-68.8 Mn), while cv. President was characterized by the smallest Fe content (38.3-53.9 mg Fe). Some earlier studies by KLEIBER, KOMOSA (2004) showed that the year of experiments can produce a considerable effect on the iron content.

The mean content of manganese in plant indicator parts ranged from 36.9mg (4-year-old plants) to 45.1 mg Mn kg\(^{-1}\) d.m. (3-year-old plants). Differences between the successive years have not been statistically proven. KLEIBER and KOMOSA (2004) confirmed significant differentiation of Mn in indicator parts in the particular years of studies. The cultivar Choco showed the highest Mn content (41.5-68.8 Mn), while cv. President was characterized by the smallest manganese content (22.1041.3 mg Mn kg\(^{-1}\) d.m.).

The mean content of zinc in the indicator parts of the analyzed cultivars was 60.3-67.6 mg Zn kg\(^{-1}\) (Table 2). The youngest plants (in 2002) were characterized by a significantly higher zinc content. The Zn content declined drastically in 3-year-old plants. In zinc, the tendencies in the plant indicator
Table 1

Coefficients of variability (CV) and mean content of iron and manganese in anthurium indicator parts

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>mg Fe kg(^{-1}) d.m.</th>
<th>mg Mn kg(^{-1}) d.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2002</td>
<td>2003</td>
</tr>
<tr>
<td>Baron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>53.9a</td>
<td>67.2a</td>
</tr>
<tr>
<td>min</td>
<td>37.3</td>
<td>57.1</td>
</tr>
<tr>
<td>max</td>
<td>64.8</td>
<td>83.9</td>
</tr>
<tr>
<td>CV (%)</td>
<td>25.1</td>
<td>18.8</td>
</tr>
<tr>
<td>Choco</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>38.3c</td>
<td>45.8b</td>
</tr>
<tr>
<td>min</td>
<td>28.0</td>
<td>36.9</td>
</tr>
<tr>
<td>max</td>
<td>45.3</td>
<td>51.1</td>
</tr>
<tr>
<td>CV (%)</td>
<td>19.5</td>
<td>13.5</td>
</tr>
<tr>
<td>Midori</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>42.9c</td>
<td>47.8b</td>
</tr>
<tr>
<td>min</td>
<td>28.2</td>
<td>34.4</td>
</tr>
<tr>
<td>max</td>
<td>57.6</td>
<td>64.2</td>
</tr>
<tr>
<td>CV (%)</td>
<td>29.6</td>
<td>24.8</td>
</tr>
<tr>
<td>Pistache</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>44.7bc</td>
<td>61.5a</td>
</tr>
<tr>
<td>min</td>
<td>38.6</td>
<td>46.6</td>
</tr>
<tr>
<td>max</td>
<td>53.3</td>
<td>84.1</td>
</tr>
<tr>
<td>CV (%)</td>
<td>17.0</td>
<td>28.6</td>
</tr>
<tr>
<td>President</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>53.9a</td>
<td>64.1a</td>
</tr>
<tr>
<td>min</td>
<td>31.5</td>
<td>51.8</td>
</tr>
<tr>
<td>max</td>
<td>78.3</td>
<td>78.8</td>
</tr>
<tr>
<td>CV (%)</td>
<td>31.9</td>
<td>15.7</td>
</tr>
<tr>
<td>Tropical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>51.9ab</td>
<td>61.7a</td>
</tr>
<tr>
<td>min</td>
<td>30.7</td>
<td>50.6</td>
</tr>
<tr>
<td>max</td>
<td>66.6</td>
<td>71.5</td>
</tr>
<tr>
<td>CV (%)</td>
<td>37.1</td>
<td>18.2</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>47.6c</td>
<td>58.0a</td>
</tr>
<tr>
<td>CV (%)</td>
<td>31.4</td>
<td>25.1</td>
</tr>
</tbody>
</table>

Mean values in columns marked with the same letter do not differ significantly.
Mean values from all the tested varieties (in rows) marked with the same letter do not differ significantly.
<table>
<thead>
<tr>
<th>Cultivar</th>
<th>mg Zn kg(^{-1}) d.m.</th>
<th>mg Cu kg(^{-1}) d.m.</th>
<th>mg B kg(^{-1}) d.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baron</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>55.4(c)</td>
<td>58.5(a)</td>
<td>64.5 (a)</td>
</tr>
<tr>
<td>min</td>
<td>49.3</td>
<td>40.6</td>
<td>34.1</td>
</tr>
<tr>
<td>max</td>
<td>61.3</td>
<td>73.9</td>
<td>92.0</td>
</tr>
<tr>
<td>CV (%)</td>
<td>35.7</td>
<td>49.7</td>
<td>50.2</td>
</tr>
<tr>
<td>Choco</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>70.7 (b)</td>
<td>60.0 (a)</td>
<td>60.4 (a)</td>
</tr>
<tr>
<td>min</td>
<td>61.9</td>
<td>42.3</td>
<td>47.7</td>
</tr>
<tr>
<td>max</td>
<td>82.8</td>
<td>78.5</td>
<td>70.0</td>
</tr>
<tr>
<td>CV (%)</td>
<td>33.5</td>
<td>36.4</td>
<td>31.0</td>
</tr>
<tr>
<td>Midori</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>80.5 (a)</td>
<td>61.8 (a)</td>
<td>68.0 (a)</td>
</tr>
<tr>
<td>min</td>
<td>67.0</td>
<td>48.7</td>
<td>34.8</td>
</tr>
<tr>
<td>max</td>
<td>96.0</td>
<td>71.9</td>
<td>93.7</td>
</tr>
<tr>
<td>CV (%)</td>
<td>32.8</td>
<td>34.4</td>
<td>41.4</td>
</tr>
<tr>
<td>Pistache</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>55.0 (c)</td>
<td>56.6 (a)</td>
<td>49.3 (c)</td>
</tr>
<tr>
<td>min</td>
<td>45.5</td>
<td>43.3</td>
<td>29.3</td>
</tr>
<tr>
<td>max</td>
<td>71.0</td>
<td>67.3</td>
<td>64.8</td>
</tr>
<tr>
<td>CV (%)</td>
<td>40.8</td>
<td>46.9</td>
<td>41.4</td>
</tr>
<tr>
<td>President</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>75.0 (ab)</td>
<td>62.6 (a)</td>
<td>59.1 (b)</td>
</tr>
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<td>min</td>
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<td>49.5</td>
<td>50.8</td>
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<tr>
<td>max</td>
<td>93.7</td>
<td>73.9</td>
<td>76.1</td>
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<tr>
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<td>44.2</td>
<td>44.4</td>
</tr>
<tr>
<td>Tropical</td>
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<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>69.3 (bc)</td>
<td>62.5 (a)</td>
<td>64.9 (a)</td>
</tr>
<tr>
<td>min</td>
<td>53.9</td>
<td>49.8</td>
<td>43.1</td>
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<tr>
<td>max</td>
<td>91.2</td>
<td>70.5</td>
<td>98.7</td>
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<tr>
<td>CV (%)</td>
<td>36.8</td>
<td>20.7</td>
<td>47.5</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>67.6 (a)</td>
<td>60.3 (b)</td>
<td>61.1 (b)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>39.9</td>
<td>39.7</td>
<td>44.7</td>
</tr>
</tbody>
</table>

Explanations see Table 1
Fig. 1. Dynamics of microelements contents in the indicator parts of anthurium (means from 6 cultivars and 2 farms)
parts are described by the equation: $y = 0.0505x^3 - 1.2304x^2 + 6.9172x + 59.47$. **KLEIBER, KOMOSA (2004)** showed that the zinc content tended to decrease during a growing season. In the present tests, significant differences were shown in the Zn content among the analyzed cultivars. The highest Zn content was found in cv. Midori (61.8-80.5 mg Zn), while the lowest Zn value was shown in cv. Pistache (49.3-56.6 mg Zn kg$^{-1}$ d.m.).

The mean content of copper, depending on the year of the experiment, varied between 5.01 and 6.43 mg Cu kg$^{-1}$ d.m. in the plant indicator parts (Table 2). Three-year-old plants (in 2003) showed significantly the highest Cu content, while the two- and four-year-old plants (in 2002 and 2004) were characterized by smaller amounts of Cu. Significant differences among the analyzed cultivars were found. The smallest mean Cu content (5.39-5.82 mg Cu) was shown in cv. Pistache, while cv. Choco showed the highest Cu content (5.48-6.98 mg Cu). Regression equation in the form: $y = 0.003x^3 - 0.0936x^2 + 0.7326x + 4.90$ described the dynamics of the Cu content changing with the aging of plants. **KLEIBER, KOMOSA (2004)** found high differentiation of the copper content in the successive years of studies.

The mean boron content in plant indicator parts showed the values of 63.5-89.0 mg B kg$^{-1}$ d.m. Analogously to copper, significantly the highest B content was shown in 3-year-old plants (in 2003). The dynamics of B in plant indicator parts is described by the equation: $y = 0.0157x^3 - 0.859x^2 + 11.48x + 39.67$. **KLEIBER, KOMOSA (2004)** found an unstable boron nutritional status of plants in the successive years of studies. The smallest mean content of boron was found in cv. President (57.3-91.1 mg B), while the highest B content was shown in cv. Pistache (65.1-90.8 mg B kg$^{-1}$ d.m.).

The content of microelements found in the present study were compared with the content ranges recommended by other authors (Table 3). A high degree of agreement was found in the content of iron in the indicator parts of plants with the data reported in the literature (Table 3). In contrast to iron, the content of manganese showed little correspondence to the ranges reported in literature. Available references reported much higher Mn content in plant indicator parts. Regarding zinc, the assessed levels coincided with the ranges reported by **CHEN et al. (2003)**. The copper content was within the ranges given by **MILLS, SCOGGINS (1998)** and by **CHEN et al. (2003)**. A much higher copper content was reported by **MILLS, SCOGGINS (1998)**. The content of boron in most of the cultivars was similar to that reported by **UCHIDA (2000)** and by **CHEN et al. (2003)**. The smallest content of B appears in a paper by **MILLS, SCOGGINS (1998)**.

**Coefficients of variability**

Coefficients of variability (CV) indicating the content of microelements in the plant indicator parts were determined (Tables 1, 2). They include the total effect of a series of factors modifying amounts of components in plants, including the cultivar type, growing conditions, light conditions. It was found
### Table 3

Recommended content of microelements in indicator parts of anthurium according to different authors

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Recommended values (mg kg⁻¹ d.m.)</th>
<th>% of results in the recommended range</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>28.0 - 112.0</td>
<td>98.6</td>
<td>84.7</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>38.0 - 109.0</td>
<td>58.3</td>
<td>52.8</td>
<td>36.1</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>46.0 - 131.0</td>
<td>68.1</td>
<td>66.7</td>
<td>62.5</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>6.3 - 12.7</td>
<td>37.5</td>
<td>43.1</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>54.0 - 76.0</td>
<td>51.4</td>
<td>29.2</td>
<td>50.0</td>
<td></td>
</tr>
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</table>

**Anthura (1998)**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Recommended values (mg kg⁻¹ d.m.)</th>
<th>% of results in the recommended range</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>20.7 - 90.0 (a)</td>
<td>95.8</td>
<td>56.9</td>
<td>95.8</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>41.0 - 237.0</td>
<td>41.7</td>
<td>44.4</td>
<td>36.1</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>41.0 - 98.1</td>
<td>66.7</td>
<td>66.7</td>
<td>58.3</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>10.3 - 25.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>12.0 - 25.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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</table>

**Mills, Scogins (1998)**

<table>
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<th>Nutrient</th>
<th>Recommended values (mg kg⁻¹ d.m.)</th>
<th>% of results in the recommended range</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>28.0 - 76.6 (b)</td>
<td>81.9</td>
<td>30.6</td>
<td>68.1</td>
<td></td>
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<tr>
<td>Mn</td>
<td>44.0 - 193.3</td>
<td>38.9</td>
<td>36.1</td>
<td>30.6</td>
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</tr>
<tr>
<td>Zn</td>
<td>17.0 - 57.5</td>
<td>41.7</td>
<td>50.0</td>
<td>51.4</td>
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</tr>
<tr>
<td>Cu</td>
<td>4.0 - 13.8</td>
<td>100.0</td>
<td>43.1</td>
<td>81.9</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>11.0 - 27.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

**Uchida (2000)**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Recommended values (mg kg⁻¹ d.m.)</th>
<th>% of results in the recommended range</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>50.0 - 400.0</td>
<td>77.8</td>
<td>100.0</td>
<td>93.1</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>50.0 - 1500.0</td>
<td>27.8</td>
<td>30.6</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>25.0 - 135.0</td>
<td>100.0</td>
<td>95.8</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

**Chen et al. (2003)**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Recommended values (mg kg⁻¹ d.m.)</th>
<th>% of results in the recommended range</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>50.0 - 400</td>
<td>77.8</td>
<td>100.0</td>
<td>93.1</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>40.0 - 500.0</td>
<td>41.7</td>
<td>47.2</td>
<td>36.1</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>20.0 - 200.0</td>
<td>100.0</td>
<td>98.6</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>5.0 - 40.0</td>
<td>87.5</td>
<td>84.7</td>
<td>51.4</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>20.0 - 100.0</td>
<td>97.2</td>
<td>68.1</td>
<td>98.6</td>
<td></td>
</tr>
</tbody>
</table>

*a* – young leaves, ripe in 90%, pale-green, 10 days before full maturity

*b* – mature leaves, dark green, with a growing and in 3/4 mature flower
that a significant effect was produced by the year of experiment and the analyzed cultivar on the coefficient of microelement content variability in the indicator parts, although there is no general tendency describing these effects. During the three years of trials, copper showed the smallest variability (CV 15.4%-24.3%). Moderate variability was found for iron (CV 25.1%-31.4%) and boron (CV 20.9-26.7%), while the highest variability was achieved for manganese (CV 40.4-58.5%) and zinc (CV 39.7-44.7%). In the analyzed cultivars, differentiation of the variability coefficients was found. For example, the CV of iron in 2002 for cv. Choco cultivar was 19.5%, while for cv Midori, it reached 29.6%. This indicates that there was significant variability in the iron content in the indicator parts of cv. Midori. Considering the variability coefficients for microelement content which indicate deviations in their content versus the mean value for the total population enables researchers to attain a more precise interpretation of leaf analysis for diagnostic purposes.

**CONCLUSIONS**

1. Significant effect of a cultivar and the age of plants was found to be produced on the content of iron, manganese, zinc, copper and boron in leaves of anthurium.

2. The mean content of microelements in the analyzed cultivars was as follows: Fe 51.8-54.6, Mn 41.1-158.6, Zn 43.2-82.8, Cu 5.35-6.29, B 73.3-73.9 mg kg\(^{-1}\) d.m. in indicator parts.

3. The highest iron content in plant indicator parts was found in the cultivar Baron; manganese and copper showed the highest value in cv. Choco; the zinc content was the highest in cv. Midori while the boron content was the highest in cv. Pistache.

4. A significant effect on the coefficient of variability (CV) in the microelement content of plant indicator parts was found to be exerted by the cultivar type and plant age. Copper showed to be a component with the lowest variability (CV 15.4%-24.3%); boron was moderately variable (CV 20.9-26.7%); iron was also characterized by a medium value of variability (CV 25.1-31.4%), while zinc (CV 39.7-44.7%) and manganese (CV 40.4-58.5%) showed the highest variability.

**REFERENCES**


EFFECT OF POLYMER SUPERSORBENT ADDED TO MEDIUM ON THE CONTENT OF MINERAL ELEMENTS IN STRAWBERRY LEAVES AND FRUIT

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Abstract

The aim of the study was to evaluate the influence of polymer supersorbent AgroHydrogel, added to soil, on the content of some mineral elements in leaves and fruit of strawberry cv. Elsanta. In 2007-2008, a pot experiment set up in a system of complete randomization was carried out in four replications in a greenhouse of the West Pomeranian University of Technology in Szczecin. The experimental factor was the addition of two doses of AgroHydrogel to the medium. The two rates were 1.8 and 3.6 g dm⁻³, i.e. 15 and 30 g per a Kick's pot versus the control which was the medium with no gel. The experiment was carried out indoors. Soil moisture was measured by means of contact soil tensometers. The plants were watered with 0.5 dm³ per pot and the tensometer, fitted in the medium with 15 g of gel per pot, showed 450 hPa. The content of the elements in strawberry leaves and fruit was determined by means of the AAS method. The applied polymer supersorbent decreased the cummulation of zinc and lead in leaves and copper, nickel and lead in fruit of strawberry. This effect particularly visible in plants growing in the medium with an increased dose of hydrogel. However, the application of AgroHydrogel did not affect the content of iron, manganese, copper and nickel in leaves and manganese, zinc and iron in fruit of the analyzed variety of strawberry.

Key words: strawberry, AgroHydrogel, microelements.
Abstrakt

Jednym z głównych czynników ograniczających plon truskawki na plantacjach bez nawadniania jest okresowy niedobór wody w glebie. Korzystny wpływ na właściwości wodno-powietrzne gleby wywierają preparaty zwiększające jej pojemność wodną, tzw. hydrożele (supersorbenty). Związki te mają zdolność wiązania i magazynowania wody grawitacyjnej, co zwiększa ilość wody dostępnej dla systemu korzeniowego roślin.

Celem badań była ocena wpływu supersorbentu polimerowego, AgroHydrogelu, dodawanego do podłoża na zawartość niektórych pierwiastków w liściach oraz owocach truskawki odmiany Elsanta. W latach 2007-2008, w hali wegetacyjnej Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie, przeprowadzono doświadczenie wazonowe w układzie kompletnej randomizacji, w czterech powtórzeniach. Czynnikiem doświadczalnym był dodatek AgroHydrogelu do podłoża – zastosowano dwa poziomy: 1,8 oraz 3,6 g dm⁻³, tj. 15 i 30 g na pojemnik Kicka, oraz kontrola, którą stanowiło podłoże bez dodatku żelu. Doświadczenie przeprowadzono pod zadaszeniem. Wilgotność gleby mierzono za pomocą tensjometrów glebowych kontaktowych. Rośliny podlewano w ilości 0,5 dm³ na wazon, przy wskazaniu tensjometru, umieszczonego w podłożu z dodatkiem żelu w ilości 15 g na wazon, wynoszącym 450 hPa. Zawartość pierwiastków w liściach oraz owocach truskawki oznaczono metodą ASA. Zastosowany supersorbent polimerowy zmniejszył pobieranie oraz kumulację cynku i ołówku w liściach oraz miedzi, niklu i ołówku w owocach truskawki. Szczególnie wyraźnie zaznaczyło się to w roślinach rosnących w podłożu z większą dawką hydrożelu. Dodatek AgroHydrogelu nie wpłynął natomiast na zawartość żelaza, manganu, miedzi i niklu w liściach oraz manganu, cynku i żelaza w owocach badanej odmiany.

Słowa kluczowe: truskawka, AgroHydrogel, mikroelementy.

INTRODUCTION

Poland is one of the largest producers of strawberry fruit (Fragaria ananassa Duch.). However, the average strawberry crop does not exceed 3.7 t ha⁻¹, which is due, among other reasons, to the fact that cultivation of strawberries is carried out without irrigation and a high sensitivity of this plant to periodical water deficit considerably depressed the yield and its quality (HOLUBOWICZ, REBANDEL 1997, MAKOWSKA 2004). Hydrogels also known as supersorbents, that is preparations increasing water capacity of soil, have a beneficial influence on water and air properties of soil. Their characteristic feature is an extremely high capability of storing water which otherwise is absorbed from precipitation and some of it can be gradually conveyed to plants (GORECKI, PAUL 1993, WIERZBICKA, MAJKOWSKA-GADOMSKA 2005).

Strawberries easily take up mineral elements from the soil. They also take up macroelements and trace elements and are characterised by their relatively large content as compared to other fruit plants popular in Poland (JĘDRZEJCZAK, SZTEKE 1989, SZTEKE et al. 2006). The content of mineral elements in plants depends on many environmental factors, including accessibility to soil water.
The available literature does not contain explicit conclusions regarding the effect of hydrogel on chemical composition of berry plants. The present paper shows the results of experiments whose aim was to determine the influence of a polymer supersorbent called AgroHydrogel added to soil on the content of some mineral elements in leaves and fruit of a dessert cultivar of strawberry called Elsanta.

**MATERIAL AND METHODS**

In 2007-2008, a pot experiment set up in a system of complete randomization was carried out in four replications in a greenhouse of the West Pomeranian University of Technology in Szczecin. The object of the study was strawberry cv. Elsanta. The experimental factor was the addition of two rates of AgroHydrogel to the medium. The two rates were 1.8 and 3.6 g dm$^{-3}$, i.e. 15 and 30 g per a Kick’s pot versus the control, which was the medium with no gel. The pots of 10 dm$^3$ capacity were filled with 8 dm$^3$ of soil material. Prior to filling the pots, hydrogel was added to the medium and then all was mixed. Table 1 shows the characteristics of the soil material.

<table>
<thead>
<tr>
<th>pH H$_2$O</th>
<th>KCl</th>
<th>Percent of clay fraction</th>
<th>$S_0$ (g cm$^{-3}$)</th>
<th>Pkw (%)</th>
<th>Pkv (%)</th>
<th>Wtw (%)</th>
<th>Wtv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.58</td>
<td>5.97</td>
<td>21</td>
<td>1.24</td>
<td>24.7</td>
<td>34.0</td>
<td>32.9</td>
<td>38.0</td>
</tr>
</tbody>
</table>

$S_0$ – bulk density, Pkw – capillary weight, Pkv – capillary volume, Wtw – total water capacity in investigated soil – weight, Wtv – total water capacity in investigated soil – volume

The doses of mineral fertilization were 50, 80 and 100 kg NPK ha$^{-1}$. Potassium, phosphorus and half a dose of nitrogen were applied prior to planting. The remaining half a dose of nitrogen was top-dressed before the flowering of the plants.

In mid-April 2007, frigo seedlings were placed in containers, 1 piece/pot. The experiment was carried out indoors. The plants wintered in pots in an unheated greenhouse. The moisture of soil was measured by means of soil contact tensometers. The plants were watered with 0.5 dm$^3$ per pot, and the tensometer, fitted in the medium with 15 g of gel per pot, showed 450 hPa. Well grown, healthy leaves and ripe fruit were taken for determination. In both years, the fruit for the analysis were gathered systematically as they were ripening, from the first decade of June to the first decade of July. A collective sample was created for each replication from the gathered fruit. The leaves were gathered once a year (in both years of the experi-
ment) in the second decade of July, after the fruiting of strawberry. The content of iron, manganese, copper, zinc, nickel and lead in fruit and leaves of strawberry was determined in four replications in plant material dried at 105°C. An average weight of a laboratory sample of dried leaves and fruit for one replication was about 30 g. The plant material (5 g) was wet mineralized in a 03:01 mixture of nitrogen (V) and chloric (VII) acid. The content of the elements in leaves and fruit of strawberry was determined by means of the AAS method (in a SOLAR 939). The determination of the content of chemical elements was carried out at the Department of Soil Science of West Pomeranian University of Technology in Szczecin. The laboratory participated in interlaboratory studies on the comparison of the content of mineral components in reference material (The Report of Institute of Nuclear...

The results of the chemical composition of the plants underwent one-factor analysis of variance. The significance of differences between the averages were defined by Duncan’s test at $\alpha = 0.05$. Due to the homogeneity of the variance of error, the results of two years of experiment were expressed synthetically (WÓJCIK, LAUDAŃSKI 1989). Coefficients of linear correlation between the content of the analyzed elements in fruit and their content in leaves were calculated. When a correlation coefficient was significant at $\alpha=0.05$, the relationship was shown in a diagram.

RESULTS AND DISCUSSION

The content of iron in fruit of strawberry cv. Elsanta ranged from 59.56 to 79.66 mg kg$^{-1}$ of dry matter (Table 2). According to HAKALA et al. (2003), the average content of this microelement in the strawberry varieties they examined reached 3.2 mg kg$^{-1}$ of fresh matter. According to GAWĘDA and BEN (2004), fruit of cv. Elsanta can accumulate 103 mg Fe kg$^{-1}$ of dry matter. More iron is accumulated in leaves, where its concentration varied from 146.3 to 273.1 mg kg$^{-1}$ of dry matter. GAWĘDA and BEN (2004) observed that the content of iron in strawberry leaves reached 475 mg kg$^{-1}$ of dry matter. No significant influence of the applied supersorbent on the accumulation of this component was noticed in the fruit or leaves of cv. Elsanta.

Likewise, AgroHydrogel did not affect significantly the amount of manganese in fruit and leaves (Table 2). According to SZTEKE et al. (2006), the content of this microelement in strawberry leaves is on average 4.51 mg kg$^{-1}$ of fresh matter. These authors emphasize the relationship between soil pH and manganese cumulation by strawberry fruit, with an increase in the soil reaction being inhibitory to the uptake of this element by plants. As GAWĘDA and BEN report (2004), the content of manganese in fruit of cv. Elsanta is 54 mg kg$^{-1}$ of dry matter, whereas in leaves it can be 137 mg kg$^{-1}$ of
dry matter. The authors have also observed that the content of iron, manganese zinc and copper in strawberries decreased significantly as they grew older.

Our experiments did not show any influence of the addition of AgroHydrogel to the medium on the content of zinc in fruit. However, the superabsorbent significantly decreased the content of this element in leaves (Table 2). According to SzTeka et al. (2006), strawberry fruit accumulates on average 1.13 mg Zn kg\(^{-1}\) of fresh matter, and the uptake of this element by plants does not depend on its content in soil. Gawęda and Ben (2004) report that the concentration of this element in fruit of cv. Elsanta is 31 mg kg\(^{-1}\), whereas in leaves, it can reach 50 mg kg\(^{-1}\) of dry matter.

Hakala et al. (2003) claim that the amount of copper in strawberry fruit varies from 0.38 to 0.98 mg kg\(^{-1}\) of fresh matter. According to Gawęda and Ben (2004), the amount of copper in strawberry fruit equals 7.8 mg kg\(^{-1}\) of dry matter, and in leaves it is 8.8 mg kg\(^{-1}\) of dry matter. The supersorbent caused a significant decrease in the content of copper in fruit. The largest content of this element in leaves was found in the control plants, while the lower copper concentration in leaves appeared in plants cultivated in the medium with of 3.6 g dm\(^{-3}\) of AgroHydrogel. However, the differences were not statistically significant (Table 2).

The results of our experiment show a considerable influence of the supersorbant added to the medium, such as a decrease in the content of lead and nickel in fruit (Table 2). Significant decrease in the content of heavy metals in fruit can prove that these heavy metals are strongly absorbed by the applied preparation, which reduced amounts of these elements in plant available forms in the medium, which in turn limits their accumulation in

---

**Table 2**

<table>
<thead>
<tr>
<th>Dose of AgroHydrogel</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
<th>Cu</th>
<th>Ni</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fruit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>79.66 a</td>
<td>14.57 a</td>
<td>19.84 a</td>
<td>4.866 b</td>
<td>5.296 b</td>
<td>6.556 b</td>
</tr>
<tr>
<td>15 g per pot</td>
<td>71.47 a</td>
<td>14.36 a</td>
<td>18.46 a</td>
<td>3.742 a</td>
<td>2.970 a</td>
<td>1.851 a</td>
</tr>
<tr>
<td>30 g per pot</td>
<td>59.56 a</td>
<td>14.20 a</td>
<td>16.51 a</td>
<td>3.49 a</td>
<td>1.890 a</td>
<td>1.297 a</td>
</tr>
<tr>
<td><strong>Leaves</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>214.1 a</td>
<td>27.24 a</td>
<td>37.38 b</td>
<td>4.187 a</td>
<td>2.888 a</td>
<td>3.588 b</td>
</tr>
<tr>
<td>15 g per pot</td>
<td>273.1 a</td>
<td>29.40 a</td>
<td>34.89 ab</td>
<td>3.290 a</td>
<td>2.595 a</td>
<td>2.198 ab</td>
</tr>
<tr>
<td>30 g per pot</td>
<td>146.3 a</td>
<td>33.21 a</td>
<td>28.25 a</td>
<td>3.166 a</td>
<td>2.320 a</td>
<td>1.384 a</td>
</tr>
</tbody>
</table>

Means assigned identical letters do not differ significantly at the level of significance \(\alpha = 0.05\).
fruit and increases the biological value of the yield. According to Szteke et al. (2006), strawberry fruit contain on average 0.01 mg Pb kg\(^{-1}\) of fresh matter. Addition of 3.6 g dm\(^{-3}\) AgroHydrogel to the medium caused a significant decrease in accumulation of lead in leaves, i.e. by 61% in versus the control. A similar relationship was also observed for the content of nickel in leaves of the analyzed strawberry cultivar (at the dose of 3.6 g dm\(^{-3}\) of the gel, the nickel concentration was 19.7% lower than in the control).

Our analysis of the rectilinear correlation showed that only copper demonstrated a significant positive relationship between its content of in fruit and the amounts in strawberry leaves (Figure 1). Regarding the other elements, the coefficients of correlation \((r)\) were non-significant.

![Fig. 1. Dependence of Cu content in fruit on Cu content in leaves of cv. Elsanta strawberry](image)

### CONCLUSIONS

1. AgroHydrogel added to the medium did not significantly affect the content of Fe, Mn, Zn in fruit and the amount of Fe, Mn, Cu and Ni in leaves of cv. Elsanta.

2. The polymer supersorbent significantly decreased accumulation of Cu, Pb and Ni in strawberry fruit and Zn and Pb in leaves.

3. The analysis of the rectilinear correlation showed a strong positive relationship between the content of copper in fruit and its amount in leaves of strawberry.
REFERENCES


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EVALUATION OF THE CORRELATIONS BETWEEN MAGNESIUM CONCENTRATION AND SELECTED SERUM LIPID COMPONENTS IN WOMEN AND MEN OF DIFFERENT AGE WITH CHRONIC KIDNEY FAILURE

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Abstract

In literature of the recent years, more and more attention has been paid to the correlations between magnesium and blood lipids. Lipid balance disorders induced by hypomagnesaemia bring about changes in the liquidity of cellular membranes. Increased migration of lipids, in particular of VLDL and LDL, across arterial walls has been observed in hypomagnesaemia, with their consequential deposition in the internal membrane. Kidneys, apart from urine production and elimination, fulfil many other important functions in organism. They determine normal functioning of the whole organism, while disorders in their function lead to serious changes in homeostasis. Lipid balance disorders are a serious risk factor in the development of atherosclerotic lesions in patients with chronic kidney failure.

The aim of this study was to examine the correlations between magnesium concentration and total cholesterol and HDL- and LDL-cholesterol contents in women and men with chronic kidney failure under and over 50 years of age. Subjects were divided into four groups: K₁ – 14 women 34-50 years old, K₂ – 14 women over 50 years old, M₁ – 17 men 30-50 years old, and M₂ – 13 men over 50 years old. Tests were made using a COBAS INTEGRA analyser. High concentration of Mg, exceeding the upper limit of reference values, was observed both in two women and men groups (K₁ – 1.26 mmol(+) kg⁻¹; K₂ – 1.25 mmol(+) kg⁻¹; M₁ – 1.13 mmol(+) kg⁻¹; M₂ – 1.16 mmol(+) kg⁻¹). Also the levels of total and HDL-cholesterol in all subjects were within physiological limits (respectively: K₁ – 4.61 and 1.23 mmol(+) kg⁻¹; K₂ – 4.69 and 1.29 mmol(+) kg⁻¹; M₁ – 3.94 and 1.20 mmol(+) kg⁻¹; M₂ – 3.98 and 1.16 mmol(+) kg⁻¹). Only the LDL-cholesterol concentration in the group of younger men was below normal limit (1.93 mmol(+) kg⁻¹). Small positive correlations were obtained between Mg content and that of total and LDL-cholesterol in older
women and younger men groups as well as between Mg and HDL-cholesterol contents in both men groups. At the same time, a small negative correlation was obtained between Mg and LDL-cholesterol concentrations in the older men group. These correlations were statistically non-significant.

**Key words:** chronic kidney failure, magnesium, lipids.

**INTRODUCTION**

Chronic kidney failure (CKF) has been newly included into civilisation diseases of the 21st century, apart from cardiovascular diseases, arterial hypertension, obesity and diabetes. CKF can be a consequence or complication of all other civilisation diseases. Due to initially latent course of the dis-
ease, it is being diagnosed too rarely. The progressing disease can be a basis for development of chronic kidney failure and for the necessity of renal replacement therapy connected with that. Moreover, the mortality due to cardiovascular complications among patients with chronic renal diseases is three times higher than that resulting from direct consequences of the underlying disease itself. Therefore, early diagnostics – allowing suppression of growing kidney function failure and prevention from further complications – is very important (Go et al. 2004, Król, Rutkowski 2008).

The classification of chronic renal disease depends on the degree of renal function measured by glomerular filtration rate (GFR). There are 5 stages of that disease: stage 1 – kidney damage with normal or increased GFR, stage 2 – kidney damage with mild reduction in GFR, stage 3 – kidney damage with moderate reduction in GFR, stage 4 – kidney damage with severe reduction in GFR, and stage 5 – established kidney disease (Czekalski 2007, Mysliwiec, Hryszko 2008). Other definitions used for chronic renal disease in successive stages (according to the authors mentioned above) are as follows: stage 1 – kidney damage in the form of albuminuria, proteinuria, haematuria; stage 2 – latent (chronic) renal insufficiency, stage 3 – compensated (chronic) renal insufficiency, stage 4 – manifest uncompensated, advanced chronic renal insufficiency, and stage 5 – terminal (extreme) renal insufficiency.

In Poland, there are probably 4.24 million patients with CKF (about 11% of population), including 1.27 M in the first stage; 1.16 M in the second one; 1.66 M in the third one, and 77 thousand in the fourth stage of disease. On the other hand, the number of sick persons with the end-stage kidney failure (ESKF), requiring dialysis therapy or renal transplantation, is about 50 thousand (Rutkowski 2007, Steciwko et al. 2006).

Lipid balance disorders are one of the elements of clinical manifestation of the advanced chronic kidney failure. They is a serious risk factor for the development of atherosclerotic lesions and increase together with the degree of kidney failure intensification (Zwolińska et al. 1997). It was found that the concentration of total cholesterol and triglycerides increased with hypomagnesaemia (Altura et al. 1990, D’Eril, Trotti 1991, Lichodziejewska, Kloś 1993), while that of HDL-cholesterol decreased (Lichodziejewska, Kloś 1993).

The aim of this study was to evaluate the correlations between magnesium content and the concentration of total cholesterol and its HDL and LDL fractions in women and men with chronic kidney failure.
MATERIAL AND METHODS

Research material was the blood collected prior to dialysis from subjects with CKF who, in 2008, were patients of the Dialysis Ward of the Independent Public United Hospital in Szczecin. They were patients with the end-stage kidney failure (stage 5 CKF). The patients, due to their age and sex, were divided into four groups: K₁ – 14 women 34-50 years old, K₂ – 14 women over 50 years old, M₁ – 17 men 30-50 years old, and M₂ – 13 men over 50 years old.

In the blood serum of the examined subjects, magnesium (Mg) concentration was determined, using COBAS INTEGRA Magnesium cassette. Total cholesterol (TCh) concentration was determined by means of COBAS INTEGRA Cholesterol cassette. To determine the concentration of HDL-cholesterol (HDL-Ch) fraction, COBAS INTEGRA HDL-cholesterol plus 2 generation cassette was used. In order to determine quantitatively the concentration of LDL-cholesterol (LDL-Ch), COBAS INTEGRA LDL-cholesterol plus 2 generation cassette was used. The cassettes mentioned above were used in the COBAS INTEGRA analyser. The findings were analysed statistically, applying Statistica 8.0 computer software package.

RESULTS AND DISCUSSION

The progressing character of chronic kidney failure leads to the necessity of implementing renal replacement therapy at the last disease stage. The method of treatment for the examined subjects was long-term ambulatory haemodialysis, three times a week. In subjects with extreme renal insufficiency, different laboratory tests are required to monitor the organism state, including examination of magnesium concentration and the content of some lipid compounds.

In all groups of the examined subjects, high serum Mg concentration was observed, exceeding the upper limit of reference values (Table 1). Higher content of that chemical element was characteristic of women, both older and younger ones, when compared to men of similar age, but these differences were not significant statistically. It results from the research works carried out by many authors that both hyper- and hypomagnesaemia may occur in subjects with ESKF, but also with normal magnesium balance. Fairly high concentration of Mg (1.06 ± 0.18 mmol dm⁻³ and 1.08 ± 0.13 mmol dm⁻³) was found by Świtalski et al. (2000). The results obtained by Nasri and Kheiri (2008) and Robles et al. (1998) in patients with ESKF were within physiological limits. Low concentration of Mg in subjects aged 47.5 ± 16.6 years (on average 0.6 ± 0.3 mmol dm⁻³) was found by Nasri and Baradaran
Concentrations of magnesium (Mg), total cholesterol (TCh), HDL-cholesterol (HDL-Ch) and LDL-cholesterol (LDL-Ch) in the blood serum of examined subjects (mmol(+)/kg⁻¹)

<table>
<thead>
<tr>
<th>Parameter examined</th>
<th>Group</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K₁</td>
<td>K₂</td>
</tr>
<tr>
<td>Mg</td>
<td>1.26</td>
<td>1.25</td>
</tr>
<tr>
<td>TCh</td>
<td>4.61</td>
<td>4.69</td>
</tr>
<tr>
<td>HDL-Ch</td>
<td>1.23</td>
<td>1.29</td>
</tr>
<tr>
<td>LDL-Ch</td>
<td>2.69*</td>
<td>2.73*</td>
</tr>
</tbody>
</table>

Explanations: \(\bar{x}\) – mean value, SD – standard deviation, * – significance of differences at \(p \leq 0.05\), K₁ – women 34-50 years old, K₂ – women over 50 years, M₁ – men 30-50 years old, M₂ – men over 50 years. Reference values for magnesium in blood serum are given after Heil et al. (1999), whereas for lipids after Kokot and Kokot (2005).

(2004). Significantly higher mean concentration of that chemical element in a group of patients with chronic kidney failure (women aged 62 ± 16 years and men aged 58 ± 14 years), when compared to the control group of healthy subjects, was observed by Walasek et al. (2005).

Hypermagnesaemia occurrence in patients with ESKF is mostly caused by a decrease in glomerular filtration in kidneys, which leads to the impairment of urinary magnesium excretion. The increase of total Mg concentration in blood serum induces reduction of its absorption, probably through reduction of the expression of magnesium protein transporter (TRAM 6) in small intestine epithelium (Groenestegge et al. 2006). Nevertheless, Mg absorption from the gastrointestinal tract in haemodialysed patients exceeds frequently the possibility of its elimination and leads to retention of that chemical element in organism.

Mean total cholesterol (TCh) concentration in the blood serum in all groups of examined patients was within physiological limits. However, it was higher in younger women (group K₁) as well as older ones (group K₂) when compared to men of both age groups, in whom it was at the lower limit. The HDL-cholesterol fraction also remained at the normal level in all examined subjects, being however observed at its lowest concentration in older men (group M₂). The level of LDL-cholesterol did not reach the lower limit in younger men (group M₁). In other patients, the content of that lipid was within reference values (Table 1). Similar mean concentration of TCh and HDL-Ch was obtained by Penar et al. (2005), who examined a group of 65 haemodialysed patients with an average age of 55.6 ± 13.4 years. The results presented by Wieliczko et al. (2006) obtained in long-term dialysed
subjects were also similar to those reported for the examined patients. Nasri and Baradaran (2004), when examining 16 women and 20 men with extreme kidney insufficiency, also obtained similar values for TCh and LDL-Ch, whereas the level of HDL-Ch was below lower physiological limit. The comparison of 71 long-term dialysed subjects (with an average age of 45 years) with a group of healthy persons made by Jendryczka-Mackiewicz et al. (1999) showed significantly lower concentration of total cholesterol and HDL and LDL fractions. The analysis of lipid profile results in subjects with extreme kidney insufficiency revealed a higher mean concentration of TCh and HDL- and LDL-cholesterol in women when compared to men, which was confirmed in the authors’ study.

Low LDL-Ch concentration which was obtained in younger men (group M₁) does not necessarily have to be connected with a decrease in the number of LDL molecules. This may also be connected with a reduction in the content of cholesterol carried by molecules and be the same as a result of LDL enrichment with apoprotein B and triglycerides. The apoB-richer molecules are smaller, denser, they easily penetrate into vascular walls and are subject to oxidation, which leads to considerable atherogenicity (Wybranska, Kwasniak 2008). Development of small, dense LDL may be the result of the abnormal function of hepatic receptors for lipoproteins affecting LDL metabolism as well as the abnormal activity of cholesterol ester transporting protein (Bartus et al. 1999, Bogucki 2009).

Dyslipidaemia, occurring in patients with CKF, contributes to accelerated development of atherosclerosis, which occurs 30 times more frequently in those patients, while mortality due to its complications is several dozen times higher than in general population (Steciwko, Mastalerz-Migas 2006). In the opinion of Bogucki (2009), unfavourable changes in blood vessels may rather be a result of disorders in the lipid composition than the increase of their concentrations in blood serum.

No statistically significant effect of magnesium concentration on the content of lipids analysed in blood serum was found. Meaningful positive corre-

<table>
<thead>
<tr>
<th>Blood serum indicator</th>
<th>Group</th>
<th>K₁</th>
<th>K₂</th>
<th>M₁</th>
<th>M₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg / TCh</td>
<td>K₁</td>
<td>-0.057</td>
<td>0.397</td>
<td>0.307</td>
<td>-0.080</td>
</tr>
<tr>
<td>Mg / HDL-Ch</td>
<td>K₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg / LDL-Ch</td>
<td>M₁</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Explanations as in Table 1
lation (but statistically non-significant) was obtained between Mg and total cholesterol in older women and younger men. Positive correlations between magnesium and total cholesterol in patients with chronic kidney failure were also obtained by other authors (NASRI, BARADARAN 2004, ROBLES et al. 1998). Magnesium content in blood serum was also positively correlated with HDL-cholesterol in men of both groups. A positive effect on LDL-cholesterol was observed in the group of older women and that of younger men (Table 2). However, also small negative correlation between Mg and LDL-cholesterol contents was obtained in older men.

CONCLUSIONS

1. In all examined groups of patients with extreme kidney insufficiency, abnormally high mean magnesium concentration was found (above the upper limit of reference values). A probable cause of hypermagnesaemia in those people was a decrease in glomerular filtration, which led to the impairment of urinary magnesium excretion mechanisms.

2. Mean total cholesterol concentration in women was at a higher level when compared to men, in whom it oscillated around the lower limit of reference values. HDL-cholesterol showed mean values within physiological limits, which was a result of large individual variability in the groups of examined patients. The evaluation of HDL-cholesterol content in respective subjects was difficult due to no information referring to intake of diuretic drugs. The LDL-cholesterol concentration in younger men was below the reference range, while remaining within physiological limits in other women and men groups.

3. No statistically significant correlations were found between magnesium concentration and lipid content in the examined patients except only irregular relationships between these indicators.

REFERENCES


APPLICATION OF GEOCHEMICAL INDICES (S : Al; Mg : Al) AND PARTITION COEFFICIENT (K_d) FOR EVALUATING RESPONSE OF CROPS TO ALUMINUM TOXICITY

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Poznan University of Life Sciences

Abstract

Field trials were carried out in order to examine the role of some geochemical indices (S : Al; Mg : Al) and partition coefficient (K_d) for evaluating response of crops to aluminum toxicity under acid soil conditions. They were established in 2007/2008 at Głuszynek Lesna (52°14', N and 16°56', E), a 300-hectare agricultural farm near Poznan. Two different crop plants were tested: (i) winter oilseed rape, variety Cabriolet, (ii) maize, variety Anamur. The source of magnesium and sulphur was kieserite (MgSO_4⋅H_2O) applied at four Mg rates: 0, 25, 50, 100 kg Mg ha\(^{-1}\) in the first decade of November 2007. The results revealed that the values of partition coefficient for magnesium (K_d_{Mg}) decreased along with a rise in pH, although a reverse trend was observed for the partition coefficient of aluminum K_d_{Al}. Changes in S : Al indices observed at both sites along with increasing kieserite (Mg) rates suggest that S-SO_4 concentrations in soil may reduce Al toxicity. The introduction of S-SO_4 to soil may be intended to meet plant’s nutritional requirements and, simultaneously, react with exchangeable aluminum (Al_{ex}) in order to mitigate its phytotoxicity. On the other hand, the incorporated magnesium (Mg\(^{2+}\)) was expected to exchange with Al\(^{3+}\) ions in the soil cation exchange complex (CEC). The values of Mg : Al indices decreased with raising kieserite rates at the oilseed rape site. Changes of Mg : Al indices observed under extremely acid soil conditions (maize site) along with increasing kieserite (Mg) rates suggest that Mg : Al cannot be treated as a direct index describing the Mg_{ex} – Al_{ex} interaction, especially at 25 and 50 kg Mg ha\(^{-1}\) rates. The values of S : Al and Mg : Al indices were lower at the maize site than at the oilseed rape site because of large amounts of Al_{ex} concentrated in soil solution (amounts of Al_{ex} on the maize site were ca 3-fold higher than those determined at the oilseed rape site).
Key words: winter oilseed rape, maize, aluminum toxicity, soil acidity, S:Al and Mg:Al indices, partition coefficient ($K_d$).

**ZASTOSOWANIE GEOCHEMICZNYCH WSKAŹNIKÓW (S:Al; Mg:Al) ORAZ WSPÓLŻCZYNNIKA PODZIAŁU ($K_d$) DO OCENY REAKCJI ROŚLIN UPRAWNYCH NA TOKSYCZNOŚĆ GLINU**

**Abstrakt**

Doświadczenie założono, aby zbadać rolę niektórych wskaźników geochemicznych (S:Al oraz Mg:Al) oraz współczynnika podziału ($K_d$) w ocenie reakcji roślin uprawnych na toksyczność glinu w warunkach zakwaszenia gleby. Polowe doświadczenia przeprowadzone w sezonie wegetacyjnym 2007/2008 w gospodarstwie rolnym o powierzchni 300 ha, w Głusznym Leśnym (52°14', N and 16°56', E) k. Poznania. Doświadczenia obejmowały dwie rośliny uprawne: rzepak oziemny i kukurydza oraz cztery dawki magnezu: 0, 25, 50 i 100 kg Mg ha$^{-1}$ zastosowane w postaci kizerytu ($\text{MgSO}_4\cdot\text{H}_2\text{O}$), w pierwszej dekadzie listopada 2007. Wykazano, iż współczynnik podziału dla magnezu ($K_{\text{dMg}}$) zmniejszał się wraz ze wzrostem pH gleby, natomiast odwrotny trend zaobserwowano w przypadku współczynnika podziału dla glinu ($K_{\text{dAl}}$). Zmiany wskaźników S/Al, obserwowane na obu stanowiskach, sugerują, że zawartość $\text{S-SO}_4$ w glebie może redukować toksyczność glinu. Zastosowany $\text{S-SO}_4$ służył zarówno do zaspokojenia potrzeb pokarmowych rośliny względem siarki, jak i do neutralizowania glinu wymienionego ($\text{Al}_{\text{wym}}$), aby ograniczyć jego fitotoksyczność. Z drugiej strony wprowadzenie do gleby magnezu ($\text{Mg}^{2+}$), poza żywieniowym aspektem, może powodować wypieranie jonów $\text{Al}^{3+}$ z glebowego kompleksu sorpcyjnego ($\text{KS}$). Na stanowisku z rzepakiem ozimym wartości wskaźnika Mg/Al maleły wraz z rosnącymi dawkami kizerytu. Zmiany wartości wskaźnika Mg:Al, zachodzące wraz z rosnącymi dawkami kizerytu ($\text{Mg}$) na stanowisku z kukurydzą, świadczyły o zmniejszeniu zawartości $\text{Al}_{\text{wym}}$ w roztworze glebowym (zawartość $\text{Al}_{\text{wym}}$ na stanowisku z kukurydzą była 3-krotnie wyższa niż na stanowisku z rzepakiem ozimym).

*Słowa kluczowe:* rzepak oziemny, kukurydza, toksyczność glinu, zakwaszenie gleb, wskaźniki S:Al oraz Mg:Al, współczynnik podziału ($K_d$).

**INTRODUCTION**

Soil acidity is a major growth-limiting factor for plants, both worldwide and in Poland. Poor plant growth on acid soil has been linked to monomeric aluminum ($\text{Al}^{3+}$) toxicity (Kinraide 1993, Barcelo et al. 1996, Kidd, Proctor 2001), which usually occurs at pH $< 5.0$ or, more precisely, at pH $< 4.7$. Less phytotoxic forms, $\text{Al(OH)}^{2+}$ and $\text{Al(OH)}^{3+}$, are expected at pH between 5.0 and 6.5, when conditions suitable for mitigating aluminum phytotoxicity appear (Lindsay 1979). Due to excess of aluminum cation excess, crops, which have a smaller and shallower root system, are unable to take up sufficient amounts of many nutrients in order to cover their nutritional needs (Szatanik-Kloc, Józefaciuk 2002, Grzebisz et al. 2005).
The present state of knowledge concerning the mitigation of aluminum toxicity assumes an implementation of a practical procedure, i.e., liming (GRZEBISZ et al. 2006). However, a question arises about some other alternative methods aimed at inducing crop plants’ response to aluminum toxicity under acid soil conditions. This problem can be solved if relationships between some macronutrients, for example sulphur (S), magnesium (Mg), and aluminum cation (Al$^{3+}$) in acid soil will be considered. It is assumed that both macronutrients (S and Mg) are intended to alleviate aluminum toxicity and hence ensure a high nutrient efficiency of crop plants grown under acid soil conditions.

Sulphur (S), occurring in the soil solution as SO$_4^{2-}$ anions, may precipitate Al$^{3+}$ ions present in the soil solution (SKUBISZEWSKA, DIATTA 2008), whereas magnesium as Mg$^{2+}$ cation in the soil solution can exchange with Al$^{3+}$ ions in the soil cation exchange complex. Moreover, magnesium may exhibit a great capacity for decreasing Al$^{3+}$ accumulation in plant root apoplast (GRZEBISZ, HARDTER 2006) owing to the antagonistic effect of Mg – Al ions in acid soil.

Metals ions, including aluminum and magnesium, exhibit different affinity with naturally occurring adsorbents and the reactions between these metal ions and adsorbents are observed to be reversible as related, among others, to soil pH and their content in soils. The magnitude of this process is generally estimated by chemical tests as well as speciation studies. The ratio of Al and Mg in the solid phase to that in solution at equilibrium is defined as partition coefficient $K_d$, which is reported as $Me_{ads}$/$Me_{sol}$ where, $Me_{ads}$ – adsorbed/retained Al or Mg and $Me_{sol}$ – their concentrations in solution (DIATTA et al. 2003, 2004). High values of partition coefficients are believed to indicate that Al and Mg have been retained by the soil solid phase through sorption reactions, while low values imply that most of Al and Mg are partitioned to the ambient soil solution, where they are potentially prone to transport and biological or geochemical reactions. The mechanisms involved are related to several physical and chemical soil properties, of which soil reaction (pH), organic matter, clay and silt contents are most often indicated as the nes which control dynamic processes of metal geochemistry.

It was assumed that partition coefficients for aluminum and magnesium ($Kd_{Al}$, $Kd_{Mg}$) may play an important role in understanding the relationship between aluminum (Al) and magnesium (Mg) concentrations in the soil solid phase and the soil solution. Furthermore, $Kd_{Al}$, $Kd_{Mg}$ status in soil as well as the relationship with pH are expected to provide useful agrochemical tool for evaluating crop plants response to soil acidification.

The aim of the present study was to report the geochemical characteristics of some indices i.e., S : Al; Mg : Al and partition coefficient ($K_d$), in the assessment of crops’ response to aluminum toxicity under acid soil conditions.
MATERIAL AND METHODS

1. Characteristics of experimental field, designs and agrochemical soil properties

a) Field trials and soil sampling

Field trials were established in 2007/2008 at Gluszyna Lesna (52°01'4, N and 16°05'6, E), a 300-hectare agricultural farm, near Poznan. Soils under these trials belong to the agronomical category covering the range from class IV to V. Two different crops were tested: (i) winter oilseed rape, variety Cabriolet, and (ii) maize variety Anamur. The source of magnesium and sulphur was kieserite (MgSO$_4$·H$_2$O) applied at four Mg rates: 0, 25, 50, 100 kg Mg ha$^{-1}$ in the first decade of November 2007. Soil samples were collected at the depths 0-20 cm and 20-40 cm as follows: (i) initial samples – just before kieserite application, both for oilseed rape and maize site, (ii) in spring – at the plant regrowth phase for winter oilseed rape, (iii) during the flowering phase for maize.

b) Soils chemical analysis

Prior to chemical analyses, soil samples were air-dried at room temperature for 4 days, crushed to pass through a 1.0 mm screen and stored in plastic bags before chemical analyses. The pH was determined potentiometrically (w/v, 1:5) according to the Polish Standard (1994) in 1.0 mole KCl dm$^{-3}$. Cation exchange capacity (CEC) was obtained by summation of 1 mole KCl dm$^{-3}$ extractable acidity and exchangeable alkaline cations (Ca$^{2+}$, Mg$^{2+}$, Na$^+$ and K$^+$) extracted by 1 mole CH$_3$COONH$_4$ dm$^{-3}$ (pH 7.0), as described by Thomas (1982). These elements were determined by the FASS method (Varian Spectra AA – 250 Plus).

Exchangeable aluminium was determined according Sokolov’s method (Moczek et al., 2000) by using 1 mole KCl for displacing Al ions. The recovered extracts were divided into two aliquots, of which one was directly titrated for determining the concentrations of H and Al, whereas the second one was titrated after Al precipitation with NaF. Exchangeable Al was obtained from the difference between these chemical tests. Sulphur was assessed by the turbidimetric method based on the extraction of S-SO$_4$ compounds by 2% CH$_3$COOH and further precipitation S-SO$_4$ with a 20% BaCl$_2$ solution (Sparks 1996). Sulphur concentrations were determined by using Specord 40 AnalytikJena equipment. All analyses were performed in duplications.

2. Characteristics of geochemical indices and partition coefficient

In this study, the following indices have been suggested for estimating Al, Mg and S geochemical changes as induced by oilseed rape and maize growth.
\[ K_{dAl} = \frac{Al_{total} \ (mg \ kg^{-1})}{Al_{ex} \ (mg \ dm^{-3})} \]  (1)

\[ K_{dMg} = \frac{Mg_{total} \ (mg \ kg^{-1})}{Mg_{ex} \ (mg \ dm^{-3})} \]  (2)

\[ S : Al = \frac{S-SO_4 \ (mg \ kg^{-1})}{Al_{ex} \ (mg \ kg^{-1})} \]  (3)

\[ Mg : Al = \frac{Mg_{ex} \ (mg \ kg^{-1})}{Al_{ex} \ (mg \ kg^{-1})} \]  (4)

where:

- \( K_{dAl} \) – aluminum partition coefficient, (dm\(^3\) kg\(^{-1}\));
- \( K_{dMg} \) – magnesium partition coefficient, (dm\(^3\) kg\(^{-1}\));
- \( S : Al \) – index expressing the relationship between S-SO\(_4\) and \( Al_{ex} \) (mass ratio basis);
- \( Mg : Al \) – index expressing the relationship between \( Mg_{ex} \) and \( Al_{ex} \) (mass ratio basis);
- \( Al_{ex} \) – exchangeable aluminum (mg kg\(^{-1}\));
- \( Mg_{ex} \) – exchangeable magnesium (mg kg\(^{-1}\)).

Additional estimation of potential aluminum phytotoxicity was undertaken as reported below (DIATTA et al. 2009):

- < 36.0 mg \( Al_{ex} \) kg\(^{-1}\) soil, slight effect
- 36.0 – 45.0 mg \( Al_{ex} \) kg\(^{-1}\) soil, negative effect
- > 45.0 mg \( Al_{ex} \) kg\(^{-1}\) soil, phytotoxic effect

Indices as well as partition coefficients were represented graphically using EXCEL\(^{®}\) spreadsheets.

**RESULTS AND DISCUSSION**

1. Soil chemical properties

The chemical properties reported in Table 1 showed that before establishment of the trials, soils under oilseed rape were acid (pH 4.65-4.70) while the maize site contained extremely acid soils (pH 3.70-3.75) (GRZEBISZ et al. 2005, STRZEMSKI et al. 1973), irrespective of the sampling depth. The amounts
of exchangeable aluminum (Al\textsubscript{ex}) at the maize site varied within the range 120.45-150.03 mg kg\textsuperscript{-1} and were ca 3-fold higher than those determined at the oilseed rape site (38.67-49.60 mg kg\textsuperscript{-1}). These amounts fluctuated within the negative (oilseed rape site) and phytotoxic (maize site) effect range of Al\textsubscript{ex}, which means that phototoxicity most probably did occur, mainly under acid conditions. Both soils (under oilseed rape and under maize) were characterized by the sulphur (S-SO\textsubscript{4}) content of 14.93-19.68 mg kg\textsuperscript{-1} and similar levels of exchangeable magnesium (Mg\textsubscript{ex}) at both sites. The agrochemical properties of the sites were in general less favorable (mainly pH) to oilseed rape and maize yields, but suitable for verifying the geochemical-based indices (S/Al; Mg/Al) and partition coefficient (Kd\textsubscript{Al}; Kd\textsubscript{Mg}) concept.

2. Aluminum (Kd\textsubscript{Al}) and magnesium (Kd\textsubscript{Mg}) partition coefficients

The partition coefficient (K\textsubscript{d}) expresses the relationship between an element contained in the soil solid phase and its concentration in the soil solution. Higher Kd\textsubscript{Al} values imply that when more Al is retained (immobilized) in the soil solid phase, Al concentration in the soil solution is lower, and vice versa. The same applies to Kd\textsubscript{Mg}, although high levels of Mg concentrations in the soil solution are most frequently expected to be caused by agrochemical practice. The geochemical characteristics of these parameters are closely linked with pH changes. From the data found in the scientific literature, it may be concluded that aluminum generates protons (H\textsuperscript{+}), unlike magnesium generating hydroxide anions (OH\textsuperscript{-}). These geochemical features were outlined by relating the respective partition coefficients to soil pH, as illustrated in Figures 1 and 2.

The above capacity to generate H\textsuperscript{+} and OH\textsuperscript{-} is expressed on the basis of a (moles H\textsuperscript{+} or OH\textsuperscript{-} produced by Al\textsuperscript{3+} and Mg\textsuperscript{2+}, respectively), which is a product of the linear coefficient value (y = ax + b, where: y = K\textsubscript{d}; x = pH; b = moles of negative charges produced by soil).
The linear relationship between Kd_{Al} and soil pH for both sites suggests more intensive generation of hydrogen ions (H\(^+\)) by Al\(^{3+}\) along with an increasing pH value (Figure 1). Aluminum (Al\(^{3+}\)) starts to produce more H\(^+\) in order to decrease soil pH through H\(^+\) soil acidification. The proton generation capacity index \((a_H)\) reached 0.84 and 0.73 moles H\(^+\) per mole Al\(^{3+}\) for the oilseed rape and maize sites, respectively. This is particularly important in terms of Al proton generation capacity in soils. The reported values indicate that for the acid site (i.e. oilseed rape), more protons are required to decrease the soil pH in order to ensure more Al activity. This action was not necessary at the maize site, characterized by extremely acid conditions. Higher Al concentrations were found within the pH range 4.0-4.7, and this corresponded to aluminum partition coefficients (Kd_{Al}) varying from 2.4-3.0 dm\(^3\) kg\(^{-1}\) at the oilseed rape site.

At the maize site, the highest concentration of Al\(_{ex}\) varied in the pH range 3.55-3.85, and this corresponded to partition coefficients (Kd_{Al}) of 1.8-2.2 dm\(^3\) kg\(^{-1}\). The lower values of (Kd_{Al}) at the maize site as compared to the oilseed rape site are attributable to the extremely high amounts of exchangeable aluminum (Al\(_{ex}\)) – Table 1. The values of coefficients of determination \((R^2)\) at both sites are high (0.82 and 0.71, respectively for oilseed rape and maize sites). This means that relationships between partition coefficients for Al (Kd_{Al}) and soil pH demonstrated a fairly strong dependence.
Relationships between the partition coefficient for Mg (Kd_{Mg}) and soil pH at the oilseed rape and maize sites are illustrated in Figure 2. Most interesting is the occurrence of a reverse trend observed for Kd_{Mg} compared to Kd_{Al}. Magnesium generated lower amounts of hydroxide anions (OH\(^{-}\)) along with a pH raise and the overall geochemical reaction is expected to overcome aluminum-induced soil acidification. This is directly related to the rise in Mg concentrations in the soil solution, which further leads to a decrease in the Kd_{Mg} values. This assumption is supported by low Kd_{Mg} values in the range from 0.30 to 1.05 dm\(^{3}\) kg\(^{-1}\), just half of the values of Kd_{Al} (maize site). The same pattern was observed at oilseed rape site with Kd_{Mg} values varying from 0.80 to 1.40 dm\(^{3}\) kg\(^{-1}\), i.e., also half the value of Kd_{Al} (at oilseed rape site).

Hydroxide anion generation capacity (a_{OH}) varied from -0.54 to -0.61 moles OH\(^{-}\) per mole Mg\(^{2+}\) for the oilseed rape and maize sites respectively, confirming a decrease in OH\(^{-}\) ion generation by Mg\(^{2+}\) along with a pH increase. This situation is reverse to the one reported for the Al proton generation capacity in soils. It means that acid conditions strengthen OH\(^{-}\) production in contrast to slightly alkaline or alkaline soils.
3. Sulphur and magnesium versus aluminum: S : Al, Mg : Al geochemical indices

Figure 3 illustrates changes related to S : Al indices, accordingly to kieserite (Mg) rates during the oilseed rape regrowth and maize flowering stages. The values of S: Al decreased generally at the depth 20-40 cm, as compared to 0-20 cm, irrespective of Mg rates and type of site. This finding may be attributed to high concentration of Al in the subsoil (20-40 cm). The occurrence of high Al concentration under such conditions (Table 1) seems to be expected since, based on a diagram of Al dissolution as described by Lindsay (1979), monomeric Al forms (i.e., Al$^{3+}$) appear mostly at pH < 4.7. Such approaches have been reported by Walker et al. (1990) and confirmed by Dijkstra and FitzHugh (2003) in different soil ecosystems. Changes of S : Al indices as observed at both sites along with increasing kieserite (Mg) rates suggest that the S-SO$_4$ concentration in soil may reduce Al toxicity. The introduction of S-SO$_4$ to soil may be intended to satisfy plants’ nutritional requirements and, simultaneously, cause a reaction with exchangeable aluminum (Al$_{ex}$) in order to mitigate its phytotoxicity. The results caused by 50 and 100 kg Mg ha$^{-1}$ rates at the oilseed rape site made it more evident that large amounts of S-SO$_4$ have reacted with Al$_{ex}$. This may be attributed to
the low S:Al values in these treatments, as compared to the ones with 25 kg Mg ha\(^{-1}\). The same trend was observed at the maize site, but only for the 100 kg Mg ha\(^{-1}\) treatment, where S:Al indices were lower as compared to the 50 kg Mg ha\(^{-1}\) rate. With respect to the oilseed rape site, values of S:Al mostly varied within the range from 0.10 to 0.30. It was only the control treatment that was characterized by an S:Al value below 0.10. The low content of S-SO\(_4\) has prevented the S-SO\(_4\) – Al\(_{ex}\) reaction. At the maize site, S:Al indices may be divided as follows:

S:Al < 0.045 – the S-SO\(_4\) – Al\(_{ex}\) reaction limited due to large amounts of Al\(_{ex}\).

S:Al > 0.045 – interaction between S-SO\(_4\) and Al\(_{ex}\) more efficient because of relatively low amounts of Al\(_{ex}\).

Changes of Mg:Al indices as influenced by kieserite application on the oilseed rape and maize sites are illustrated by Figure 4. The Mg:Al values were the most interesting as they were decreasing along with the rising kieserite rates applied on the oilseed rape site. The introduction of magne-
sium Mg$^{2+}$, in additional to nutritional purposes, is intended for an exchange of Mg$^{2+}$ with Al$^{3+}$ ions in the soil cation exchange complex (CEC), especially when total Al content is considered (Table 1).

The Mg : Al indices at the maize site proved to be reverse to the ones observed at the oilseed rape site, i.e. the Mg : Al values increased along with the rising kieserite rates. This particular finding may be attributed to a smaller and shallower root system, which was unable to efficiently take up magnesium in order to cover maize’s nutritional requirements affected by Al$^{3+}$ toxicity. Changes in the Mg : Al indices observed under extremely acid soil conditions (the maize site) along with increasing kieserite (Mg) rates suggest that Mg : Al may not be the only index describing the Mg$_{ex}$ – Al$_{ex}$ interaction, especially at 25 and 50 kg Mg ha$^{-1}$.

The values of Mg : Al were almost identical at both depths (0-20, 20-40 cm), irrespective of the site. A reverse pattern was observed for the S : Al indices, where values of the S : Al ratio generally decreased at the depth 20-40 cm, as compared to 0-20 cm, irrespective of Mg rates and site characteristics.

Generally, values of the S : Al and Mg : Al indices were lower at the maize site than at the oilseed rape plot due to the high levels of Al$_{ex}$ (the amounts of Al$_{ex}$ at the maize site were ca 3-fold higher than those determined at the oilseed rape site).

**CONCLUSIONS**

1. Values of the partition coefficient for aluminum (Kd$_{Al}$) rose along with a pH increase, irrespective of the site. At higher Kd$_{Al}$, more Al was retained in the soil solid phase and less in the soil solution.

2. Magnesium partition coefficients (Kd$_{Mg}$) decreased with a pH rise (a reverse trend than that of Kd$_{Al}$). Magnesium generated fewer hydroxide anions (OH$^{-}$) along with a pH growth. OH$^{-}$ groups are expected to overcome aluminum-based soil acidification.

3. Changes of the S : Al indices observed at both sites with increasing kieserite (Mg) rates suggest that the S-$SO_{4}$ concentration in soil may have reduced Al toxicity. The introduction of S-$SO_{4}$ to soil is intended to meet plants’ nutritional requirements and simultaneously react with exchangeable aluminum (Al$_{ex}$) in order to mitigate its phytotoxicity.

4. Changes in the Mg : Al indices observed under extremely acid soil conditions (the maize site) imply that Mg : Al may not be the only index describing the Mg$_{ex}$ – Al$_{ex}$ interaction, especially at 25 and 50 kg Mg ha$^{-1}$ rates.

5. Values of the S : Al and Mg : Al indices were lower at the maize site, as compared to the oilseed rape site, due to high levels of Al$_{ex}$ (amounts of Al$_{ex}$ at the maize site were ca 3-fold higher than at the oilseed rape site).
REFERENCES


EFFECT OF PLANT BIOSTIMULATION WITH PENTAKEEP V FERTILIZER AND NITROGEN FERTILIZATION ON THE CONTENT OF MACRO- AND MICRONUTRIENTS IN SPINACH*

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Abstract

The aim of the research was to determine the influence of foliar nutrition with Pentakeep V as well as differentiated nitrogen fertilisation on the content of macro- (Ca, K, Mg, Na, P and S) and micronutrients (B, Cu, Fe, Mn and Zn) in spinach leaves. Pentakeep V is a fertilizer containing 5-aminolevulinic acid (5-ALA), which is the direct precursor of chlorophyll in plants. In 2006-2007, a pot experiment with spinach Spinacia oleracea L. cv. Spinaker F1 was carried out. The plants were cultivated in 60 × 40 × 20 cm containers placed in an open field under a shade-providing fabric. Containers were filled with loamy clay soil (35% of sand, 28% silt, 37% clay) with the organic matter content of 2.44% in 2006 and 2.52% in 2007. The experiment design included 2 sub-blocks: with and without foliar nutrition. The plants were sprayed twice with Pentakeep V fertilizer in a dose of 0.02% w/v (16 ml 100 dm⁻³ – 3000 dm³ per 1 ha). In each sub-block, soil fertilization with nitrogen was applied: 1 – control (without N fertilization), 2 – 25 mg N dm⁻³ of the soil (50% of N dose), 3 – 50 mg N dm⁻³ of the soil (100% of N dose). Nitrogen fertilization was applied in the form of ammonium nitrate prior to seed sowing. Among all of the determined nutrients, a significant interaction between foliar nutrition and soil application of nitrogen was observed in the case of Ca and Fe content in spinach leaves. Foliar application of Pentakeep V decreased the content of Ca in plants without N fertilization as well as increased the amount of this element in plants fertilized with full dose of nitrogen (100% of N dose). These observations were further verified by the changes of Ca content in soil after plant cultivation. Increased uptake of Ca from soil was observed for plants treated with Pentakeep V and fertilized with the full dose of nitrogen. A higher content of this

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element in soil was noted after cultivation of plants receiving only Pentakeep V (without N fertilization). Specific interaction of Pentakeep V on the increased content of Fe in spinach fertilized with 50% dose of N was observed. In comparison with the control, N fertilization in both doses (regardless of the foliar nutrition) led to the decrease of the plant content of Ca, Na and Fe as well as resulted in the increased concentration of K in spinach leaves. Plants fertilized with half-dose of N were characterized by lower content of Mn but plants treated with 100% of nitrogen had higher concentration of this element in comparison to the control. Foliar application of Pentakeep V (considered independently of N fertilization) did not significantly influence the content of these nutrients in spinach leaves. Weather conditions throughout both years of cultivation had no effect on the interaction between foliar nutrition with Pentakeep V and N fertilization on the content of analyzed nutrients.

Key words: biostimulation, 5-aminolevulinic acid, macronutrients, micronutrients, mineral nutrition.

WPŁYW BIOSTYMULACJI ROŚLIN NAWOZESEM PENTAKEEP V ORAZ NAWOŻENIA AZOTEM NA ZAWARTOŚĆ MAKRO- I MIKROSKŁADNIKÓW W SZPINAKU

Abstrakt

Celem badań było określenie wpływu dokarmiania dolistnego nawozem Pentakeep V oraz zróżnicowanego pod względem dawki nawożenia azotem na zawartość makro- (Ca, K, Mg, Na, P and S) i mikroskładników pokarmowych (B, Cu, Fe, Mn i Zn) w szpinaku. Nawóz Pentakeep V zawiera kwas 5-aminolewulinowy (5-ALA), który w roślinach jest m.in. bezpośrednim prekursorem cząsteczek chlorofilu. W latach 2006-2007 przeprowadzono doświadczenie wagonowe z uprawą szpinaku Spinacia oleracea L. Spinaker F1. Szpinak uprawiano w pojemnikach ażurowych o wymiarach 60×40×20 cm umieszczonych na terenie otwartym pod cieniówką. Pojemniki wypełniono gliną średnią pylastą (35% piasku, 28% pyłu i 37% ilu) zawierającą 2,44% i 2,52% materii organicznej odpowiednio w 2006 i 2007 roku. Badaniami objęto dwa podbloki z dolistnym i bez dolistnego dokarmiania roślin. Rośliny dokarmiano dolistnie dwukrotnie nawozem Pentakeep V w dawce 0,02% m/o (16 ml 100 dm–3 – stosując w przeliczeniu 3 000 dm3 wody na 1 ha. W obrębie podbloków zastosowano doglebowe nawożenie azotem: 1 – kontrola (nienawożona azotem), 2 – 25 mg N dm –3 gleby (50% dawki N), 3 – 50 mg N dm –3 gleby (100% dawki N). Nawożenie azotem zastosowano przedsięwzięcie w formie saletry amonowej. Spośród wszystkich oznaczonych pierwiastków istotny wpływ współdziałania dokarmiania dolistnego z doglebowym nawożeniem azotem stwierdzono jedynie w odniesieniu do zawartości Ca i Fe w szpinaku. W roślinach nienawożonych azotem dokarmianie dolistne Pentakeep V powodowało zmniejszenie zawartości Ca w szpinaku, w roślinach zaś nawożonych 100% dawką N zwiększenie. Wykazane zmiany zawartości Ca w szpinaku pod wpływem Pentakeep V i nawożenia azotem znajdują uzasadnienie w kierunku zmian zawartości tego pierwiastka w glebie wykazanych po zakończonej uprawie. Świadczają one (odpowiednio w przypadku kontroli i nawożenia 100% dawką N) o zmniejszonym i zwiększonym pobieraniu Ca z gleby przez rośliny dokarmiane dolistnie Pentakeep V powodowało zmniejszenie zawartości Ca w szpinaku, w roślinach zaś nawożonych 100% dawką N zwiększenie. Wykazano specyficzne oddziaływanie Pentakeep V na zwiększenie zawartości Fe w roślinach szpinaku nawożonych 50% dawką N. W porównaniu z kontrolą nawożenie dwiema zastosowanymi dawkami azotu (rozpatrywane niezależnie od dokarmiania dolistnego) powodowało zmniejszenie zawartości Ca, Na i Fe, a wzrost zawartości K w szpinaku. Nawożenie 50% dawką N powodowało zmniejszenie zawartości Mn w szpinaku, a 100% dawką N zwiększenie. Zabieg dokarmiania dolistnego Pentakeep V, rozpatrywany niezależnie od nawożenia azotem, nie powodował istotnych zmian w zawartości badanych pierwiastków w szpinaku. Przebieg warunków klimatycznych w obydwu latach
INTRODUCTION

Plant biostimulation has recently become an increasingly more common treatment in modern agricultural production, carried out to intensify the quantity and improve the quality of crop yield. This procedure can be performed independently or along with foliar nutrition. Biostimulation is carried out using growth stimulators (bioactivators, biostimulators). According to the Act of 10 July, 2007 on Fertilizers and Fertilization (Journal of Laws, 2007 no 147, item 1033), a growth stimulator is an organic or mineral compound or its mixture, which has positive impact on plants’ growth or other metabolic processes in plants, excluding a growth regulator, which is a plant protection product in the sense defined in the provisions on plants protection. One of the compounds used in plant biostimulation is 5-aminolevulinic acid (ALA), a common precursor to tetrapyrrole compounds found in chlorophyll and hemes. ALA is also a natural organic acid presented in all living organisms (TANAKA et al. 2005). HOTTA et al. (1997) suggest that ALA has plant growth regulating properties at low concentrations and may enhance agricultural productivity. Foliar application of this chemical compound leads to an increased content of photosynthetic pigments in leaves and higher photosynthetic activity. YARONSKAYA et al. (2006) found a positive relation between ALA content and carbon dioxide assimilation in barley seedlings. Foliar application of ALA resulted in a higher content of chloroplast pigments as well as increased photosynthetic and antioxidant activity in pakchoi (MEMON et al. 2009). The effect of ALA or fertilizers containing this compound (e.g. Pentakeep® fertilizers) on mineral nutrition is still vaguely defined and only few reports concern this question. Diverse effects of foliar application of Pentakeep® on the content of N, Cu and Zn in Phoenix dactylifera L. palm leaves were described (AWAD 2008). Studies by WATANABE et al. (2000) showed that ALA improves salt tolerance in cotton seedlings through the reduction in sodium uptake. The aim of this research was to determine the influence of foliar nutrition with Pentakeep V and different nitrogen fertilization on the content of macro- (Ca, K, Mg, Na, P and S) and micronutrients (B, Cu, Fe, Mn and Zn) in spinach leaves.
MATERIAL AND METHODS

Spinach (*Spinacia oleracea* L.) cv. Spinaker F₁ was cultivated in 2006-2007 in open containers 60x40x20 cm in size, placed in an open field under a shade-providing fabric. The containers were filled with silt loam soil (35% sand, 28% silt and 37% clay) with the content of organic matter of 2.44% in 2006 and 2.52% in 2007, and the following concentrations of the available nutrient forms soluble in 0.03 M acetic acid (for 2006 and 2007, respectively): N (NO₃-N+NH₄-N) – 16.6-86.3 mg, P – 16.6-64.8 mg, K – 37.6-53.1 mg, Mg – 121.4-158.3 mg and Ca – 1032.2-2342.9 mg dm⁻³ soil. In 2006 an 2007, soil pH(H₂O) was 6.38-6.99, while the total concentration of salt in soil (EC) was 0.19-0.41 EC mS cm⁻¹, respectively. The content of available forms of phosphorus and potassium was supplemented before the cultivation to the following levels: 60 mg P (in 2006) and 200 mg K dm⁻³ (in 2006 and 2007) of soil.

The research comprised two sub-blocks: with and without plant foliar nutrition. In the sub-block with foliar application, plants were sprayed twice (on 5 and 12 September 2006 as well as on 3 and 14 September 2007) with Pentakeep V in a dose of 0.02% w/v (16 ml 100 dm⁻³). The solution was applied in the amount of 3 000 dm³ per hectare according to the manufacturer’s recommendation (Cosmo Seiwa Agriculture Co., LTD. Japan). The following combinations with soil fertilized with nitrogen were distinguished within the sub-blocks: 1 – control (without N fertilization), 2 – 25 mg N dm⁻³ of the soil (50% of N dose: equal 50 kg N ha⁻¹ in field fertilization), 3 – 50 mg N dm⁻³ of the soil (100% of N dose: equal 100 kg N ha⁻¹ in field fertilization). Nitrogen fertilization was carried out prior to seed sowing using ammonium nitrate. Pentakeep V contains (in gravimetric percent): 9.5% N (3.8% NO₃-N, 5.7% NH₄-N), 5.7% MgO, 0.14% B, 0.02% Cu, 0.6% Fe-DTPA, 0.23% Mn, 0.02% Mo, 0.16% Zn and 5-aminolevulinic acid in concentration not declared by the producer.

The experiment was carried out using a split-plot method in four replicates. Each replicate (one container) consisted of 4 rows of plants. In both years of the experiment, seeds were sown on 1 August using 15 seeds in a row. After germination plants were thinned out leaving 10 seedlings in one row (40 plants per one container). Spinach plants were harvested on 19 and 18 September in the subsequent years.

In each year, shredded plant material (spinach leaves) was dried at 70°C, ground and mineralized in 65% super pure HNO₃ (Merck no. 100443.2500) in a CEM MARS-5 Xpress microwave oven (PASŁAWSKI, MIGASZEWSKI 2006). Concentrations of Ca, K, Mg, Na, P, S, B, Cu, Fe, Mn and Zn were determined in the mineralized plant material using the ICP-OES technique with the use of a Prodigy Teledyne Leeman Labs USA spectrometer.
In both years, prior to the experiment, organic matter concentration in soil was determined using Tiurin method modified by Oleksynova. Soil pH$_{\text{H}_2\text{O}}$ was assessed with a potentiometer, while the total concentration of salt in soil EC was measured conductometrically. Prior to the experiment and after the harvest, the content of Ca, K, Mg and P in soil was determined after extraction with 0.03M CH$_3$COOH. Concentrations of Ca, K and Mg were assessed by the AAS method and P was measured by the vanadium-molybdenum method. It was only after the harvest in 2007 that the concentrations of Na and S (after extraction with 0.03 M CH$_3$COOH) as well as B, Cu, Fe, Mn and Zn after extraction with 0.01 M CaCl$_2$ in soil were determined by the ICP-OES method.

The results were verified statistically using the ANOVA module of Statistica 8.0 PL programme at the significance level $P < 0.05$. The significance of changes was assessed with the use of variance analysis. Whenever significant changes were detected, homogenous groups were determined by Duncan’s test.

**RESULTS AND DISCUSSION**

The research conducted by Awad (2008) indicated that during acclimatization of young plants from *in vitro* conditions, soil application of Pentakeep V in concentrations of 0.02%, 0.04% and 0.08% improved growth capacity and increased biomass of *Phoenix dactylifera* L. plants. Higher concentrations of chlorophyll a, accompanied by an increased content of N, Cu and Zn, were observed in palm leaves. In contrast, no significant effect of Pentakeep V on the leaf content of P, K, Fe and Mn was found. It was only in the case of Zn that its higher accumulation in plants correlated with an increasing concentration of this element in soil after Pentakeep V application. As no differences were observed in the soil concentrations of N and Cu, the increase in leaf content of these nutrients was caused exclusively by stimulating the activity of ALA.

Foliar treatment, among other physiological responses, can induce more effective root uptake of mineral nutrients (Adamiec 2002, Barczak et al. 2007). In the present study, the results of statistical analysis indicated significant interaction of foliar nutrition with Pentakeep V and soil fertilization with nitrogen on the Ca and Fe content in spinach leaves (Tables 1 and 2). In both sub-blocks, the concentration of calcium in plants fertilized with 50% dose of N remained at the same level and was lower in comparison to the control plants (Table 1). Foliar nutrition with Pentakeep V caused a significant decrease in the Ca content in control plants without N fertilization, which can indicate a reduced calcium accumulation in plant tissues. This assumption can be supported by the increased amount of this element in
soil from this combination after spinach harvesting (Table 3), particularly in 2006 (Figure 1). Therefore, it is more interesting to have observed how Pentakeep V increased the Ca uptake and Ca accumulation in spinach plants fertilized with the full dose of nitrogen. The results of chemical analysis of soil from this combination showed a lower calcium concentration in comparison to the combination with 50 mg N dm⁻³ fertilization and without foliar nutrition. However, this dependence was observed only in 2006 (Figure 1). It should also be mentioned that differences in K concentration noted in soil after spinach cultivation in 2007 (Figure 1) were not correlated with the spinach leaf content of potassium in either year of the experiment (Table 1).

Spinach leaves of plants fertilized with the N dose of 25 mg dm⁻³ and not treated with Pentakeep V were characterized by the lowest accumulation of Fe (Table 2). Foliar nutrition with Pentakeep V contributed to a significant increase in the content of this element in plants. In other N combinations, the Fe concentration in leaves was not dependent on foliar

<table>
<thead>
<tr>
<th>Combinations</th>
<th>(% d.w. – means from 2006-2007)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
</tr>
<tr>
<td>Without foliar nutrition</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>1.59 c</td>
</tr>
<tr>
<td>25 mg N dm⁻³</td>
<td>1.29 a</td>
</tr>
<tr>
<td>50 mg N dm⁻³</td>
<td>1.26 a</td>
</tr>
<tr>
<td>Pentakeep V</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>1.43 b</td>
</tr>
<tr>
<td>25 mg N dm⁻³</td>
<td>1.29 a</td>
</tr>
<tr>
<td>50 mg N dm⁻³</td>
<td>1.38 b</td>
</tr>
</tbody>
</table>

Means for foliar nutrition

<table>
<thead>
<tr>
<th></th>
<th>(% d.w. – means from 2006-2007)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
</tr>
<tr>
<td>without foliar nutrition</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>1.38 a</td>
</tr>
<tr>
<td>Pentakeep V</td>
<td>1.37 a</td>
</tr>
<tr>
<td>Test F for interaction: foliar nutrition x year of study</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Means followed by the same letters are not significantly different for P < 0.05. Test F: n.s. – means are not significant different.
application, which makes the results obtained for the combination of 25 mg dm\(^{-3}\) N dose difficult to interpret. The concentration of Fe in soil from all combinations remained at the same level (Table 3). Higher content of this element in plant tissues can result from specific interaction of Pentakeep V with plants nourished with the lower dose of nitrogen. Nitrogen fertilization (regardless of foliar nutrition) decreased the Fe content in spinach leaves, especially in the case of 25 mg dm\(^{-3}\) N dose (Table 2).

In the present study, no significant effect of the interaction between Pentakeep V foliar nutrition and N fertilization on the content of K, Mg, Na, P, S, B, Cu, Mn and Zn in spinach leaves was observed (Tables 1 and 2). This finding is supported by the results of our previous study on foliar nutrition and N fertilization (Smolen, Sady, 2009), where only a slight interaction of foliar nutrition (alternately with 2% of urea solution, 1% of Supervit R fertilizer solution and again with 2% urea solution) and N fertilization on the mineral composition of carrot roots was found. Among all of the nutrients taken into consideration in that study, i.e. Al, As, B, Ba, Be, Bi, Ca, Co, Cr, Fe, Ga, In, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, Ti and V, it

Table 2

The effect of Pentakeep V foliar nutrition and nitrogen fertilization on the content of B, Cu, Fe, Mn, Zn in spinach

<table>
<thead>
<tr>
<th>Means for interaction: foliar nutrition \times nitrogen fertilization</th>
<th>B</th>
<th>Cu</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without foliar nutrition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>30.6 a</td>
<td>11.5 a</td>
<td>468.2 b</td>
<td>224.8 a</td>
<td>195.0 a</td>
</tr>
<tr>
<td>25 mg N dm(^{-3})</td>
<td>29.0 a</td>
<td>12.7 a</td>
<td>393.3 a</td>
<td>191.0 a</td>
<td>191.8 a</td>
</tr>
<tr>
<td>50 mg N dm(^{-3})</td>
<td>31.5 a</td>
<td>12.5 a</td>
<td>446.5 b</td>
<td>246.1 a</td>
<td>209.7 a</td>
</tr>
<tr>
<td>Pentakeep V</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>29.0 a</td>
<td>12.5 a</td>
<td>453.5 b</td>
<td>202.3 a</td>
<td>191.8 a</td>
</tr>
<tr>
<td>25 mg N dm(^{-3})</td>
<td>28.7 a</td>
<td>11.8 a</td>
<td>443.0 b</td>
<td>199.5 a</td>
<td>182.5 a</td>
</tr>
<tr>
<td>50 mg N dm(^{-3})</td>
<td>30.2 a</td>
<td>12.5 a</td>
<td>424.3 ab</td>
<td>234.2 a</td>
<td>203.7 a</td>
</tr>
</tbody>
</table>

Means for nitrogen fertilization

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Cu</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>29.8 a</td>
<td>12.0 a</td>
<td>460.9 b</td>
<td>213.5 b</td>
<td>193.4 a</td>
</tr>
<tr>
<td>25 mg N dm(^{-3})</td>
<td>28.8 a</td>
<td>12.3 a</td>
<td>418.2 a</td>
<td>195.3 a</td>
<td>187.2 a</td>
</tr>
<tr>
<td>50 mg N dm(^{-3})</td>
<td>30.9 a</td>
<td>12.5 a</td>
<td>435.4 ab</td>
<td>240.1 c</td>
<td>206.7 b</td>
</tr>
</tbody>
</table>

Means for foliar nutrition

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Cu</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>without foliar nutrition</td>
<td>30.4 a</td>
<td>12.2 a</td>
<td>436.0 a</td>
<td>220.6 a</td>
<td>198.8 a</td>
</tr>
<tr>
<td>Pentakeep V</td>
<td>29.3 a</td>
<td>12.3 a</td>
<td>440.3 a</td>
<td>212.0 a</td>
<td>192.7 a</td>
</tr>
</tbody>
</table>

Test F for interaction: foliar nutrition \times nitrogen fertilization \times year of study

|                                                      | n.s.  | n.s.  | n.s.  | n.s.  | n.s.  |

Means followed by the same letters are not significantly different for P < 0.05.
Test F: n.s. – means are not significant different.
Table 3

The content of Ca, K, Mg, Na, P, S, B, Cu, Fe, Mn and Zn in soil after spinach cultivation – means from 2006-2007

<table>
<thead>
<tr>
<th>Combinations: foliar nutrition × nitrogen fertilization</th>
<th>(mg dm⁻³ soil)</th>
<th>(mg kg⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Without foliar nutrition</strong></td>
<td>Ca</td>
<td>K</td>
</tr>
<tr>
<td>control</td>
<td>1271.2</td>
<td>43.8</td>
</tr>
<tr>
<td>25 mg N dm⁻³</td>
<td>1218.2</td>
<td>60.2</td>
</tr>
<tr>
<td>50 mg N dm⁻³</td>
<td>2667.3 c</td>
<td>52.3</td>
</tr>
<tr>
<td><strong>Pentakeep V</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>2049.8 b c</td>
<td>57.6</td>
</tr>
<tr>
<td>25 mg N dm⁻³</td>
<td>1501.5 ab</td>
<td>34.7</td>
</tr>
<tr>
<td>50 mg N dm⁻³</td>
<td>1690.0 ab</td>
<td>34.4</td>
</tr>
</tbody>
</table>

Means for nitrogen fertilization

| control | 1660.5 a | 50.7 a | 102.9 a | 13.4 a | 37.9 a | 4.7 a | <0.0013 | 1.94 a | 280.4 a | 180.5 a | 51.5 a |
| 25 mg N dm⁻³ | 1359.9 a | 47.5 a | 87.4 a | 7.7 a | 42.6 a | 4.7 a | <0.0013 | 1.97 a | 272.3 a | 172.3 a | 51.4 a |
| 50 mg N dm⁻³ | 2178.6 b | 43.4 a | 109.2 a | 7.5 a | 37.9 a | 8.6 b | <0.0013 | 2.17 a | 276.9 a | 175.7 a | 49.4 a |

Means for foliar nutrition

| without foliar nutrition | 1718.9 a | 52.1 a | 96.0 a | 7.1 a | 40.7 a | 5.4 a | <0.0013 | 2.14 a | 272.3 a | 168.4 a | 53.6 a |
| Pentakeep V              | 1747.1 a | 42.3 a | 103.6 a | 11.9 a | 38.3 a | 6.6 a | <0.0013 | 1.91 a | 280.8 a | 183.9 b | 47.9 a |

Test F for interaction: foliar nutrition × nitrogen fertilization × year of study

| * | * | n.s. | - | n.s. | - | - | - | - | - | - |

Means followed by the same letters are not significantly different for P < 0.05.

Test F> means are significantly different. n.s. - not significant, “*” for Na, S, B, Cu, Fe, Mn and Zn results only for 2007
was only the concentration of Na in cv. Kazan F₁ carrot that was affected by the interaction of the tested factors.

The effect of N fertilization on mineral nutrition depends on the form of applied N, its dose as well as the cultivated species (JURKOWSKA et al. 1981, SORENSEN 1999). JURKOWSKA et al. (1981) demonstrated that increasing N fertilization led to a higher content of N, S, Ca, Na and Mg as well as a decreased concentration of P, Cl and K in oat and sorrel plants. In a study conducted by SORENSEN (1999), higher doses of nitrogen nutrition resulted in lower (P and K) or higher (Na) accumulation of macronutrients in cabbage and carrot. In the present research, nitrogen fertilization (in both doses) contributed to the reduction in the leaf content of Ca, Na and Fe (Tables 1 and 2) as well as increased plant concentration of potassium. Variable interaction of N nutrition with Mn accumulation was observed as the application of 25 mg N dm⁻³ decreased and that of 50 mg dm⁻³ N dose increased the Mn concentration in leaves. The demonstrated changes in the Na and Mn content in plants did not coincide with the results of soil analysis (Table 3), as the content of these nutrients in soil remained at the same level.

In the research conducted by SMOLEN and SADY (2009), foliar nutrition (analyzed independently of nitrogen fertilization) considerably increased Bi and Be concentrations, although it did not affect the content of the other twenty-three nutrients in storage roots. The results from the present study indicate that foliar application of Pentakeep V (regardless of N nutrition) had no significant effect on the content of all the analyzed nutrients in spinach leaves (Tables 1 and 2). Except for manganese, the mean values of concentrations of elements in soil from both sub-blocks (with and without foliar nutrition) remained at the same level (Table 3).

AWAD (2008) informed that the effectiveness of ALA influence on plants is closely related to weather conditions during cultivation. In our study, the total rainfall in 2006 was 2.6-fold lower than in 2007 but its distribution was...
much more uniform throughout the growing season (Table 4). The first decade of August 2007 was characterized by relatively small rainfall and nearly twice as many sunshine hours as in 2006. In the first decade of September 2007, the number of sunshine hours was 4.6-fold lower and the rainfall was 18.8-fold higher than in 2006. The average relative humidity of air in the first decade of August 2006 was higher, and in the third decade lower, in comparison to the respective periods in 2007. In both years, the total number of sunshine hours remained at the same level. Despite variable climatic conditions in both years, the content of the analyzed nutrients in spinach leaves was not related to this factor. This observation suggests that there is no significant interaction between foliar nutrition, nitrogen fertilization versus the cultivation years, the third factor included in our statistical analysis.

The influence of Pentakeep V application and nitrogen fertilization on yield, nitrogen metabolism and the content of heavy metals and trace nutrients in spinach plants will be presented in a separate publication.

<table>
<thead>
<tr>
<th>Month</th>
<th>Decade</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>average air temperature (°C)</td>
<td>rainfall (mm)</td>
<td>sunshine (h)</td>
</tr>
<tr>
<td>August</td>
<td>1</td>
<td>18.8</td>
<td>35.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20.2</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16.4</td>
<td>58.7</td>
</tr>
<tr>
<td>September</td>
<td>1</td>
<td>16.8</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>120.4</td>
<td>287.9</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

1. Among all of the tested nutrients (Ca, K, Mg, Na, P, S, B, Cu, Fe, Mn and Zn), a significant effect of interaction between foliar nutrition and nitrogen fertilization was found only for the content of Ca and Fe in spinach leaves.

2. Pentakeep V application on plants unfertilized with N resulted in a decrease in the Ca content, although foliar nutrition increased the leaf concentration of this element in plants fertilized with 50 mg N dm⁻³.
3. Foliar nutrition of plants fertilized with the 25 mg N dm$^{-3}$ dose led to an increase in the accumulation of Fe in spinach.

4. Nitrogen fertilization at both levels (regardless of foliar nutrition) caused a reduction in the Ca, Na and Fe content as well increased accumulation of K in spinach leaves.

5. Soil application of 25 mg N dm$^{-3}$ resulted in a decrease, while the 50 mg dm$^{-3}$ N dose increased the Mn concentration in plants.

6. Foliar application of Pentakeep V (regardless of N nutrition) had no significant effect on the content of Ca, K, Mg, Na, P, S, B, Cu, Fe, Mn and Zn in spinach leaves.

7. The weather conditions throughout the cultivation period had no significant impact on the interaction Pentakeep V with nitrogen fertilization on the content of Ca, K, Mg, Na, P, S, B, Cu, Fe, Mn and Zn in spinach plants.

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FORMULATION OF ADENYLATE ENERGY CHARGE (AEC) VERSUS THE FLUORINE CONTENT IN FOREST SOIL IN THE AREA AFFECTED BY EMISSION FROM POLICE CHEMICAL PLANT

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Chair of Biochemistry
West Pomeranian University of Technology in Szczecin

Abstract

Activity of most enzymes of the key-metabolic pathways depend on the content of adenine nucleotides, such as ATP, ADP and AMP, in cells. Based on the level of these nucleotides, the adenylate energy charge (AEC) was defined as $\text{AEC} = \frac{[\text{ATP}] + 0.5[\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]}$. Theoretically, AEC values can range from 0 to 1 and represent the physiological state of a soil microbial population.

Soil microorganisms and the enzymes they secrete are connected with biological processes which form soil fertility in all ecosystems, including forests. Forests are such ecological systems which are an integral complex and their dysfunction could be caused by permanent influence of anthropogenic factors, including industrial emission of gases and dusts.

The aim of this study was the determination of changes in adenylate energy charge values and fluorine content in the humus layer of forest-podsoil soils affected by the emission of Police Chemical Plant. During a year, soil samples were taken five times (in October 2007, February, April, June and September 2008) from five different sites Wkrzańska Forest near Węgornik, Tatynia, Tanowo, Trzeszczyn and Mścięcino. In the samples, concentration of fluorine, both water-soluble (extracted by 0.01 M CaCl$_2$) and potentially accessible to plants (extracted by 2 M HClO$_4$), was assayed by potentiometry. Additionally, the content of adenine nucleotides was assayed by chromatography. Based on the content of nucleotides, adenylate energy charge values in soil were calculated. The AEC values and fluorine content in soil depended on a distance from the emitter and the dates on which the samples were taken. In order to determine the relationships between the fluorine content and AEC values, Pearson’s correlation coefficients were calculated. Between
the fluorine (both, water-soluble and plant available) content and AEC values there was a significant negative correlation, which could mean that AEC is a very good indicator of the fluorine content in soil.

Key words: fluorine, adenylate energy charge, soil, forest.

**INTRODUCTION**

Forest ecosystems as ecological structures are an integral complex. They include organisms and their abiotic environment. Dysfunction of their functioning could be caused by permanent influence of anthropogenic factors, including industrial emission of gases and dusts (Telesiński et al. 2008).
Soil is one of the environmental elements which are strongly affected by pollution. Soil is highly biologically active and depressing soil activity indicates soil contamination. The activity of most enzymes of key-metabolic pathways depends on the content of adenine nucleotides, such as adenosine tri- (ATP), di- (ADP) and monophosphates (AMP) in cells. These three adenine nucleotides give information about adenylate energy charge, which was defined by Atkinson and Walton (1977) as \((\text{[ATP]} + 0.5 \text{[ADP]}) : (\text{[ATP]} + \text{[ADP]} + \text{[AMP]})\).

Theoretically, AEC values can range from 0, corresponding to a totally dephosphorylated adenine nucleotide pool of heavily impaired microorganisms, to 1, corresponding to a completely phosphorylated adenine nucleotide pool of viable microorganisms under optimal growth conditions (Wiese, Seydel 1995). AEC values above 0.8 are the evidence of intense growth; AEC values between 0.5 and 0.7 represent cells incapable of reproduction and AEC values under 0.4 occur in a dying microorganisms population (Jørgensen, Raubuch 2002). Fluorine is a chemical element especially dangerous to the environment. It belongs to the elements defined as having a small range of safe levels (Evdokimova 2001). Nevertheless, it activates some enzymes. More often, however, it contributes to disturbances of biochemical functions in cells.

The aim of study was to determine changes in the fluorine content and AEC values in forest-podsol soils affected by emission from Police Chemical Plant and located at different distance from the source of emission, and to assess the relationship between the fluorine content and AEC.

**MATERIAL AND METHODS**

The material for analyses consisted of soil samples taken from the humus layer of forest-podsol soils affected by the emission of Police Chemical Plant. During a year, soil samples were taken five times (in October 2007, February, April, June and September 2008) from five different sites in Wkrzańska Forest near Węgornik, Tatynia, Tanowo, Trzeszczyn and Mścięcino. The location of the sites is presented in Table 1. The organic carbon content in these soils was between 10-12%.

In the soil samples, concentration of water-soluble fluorine (extracted by 0.01 M CaCl₂) and plant available fluorine (extracted by 2 M HClO₄) was assayed by potentiometry with a pH/mV Orion 920 A device with an ion-selective fluorine electrode, according to Larsen and Widdowson (1971) and according to Ogonski and Samujlo (1996) as modified by Nowak and Kuran (2000).

Measurements of adenine nucleotides and calculations of the adenylate energy charge were made according to the procedure elaborated by Bai et al.
As described by D'YCKMANS and RAUBUCH (1997). Dimethylsulphoxide (DMSO), Na₃PO₄ (10 mM) buffer + EDTA (20 mM) at pH 12 were used as extractants. After derivatisation with chloracetaldehyde, adenine nucleotides were determined by HPLC. The separation was carried out on a Hypersil C18 5µ ODS (250 × 4.6 mm) column. Chromatography was performed isocratically (2 cm³ min⁻¹) with 50 mM ammonium acetate buffer containing 1 mM EDTA and 0.4 mM TBAHS mixed with methanol (90 : 10 v : v) as a mobile phase. Fluorometric emission was measured at a wavelength of 410 nm with 280 nm as the excitation wavelength. The AEC was calculated as ([ATP] + 0.5[ADP]) : ([ATP] + [ADP] + [AMP]).

The results were processed statistically using two-way analysis of variance. The least significant differences (LSD) were determined by Tukey's test at α = 0.05. In order to determine relationships between the fluorine content and AEC values, the results were analysed statistically using Pearson's linear correlation coefficients. Statistical calculations were carried out with the use of Statistica 8.0 programme.

**RESULTS AND DISCUSSION**

During the study, significant differences of water-soluble and plant available fluorine in forest soils were noticed for all sampling dates and sites. The water-soluble fluorine content was in the range of 1.259 to 10.272 mg kg⁻¹ d.w. soil, and the content of fluorine potentially available to plants was in the range from 59.3 to 302.1 mg kg⁻¹ d.w. soil (Table 2). These values are similar to the fluorine content in garden soils affected by emissions from Police Chemical Plant, which was determined by KLÓDKA et al. (2008). The smallest mean content of fluorine, both water-soluble and potentially accessible to plants, was recorded near Trzeszczyń, in the SW direction. The largest water-soluble fluorine content was found in soil samples taken from
Fluorine content in forest soil near Police Chemical Plant (mg kg\(^{-1}\) d.w. soil)

<table>
<thead>
<tr>
<th>Date (A)</th>
<th>Tatynia</th>
<th>Trzeszczyn</th>
<th>Mścięcino</th>
<th>Tanowo</th>
<th>Węgornik</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.02.2008</td>
<td>1.728</td>
<td>1.598</td>
<td>1.259</td>
<td>1.603</td>
<td>1.541</td>
<td>1.546</td>
</tr>
<tr>
<td>16.06.2008</td>
<td>1.161</td>
<td>1.132</td>
<td>3.792</td>
<td>1.699</td>
<td>3.637</td>
<td>2.284</td>
</tr>
<tr>
<td>27.09.2008</td>
<td>1.898</td>
<td>2.280</td>
<td>3.947</td>
<td>2.262</td>
<td>3.750</td>
<td>2.827</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>A = 0.302</td>
<td>B = 0.248</td>
<td>A × B = 0.602</td>
<td>B × A = 0.561</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fluorine potentially accessible to plants

<table>
<thead>
<tr>
<th>Date (A)</th>
<th>Tatynia</th>
<th>Trzeszczyn</th>
<th>Mścięcino</th>
<th>Tanowo</th>
<th>Węgornik</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.10.2007</td>
<td>136.3</td>
<td>145.1</td>
<td>156.5</td>
<td>145.2</td>
<td>137.9</td>
<td>144.2</td>
</tr>
<tr>
<td>27.02.2008</td>
<td>84.8</td>
<td>72.2</td>
<td>59.3</td>
<td>74.6</td>
<td>62.9</td>
<td>70.8</td>
</tr>
<tr>
<td>23.04.2008</td>
<td>292.0</td>
<td>237.8</td>
<td>282.7</td>
<td>266.6</td>
<td>302.1</td>
<td>276.2</td>
</tr>
<tr>
<td>16.06.2008</td>
<td>163.5</td>
<td>197.2</td>
<td>234.7</td>
<td>233.6</td>
<td>211.9</td>
<td>208.2</td>
</tr>
<tr>
<td>27.09.2008</td>
<td>196.3</td>
<td>178.4</td>
<td>297.7</td>
<td>264.6</td>
<td>229.8</td>
<td>233.4</td>
</tr>
<tr>
<td>Mean</td>
<td>174.6</td>
<td>166.1</td>
<td>206.2</td>
<td>196.9</td>
<td>188.9</td>
<td>186.5</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>A = 5.921</td>
<td>B = 4.968</td>
<td>A × B = 11.82</td>
<td>B × A = 11.21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The farthest site from the emitter (Węgornik, 10 km W), whereas fluorine potentially accessible to plants was the most abundant in Mścięcino, which is remote from Police Chemical Plant. Nevertheless, results of many studies have shown that fluorine content in soil diminishes with a growing distance from the emitter of this element (FRANZARING et al. 2006). A reverse relationship has been determined for the water-soluble fluorine content. However, in the present study, same as in the investigations completed by KLÓDKA et al. (2008), no relationship has been found between the content of fluorine potentially accessible to plants and the distance from Police Chemical Plant.

Fluorine content in forest soil was the highest in spring, which could have been caused by decay of shed leaves and needles, which accumulated fluorine from air (FRANZARING et al. 2006). Afterwards, the fluorine content in forest soil decreased. KLÓDKA et al. (2008) showed that the highest fluorine
AEC values in forest soil near Police Chemical Plant

<table>
<thead>
<tr>
<th>Date</th>
<th>Tatynia</th>
<th>Trzeszczyn</th>
<th>Mścięcin</th>
<th>Tanowo</th>
<th>Węgornik</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.10.2007</td>
<td>0.791</td>
<td>0.800</td>
<td>0.782</td>
<td>0.822</td>
<td>0.811</td>
</tr>
<tr>
<td>27.02.2008</td>
<td>0.889</td>
<td>0.908</td>
<td>0.894</td>
<td>0.903</td>
<td>0.882</td>
</tr>
<tr>
<td>23.04.2008</td>
<td>0.643</td>
<td>0.760</td>
<td>0.657</td>
<td>0.642</td>
<td>0.589</td>
</tr>
<tr>
<td>16.06.2008</td>
<td>0.922</td>
<td>0.932</td>
<td>0.789</td>
<td>0.899</td>
<td>0.827</td>
</tr>
<tr>
<td>27.09.2008</td>
<td>0.878</td>
<td>0.838</td>
<td>0.811</td>
<td>0.856</td>
<td>0.840</td>
</tr>
</tbody>
</table>

Fig. 1. Relationship between content of fluorine: water-soluble (a) and potentially accessible to plants (b), and adenylate energy charge in forest soil near Police Chemical Plant

(* – significant at p = 0.05, ** – significant at p = 0.01)
content in garden soils attributable to emission from Police Chemical Plant was in June, declining during the plant growing season. This might have been caused by the fact that fluorine was taken up by growing plants.

The adenylate energy charge (AEC) values in the humus layer of forest soils near Police Chemical Plant varied from 0.589 to 0.932 (Table 3) and depended on the fluorine content. Many authors showed that AEC decreased in the presence of xenobiotics in soil, for example heavy metals (CHANDER et al. 2001) and pesticides (LEHR et al. 1996, NOWAK et al. 2006).

The calculated coefficients of Pearson’s linear correlation showed a significant negative relationship between the fluorine content in soil and adenylate energy charge (Figure 1). However, the correlation coefficients between the water-soluble fluorine content and AEC were significant at $p = 0.01$, and between the content of fluorine potentially accessible to plants and AEC were significant at $p = 0.05$. Thus, AEC could be a very good indicator of fluorine (especially in its water-soluble form) content in soil. Other biochemical parameters could be used for bioindication of soils contaminated with fluorine. NOWAK et al. (2005) showed that inhibition of the activity of phosphatases, β-glucosidase and dehydrogenase was significantly positively correlated with fluorine content in soil. Earlier studies by other authors showed that soil enzymatic activity, especially that of acid phosphatase, could also be a very good indicator of soil contamination with this element (TELESIŃSKI et al. 2008).

CONCLUSIONS

1. Fluorine content in the humus layer of forest-podsol soils depended on the distance from Police Chemical Plant and increased with a growing distance from the emitter.

2. Adenylate energy charge values depended on the fluorine content in soil, especially in its water-soluble form, and significantly decreased with an increasing concentration of this element.

3. Adenylate energy charge could be a very good indicator of fluorine content in forest soil.

REFERENCES


LEAD AND CADMIUM CONTENT IN HUMAN HAIR IN CENTRAL POMERANIA (NORTHERN POLAND)

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Abstract

Samples of hair collected in 2004-2007 from 416 persons living in Central Pomerania were analyzed. The subjects donating hair represented a vast spectrum of age, from a ten-month-old child to a 75-year-old person. The subjects were selected randomly. Lead and cadmium were determined by atomic absorption spectrophotometry using an ASA-3 spectrometer. The average content of the metals in the hair samples was 3.20 µg g⁻¹ (Pb) and 0.284 µg g⁻¹ (Cd). The highest concentration of lead in human hair (about 3.88 µg g⁻¹) was determined for the age group 61-75 years, and that of cadmium (0.406 µg g⁻¹) – for the age group 26-50 years. The lowest concentrations of these metals in human hair (2.07 and 0.152 µg g⁻¹, respectively) were determined for the age group of 0-15 years. Most hair samples (50%) contained 2.01-4.00 µg g⁻¹ Pb, while 45% of the samples contained 0.001-0.300 µg g⁻¹ of cadmium. Studying the dependence of the content of lead and cadmium in hair on the gender of subjects, it was discovered that in all age groups males had more lead and cadmium (3.79 and 0.334 µg g⁻¹, respectively) than females (2.63 µg g⁻¹ and 0.236 µg g⁻¹). This study has also demonstrated that the environment affects the content of the analyzed metals in hair. The average value of lead and cadmium concentrations for people living in the country were 2.39 µg g⁻¹ for Pb and 0.214 µg g⁻¹ for Cd, while for the people living in towns and cities, the respective values were 4.17 and 0.361 µg g⁻¹. The present study has demonstrated how nutrition affects lead and cadmium content in human hair. Among the subjects, 17% had been on some kind of a diet, predominantly easily digestible and light foods. The lowest content of these metals (on average, 2.08 µg g⁻¹ Pb and 0.141 µg g⁻¹ Cd) was found in hair of people on a diet, while the highest levels (3.54 µg g⁻¹ Pb and 0.315 µg g⁻¹ Cd) were determined in people who did not limit consumption of meat and dairy products. Among the analyzed population, 241 persons suffered from chronic disease. The average content of lead and cadmium in hair of healthy subjects was 3.05 µg g⁻¹ Pb and 0.257 µg g⁻¹ Cd, but in patients suffering from artherosclerosis, allergy and hyperplasia prostate the levels of lead and cadmium in hair reached the upper values of the-
se limits. Hair of the patients who suffered from cardiovascular disease showed deficiency of these metals (on average, 1.73 µg g⁻¹ Pb and 0.182 µg g⁻¹ Cd).

Key words: lead, cadmium, hair, personal features, environment, nourishment, chronic diseases.

ZAWARTOŚĆ OLOWIU I KADMU WE WŁOSACH LUDZI Z POMORZA ŚRODKOWEGO (PÓŁNOCNA POLSKA)

Abstrakt

W latach 2004-2007 dokonano analizy włosów 416 osób pochodzących z Pomorza Środkowego, w szerokim zakresie wiekowym, od kilkumiesięcznych dzieci do osób w wieku 75 lat. W badanych włosach oznaczano zawartość kadmu i ołowiu metodą spektrofotometrycznej absorpcji atomowej. Średnia ich zawartość wyniosła odpowiednio 3,20 µg g⁻¹ (Pb) i 0,284 µg g⁻¹ (Cd). Najwyższe stężenie ołowiu (średniok 3,88 µg g⁻¹) we włosach stwierdzono w grupie wiekowej 61-75 lat, a kadmu (0,406 µg g⁻¹) w grupie 26-50 lat. Natomiast najmniejszą koncentrację tych metali (odpowiednio 2,07 i 0,152 µg g⁻¹) odnotowano wśród dzieci 0-15 lat. Włosy większości badanych osób zawierały od 2,01 do 4,00 µg g⁻¹ Pb i od 0,001 do 0,300 µg g⁻¹ Cd. Badając zależność zawartości metali we włosach od płci, stwierdzono, że we wszystkich grupach wiekowych u płci męskiej stwierdzono więcej ołowiu i kadmu (3,79 i 0,334 µg g⁻¹) niż u płci żeńskiej (2,63 i 0,236 µg g⁻¹). Na zawartość analizowanych metali we włosach istotny wpływ wywiera środowisko. U osób mieszkających na wsi stwierdzono we włosach znacznie mniej tych metali (średnio: 2,39 µg g⁻¹ Pb i 0,214 µg g⁻¹ Cd) niż u osób mieszkających w mieście (odpowiednio 4,17 i 0,361 µg g⁻¹). Znaczący wpływ na koncentrację ołowiu i kadmu we włosach wywiera rodzaj spożywanych pokarmów. Stwierdzono, że osoby, które nie spożywały mięsa i wyrobów mięsnych oraz mleka i jego produktów miały we włosach najmniej tych metali (średnio 2,08 Pb i 0,141 µg g⁻¹ Cd), a u osób, które nie unikały tych produktów, włosy zawierały najwięcej ołowiu i kadmu (3,54 i 0,315 µg g⁻¹). Wykazano, że może istnieć związek między niektórymi przewlekłymi chorobami u badanych osób a poziomem wymienionych pierwiastków w ich włosach. Przeciętna zawartość ołowiu i kadmu we włosach ludzi zdrowych wynosiła odpowiednio 3,05 i 0,257 µg g⁻¹. U alergików i osób chorujących na rozrost gruczołu krokowego lub niedokrwistość stwierdzono we włosach więcej tych metali niż u ludzi zdrowych, a u osób chorujących na nadciśnienie tętnicze znacznie mniej.

Słowa kluczowe: ołów, kadm, włosy, cechy osobiste, środowisko, odżywianie, choroby przewlekłe.

INTRODUCTION

Heavy metal pollution has become a serious health concern in recent years. Continuous exposure to low levels of heavy metals may result in bioaccumulation and health deterioration in humans. Toxic heavy metals of greatest concern are cadmium, lead and mercury. The exposure to these metals is a continuous daily process, as they can be found at the place of work, in potable water, in food and in the air (Goyer 1996). Moreover, metals can enter an organism via different routes, i.e. they are transferred
from the air, water, food or pharmaceuticals applied through skin and the respiratory tract. Afterwards, they are transported and distributed through blood into organs (i.e. liver, kidney) and removed from the organism through the following excretory pathways: sweat, hair, urine and faeces (APOSTOLI 2002, LEE et al. 2000). Measuring levels of metals in human bodies is usually done by analyzing human fluids. Fluids, such as blood and urine, are often considered the best specimens for evaluation of undue exposure, but the results reflect a transient situation (WILHELM et al. 2002). Other materials such as scalp hair clippings can be used as biomonitors because human hair is an excretory system for trace metals and can act as an accumulating tissue, therefore the metal content in hair can reflect the body status over a long period, including exposure to metals in time (ALMEIDA et al. 1999, APOSTOLI 2002, D’HAVE et al. 2006). Besides, concentration of metals in human hair may be to 10-fold higher than the amount found in blood or urine (MORTADA et al. 2002, SANNA et al. 2003). The high affinity of hair to metals is mainly due to the presence of cysteine, which makes up approximately 14% of human hair (MORTON et al. 2000).

Hair analysis is a promising tool for routine clinical screening and diagnosis of heavy metal exposure and essential trace element states in the human body (CONTIERO, FOLIN 1994, CAROLI et al. 1998, STZIELCZYK et al. 2001). Deficiency or excess of essential elements such as Ca, Zn, Cu and Fe in hair have been correlated with diseases and nutritional status (MAN et al. 1996, BOCCA et al. 2006). It has been shown that there is a relationship of hair assays with some imbalance of various metals in patients (MIEKELEY et al. 2001, FORTE et al. 2005). The trace element profiles in hair of cancer patients were also found to be different from those of healthy people (KOLMOGOROW et al. 2000). They are also linked to neurological disease (BOCCA et al. 2006).

The impact of environmental exposure has been discussed in numerous publications for a long time, especially with respect to lead and cadmium (BENCO 1995, KUBOVA’ et al. 1997, NOWAK, CHMIELNICKA 2000). Data on concentrations of these metals in hair are found in the following papers from various countries: SUKUMURA, SUBRAMANIAN 1992, CONTIERO, FOLIN 1994, KOZIELEC, DRYBAŃSKA-KALITA 1994, ZABOROWSKA, WIERCIŃSKI 1997, HäC et al. 1998, NOWAK, CHMIELNICKA 2000, CHOJNACKA et al. 2006. Cadmium and lead consumed in moderate amounts over many years can become detrimental to human health.

The aim of this work has been to assess the influence of personal features, environment, nourishment and health state on lead and cadmium content in hair of a population living in Central Pomerania.
MATERIAL AND METHODS

In 2004-2007, a study was conducted on hair samples from 416 individuals living in Central Pomerania. These people represented a wide age spectrum, i.e., from a 10-month-old infant to a 75-year-old elderly person. They were selected for the study in a random manner. An interview was conducted using a chart prepared in advance, including personal data, the place of residence, profession (for children – the profession of their parents), diet, health state and hair colour. Children were interviewed in the presence of their parents. All respondents were divided into four age groups: children (up to 15 years of age), young people (aged 16-25 years), adults (26-50 years) and elderly people (51-75 years of age).

Analyses were conducted on hair not subjected previously to hairdressing procedures. Hair samples were cut at the scalp at 6 different locations on the head. Hair sections of 3 cm from the scalp were analyzed chemically. The total amount of hair collected from each individual and then analyzed ranged from 0.3 to 0.5 g. Hair samples were rinsed with acetone followed by triple rinsing with water and then a repeated rinsing with acetone. Washed hair was dried to constant weight at 105°C. Dried samples were weighed and then mineralized using a mixture of concentrated nitric and perchloric acids (mixed at a ratio of 5:1). The content of lead and cadmium in the solution was determined by atomic absorption spectrometry with an ASA-3 spectrometer equipped with an attachment consisting of an EA3 electromagnetic atomizer and an automatic sample injector port. For all measurements identical analytical parameters were applied, i.e. wavelength of 324.8 nm (Pb) and 228.8 nm (Cd), lamp current 6 mA and a 10 mm slit. The results were read from the plotted analytical curve and expressed in µg g⁻¹ hair dry matter. The precision and repeatability of the method were verified (using 10 uniform hair samples) and the recovery of cadmium and lead was analyzed (by adding a known amount of the standard solution to 10 uniform hair samples and at the same time investigating Cd and Pb contents in these samples with no standard added). The correlation coefficient of the analytical curve was 0.992 (Cd) and 0.997 (Pb). In turn, the coefficient of variation for repeatability of the applied method was 6.41 and 3.52%, respectively, mean recovery was 99.0 and 99.5%, while the mean error of method was 7.8 and 4.2%, respectively.

The results were developed statistically using Student’s t-test and values of correlation coefficients.
RESULTS AND DISCUSSION

It results from data reported in literature that the level of minerals in hair is many times higher than in blood or urea (MAUGH 1978, ELTAYEB, VAN GRIEKEN 1990, RADOVSKA et al. 1993, WILHELM et al. 2002, BOCCA et al. 2006). Thus, analysis of hair samples considerably facilitates the entire analytical procedure in terms of the detectable content of these substances in the organism and reduces analytical errors. Another very important advantage of this method is its non-invasiveness. Chemically, hair is composed of protein rich in cystein, which, owing to hydrogensulfide groups, has the ability to bind metal ions, therefore hair analysis relatively precisely determines content of metals in the organism (CONTIERO, FOLIN 1994, BORZECKA et al. 1999, ZABOROWSKA, WIERCIŃSKI 1997, HARKINS, SUSTEN 2003). Also melanin binds certain metals (BILIŃSKA 2001).

In 2004-2007, hair samples from 210 females and 206 males were analyzed in terms of their lead and cadmium contents.

Table 1 contains mean contents of analyzed metals, the range of data, standard deviations, coefficients of variation and numbers of individuals in each age group as well as percentages in the total population. A considerable range of fluctuations was observed in all age groups in terms of the content of these metals in hair. In turn, small values were recorded for standard deviation, which reflected average variation of results. The least numerous groups comprised children, who accounted for approx. 19% of the analyzed population, whereas the other age groups were comparable in size (over 25%).

<table>
<thead>
<tr>
<th>Age groups</th>
<th>n</th>
<th>U%</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$x^*$</td>
<td>$x_{	ext{min}}$</td>
</tr>
<tr>
<td>0 - 15</td>
<td>80</td>
<td>19.2</td>
<td>2.07</td>
<td>0.10</td>
</tr>
<tr>
<td>16 - 25</td>
<td>106</td>
<td>25.5</td>
<td>2.84</td>
<td>0.60</td>
</tr>
<tr>
<td>26 - 50</td>
<td>109</td>
<td>26.2</td>
<td>3.71</td>
<td>1.20</td>
</tr>
<tr>
<td>61 - 75</td>
<td>121</td>
<td>29.1</td>
<td>3.88</td>
<td>0.62</td>
</tr>
<tr>
<td>0 - 75</td>
<td>416</td>
<td>100.0</td>
<td>3.20</td>
<td>0.10</td>
</tr>
</tbody>
</table>

$n$ – number of subjects, $U\%$ – share, $x^*$ – average value

The results showed that the mean concentration of lead and cadmium in hair of the population of inhabitants of Central Pomerania, aged 0-75 years, was $3.20 \pm 0.28$ and $0.284 \pm 0.025 \, \mu g \, g^{-1}$, respectively. In order to verify whether this level of analyzed metals is safe it would be necessary to compare it with analogous concentrations in hair of people living in areas not exposed to the action of these metals, since metal concentrations in the
organism, including hair, depend on the geographic zone, or even the region of a country. Moreover, detected levels of heavy metals change with the improvement of testing methods, facilitating a more precise determination of admissible concentrations. Thus, it is advisable to compare recorded values with literature data concerning individuals not exposed to the action of lead and cadmium compounds. Table 2 presents mean concentrations of these metals in hair of people not living in urban areas and not exposed to metals in their workplace. The values for Pb ranged from 0.3 to 10.8 µg g⁻¹, while for Cd they varied from 0.028 to 0.83 µg g⁻¹; however, in 11 out of the 13 cited studies, this range was much narrower (1.0-7.0 µg g⁻¹ and 0.10-0.60 µg g⁻¹,

### Table 2

<table>
<thead>
<tr>
<th>Pb</th>
<th>Cd</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X^a$</td>
<td>range</td>
<td>$X^a$</td>
</tr>
<tr>
<td>2.85 - 11.64</td>
<td>-</td>
<td>0.18 - 0.33</td>
</tr>
<tr>
<td>4.34</td>
<td>1.2 - 11.4</td>
<td>-</td>
</tr>
<tr>
<td>1.54 - 2.60</td>
<td>0.20 - 8.7</td>
<td>0.23 - 0.31</td>
</tr>
<tr>
<td>3.4</td>
<td>1.7 - 6</td>
<td>0.23 - 0.27</td>
</tr>
<tr>
<td>1.84 - 3.31</td>
<td>0.03 - 3.09</td>
<td>0.26 - 0.37</td>
</tr>
<tr>
<td>4.47</td>
<td>-</td>
<td>0.245</td>
</tr>
<tr>
<td>0.299 - 2.81</td>
<td>-</td>
<td>0.028 - 0.150</td>
</tr>
<tr>
<td>3.7 - 5.8</td>
<td>-</td>
<td>0.5 - 0.9</td>
</tr>
<tr>
<td>4.8</td>
<td>-</td>
<td>0.55</td>
</tr>
<tr>
<td>6.82</td>
<td>2.7 - 12.2</td>
<td>0.35</td>
</tr>
<tr>
<td>1.05 - 4.99</td>
<td>-</td>
<td>0.114 - 0.610</td>
</tr>
<tr>
<td>2.6</td>
<td>-</td>
<td>0.83</td>
</tr>
<tr>
<td>10.8</td>
<td>-</td>
<td>0.5</td>
</tr>
</tbody>
</table>

respectively). Content of lead and cadmium in hair of the examined inhabitants of Central Pomerania fell within this narrower range and it was almost two-fold smaller than in the area of the city of Katowice (NOWAK, CHMIELNICKA 2000). Similar results concerning lead were also reported by BORZECKA et al. (1999) and SANNA et al. (2003). In turn, in hair of individuals exposed to these metals in their workplace, the recorded values were much higher than reference values (ZBOROWSKA et al. 1989, WASIAK et al. 1996).

In the examined population, lead content in hair was increasing with age (Table 1). Figures 1-4 present changes in concentrations of this metal depending on age in individual groups. The highest level of lead was found in hair of individuals aged approx. 60 years, fluctuating around 4.5 µg g⁻¹ (Figure 4). The lowest lead content was recorded in hair of children, while the highest one – in hair of elderly people (Table 1). This difference is statistically non-significant ($p = 0.05$). In the group of children, a decreasing
Fig. 1. Mean values of lead and cadmium (µg g⁻¹) in children’s hair (1-15 years) from Central Pomerania.

Fig. 2. Mean values of lead and cadmium (µg g⁻¹) in human hair (16-25 years) from Central Pomerania.

Fig. 3. Mean values of lead and cadmium (µg g⁻¹) in human hair (26-50 years) from Central Pomerania.
A tendency was observed for lead content in hair with age, but it was only up to 8 years of age (Figure 1). After that time, the content of this metal in hair increased from 1.04 to 3.03 µg g⁻¹ at the age of 14 years. The dependence of lead level in hair on the age of children was confirmed by significant correlation coefficients with very high values (at the age of 0-8 years $r = 0.89$, 9-15 years $r = 0.81$, $p = 0.05$). Similar changes among children (aged from 5 to 18 years) were observed by Kozielec and Drybańska-Kalita (1994), with the minimum level of this element being recorded at the age of 7 years. When examining Moroccan children aged 6 to 14 years, Lekouch et al. (1999) found that the lowest amount of lead in hair was recorded for children aged 10 years. In turn, Ashraf et al. (1995) reported that the mean content of this element in hair of Pakistani children aged 11-15 years was higher than in children aged 6-10 years. In turn, it results from a study by Chojnacka et al. (2006) that Pb content in hair of examined children decreased after 7 years.

Lead content in hair was increasing consistently in successive age groups. In age groups of 16-25 years (Figure 2) and 26-50 years (Figure 3), a trend was observed for lead content in hair to increase with age. In the group of young people (16-25 years) the content of this metal increased from the mean value of 2.17 µg g⁻¹ to 3.45 µg g⁻¹, while among adults (26-50 years), it grew from 2.90 to approx. 4.15 µg g⁻¹. In these two age groups, the dependence of lead concentration in hair and the age of individuals was characterized by correlation coefficients of 0.86 and 0.72, respectively ($p = 0.05$). It needs to be stressed that among young people up to 9 years the content of this metal increased by 59%, while among adults up to 24 years it was by 43%. Thus, statistically speaking in young people lead content increased by approx. 0.14 µg g⁻¹ annually, while in the case of adults it grew by approx. 0.05 µg g⁻¹. In elderly people (aged 51-75 years) an upward trend was also observed for lead content in hair to increase with age, but it was only to the age of 60-64 years (on average, from 3.82 µg g⁻¹ at the age
of 51 years to 4.65 µg g$^{-1}$ at 60 years, Figure 4). Afterwards, Pb content in hair was decreasing consistently with age, to drop to the value of 2.30 µg g$^{-1}$ at the age of 75 years.

When investigating the population of people aged from 11 to 100 years, Garry and Gordon (1985) also observed that the maximum lead content in hair is found at the age of 50-60 years and next it consistently decreases with age. A similar dependence was reported by Ashraf et al. (1995), but only among women. In contrast, when analyzing hair samples of individuals aged from 7 to 55 years, Chojnacka et al. (2006) reported the highest lead concentration in hair of children, which decreased consistently with age until approx. 40 years, and next it increased again. In turn, Sukumar and Subramanian (2007), when examining Indian males aged 16-75 years, recorded the highest concentration of this metal in hair of males aged 31-45 years (8.6 µg g$^{-1}$). The differences are probably caused by changes in metabolism which occur with age and by the fact that the examined individuals lived in different regions.

The mean content of cadmium in hair of the analyzed individuals (0.284 µg g$^{-1}$) was comparable to that observed by Garry and Gordon (1985) and Wasiak et al. (1996). It fell within the range of normal concentrations of this element in hair, i.e. below 1.0 µg g$^{-1}$ (Chojnacka et al. 2005). An almost two-fold smaller concentration of cadmium in comparison to that of the examined individuals living in Central Pomerania was reported by Chojnacka et al. (2006) in hair of inhabitants in the city of Wrocław. In turn, inhabitants of Katowice had two-fold higher concentrations of this metal in their hair (Nowak, Chmielnicka 2000), while hair of inhabitants of New Delhi had almost three times as much cadmium (Sukumar, Subramanian 2007). A ten-fold or even one hundred-fold higher content of this metal was recorded in hair of individuals exposed to cadmium in their workplace (Wasiak et al. 1996). The concentration of this metal in hair changed with age of the discussed group of individuals. Analogously to lead, the lowest content of cadmium was found in hair of children, while the highest – in hair of adults (Table 2). The difference between these levels was statistically significant ($p = 0.05$). As shown in Figure 1, Cd content in hair of children (0-15 years) increased consistently with age, from 0.110 to 0.218 µg g$^{-1}$. This dependence was characterized by the correlation coefficient of 0.73 ($n = 80$, $p = 0.05$). Also in hair of young people (16-25 years) the content of this metal increased with age, reaching the highest mean of 0.334 µg g$^{-1}$ in individuals aged 24 years (Figure 2), where this dependence was characterized by a high value of correlation coefficient of 0.86 ($n = 106$, $p = 0.05$). The estimated statistical annual increase of cadmium level in hair of children was 0.007 µg g$^{-1}$, while in hair of young people it was almost twice as high, i.e. 0.017 µg g$^{-1}$. The content of this metal in hair of adults (26-50 the lowest in years) also increased with age, but only to the age of approx. 42 years (Figure 3), when the mean cadmium content reached the highest val-
ue of 0.500 µg g\(^{-1}\). The difference between this value and the mean concentration of this metal at the age of 26 years (0.307 µg g\(^{-1}\)) was statistically significant (\(p = 0.005\)). The observed increase of Cd content in hair in that age group was characterized by a correlation coefficient of 0.79 (\(n = 109, p = 0.05\)). After 42 years of age, the content of cadmium was found to decrease to the mean content of 0.389 µg g\(^{-1}\) at the age of 50 years. A further decrease in cadmium content in hair was observed among elderly people (51-75 years) from the mean value of 0.402 µg g\(^{-1}\) at the age of 51 years to 0.230 µg g\(^{-1}\) at the age of 70-75 years (Figure 4). This dependence was characterized by a correlation coefficient of 0.70 (\(n = 121, p = 0.05\)). The estimated statistical loss of cadmium was on average 0.012 µg g\(^{-1}\) within one year.

Similar age-related changes in the concentration of cadmium in hair were observed by Sukumar and Subramanian (2007). In turn, Chojnacka et al. (2006) did not report significant changes with age. The highest concentrations of cadmium were found among individuals aged 20 years.

In the investigated population, in majority of the subjects (209 individuals, 51% of the analyzed population) the content of lead in hair ranged from 2.01 to 4.00 µg g\(^{-1}\) Pb (Table 3). In individual age groups, this distribution was as follows. Among children, 38 individuals (48%) had Pb content in their hair of 1.0-3.00 µg g\(^{-1}\), while for 24 individuals (30%) it was below 1.01 µg g\(^{-1}\). In the age group of young people (aged 16-25 years), the most numerous group of individuals (33%) had from 2.01 to 3.00 µg g\(^{-1}\) lead in their hair. The data found in Table 2 shows that the highest number of individuals among adults and elderly people (33 and 35%, respectively) had from 3.01 to 4.00 µg g\(^{-1}\) Pb in their hair. However, we need to remember that for the second in size group of adults (23%) the value ranged from 2.01 to 3.00 µg g\(^{-1}\), while among elderly people, i.e. a group comprising 25% population, the content ranged from 4.01 to 5.00 µg g\(^{-1}\).

With respect to cadmium, 49% of the entire population (201 individuals) had from 0.101 to 0.300 µg g\(^{-1}\) Cd in their hair (Table 4). In turn, for most
children (82%) the level was below 0.200 µg g⁻¹ cadmium. Among young people and elderly individuals the most numerous group (34 and 37%, respectively) comprised those with cadmium levels in their hair ranging from 0.201 to 0.300 µg g⁻¹ Cd. In turn, 80% adult population had more than 0.300 µg g⁻¹ of this element in their hair.

The fact that the level of both analyzed metals in hair of individuals living in Central Pomerania was observed to increase with age can most probably be attributed to environmental factors. Regression analysis showed significant correlation between content of lead and cadmium, characterized by a high correlation coefficient (r = 0.52, p = 0.05), and the regression equation y = 0.065x + 0.079. This dependence confirms the above conclusion and suggests that both metals probably come from the same source.

Table 5 presents lead contents in hair depending on gender. The investigations showed the effect of sex on Pb content in hair of the analyzed popu-

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Number of persons with specific cadmium concentration in hair</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 0.101</td>
</tr>
<tr>
<td>0 - 15</td>
<td>29 (36)</td>
</tr>
<tr>
<td>16 - 25</td>
<td>6 (6)</td>
</tr>
<tr>
<td>26 - 50</td>
<td>2 (2)</td>
</tr>
<tr>
<td>51 - 75</td>
<td>10 (8)</td>
</tr>
<tr>
<td>0 - 75</td>
<td>47 (11)</td>
</tr>
</tbody>
</table>

Table 4

Number of persons in age groups with cadmium content in hair (µg g⁻¹) according to range brackets. The per cent share to the whole population given in parentheses.

Table 5

Dependence of lead content (µg g⁻¹) in analyzed hair samples, in age groups, on gender.
The mean content of this metal in hair of female patients was by 44% lower than in hair of males. The difference between these values was statistically significant ($p = 0.05$). The biggest differences were observed between females and males in the group aged 26-50 years (on average by 1.40 µg g$^{-1}$). The differences recorded between concentrations of this element depending on gender were confirmed by the data published by other researchers (Garry and Gordon 1985, Chojnacka et al. 2006). Although Ashraf et al. (1995) found a similar dependence only among children aged up to 10 years, in those over 10 years they observed higher lead concentrations in hair of females than males. Also Sukumar and Subramanian (1996) reported higher concentrations of this metal in hair of females.

In each age group, over 90% females had 1.0-5.0 µg g$^{-1}$ lead in their hair. For a considerable percentage of girls (35%), the content of this element was below 1.0 µg g$^{-1}$. In turn, among boys, only 21% had less than 1.0 µg g$^{-1}$ Pb in their hair, while for most, as in the other age groups, its content ranged from 1.0 to 5.0 µg g$^{-1}$. However, results contained in Table 5 indicate that with age the proportion of males with the content of this metal in hair of more than 5.0 µg g$^{-1}$ Pb is increasing.

Also in the case of cadmium, its content in hair of females was lower than in hair of males (Table 6). However, the difference between these values was not statistically significant ($p = 0.05$). It was only in the group of adults that the difference was on average 0.169 µg g$^{-1}$ and as suchy was statistically significant. Also Garry and Gordon (1985), Reeves et al. (2001) and Chojnacka et al. (2006) reported higher content of this metal in hair of females than males. The smallest difference in cadmium content in hair was observed between girls and boys (0.035 µg g$^{-1}$) and it may be assumed

<table>
<thead>
<tr>
<th>Range of content</th>
<th>Number of persons in individual age groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>female</td>
</tr>
<tr>
<td></td>
<td>0-15</td>
</tr>
<tr>
<td>&lt; 1.00</td>
<td>9</td>
</tr>
<tr>
<td>1.00 - 5.00</td>
<td>28</td>
</tr>
<tr>
<td>&gt; 5.0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
</tr>
</tbody>
</table>

$x^*$ – average value, SD – standard deviation, V% – coefficient of changeability
that in children the accumulation of this metal in hair is not sex-dependent. A vast majority of examined individuals, both females and males, had 0.10-0.40 µg g⁻¹ cadmium in their hair, whereas in the age group of 26-50 years only 26% males had the same range of values, while 74% males had over 0.40 µg g⁻¹ Cd in their hair.

When investigating the effect of lead and cadmium content in hair of the examined population, statistically significant differences were recorded only in the age group of 0-15 years. The lowest amounts of lead were detected in hair of children with the height of 101-120 cm, while the highest amounts were found in hair of children below 90 cm and over 140 cm tall (Figure 5). As for cadmium, its lowest amount was reported in hair of children less than 70 cm tall, while the highest for those of 161-170 cm in height. The difference in Pb and Cd content in hair observed between children with the lowest and highest amounts of these metals was approx. 1.9 µg g⁻¹ and 0.19 µg g⁻¹, respectively. Among 80 examined children, the most numerous group comprised those between 131-140 cm tall (13 individuals), who made up 16.2% of all children, characterized by the mean Pb content of 2.18 µg g⁻¹ and mean Cd content of 0.161 µg g⁻¹. In turn, in the other analyzed age groups no correlation was found between content of lead and cadmium in hair and the height of examined individuals.

In the examined individuals aged from 16 to 75 years, analogously to height, no effect of weight on content of lead and cadmium in hair was observed. A similar dependence for weight as that for height was found only in children. Children who weighed 36-40 kg had the lowest lead levels in hair, while in children of less than 15 kg and more than 55 kg it was on average more than 3.5 µg g⁻¹ (Figure 6). The most numerous group (25 individuals) comprised children of 46-55 kg of weight, with a mean of 2.33 µg g⁻¹ Pb and 0.188 µg g⁻¹ Cd in their hair.
When determining for each individual the obesity index (BMI) the effect of obesity on cadmium level in hair was observed, while no such correlation was recorded for lead. In all age groups, irrespective of the sex of individuals, an increase in cadmium content in hair was found with an increase in the degree of obesity (Table 7). For approx. 80% individuals in all age groups normal weight or overweight without obesity was recorded. Only in the age group of 51-75 years there were fewer such people (almost 70%), while as many as 28% individuals were classified as obese. The smallest differences in cadmium content in hair between individuals with the highest and the lowest obesity indexes were found between children, whereas the biggest differences were recorded between adults (26-50 years). Results showed that when penetrating the organism cadmium is accumulated in the adipose tissue to a much higher extent than lead.

Metal contents in different organs and tissues, including hair, are affected by several factors, such as e.g. content of these elements in the soil, drinking water and foodstuffs. As far as foodstuffs are concerned, the everyday diet is essential in this respect. Content of metals in foodstuffs and individual diet determine the supply of these elements in everyday diet. Most metals, consumed with food, are absorbed mainly in the small intestine. All chronic diseases of the alimentary tract reduce or even prevent its uptake. In turn, all permanent stressful situations also reduce the ability of the organism to absorb metals (RADOMSKA et al. 1991).

Thus, the adopted diet has a significant effect on content of lead and cadmium in hair, i.e. the organism. This pertains also to other metals (KALUŻA et al. 2001). When conducting research in this area, all individuals were divided into four groups: individuals who abstained from both meat and dairy products, individuals who consumed both these types of products, the group...
<table>
<thead>
<tr>
<th>Metals</th>
<th>BMI</th>
<th>0 – 15 years</th>
<th>16 – 25 years</th>
<th>26 – 50 years</th>
<th>51 – 75 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>M</td>
<td>total</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt; 18.4</td>
<td>-</td>
<td>2.04 (1)</td>
<td>2.04 (1)</td>
<td>2.04 (1)</td>
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<tr>
<td></td>
<td>18.5-24.9</td>
<td>1.10 (17)</td>
<td>2.82 (17)</td>
<td>2.04 (17)</td>
<td>2.82 (17)</td>
</tr>
<tr>
<td></td>
<td>25.0-29.9</td>
<td>2.31 (13)</td>
<td>2.62 (17)</td>
<td>2.31 (13)</td>
<td>2.62 (17)</td>
</tr>
<tr>
<td></td>
<td>30.0-34.9</td>
<td>0.65 (6)</td>
<td>1.49 (8)</td>
<td>0.65 (6)</td>
<td>1.49 (8)</td>
</tr>
<tr>
<td></td>
<td>35.0-39.9</td>
<td>2.06 (1)</td>
<td>-</td>
<td>2.06 (1)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>&gt; 39.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.5 (2)</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt; 18.4</td>
<td>-</td>
<td>0.112</td>
<td>0.112</td>
<td>0.112</td>
</tr>
<tr>
<td></td>
<td>18.5-24.9</td>
<td>0.112</td>
<td>0.141</td>
<td>0.127</td>
<td>0.226</td>
</tr>
<tr>
<td></td>
<td>25.0-29.9</td>
<td>0.146</td>
<td>0.183</td>
<td>0.167</td>
<td>0.256</td>
</tr>
<tr>
<td></td>
<td>30.0-34.9</td>
<td>0.154</td>
<td>0.201</td>
<td>0.181</td>
<td>0.289</td>
</tr>
<tr>
<td></td>
<td>35.0-39.9</td>
<td>0.185</td>
<td>-</td>
<td>0.185</td>
<td>0.312</td>
</tr>
<tr>
<td></td>
<td>&gt; 39.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.43</td>
</tr>
</tbody>
</table>

F – female, M – male

Table 7

Influence of obesity on content of lead and cadmium in hair of the subjects divided according to gender and age
Number of persons given in parentheses
of individuals who did not eat meat products, but consumed dairy products, and the group of individuals who did the opposite (Table 8). A statistically significant difference was found between individuals who did not use either dairy nor meat products in their diet and, finally, other individuals, with the exception of individuals who consumed meat products but abstained from dairy products. Individuals whose diet contained both meat and dairy products had over 3.5 µg g⁻¹ lead and over 0.31 µg g⁻¹ cadmium in their hair, whereas for the others the figures were lower. The lowest amounts of these metals were detected in hair of individuals, who abstained from both these types of products, and the recorded values in this group exhibited the low-

<table>
<thead>
<tr>
<th>Consumption of meat products</th>
<th>n</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x*</td>
<td>A</td>
</tr>
<tr>
<td>+</td>
<td>69</td>
<td>3.54</td>
<td>0.94-9.74</td>
</tr>
<tr>
<td>+</td>
<td>127</td>
<td>2.74</td>
<td>0.60-9.39</td>
</tr>
<tr>
<td>-</td>
<td>77</td>
<td>3.10</td>
<td>0.10-7.90</td>
</tr>
<tr>
<td>-</td>
<td>143</td>
<td>2.08</td>
<td>0.72-7.15</td>
</tr>
</tbody>
</table>

n – number of persons, x* – average values, A – range

Table 8

Influence of a diet on the content of lead and cadmium in hair of persons living in Central Pomerania

est variation (8.2% and 8.5%, respectively). This is consistent with the results of studies by NABRZYSKI and GAJEWSKA (1984). They showed that milk contains approx. 14 µg dm⁻³ Cd and 9 µg g⁻¹ Pb, acid tvarog approx. 1.6 µg g⁻¹ Cd and 2.0 µg g⁻¹ Pb, hard cheese 4.8 µg g⁻¹ Cd and 5.9 µg g⁻¹ Pb, meat from approx. 2 to 10 µg g⁻¹ Cd and from 4 to 38 µg g⁻¹ Pb, while sausages and processed meats from 3 to 9 µg g⁻¹ Cd and from 2 to 9 µg g⁻¹ Pb, respectively. The uptake of Cd for an adult consuming dairy products during a week can reach 10 µg Cd and 13 µg Pb, while for one consuming meat and its processed products the uptake is approx. 47 µg Cd and approx. 77 µg Pb.

Thus, an overall living standard, including the environment, may have a significant effect on concentrations of metals in the human organism (BENČKO 1995). For this reason, hair may constitute a suitable material for the evaluation of environmental metal exposure, including lead and cadmium. In this study, the type of environment is connected with the place of residence (rural vs. urban areas) of individuals in the analyzed population. Since inhabitants of big cities are more at risk of being exposed to metals in different forms, the levels of lead and cadmium in hair of examined individuals living in big cities were higher than those in inhabitants of rural areas, irrespective of their age group (Table 9), with the differences being
### Table 9
Influence of the environment on content of lead and cadmium in hair of the subjects divided according to gender and age.
Number of persons given in parentheses

<table>
<thead>
<tr>
<th>Metals</th>
<th>Place of living</th>
<th>Female</th>
<th></th>
<th></th>
<th>Male</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-15 years</td>
<td>16-25 years</td>
<td>26-50 years</td>
<td>51-75 years</td>
<td>0-75 years</td>
<td>0-15 years</td>
</tr>
<tr>
<td>Pb</td>
<td>village town</td>
<td>0.86 (20)</td>
<td>1.65 (30)</td>
<td>2.22 (23)</td>
<td>2.88 (34)</td>
<td>2.02 (107)</td>
<td>1.51 (23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.21 (17)</td>
<td>2.96 (25)</td>
<td>3.48 (31)</td>
<td>4.32 (30)</td>
<td>3.39 (103)</td>
<td>3.98 (20)</td>
</tr>
<tr>
<td>Cd</td>
<td>village town</td>
<td>0.085</td>
<td>0.174</td>
<td>0.246</td>
<td>0.156</td>
<td>0.174</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.172</td>
<td>0.313</td>
<td>0.385</td>
<td>0.284</td>
<td>0.303</td>
<td>0.246</td>
</tr>
</tbody>
</table>

### Table 10
The content of lead and cadmium in hair of ill and healthy subjects

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x̄</td>
<td>A</td>
</tr>
<tr>
<td>Prostatic hypertrophy</td>
<td>38</td>
<td>4.83</td>
<td>0.51-9.05</td>
</tr>
<tr>
<td>Arteriosclerosis</td>
<td>59</td>
<td>3.24</td>
<td>0.43-7.15</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>32</td>
<td>2.11</td>
<td>0.26-7.08</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>85</td>
<td>1.73</td>
<td>0.10-6.38</td>
</tr>
<tr>
<td>Coronary disease</td>
<td>47</td>
<td>3.18</td>
<td>0.38-8.02</td>
</tr>
<tr>
<td>Anaemia</td>
<td>36</td>
<td>5.09</td>
<td>0.47-9.24</td>
</tr>
<tr>
<td>Renal hypofunction</td>
<td>28</td>
<td>3.30</td>
<td>0.31-7.26</td>
</tr>
<tr>
<td>Allergie</td>
<td>43</td>
<td>5.47</td>
<td>0.55-9.74</td>
</tr>
<tr>
<td>Without the symptoms of sick leave</td>
<td>175</td>
<td>305</td>
<td>0.32-9.39</td>
</tr>
</tbody>
</table>

n – number of persons, x̄ – average values, A – range
It was only between children living in these two diverse environments no significant difference in cadmium levels were found. Moreover, it was observed that in each age group, the differences between males living in these two different environments were higher than in the case of women. The effect of the environment on concentrations of lead and cadmium in human hair was shown in studies by many authors (ZABOROWSKA, WIERCINSKI 1997, NOWAK, CHMIELNICKA 2000, MORTADA et al. 2002, CHOJNICKA et al. 2005). Inhabitants of more urbanized or industrialized areas had much higher levels of these metals in their hair than people living in rural or even suburban areas.

Analysis of hair may be a suitable method for determination of the mineral composition of the organism (RADOMSKA et al. 1991). Both, excess and deficiency of metals in the body are conducive to the development of different diseases. This problem has been investigated by many researchers (ŁUKASIAK et al. 1998, KOŁMOGOROW et al. 2000, FORTE et al. 2005, BOCCA et al. 2006). Based on elemental analysis, conclusions may be drawn on a possible relationship between a specific disease and detected deficiency or surplus of bioelements. Inferences may also be presented regarding increased risk of incidence of specific diseases, suggesting a potentially harmful effect of toxic elements on the metabolism of a patient. REN et al. (1997) and STRZELCZYK et al. (2001) showed a significant correlation between content of certain metals in hair and development of malignant cells. With respect to copper deficiency, formation of elastin in walls of blood vessels and collagen in the skeletal system are disturbed and hypochromic anaemia is frequently observed (KALUZA et al. 2001). Analysis of the mineral composition of hair is an analytical test, which in combination with other laboratory analyses and the clinical picture, may be applied in diagnostics of pathological conditions (GUTTER-TIGE 1990, MIEKELEY et al. 2001). Within this study, the effect of certain chronic diseases on content of lead and cadmium in hair has also been investigated (Table 10). As suggested by the collected data, there is a relationship between certain chronic diseases among the examined patients and the level of the above-mentioned elements in their hair. Individuals suffering from allergies had the highest levels of these metals in their hair. Patients suffering from prostatic hypertrophy or anaemia had much more lead (4.83 and 5.09 µg g⁻¹, respectively) in their hair than healthy individuals. These differences were statistically significant (p = 0.05). In turn, patients suffering from hypertension or type II diabetes had much lower levels of lead than people with no disease symptoms. In patients with renal hypofunction, a much higher level of cadmium was observed than that in healthy individuals, in whom much higher metal concentrations were detected in comparison to patients suffering from anaemia or arterial hypertension. These differences were statistically significant (p =0.05). An elevated level of cadmium compared to that found in healthy people was recorded in hair of patients with arteriosclerosis or prostatic hypertrophy.
From the medical point of view, one of the factors affecting levels of heavy metals in hair may be consumption of vitamins or hormones (KUTSKY 1981), or medications (ROE 1976). Based on hair analysis, STANBURY et al. (1983) and ŠTUFA et al. (2007) investigated the metabolism of metals under the influence of certain hereditary diseases. Each new observation during such analysis contributes to our improved understanding of metal metabolism. Broadening of the knowledge to include the physiology of trace elements facilitates clinical applications or makes them possible. Results of mineral analysis of hair is used at present more frequently than in the past. The application of mineral hair analysis in healthy individuals may indicate certain disorders in the organism and potential diseases. In combination with other analytical data, it may constitute a complementary diagnostic method for practicing physicians. It would be complementary since in clinical practice it may not be used in individual evaluations due to difficulties with reliable interpretations. These problems result from a large number of factors affecting metal contents in hair. The effect of some of such factors are a subject of this paper.

CONCLUSIONS

1. The mean content of lead and cadmium in hair of individuals living in Central Pomerania is 3.20 µg g⁻¹ and 0.284 µg g⁻¹, respectively, and it increases with age, reaching the highest level at the age of approx. 40 years in case of cadmium, and at the age of approx. 60 years in case of lead.

2. Most of the examined individuals had from 2 to 4 µg g⁻¹ lead and from 0.1 to 0.3 µg g⁻¹ cadmium in their hair.

3. Examined females had lower content of lead and cadmium in their hair than males.

4. Content of analyzed metals in hair was significantly affected by diet. People abstaining from dairy and meat products had much lower contents of lead and cadmium in their hair than those using these products in their diet.

5. The place of residence also has an effect on the concentrations of these metals in hair of examined people. Inhabitants of rural areas had lower metal contents in their hair than those living in big cities.

6. The height and weight of examined individuals did not have an effect on concentrations of lead and cadmium in their hair. It was only among children that significant dependence was found between these parameters.

7. The bigger the obesity of examined individuals, the more cadmium detected in their hair.
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ZABOROWSKA W., WIERCIŃSKI J. 1997. Zawartość ołowiu, kadmu, miedzi i cynku we włosach dzieci szkolnych z wybranych terenów wiejskich Lubelszczyzny [Content of lead, cadmium, copper and zinc in hair of schoolchildren from some rural areas in the region of Lublin]. Roczn. PZH, 48: 337-342. (in Polish)
CONTENT AND UPTAKE OF PHOSPHORUS AND CALCIUM WITH THE YIELD OF POTATO TUBERS DEPENDING ON CULTIVATION OPERATIONS

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Chair of Plant Cultivation
University of Podlasie

Abstract

In 2002-2004, a study was carried out, based on a field experiment set up on soil of very good rye complex. The experiment was designed as randomized sub-blocks with three replications. Two methods of soil tillage, conventional and simplified tillage, and seven weeding methods such as application of the following herbicides 1) control object – without herbicides, 2) Plateen 41,5 WG, 3) Plateen 41,5 WG + Fusilade Forte 150 EC, 4) Plateen 41,5 WG + Fusilade Forte 150 EC + adjuvant Atplan 80 EC, 5) Barox 460 SL, 6) Barox 460 SL + Fusilade Forte 150 EC, 7) Barox 460 SL + Fusilade Forte 150 EC + adjuvant Atplan 80 EC), constituted experimental factors. Phosphorus content in the tubers of cultivar Wiking potato was determined by colorimetry and calcium was tested by the atomic absorption spectrophotometry method (AAS). Phosphorus and calcium content and their uptake with tubers yield significantly depended on the tillage methods and weed control methods as well as weather conditions in the study years. Potato tubers from the simplified tillage cultivation had more phosphorus than tubers whose cultivation was based on the conventional tillage, in contrast to calcium, which was more abundant in tubers from the conventional method. The herbicides significantly reduced the phosphorus content and increased the calcium content compared with the control object. Phosphorus and calcium uptake by potato was significantly higher in the conventionally tilled treatments compared with the simplified method, and in the herbicide-treated plots compared with the mechanically cultivated control treatment.

Key words: potato, phosphorus, calcium, content, uptake.
ZAWARTOŚĆ ORAZ POBRANIE FOSFORU I WAPNIA Z PLONEM BULW ZIEMNIAKA W ZALEŻNOŚCI OD ZABIEGÓW AGROTECHNICZNYCH

Abstrakt

W latach 2002-2004 przeprowadzono badania oparte na doświadczeniu polowym założonym na glebie komplexu żytniego bardzo dobrego. Doświadczenie założono metodą losowych podbloków w trzech powtórzeniach. Badanymi czynnikami były 2 sposoby uprawy roli – tradycyjna i uproszczona oraz 7 sposobów pielęgnacji z zastosowaniem herbicydów 1) obiekt kontrolny – bez herbicydów, 2) Plateen 41,5 WG , 3) Plateen 41,5 WG + Fusilade Forte 150 EC, 4) Plateen 41,5 WG + Fusilade Forte 150 EC + adiuwant Atpolan 80 EC, 5) Barox 460 SL, 6) Barox 460 SL + Fusilade Forte 150 EC, 7) Barox 460 SL + Fusilade Forte 150 EC + adiuwant Atpolan 80 EC). Zawartość fosforu w suchej masie bulw ziemniaka oznaczono kolorymetrycznie, a wapnia – metodą absorpcyjną spektrofotometriiatomicowej (ASA). Zawartość fosforu i wapnia i ich pobranie z plonem bulw zależały istotnie od sposobów pielęgnacji i warunków pogodowych w latach badań. Więcej fosforu zawierały bulwy ziemniaka z uprawy uproszczonej w porównaniu z tradycyjną, a więcej wapnia z uprawy tradycyjnej. Herbicydy zastosowane w pielęgnacji wpłynęły na obniżenie zawartości fosforu i podwyższenie zawartości wapnia w porównaniu z bulwami z obiektu kontrolnego. Pobranie fosforu i wapnia z plonem bulw ziemniaka było istotnie większe na obiektach z uprawą tradycyjną niż uproszczoną oraz na obiektach odchwaszczanych chemicznie w stosunku do obiektu kontrolnegopielęgowanego wyłącznie mechanicznie.

Słowa kluczowe: ziemniak, fosfor, wapń, zawartość, pobranie.

INTRODUCTION

Application of herbicide to potato fields limits harmful effect of weeds. However, herbicides can cause changes in the chemical composition of potato tubers (Leszczyński 2002, Richardson et al. 2004, Zarzecka, Myszkowska 2004). Quality of potato tubers depends mainly on a potato genotype (Mazurczyk 1994, Tekalign, Hammes 2005), soil (Czełajka, Gładyshiak 1995, Kołodziejczyk, Szmigiel 2005) and weather conditions (Nowak et al. 2004, Wichrowska 2008) as well as cultivation technology (Klikocka 2001) and methods of weed control (Ceglarak, Książek 1992, Klikocka 2001, Zarzecka et al. 2002). According to Mazurczyk (1994), the content of macronutrients in tubers is variable, closely connected with climatic conditions during the vegetation. With respect to the influence of plant protection products on the chemical composition of tubers, researchers seem to disagree (Wichrowska 2008, Wyszkowski, Ciecko 2001, Zarzecka et al. 2002). Therefore, the aim of this research was to determine the effect of herbicides and tillage systems on the content and uptake of phosphorus and calcium with the yield of potato tubers.
MATERIAL AND METHODS

The study was carried out on the basis of a field experiment set up on soil of very good rye complex. Selected soil chemical properties prior to the experiment are shown in Table 1. Two method of soil tillage, the conventional and simplified tillage, and seven weeding methods such as application of the following herbicides (1) control object – without herbicides, 2) Plateen 41,5 WG , 3) Plateen 41,5 WG + Fusilade Forte 150 EC, 4) Plateen 41,5 WG + Fusilade Forte 150 EC + adjuvant Atoholan 80 EC, 5) Barox 460 SL, 6) arox 460 SL + Fusilade Forte 150 EC, 7) Barox 460 SL + Fusilade Forte 150 EC + adjuvant Atoholan 80 EC), constituted experimental factors. Organic fertilization was applied in the form manure at 25 t·ha⁻¹. Mineral fertilizers were applied at the following concentrations: 90 kg N, 32.9 kg P and 112.1 K·ha⁻¹. Each plot was 25 m² in surface area. Phosphorus content in tubers of cv. Wiking potato was determined by colorimetry and calcium - by atomic absorption spectrophotometry (AAS). The results were statistically processed with the analysis of variance and the significance of differences was determined using Tukey's test. Meteorological conditions over the period of the study varied and are presented in Table 2.

Table 1

<table>
<thead>
<tr>
<th>Specification</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter (g kg⁻¹)</td>
<td>11.3</td>
<td>11.3</td>
<td>11.5</td>
</tr>
<tr>
<td>Soil pH (1 M KCl)</td>
<td>6.5</td>
<td>6.4</td>
<td>5.6</td>
</tr>
<tr>
<td>Content of available nutrients (mg kg⁻¹):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- P</td>
<td>38.8</td>
<td>43.0</td>
<td>62.5</td>
</tr>
<tr>
<td>- K</td>
<td>150.3</td>
<td>102.2</td>
<td>103.9</td>
</tr>
<tr>
<td>- Mg</td>
<td>70.0</td>
<td>157.0</td>
<td>159.0</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Year</th>
<th>Meteorological conditions from April to September</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rainsfalls sum (mm)</td>
</tr>
<tr>
<td>2002</td>
<td>310.1</td>
</tr>
<tr>
<td>2003</td>
<td>132.5</td>
</tr>
<tr>
<td>2004</td>
<td>320.9</td>
</tr>
<tr>
<td>1981-1995</td>
<td>343.7</td>
</tr>
</tbody>
</table>
Content and uptake phosphorus with yield of tubers. Phosphorus content in the tubers of cv. Wiking edible potato ranged from 2.310-2.920 g kg\(^{-1}\) d. m., and significantly depended on soil tillage systems and weed control methods as well as precipitation and temperature in the study years (Tables 3, 5). Phosphorus concentration in tubers was similar to the values reported by other authors (Karim et al. 1997, Kołodziejczyk, Szmigiel 2005, Tekalign, Hammes 2005, Wyszkowski, Ciećko 2001). Higher phosphorus content was found in tubers of potato under the simplified tillage compared with the conventional tillage. Similar changes were observed by other authors who tested simplified tillage in potato cultivation (Ekeberg, Riley 1996, Kliocka 2001). The herbicides reduced the phosphorus content from 0.176 to 0.235 g kg\(^{-1}\) d.m., which was less than the phosphorus level in tubers from the mechanically controlled treatment, Kliocka (2001), Zarzecka, Mysztowska (2004) observed less phosphorus in tubers following application of Sencor 70 WG and Basagran 600 SL.

In our tests, was found that phosphorus uptake by potato tubers was significantly higher in the conventionally tilled treatments (on average 21.18 kg ha\(^{-1}\)) than in the treatments where some cultivation operations had been abandoned (on average 20.08 kg ha\(^{-1}\)) – Table 3. Increased phosphorus uptake was also recorded after the application of herbicides, where it was on average higher by 0.42 kg ha\(^{-1}\) than in the mechanically weeded object. Phosphorus uptake with the yield of tubers was higher about 5 kg ha\(^{-1}\) than the amount cited by Nowak et al. (2004).

Content and uptake of calcium with yield of tubers. Calcium content in tubers ranged from 0.710 to 0.850 g kg\(^{-1}\) d. m. (Tables 4, 5) and was comparable to the content reported by other authors (Prośba-Bialczyk et al. 2002, Tekalign, Hammes 2005, Wyszkowski, Ciećko 2001). Calcium concentration in the examined potatoes depended on tillage and weed control methods, as well as weather conditions during the years of the study. Owing to more tillage operations in the conventional tillage, the calcium content in tubers was superior to that from the simplified cultivation, an observation which has been reported in other studies (Kliocka 2001). The herbicides applied in potato cultivation increased calcium concentration (from 0.038 to 0.110 g kg\(^{-1}\)) compared with the tubers of mechanically cultivated potatoes. Also, Zarzecka et al. (2002) demonstrated that herbicides slightly increased the calcium content in tubers, but the difference was not statistically significant. In contrast, Prośba-Bialczyk et al. (2002), Wyszkowski, Ciećko (2001) fund that levels of phosphorus, magnesium and calcium remained relatively stable and were not affected by plant protection chemicals.

Calcium uptake, coupled with yield formation, was significantly affected by the experimental factors and thermal and precipitation conditions over the studied years (Tables 4, 5). Higher calcium uptake from per 1 ha and
## Table 3

Content and uptake of phosphorus with the yield of potato tubers

<table>
<thead>
<tr>
<th>Weed control methods</th>
<th>Content of P (g kg(^{-1}) d.m.)</th>
<th>Uptake of P (kg ha(^{-1}))</th>
<th>Uptake of P (kg t(^{-1}) of yield d.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tillage systems</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>conventional</td>
<td>simplified</td>
<td>mean</td>
</tr>
<tr>
<td>1.</td>
<td>2.727</td>
<td>2.773</td>
<td>2.750</td>
</tr>
<tr>
<td>2.</td>
<td>2.510</td>
<td>2.637</td>
<td>2.574</td>
</tr>
<tr>
<td>3.</td>
<td>2.467</td>
<td>2.570</td>
<td>2.519</td>
</tr>
<tr>
<td>4.</td>
<td>2.497</td>
<td>2.540</td>
<td>2.519</td>
</tr>
<tr>
<td>5.</td>
<td>2.520</td>
<td>2.537</td>
<td>2.529</td>
</tr>
<tr>
<td>6.</td>
<td>2.493</td>
<td>2.537</td>
<td>2.515</td>
</tr>
<tr>
<td>7.</td>
<td>2.507</td>
<td>2.577</td>
<td>2.542</td>
</tr>
<tr>
<td>Mean</td>
<td>2.532</td>
<td>2.596</td>
<td>2.564</td>
</tr>
<tr>
<td>Mean for 2-7 object</td>
<td>2.499</td>
<td>2.566</td>
<td>2.533</td>
</tr>
<tr>
<td>LSD(_{0.05}) between:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tillage systems (I)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>weed control methods (II)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>interaction I x II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.020</td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>0.040</td>
<td></td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>0.044</td>
<td></td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s. – non-significant differences
## Table 4

Content and uptake of calcium with the yield potato tubers

<table>
<thead>
<tr>
<th>Weed control methods</th>
<th>Content of Ca (g kg(^{-1}) d.m.)</th>
<th>Uptake of Ca (kg ha(^{-1}))</th>
<th>Uptake of C (kg t(^{-1}) of yield d.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tillage systems mean</td>
<td>tillage systems mean</td>
<td>tillage systems mean</td>
</tr>
<tr>
<td></td>
<td>conventional</td>
<td>simplified</td>
<td>conventional</td>
</tr>
<tr>
<td>1.</td>
<td>0.737</td>
<td>0.710</td>
<td>0.724</td>
</tr>
<tr>
<td>2.</td>
<td>0.767</td>
<td>0.757</td>
<td>0.762</td>
</tr>
<tr>
<td>3.</td>
<td>0.810</td>
<td>0.777</td>
<td>0.794</td>
</tr>
<tr>
<td>4.</td>
<td>0.787</td>
<td>0.753</td>
<td>0.770</td>
</tr>
<tr>
<td>5.</td>
<td>0.800</td>
<td>0.773</td>
<td>0.787</td>
</tr>
<tr>
<td>6.</td>
<td>0.850</td>
<td>0.817</td>
<td>0.834</td>
</tr>
<tr>
<td>7.</td>
<td>0.787</td>
<td>0.753</td>
<td>0.770</td>
</tr>
<tr>
<td>Mean</td>
<td>0.791</td>
<td>0.763</td>
<td>0.777</td>
</tr>
<tr>
<td>Mean for 2-7 object</td>
<td>0.800</td>
<td>0.772</td>
<td>0.786</td>
</tr>
</tbody>
</table>

\textit{LSD}_{0.05} between:
- tillage systems (I)
- weed control methods (II)
- interaction I x II

n.s. – non-significant differences
converted into 1 tone of tuber yield was determined for the conventional tillage compared with the simplified method, and for the herbicide-treated plots compared with the control (mechanical weeding). Accumulation of elements in plant material is a function of yield and the content of the element determined. Also, Nowak et al. (2004) found that uptake of nutrients depended on the yield and analyzed component.

**Table 5**

<table>
<thead>
<tr>
<th>Year</th>
<th>Content (g kg⁻¹ d.m.)</th>
<th>Uptake (kg ha⁻¹)</th>
<th>Uptake (kg t⁻¹ of yield d.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>Ca</td>
<td>P</td>
</tr>
<tr>
<td>2002</td>
<td>2.310</td>
<td>0.760</td>
<td>23.35</td>
</tr>
<tr>
<td>2003</td>
<td>2.470</td>
<td>0.845</td>
<td>16.93</td>
</tr>
<tr>
<td>2004</td>
<td>2.920</td>
<td>0.725</td>
<td>21.62</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td>0.030</td>
<td>0.009</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Phosphorus and calcium content and their uptake coupled with yield formation varied during the research (Table 5). In the 2004 season, the content of phosphorus was the highest as the precipitation and the temperature were favourable for potato growth. In contrast, tubers harvested in the warm and dry 2003 season were the richest in calcium. Phosphorus and calcium uptake was stimulated by the weather conditions during the hottest year 2002. These findings are supported by studies of other authors (Czekala, Gladysia 1995, Kołodziejczyk, Szmigiel 2005).

**CONCLUSIONS**

1. Introduction of simplifications to soil tillage result in increased phosphorus and decreased calcium content in tubers, and in a reduced uptake of these elements by potato yield, compared with the conventional tillage.

2. When potatoes were cultivated using herbicide-based weed control, the phosphorus content in tubers was smaller and calcium content higher than in the tubers weeded mechanically.

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REVIEW PAPERS

LITHIUM THERAPY –
THE EFFECTIVENESS OF THE MEDICINE,
SIDE SYMPTOMS, COMPLICATIONS
AND THEIR INFLUENCE ON THE QUALITY
OF THE LIFE IN AFFECTIVE DISEASES

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Abstract

Lithium is a medicine of the first choice in the preventive treatment of bipolar affective disorder. It is also used to enhance the treatment of drug resistant depression. How exactly this element acts is not yet fully understood. Lithium influences the transportation of sodium via cellular membranes (sodium-potassium ATPase dependant), has an inhibitory influence on the second transmitter system (connected with phosphatidylinositol), thus probably acting as a stabiliser of inter cellular processes. Lithium does not associate with plasmatic proteins and is almost entirely excreted by kidneys. The side effects of the medicine are linked to its influence on the central nervous system and on the renal transportation of electrolytes as well as the narrow therapeutic index of the medicine, which can cause intoxication if the recommended doses are not when medical recommendations are not observed. The undesirable effects are more intensive when the level of lithium in the blood plasma increases. Among the most common side effects are stomachaches, nausea, diarrhoea, lack of appetite, polydipsia, polyuria, shaking hands, headaches, sleepiness or deterioration of memory. Complications during lithium therapy listed in lite-
rature are ataxia, dysarthria, nystagmus and extrapyramidal symptoms, but the most severe complication is lithium poisoning. Lithium can be applied for a long-term maintenance treatment, which limits recurrence of the disease and improves the patient’s family, social and occupational life. The inferior quality of life among patients with affective disease can result from the disorder itself or can develop on the somatic grounds, appear due to abuse of tobacco or alcohol, or else be a side effect of other medicines taken by the patient. Good co-operation with the patient during the therapy can lessen the pronouncement of undesirable symptoms and complications of a lithium treatment, and this in turn can improve of the quality of the patient’s life.

Key words: lithium therapy, affective diseases.

INTRODUCTION

First attempts at using lithium for therapeutic purposes were made in the 1900s, but it was not until the second half of the 20th century that some clinical evidence for therapeutic effect of lithium in affective disorders was obtained, including the prophylaxis of bipolar affective disease recurrences, therapy of manic and depressive episodes and potentiation of antidepressant action. Mood disorders affect 10% of the general population...
In these disorders, there are alternately occurring depressive and manic episodes (bipolar affective disease) or depressive alone (unipolar affective disease). Mood disorders can be chronic and recurrent, and they are associated with high preterm mortality due to a high risk of suicidal attempts and increased exposure to somatic illnesses compared to the general population (Pużyński 2002). Mood disorders have serious consequences, like the deteriorated quality of family and professional life among patients and their families.

Lithium is a metallic element widespread in nature. In psychiatry, lithium is used in the form of easily dissociating salts, and the most common preparations are lithium carbonates and citrates. The therapeutic action is displayed by lithium cations, which are easily absorbed in the gastrointestinal tract after oral intake, are not bound by plasma proteins and are not metabolised so that almost all of the amount taken by the patient is excreted by the kidneys. The maximum blood concentration appears 2-4 hours after the intake (Pużyński, Beręsewicz 1993, Rybakowski 2003, Schou 2006).

THE AIM OF THE ARTICLE

This paper contains our analysis of the data available in the literature data concerning the efficacy of lithium, side effect occurring during lithium therapy and complications disturbing the course of the therapy and deteriorating the quality of life of patients with bipolar or unipolar affective disease.

MECHANISM OF LITHIUM ACTION AND ITS EFFECTIVENESS

The complex mechanism of lithium ionic action at the cellular level is not yet fully understood. There are reports about lithium regulating transport of sodium via cellular membranes with the help of sodium-potassium adenosine-5’-phosphatase. Lithium modifies intracellular transmission through its effect on the second transmitter system: phosphatidylinositol and adenyly cyclase (Rybakowski 2003). Adenyl cyclase stimulates synthesis of cyclic adenosine monophosphate (cAMP), which in turn activates cellular albininous kinase A, responsible for phosphorylation of many intracellular proteins (Bullock et al. 1997). In the process of inositol transformation, enzyme phospholipase C hydrolyzes phosphatidylinositol diphosphate (PIP 2) into myoinositol triphosphate (IP 3) and diacylglycerol (DAG). IP 3 stimulates releasing calcium ions from intracellular tanks; DAG in turn stimulates ac-
tivity of the cytosol enzyme, protein kinase C, which phosphorylates cellular proteins leading to its activation (BULLOCK et al. 1997). Re-synthesis of phosphatidylinositol requires participation of inositol monophosphatase in the breakdown of inositol monophosphate into alcohol. Blockage of this enzyme’s action would make it impossible to synthesize IP₃ and DAG. Lithium decreases the activity of both inositol monophosphatase and protein kinase C, stabilizing cellular processes and expression of genes related to neurotransmitters (STAHLMANN 2007). The normalizing influence of lithium on the phosphatidylinositol system has been demonstrated, for example, by was shown SILVERSTONE et al. (2002). Lithium probably activates the serotonin neurotransmitter system, inhibits the dopaminergic system and also changes the catecholamine metabolism (RYBAKOWSKI 2003). ANAND et al. (1999) proved that the mechanism of prevention of manic episodes was also stimulated by the stabilizing influence of lithium on the catecholamine system.

In these complex intracellular functions, lithium also leads to an increase in the cytokine concentration, activates cells of the immunity system, including granulocytes, and reveals the antiviral action. Neuroprotective and neurotrophic action of this element is probably linked to its influence on the growth factors being activated the central nervous system as well as the increase in the grey matter amount by activating neurogenesis processes (RYBAKOWSKI 2003, STAHLMANN 2007). This fact seems to play a significant role, as suggested by the literature reports on functional and morphological abnormalities of brain cortex gyri in patients with affective diseases. SASSI et al. (2004) showed that in patients taking lithium there were no statistical differences in the volume of anterior brain gyrus volume compared to healthy controls. The authors relate this effect to the neuroprotective nature of lithium (SASSI et al. 2004). SILVERSTONE et al. (2003) and MOORE et al. (2000) report that in patients who continuously take lithium, there is an increase in the cortex N-acetyl-aspartate (NAA), which is a marker of neuronal viability and functionality.

Despite introducing new agents to stabilize mood in therapy of affective disorders, lithium remains a medicine of the first choice (SHARMA et al. 1997, COMPTON, NEMEROFF 2000, SCULLY 2003). Some data show that lithium’s efficacy in the treatment of depressive and manic episodes reaches 80% (SCULLY 2003). However, lithium salts have a delayed effect and in the early stages of acute manic episodes treatment addition of other stabilizing agents is recommended (REISCHIES et al. 2002). Lithium is more effective in affective disorders following a classical course, i.e. with an average frequency of episode recurrences and moderate symptoms (KLEINDENST, GREIL 2000, RYBAKOWSKI 2001). Lithium also seems to be more effective in the case of sequences of mania – depression (KLEINDENST, GREIL 2000) and the coexistence of other psychiatric disorders worsens the response to this medication (KELLER et al. 2006). The Polish data show that in 30% of patients there is no recurrence of the disease and no burdensome side effects during the thera-
apy with lithium (RYBAKOWSKI 2003). As far as the potentiation of antidepressant action is concerned, clinical improvement is reached in half of all the cases (RYBAKOWSKI 1999). The anti-suicidal effect of lithium has been broadly documented in the literature. Meta-analysis by TONDO et al. (2001) showed that a long-term treatment with lithium salts decreases the risk of a suicidal attempt in all the analyzed cases.

**SIDE EFFECTS AND COMPLICATIONS**

Side effects of lithium involve its influence on the central nervous system, on the renal transport of electrolytes and the narrow therapeutic index of the medicine, which can be dangerous in the case of non-tolerance and may cause intoxication (SCULLY 2003, STAHL 2007). Severity of side effects increases together with an increase in the blood level of the medicine. GELENBERG et al. (1989) showed lithium salts were more effective in the concentration of 0.8-1.0 mmol dm\(^{-3}\) in the plasma than at the level of 0.4-0.6 mmol dm\(^{-3}\). However, higher lithium concentrations caused the occurrence and intensification of side effects, including tremor of extremities, pollakiuria, diarrhoea, body weight increase and a metallic taste in the mouth. A study conducted by ABOU-SALEH and COPPEN (1989) confirmed this relationship.

Another significant and common side effect of the administration of lithium is some disturbance of the water balance. It is manifested by polydipsia (excessive thirst) and polyuria (excessive urination) and occurs because lithium impaires the renal ability to concentrate urine through blockage of the antidiuretic hormone in renal tubules. The literature reports cases of nephrogenic diabetes insipidus during the therapy with lithium salts (PUŻYŃSKI, BERĘSEWICZ 1993, STAHL 2007). Usually, after a dose decrease, compensation of renal functions appears in a few months (SCHOU 2006). Cases of renal failure due to interstitial nephritis in the course of lithium therapy are very rare (STAHL 2007). Some early experiments on the lithium influence on the kidneys showed that in the first days of the treatment, cytoplasm vacuolization and glycogen cumulation in the cells of the renal distal nephrons and collecting tubules appeared (WALKER et al. 1983), although there is no evidence that lithium causes permanent renal lesion (RYBAKOWSKI 2003).

There are some initial and transient adverse event appearing during lithium therapy at the same frequency, like nausea, stomachaches, diarrhoea and loss of appetite and weakness (PUŻYŃSKI, BERĘSEWICZ 1993).

Some disturbances in the cardiovascular system are more seldom and appear at the beginning of lithium therapy (RYBAKOWSKI 2003). The literature reports cases of bradycardia, decrease in the arterial blood pressure, cardiac dysrhythmias and a sick-sinus syndrome (STAHL 2007). The non-specific ECG changes observed at the beginning of the therapy tend to regress during a long-term lithium therapy (RAJEWSKA, RYBAKOWSKI 1995).
There are some reports on an adverse effect of lithium adverse on the bone metabolism (Misra 2004). Tests completed by El Khoury et al. (2002) showed that a long-term lithium therapy leads to disturbances in the calcium metabolism manifested as mild hypercalcemia. Using lithium for more than 6 months also causes a decrease in prolactin (Basturk et al. 2001). The effect of lithium on aldosterone secretion may be a reason for oedema, which sometimes appears during lithium therapy (Puzyński, Beręsewicz 1993).

Lithium may cause a decrease in libido and erectile dysfunctions in men. However, such negative effects are rare and it has not been conclusively established if they are not due to possible symptoms of depression (Rybakowski 2003, Schou 2006).

Depression can also intensify cognitive disturbances that appear during lithium therapy. Despite the reports suggesting that lithium worsens thinking capabilities and the ability to remember or even inhibits creativity (Schou 2006), long-term observations concerning people taking this medicine prove that by maintaining remission of mood disorders, lithium leads to improvement of psychosocial (including occupational and interpersonal) functions in patients. The better lithium level in blood (according to standards), the better the effect (Solomon et al. 1996). Inferior cognitive functions can be attributed to the toxic lithium influence on the central nervous system, similarly to somnolence, headaches and vertigos (Rybakowski 2003). Serious neurotoxic symptoms of lithium are nystagmus, extrapyramidal symptoms, akathisia and, most often, ataxia and dysarthria. Such symptoms significantly worsen the patients’ quality of life, require quick medical intervention and a cautious assessment in terms of possible lithium intoxication (Puzyński, Beręsewicz 1993, Koreš, Lader 1997).

Lithium intoxication can be accidental (due to lack of control of the lithium blood level), suicidal or caused by the inferior renal elimination of lithium (in renal and circulatory dysfunctions, dehydration or excessive loss of sodium). It is the most serious complication of lithium therapy. According to the literature, is appears when the lithium level in plasma exceeds 1.6-1.8 mmol dm⁻³. Symptoms of lithium intoxication are the aforementioned neurological symptoms and nausea, vomiting, diarrhoea, tremor, great weakness, consciousness disturbances, cardiac dysrhythmias, convulsions, circulatory and renal failure (Puzyński, Beręsewicz 1993, Rybakowski 2003, Puzyński 2009).

There are some conditions when lithium therapy requires great care or even discontinuation, which is due to the narrow therapeutic index of this element. These are the conditions when rapid accumulation of lithium in the body is possibile or when the toxic effect of lithium appears even though its level in plasma is within the normal values. Such conditions include dehydration, renal and circulatory disorders, lesions of the central nervous system with dementia, Parkinson’s disease, epilepsy and hypothyroidism (Stahl 2007, Puzyński 2009).
Lithium can cause a non-toxic goitre or a goitre involving hypothyroidism. This effect is due to the inhibition of the secretion of thyroid hormones caused by lithium and can lead to the appearance of the symptoms of hypothyroidism (Schou 2006, Stahl 2007).

Among more common adverse symptoms during lithium therapy is weight gain. On average, patients put on about 4 kg, usually during the first year of the therapy and this effect more often concerns women (Schou 2006, Stahl 2007).

Lithium can cause exacerbation of the existing dermatological disorders, for example psoriasis, and can reduce the response of these disorders to pharmacotherapy. Lithium can also cause new dermal lesions such as acneform, psoriasiform lesions, sycosis, maculopapular eruption. Alopecia areata appears in 10% patients treated with lithium (Gupta et al. 1995, McKinney et al. 1996). Lithium administration can also lead to hyperglycemia and leukocytosis (Pużyński, Beręsewicz 1993, Pużyński 2009).

Most of the aforementioned side effects appear at the beginning of lithium therapy and disappear spontaneously or after adjusting the lithium level in plasma. Mauri et al. (1999) showed that there were no statistically significant differences in lithium tolerance in any period of its administration, from the first day to twenty-first year of therapy. Complications can be prevented by appropriate classification of patients for lithium therapy, which takes into consideration relative and unconditional contraindications. According to the literature, renal and circulatory dysfunctions, inadequate water-electrolyte balance, hypothyroidism, pregnancy and breast-feeding are unconditional contraindications for lithium therapy (Pużyński, Beręsewicz 1993).

Lithium ions pass through the placenta so that their level in the fetus’s plasma is equal to the level in the mother’s plasma (Pużyński, Beręsewicz 1993). Pinelli et al. (2002) reviewed the literature concerning the involvement of lithium in perinatal complications. Newborns of mothers treated with lithium most often suffer from congenital heart diseases, especially Ebstein’s syndrome, cardiac dysrhythmias, decreases in the plasma glucose level, decreases of arterial blood pressure, respiratory failure, cyanosis, coma, disturbances of the thyroid function and hyperbilirubinemia. Most of the side effects of lithium’s toxic influence on a fetus are transient and have no effect in the later childhood.

Adolescents are a specific target group for lithium therapy. Lithium is registered for therapy of persons above 12 years of age. Administration of lithium in the developmental period requires particular caution because of the altered pharmacokinetics of this medicine. High vulnerability of young organisms to disorders in the hormonal balance (Rajewski 2003), larger volumes of systemic water, higher speed of filtration in renal glomerules and high risk of lithium toxic effect on the developing central nervous system can limit administration of lithium in this age group (Tueth et al. 1998). On the other hand, elderly people have smaller volumes of systemic water, slow-
er metabolism and, often, coexisting somatic disorders complicating this therapy and exposing them to a higher risk of adverse events, thus lithium therapy in this group requires lower lithium concentrations in plasma (Tueth et al. 1998, Stahl 2007).

Simultaneous administration of medicines that interact with lithium requires great caution in lithium therapy. Medicines increasing the plasma level of lithium and thus increasing the risk of its toxicity are, for example, non-steroid anti-inflammatory agents, inhibitors of angiotensin converting enzyme and thiazides. Lithium should be used with caution also in the case of simultaneous therapy with metronidazol, methyldopa, phenytoin, haloperidol, carbamazepin and antidepressant agents with serotonergic activity (Rybakowski 2003, Stahl 2007).

QUALITY OF LIFE OF PATIENTS TAKING LITHIUM

Good co-operation with patients during lithium therapy diminishes the risk adverse events and complications and thus can improve patients’ quality of life. On the other hand, patients’ negative attitude to lithium therapy, partial response or, on the contrary, great improvement which gives a feeling of absolute recovery, can cause termination of therapy. Discontinuation of lithium therapy can also be caused by some burdensome side effects or the patient’s disapproval of the need of periodical laboratory and clinical tests, which are necessary for appropriate lithium therapy (Schou 2006). Vieta (2005) reports that psychoeducational interventions concerning identification of symptoms of disease recurrence, requirement of systematic maintenance treatment, conducting stabilised lifestyle improve the response to lithium therapy, decrease the number of disease recurrences and hospitalisations and improve patients’ quality of life. Dogan and Sabanciogullari (2003) studied the influence of education about lithium therapy on the severity of symptoms and patients’ quality of life. At the end of a three-month-long observational period, the group receiving psychoeducational instruction had better knowledge about the medicine, improved its taking, had fewer side effects and improved their quality of life. Scott and Tacchi (2002) proved that compliance with and acceptance of lithium therapy means maintaining optimal levels of the lithium concentration in plasma.

Many authors report that the presence of depressive symptoms prevails in the shaping of the quality of life in people with affective disorders. Montes et al. (2008) assessed a group of 115 patients with bipolar affective disorder, in which 71.3% were treated with lithium salts. The authors showed correlation between worse subjective assessment of own quality of life and depressive symptoms. Lower quality of life in patients with affective disorder can be caused by the disorder itself, somatic reasons, using tobacco or
alcohol as well as side effects of the administered medicines. Chand et al. (2004) showed that the quality of life in patients with bipolar affective disorder being stabilised with lithium was comparable with healthy controls. The authors relate this good result to fewer side effects during lithium therapy in comparison to the other mood stabilizers. Attaining an optimal response to treatment and preventing side effects as well as maintaining stabilizing therapy result in good quality of life. Revicki et al. (2005) showed that patients treated with lithium for one year experienced improvement of mental health, which was accompanied by lower medical costs (Revicki et al. 2005).

CONCLUSIONS

Although lithium has been used in medicine for many years, during which new medications producing similar effects have been introduced, it remains a medicine of the first choice long-term stabilizing therapy, although, in the light of current research, lithium monotherapy in acute manic conditions or severe and moderate depressive episodes is not recommended (Rybakowski 2003). We can prevent complications related to lithium therapy and achieve satisfactory improvement as well as good quality of life, having considered in great detail possible contraindications for lithium therapy and later monitoring of its plasma level and observing the patient to detect any side effects.

REFERENCES


BIOELEMENTS AND EATING DISORDERS
– ASPECTS OF THE QUALITY OF LIFE

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Abstract

Anorexia nervosa and bulimia are emotional disorders which are a serious hazard to the physical health or life. They most often affect girls and young women and disorganize their mental and social life. In this paper, complications caused by eating disorders as a result of deficiency or excessive loss of bioelements by an organism are reviewed along their influence on the quality of life. The symptoms of anorexia nervosa are the following: weight loss over 15% of the standard body mass for the age and height, severe fear of body weight gain despite clear evidence of weight deficiency. The main symptoms of bulimia involve uncontrolled overeating and counteracting weight gain which could occur after overeating episodes by self-induced vomiting or overuse of laxatives and diuretics.

Medical complications of bulimia are related to the method and frequency of purgation, while in anorexia they are caused by starvation and weight loss. The following deviations are observed in both restrictive and bulimic forms on anorexia: hypokalemia, hypocalcemia, hypophosphatemia and sometimes also hyponatremia, hypomagnesemia and hypochloreemic alkalosis. Many electrolytic and acid abnormalities are found in bulimia depending on the method for laxation (self-induced vomiting, misuse of laxatives or diuretics). Most patients adapt well for a relatively long time to low levels of potassium in plasma but sometimes the situation may cause life threatening consequences, like dysrhythmia, paralytic ileus, neuropathy, muscle weakness and paresis. Physicians and patients should understand that anorexia nervosa is a systemic disease and can affect all body organs. Full knowledge about possible complications of anorexia nervosa allows physicians to achieve precise assessment and conduct appropriate treatment of patients when the diagnosis has already been made.

Key words: anorexia nervosa, bulimia, bioelements, somatic complications

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INTRODUCTION

Eating disorders like anorexia nervosa and bulimia are two emotional disorders that seriously threaten physical health or life. They mostly affect girls and young women and disorganize their mental and social life (Namysłowska 2000). In this paper, we discuss complications of eating disorders due to deficiency or excessive loss of bioelements by an organism and the influence of these complications on the quality of life.

ANOREXIA NERVOSA

The term “anorexia” originates from Greek and consists of two words: “an” – lack, “oreksis” – appetite. The oldest reports of girls starving until dangerous weight lost date back to the Middle Ages (Rabe-Jabłońska 2006).

The most characteristic symptom of anorexia nervosa is persistent striving for weight loss. Patients do not stop to slim down even when they are seriously physically cachetic. Most cases of anorexia nervosa occur be-
between the age of 13-14 and 17-25 years. About 1% of school girls are affected by this disorder. Despite numerous studies, precise causes of anorexia nervosa remain unclear. Most researchers assume a multifactorial model of this disorder, which involves individual, family, social and cultural factors.

Symptoms of anorexia nervosa are the following: weight loss more than 15% of standard body mass for the age and height, severe fear of body weight gain despite clear evidence of severe weight deficiency, an aberrated manner in which patients experience their body weight and dimensions; excessive influence of body weight and dimensions on self-estimate or negation of currently low body weight; lack of at least 3 menstrual cycles in menstruating women (Namysłowska 2000, Dubelt, Szewczyk 2007).

In 1993, Garner distinguished two types of anorexia: restrictive and bulimic. Restrictive type is characterised by body weight loss and persistent limiting of calorie supply; bulimic type is characterised by occasional overeating episodes and using of many purgation methods (vomiting, laxatives, diuretics, enemas) and intensive physical exercise (Rabe-Jablońska 2006).

Anorexia nervosa has the highest mortality among all psychiatric disorders. The index of annual mortality is 5.6%, which is 12-fold higher than the annual mortality index in women aged 15-24 years among the general population (Athey 2003). Unfortunately, very much time passes between the first occurrence of the symptoms and the diagnosis followed by a treatment. When anorectic patients finally present at the doctor’s, they are usually urged to do so due to medical problems secondary to malnutrition and starving, e.g. lack of menstruation or infertility (Becker et al. 1999). Physicians should remember the underlying reason for these symptoms (Athey 2003).

**BULIMIA**

Bulimia was first identified as a separate disorder by Dr Gerald Russell in the late 1970s (Mehler et al. 2004). Analogously to anorexia, bulimia is a disorder that affects mainly young women aged 12-34, although there is now more evidence of higher incidence of this disorder among middle-aged women. The morbidity rate is 1-4%, but can reach up to 19% in some groups of patients from secondary schools (Kendler et al. 1991, Garfinkel et al. 1995).

The main signs of bulimia are attacks of uncontrolled overeating and using methods of counteracting consequences of these attacks by provoking vomiting, overuse of laxatives, diuretics and starving. Another symptom is the self-assessment dependence of body weight and dimensions (Namysłowska 2000).

Excessive interest in body weight is the prevailing symptom of both anorexia and bulimia and many patients show a mixture of anorectic and bulimic symptoms. Up to 50% patients with initial bulimia develop anorexia symptoms later (Mehler et al. 2004). Death risk is significantly lower in
bulimia compared to anorexia, although it is still higher than in the general population of women matched by age (Keel, Mitchell 1970). Pathogenesis of bulimia can be best understood with a biopsychosocial model.

SOMATIC COMPLICATIONS OF EATING DISORDERS

Although anorexia and bulimia are originally psychological disorders, there are serious nutritional, biochemical and other somatic disturbances here. Medical complications in bulimia are related to the way and frequency of purgation, while in anorexia they are caused by starving and body weight loss (Mehler et al. 2004). Metabolic disturbances are common, but usually they increase slowly and the patients may not experience any symptoms despite serious abnormalities (Herzog et al. 1997). These disturbances occur mainly in patients with a great body loss in a few months, especially in the ones who were often vomiting and/or taking laxatives and/or diuretics. They can also occur in patients who have been ill for a long time, become extremely cachetic due to nutritional restrictions and sometimes, albeit much less often, due to restrictions in fluid intake (Rabe-Jablonska, Melcer 2006). Electrolyte abnormalities often occur in the disorder and are a risk factor leading to ventricular arrhythmia and sudden death (Cooke, Chambers 1995, Dubelt, Szewczyk 2007). Other reported biochemical abnormalities involve increase in hepatic enzymes and loss of thiamin and zinc (Kovacs, Winston 2003). Hypoalbuminemia was proved to be the most valid biochemical marker of the risk of life loss in anorexia (Herzog et al. 1997). Endocrinological abnormalities in anorexia involve decreased gonadotrophin secretion, hypocortisolemia and sick euthyroid syndrome (euthyroid is a state of normal thyroid function) (Kovacs, Winston 2003). Moreover, hyperthyroidism and Addison disease are important differential diagnosis in this disorder. The patients with serious eating disorders should be fully diagnosed clinically and via laboratory tests as part of the initial assessment (Mitchell, Specker, de Zwana 1991, Kovacs, Winston 2003). Late complications of the disorder involve decrease of mineral bone density (osteopenia and osteoporosis), tissue composition disturbances and structural (atrophy) and functional changes in the central nervous system (Rabe-Jablonska, Melcer 2006).

BIOELEMENTS AND ANOREXIA NERVOSA

Pathogenesis of the water-electrolyte and acid-base equilibrium disturbances involve deficiency in nutritional intake, sometimes coupled with impaired nutrient absorption and also a fairly characteristic behaviour of anorectic patients, which provoke vomiting, use laxatives, limitat fluid intake and use diuretics, but in some cases act adversely and drink too much fluid. All of these practices significantly influence the quality of life, but interestingly patients feel well for quite a long time. This is due to compensatory abilities of an organism. Both in the restrictive and bulimic type of
anorexia the following disturbances can be present: hypokalemia, hypocalcemia, hypophosphatemia, less often hyponatremia, hypomagnesemia and hypochloremic alkalosis. Most patients adapt to low potassium level in plasma for a long time, but sometimes it can cause dangerous consequences like cardiac arrhythmia, paralytic ileus, muscle weakness and paresis (RABE-JABŁOŃSKA, MELCER 2006).

Hypokalemic nephropathy occurs in patients taking laxatives or diuretics. Symptoms of chronic renal failure appear (decrease of specific weight of urine, polyuria, increase of creatinine level in plasma). These complications have a definitely negative influence on the quality of life.

Hyponatremia occur in patients with hyponatremic dehydration during chronic purgation and can be manifested by orientation disturbances, muscle weakness and circulatory disturbances (CAREGARO et al. 2005). Hypophosphatemia was observed in extremely cachetic patients due to overuse of diuretics and renal failure, although it can also be caused by excessively rapid re-alimentation, especially with a high glucose supply, because it leads to increased penetration of phosphate ions into cells (HAGLIN 2001, RABE-JABŁOŃSKA, MELCER 2006).

Hypophosphatemia and hypocalcemia can also be caused by too little supply and absorption dysfunction. Hypophosphatemia is said to be a factor worsening prognosis, because it reflects depletion of body energetic resources and may be a predictor of sudden deterioration – due to rhabdomyolysis, congestive heart failure, red blood cells dysfunction secondary to adenosine-5'-triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) deficits (RABE-JABŁOŃSKA, MELCER 2006). Hypocalcemia in anorexia nervosa can be caused by both alimentary deficits, absorption disturbances in intestines and alkalosis; it can result in heart dysfunction (visible in ECG) or in tetany symptoms. Hypomagnesemia can be increased by hypophosphatemia and alcohol and can result in abnormalities in ECG, muscle weakness and convulsions. It is also related to increased hypocalcemia and hypokalemia, which cannot be compensated until magnesium depletion is supplemented (ATHEY 2003). Electrolytic disturbances are rather seldom when purgation is absent.

Hypomagnesemia is present in ¼ of patients with anorexia nervosa and most often is related to treatment resistant hypocalcemia and hypokalemia. Patients with this kind of electrolytic disturbance have the following symptoms: cramps, crampy abdominal pains and cardiac dysrhythmia. Risk of development of nephrolithiasis is also increased, likewise renal and electrolytic disturbances (RABE-JABŁOŃSKA, MELCER 2006).

**BIOELEMENTS AND BULIMIA**

Many electrolytic and acid abnormalities can be present in bulimia, depending on the used purgation method (self-induced vomiting, misuse of diuretics or laxatives). Hypokalemia is the most common abnormality and
can result in arrhythmia, rhabdomyolysis, muscle weakness, hypokalemic cardiomyopathy and tetany. Hypokalemia is not often present (in 4.6% of bulimic patients) and it occurs first of all in people with a low body weight who vomit or use laxatives or do both (GREENFELD et al. 1995, MEHLER 1998). Some mechanisms are important for the occurrence of hypokalemia. These involve a direct loss of potassium caused by vomiting. Loss chloride ions and gastric acid accompanies hypokalemia and result in metabolic hypokalemic-hypochloremic alkalosis. Overuse of laxatives cause loss of potassium and bicarbonate with stools, which in turn results in hypokalemia and metabolic acidosis. Some diuretics cause renal loss of potassium. More significant potassium loss occurs when any of the purgation methods leads to a significant loss of volume. Then, renin-angiotensin system is activated, leading to high levels of these hormones. This in turn causes renal sodium retention in place of hydrogen and potassium ions loss, which are secreted to urine. The result is metabolic alkalosis in bulimic patients who excessively purge their gastrointestinal tract by self-induced vomiting or diuretic misuse. The most serious cases of metabolic alkalosis are observed in self-induced vomiting. Normotensive hypokalemic hypochloremic metabolic alkalosis, known as the pseudo-Bartter syndrome, is observed in many patients with bulimia, wheer it has some significant therapeutic implications. Effectiveness of potassium supplementation is low until normalization of hypovolemia is reached (MEHLER et al. 2004). Sometimes bulimic patients with who seek help in admission rooms are diagnosed with severe hypokalemia. Despite massive supplementation of potassium, these patients remain hypokalemic because the level of fluids is not normalized. Improvement of fluid volume improves metabolic alkalosis and inactivates renin-angiotensin axis, allowing for effective potassium supplementation.

The biochemical disturbances described above have an unquestionably very significant meaning for the patients’ life quality, therefore the research conducted by KOVACS and WINSTON (2003) is very interesting. The authors assessed which diagnostic method of for electrolytic disturbances in people with eating disorders is the most suitable. They showed that potassium and calcium phosphatc levels are often been measured in patients with anorexia, in some of them, regularly. Electrolyte levels are much less often measured in bulimia, especially as far as magnesium is concerned; levels of calcium and phosphates are also less often measured compared with patients suffering from anorexia. It is an important observation because electrolytic abnormalities (which are often caused by self-induced vomiting and laxative overuse) may be more frequently present in patients with bulimia than anorexia (especially in the restrictive type) (GREENFELD et al. 1995, KOVACS, WINSTON 2003). Physicians should check laboratory investigations every 1-2 days during the first stage of return to food intake (ATHEY 2003).
SUMMARY

When patients with anorexia finally come to the doctor, they are usually urged to do so because of medical problems secondary to malnutrition and starving, e.g. lack of menstruation or infertility (Becker et al. 1999, Atthey 2003). Physicians should remember about the true reasons for these symptoms. Physicians can more easily diagnose anorexia nervosa by recognizing a full spectrum of medical consequences of eating disorders. Both physicians and patients should understand that anorexia nervosa is a systemic disease and can affect all body organs. Full knowledge about possible complications of anorexia nervosa allows physicians to assess precisely the patient’s condition and to conduct an appropriate treatment of patients when the diagnosis has already been made. It also allows doctors to educate patients about possible complications of anorexia. Objective data about medical complications of anorexia can even help patients who deny their disorder to accept it and to adhere to its treatment (APA 2000).

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