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ORIGINAL PAPERS

PRODUCTION OF ZINC-ENRICHED BIOMASS OF *SACCHAROMYCES CEREVISIAE*

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Abstract

Zinc accumulation and the growth of *Saccharomyces cerevisiae* were investigated in a culture with zinc sulfate-supplemented medium. The cultivations were performed on Sabouraud dextrose broth medium in aerobic conditions, without the addition of zinc (control culture) and with the addition of zinc sulfate (5, 10, 15, 30 and 60 mg $ZnSO_4$ l^{-1} medium) at 28°C for 72 hours. The results showed similar trends of yeast growth rates at 24, 48, and 72-hour interval, with concentrations above 10 mg l^{-1} $ZnSO_4$ in the nutritional medium significantly decreasing the yeast growth rate and the biomass yield (P<0.05). Substantial differences between the initial $ZnSO_4$ concentrations in the growth medium were demonstrated in the overall adsorption of Zn ions (Zn^{2+}) in yeast cells by a colorimetric assay (P<0.05). Similarly, the content of total accumulated zinc, as well as the fractions of Zn present in cells depended mainly on the zinc concentration in the medium, as the total Zn accumulation and organically bound Zn fractions were increased by elevating the $ZnSO_4$ supplementation in the culture medium up to 30 mg l^{-1}, but gradually reduced by any further addition of $ZnSO_4$ determined by an ICP-MASS assay (P<0.05). In the presence of 30 mg l^{-1} $ZnSO_4$, the Zn content in the biomass increased by 24-fold, to 4132.34 μg g^{-1} in comparison to 171.9 μg g^{-1} achieved in the basal medium. Thus, the ability of *S. cerevisiae* to accumulate zinc can be used for production of a zinc-rich ingredient for functional food products.

Keywords: accumulation, growth rate, organically bound Zn, *Saccharomyces cerevisiae*, yeast, zinc.

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INTRODUCTION

As structural and functional constituents of numerous metalloproteins, enzymes and hormones, microelements are involved in several biological processes of living organisms (BERG et al. 2002). Also certain ions neutralize electrostatic forces present in various cellular anionic units, especially in the DNA double helix and cell membranes (JONES, GREENFIELD 1984). On the other hand, it has been demonstrated that better bioavailability and consequently more efficient absorption of organically bounded microelements are achieved when administered in the form of protein complexes and obtained from the digestive tract of humans and animals (DOBRZANSKI, JAMROZ 2003).

Yeasts are known for their ability to bioaccumulate minerals from aqueous solutions, from which they permanently incorporate these metal ions into own cellular structures owing to their high concentration of protein (BLACKWELL et al. 1995, TEMPLETON et al. 2000, CORNELIS et al. 2001). Yeast cells incorporate double-valence metals through mechanisms including production of metalloproteins, mineralization and capture of metals into vacuoles (ECKER et al. 1986, WELCH 1993, EIDE 1997). Numerous *studies* about the processes involved in the uptake of trace metals by the yeast *Saccharomyces cerevisiae* as a model organism for investigating metal transporters and their accumulation in the cells have been conducted in recent years (NELSON 1999, COHEN 2000).

Zinc is an essential element required for *Saccharomyces cerevisiae* growth and metabolism. Also, zinc metalloenzymes are recognized in all 6 enzyme classes as identified by the IUBMB* (VALEE, FALCHUK 1993). Moreover, zinc is involved in the structure and function of proteins and nucleic acids, gene expression and immune system development (CASTRO, SEVALL 1993, DARDENNE, BACH 1993, REBAR, MILLER 2004).

The bioaccumulation of zinc ions by yeast proceeds in two stages (MOWLL, GADD 1983). The first, known as biosorption or "passive capture", is independent of microbial metabolism and is related to the cation accumulation on the outer surface of a cell wall, so that the metals retained there are subsequently adsorbed on anionic binding sites present in the cell intramicrofibrillar structures (VOLESKY, MAY-PHILLIPS 1995, GAUDREAU 2001). The second stage, called bioaccumulation or "active capture", is metabolism-dependent intracellular uptake and involves the penetration of metal ions to the cell interior using specific membrane transporters and the cell's metabolic cycles (BRADY, DUNCAN 1994). Metal ions are then primarily accumulated in the yeast vacuole (MACDIARMID 2002). The optimal concentration of zinc is yeast strain dependent. Generally, for *S. cerevisiae*, 0.25-0.50 mg l^{-1} appears to be required for the cell growth, and 1-2 mg l^{-1} is optimal for glycolysis (JONES, GREENFIELD 1984).

* International Union of Biochemistry and Molecular Biology

Owing to its ability to incorporate metals, yeast biomass has been used frequently as a delivery vehicle for many minerals. Zinc enriched yeast is produced by growing yeast cells in Zn high medium. The uptake of zinc by yeast cells may be affected by numerous parameters including the chemistry of the metal ions, cell physiology, specific surface properties of the organisms and physicochemical influence from the environment (Godlewska-Zlkiewicz 2006). However, high concentration of zinc ions in a nutrient substrate may be toxic, since zinc affects the permeability of membranes to potassium, causing a decrease of the yeast growth (Liu 1997). Thus, it seems that an optimal concentration of Zn is critical. Few studies have been completed on the optimal concentration of Zn in yeast and its effects on yeast characteristics (De Nicola et al. 2009 *a*,*b*).

Therefore, the aim of the present study was to produce high quantities of Zn-enriched yeast biomass in the presence of this metal using an industrially important strain of *S. cerevisiae*. Other objectives were to determine the zinc *concentration* in Zn-enriched biomass by rapid colorimetric assay and atomic absorption spectrometry, and to assess the effect of different Zn concentrations in culture medium on the yeast growth rate and Zn content in yeast biomass.

MATERIAL AND METHODS

Organisms, media and culture conditions

The *Saccharomyces cerevisiae* (strain PTCC 5209) used in this study was obtained from the collection of the Industrial Microorganisms Laboratory of the Iranian Research Organization for Science and Technology (IROST). Agar plates for storing and maintaining the viability of yeast culture were prepared by addition of 15 g l^{-1} agar to the Sabouraud dextrose broth (SB).

The composition of the basal medium* (BM) for yeast cultivation was (g l^{-1}): Dextrose – 20.0; Peptic digest of animal tissue – 5; Pancreatic digest of casein – 5. The batch processes were performed in 500 ml Erlenmeyer flasks with 200 ml of BM. Cultivation was performed in a shaker agitated at 200 strokes min^{-1} at 28°C for 72 h. The pH of the medium was initially adjusted to 4.5 using HCl at 0.1M. Zinc concentrations of the SB medium were adjusted to 0, 5, 10, 15, 30 and 60 mg l^{-1}, using a 1,000 mg l^{-1} sterile stock solution of zinc sulfate heptahydrate (Merck, Germany). The time of the addition of the $ZnSO_4$ solution was 0 h of inoculation. After cultivation, samples were centrifuged at 4000 rpm for 10 min at 20°C. The supernatant fluid was discarded and the solid phase was washed with deionized water thrice in order to remove residues of the medium and surface-bound zinc.

* Sabouraud Dextrose Broth; Liofilchem, Italy

Growth rate evaluation

In the course of cultivation, the yeast growth was determined at 24-hour intervals (0, 24, 48, and 72 hours) turbidimetrically, by measuring the suspension optical density at 600 nm wavelength (OD_{600}). Also, at the end of cultivation, the dry biomass yield was determined by centrifuging 25 ml of samples at 4000 rpm for 10 min in a portable centrifuge. Immediately after centrifugation, the supernatant was removed and the content of dry matter was determined using two-stage drying, first at the temperature of 60°C for 2 h, and then finish drying at 105°C until the constant weight was reached. The yield of yeast cell biomass was expressed in g dry weight l^{-1} medium (NOWAK et al. 2005).

Measurement of metal content in yeast cells

After the fermentation was performed, two methods were applied for the assessment of the total zinc accumulation: colorimetric assay and ICP-MASS assay.

Colorimetric assay for zinc biosorption evalution

The concentrations of zinc in supernatant were determined spectrophotometrically (UV-visible S2100, Scinco, Korea) by using commercial kits (Giesse diagnostics, Italy).

The amount of Zn^{2+} adsorbed by cells was calculated as follows:

$$q\ (\mu g\ g^{-1} \text{ of yeast}) = 1000\ (c_0 - c_t) \cdot V\ W^{-1}$$

where: t – the incubation time (h),

c_0 – the initial concentration of $ZnSO_4$ at t = 0 (mg l^{-1}) before inoculation of yeast,

c_t – the concentration of residual Zn^{2+} in supernatant at the time of t (mg l^{-1}) after removal of biomass at the end of cultivation,

V – the volume of medium (ml),

W – the dry weight of yeast (g).

In addition, yeast cells were washed with ddH_2O to eliminate residues of Zn^{2+} adsorbed to the surface of the biomass, and amount of surface-bound Zn was determined by the above method.

Zn Incorporation Efficiency: The final Zn content (mg) in the produced yeast was divided by the initial amount of $ZnSO_4$ (mg) added to the medium and multiplied by 100.

Determination of total accumulated zinc in yeast cells

The content of zinc in whole yeast cells was assessed according to the modified AOAC method based on YANG et al. (2005). Briefly, dry yeast biomass was digested by the addition of 5 ml of 65% (v v^{-1}) nitric acid* to 1 mg dry biomass in 25 ml digestion tubes. The pre-digested samples were heated for 1 h at 90°C, then the temperature was raised to 140°C and hydrogen peroxide** (30%) was added to the tubes. After cooling down, the samples were diluted with bidistilled water up to 10 ml and the zinc content was analyzed by ICP-MASS (Yokogawa Analytical Systems HP 4500, Japan).

Zinc standard solutions were prepared by using appropriate dilutions of a stock zinc standard solution for ICP-MASS analysis (1000 ± 2 mg $ZnSO_4$ l^{-1} in 5% HNO_3, Merck). The amount of total zinc was calculated per gram of dry weight (μg Zn g^{-1} d.w. of yeast).

Moreover, different forms of Zn (organic and inorganic forms) present in the biomass were quantified as described by ROEPCKE et al. (2011).

All glassware was washed in 10% HCl*** and rinsed 3 times with distilled water to avoid any mineral contamination.

Statistical analysis

The experiment was carried out in a completely randomized design with 4 replications. Each variable was compared using the Anova procedure of SAS (1996) to determine statistical significance. Differences among treatment means were separated using the Duncan's multiple range test. Verification of significance was based on $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth rate assay

The effects of supplementing the culture medium with zinc sulfate on the yeast growth rate are *illustrated* in Figure 1. As expected, the growth rate was higher up to 24 hours after inoculation but lower in comparison to 0-24 hour inoculums ($P<0.05$). Also, the OD of the cell culture increased correspondingly with the incubation time and leveled off at around 60 h after inoculation, indicating the end of the yeast cell growth as the number of yeast cells present in the broth remained constant (Figure 1).

The above trend was in agreement with the growth curve for yeast cells reported by other investigators (DALGAARD, KOUTSOUMANIS 2001). According to VASANTHY (2004), the growth rate of yeast biomass rose with the incubation

* HNO_3 65%, Merck, Germany

** H_2O_2 30% Merck, Germany

*** HCl 37% Merck, Germany

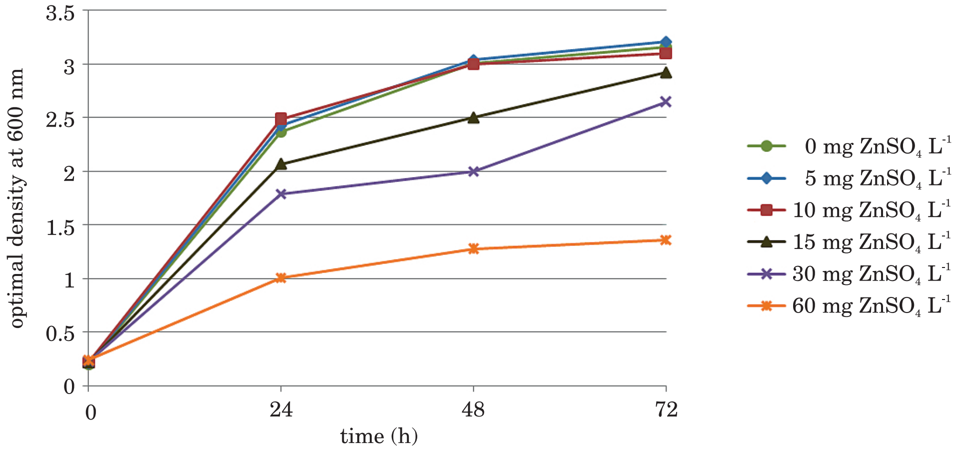

Fig. 1. The effect of zinc sulfate on the growth of *S. cerevisiae* yeast

time, reached the maximum at an equilibrium time and afterwards remained constant.

A similar upward trend in the yeast growth rate was found at 24, 48, and 72 hours after inoculation across treatments. The continuation of the growth rate was consistent and dose related. However, in the presence of 5 and 10 mg l^{-1} of $ZnSO_4$, the growth curves and the duration of the lag-period were almost the same as in the control culture (Figure 1). Furthermore, the concentration of $ZnSO_4$ 5 mg l^{-1} resulted in a slight OD_{600} increase in the growth phase. As the $ZnSO_4$ concentration rose up to 15 mg l^{-1}, a decrease in the growth rate during the cultivation was observed. Higher $ZnSO_4$ concentrations, i.e. 30 and 60 mg l^{-1}, more drastically inhibited the yeast growth rate during the linear phase ($P<0.05$). The maximum OD for these treatments did not exceed 43% of that in the control culture.

Zinc added to culture medium Zn may enhance the cell growth yeast (Jones, Gadd 1990) because this element is involved in the regulation of structure and metabolic activity of yeast and other processes such as flocculation and cell division (Bromberg 1997).

Our results show that the addition of $ZnSO_4$ up to 5 mg l^{-1} of nutrient media slightly increased the growth of *S. cerevisiae*. This growth stimulating effect of Zn in low concentrations can be explained by the increased synthesis of riboflavin and stimulation of cell proliferation, which lead to a more intensive cell growth (Greco et al. 1990). However, the growth rate of yeast cells and the final biomass yield were reduced drastically by increasing the $ZnSO_4$ concentration above 10 mg l^{-1} of medium. The inhibitory effect of high concentrations of Zn salts in nutrient media on the growth of yeast cells and/or on the final biomass yield is due to the harmful effects caused by the toxic nature of zinc. Zinc toxicity inevitably results in the biological inactivation of cells and loss of viability.

Since toxicity depends on the Zn concentration, its manifestation in metabolism is proportional to the amount of zinc capable of permeating into the cell and interfering with its biological functions. On the other hand, an increase in the $ZnSO_4$ concentration lengthened the lag-phase and shortened the exponential phase duration, thereby decreasing the yield of yeast biomass. These results are consistent with the observation of KONOPKA et al. (1999), who confirmed that the microbial biomass generation diminished as the concentration of the heavy metal increased.

It can be assumed that the presence of large amounts of Zn ions taken up by *S. cerevisiae* causes extensive K^+ release, as well as Mg^{2+}, Na^+ and H^+ efflux from the biomass, which may be an essential component of the physiological mechanism responsible for maintaining the ionic balance, or may be a symptom of membrane disruption and irreversible viability loss (PASSOW, ROTHSTEINA 1960, NORRIS, KELLY 1977, GADD, MOWLL 1983).

Table 1

Yields of yeast biomass and accumulation of zinc in biomass of *S. cerevisiae* yeast with the addition of different concentrations of $ZnSO_4$ into medium

Treatment*	Yeast biomass yield (g l^{-1})	Zinc accumulation (μg g^{-1} of yeast)		
		total Zn accumulation	organically bound Zn	inorganically bound Zn
1	4.342^{b}	171.8^{e}	163.2^{e}	8.590^{f}
2	4.995^{a}	1888.5^{d}	1756.4^{d}	132.2^{e}
3	4.260^{bc}	3265.9^{c}	3004.7^{b}	261.2^{d}
4	3.863^{c}	3795.7^{b}	3302.3^{a}	493.4^{c}
5	3.020^{d}	4132.3^{a}	3140.5^{ab}	991.8^{b}
6	1.741^{e}	3895.9^{ab}	2415.5^{c}	1480.5^{a}
P value	0.001	0.001	0.001	0.001
SEM	0.260	33.32	27.54	7.390

Means with same superscripts in the same column are not significantly different ($P<0.05$).
SEM: Standard error of the means.
* Treatment: 1 – without the addition of zinc (control); 2–5; 3–10; 4–15; 5–30; 6–60 mg zinc l^{-1} of medium.

Moreover, one of the major Zn detoxification mechanisms in *S. cerevisiae* is the induced synthesis of metallothioneins (MT), which restrains the binding of free Zn ions in the cytosol (WELCH et al. 1983). Metallothioneins can be induced by heavy metal ions, such as Zn, Cu, Cd and Co. However, the MT in *S. cerevisiae* can only be induced by Cu (VIJVER et al. 2004), hence it iis called Cu-MT. Therefore, the absence of MT synthesis and consequently loss of Zn homeostasis under high Zn concentrations result in toxicity and retarded cell growth.

In terms of biomass production, the results shown in Table 1 demonstrate that as the $ZnSO_4$ concentration increased above 15 mg l^{-1} in the medium, the biomass yield significantly diminished ($P<0.05$), whereas an increase in the concentrations of $ZnSO_4$ in a medium from 0 to 10 mg l^{-1} had no influence on the yeast biomass production ($P<0.05$). The minimum and maximum biomass production was 4.99 and 1.74 mg l^{-1} biomass in media containing $ZnSO_4$ at concentrations of 5 and 60 mg l^{-1}, respectively (Table 1).

Excessive $ZnSO_4$ (above 15 mg l^{-1}) appeared to have a detrimental effect on the yeast growth. HAMMOND (2004) reported that excessive Zn could depress the growth of brewing yeast unless the concentrations of manganese were correspondingly high. Therefore, the results indicate that although Zn is an essential element for *S. cerevisiae*, Zn^{2+} added in high concentrations had inhibitory effects on the yeast cell growth.

The results of the current study suggest that zinc ion concentrations in medium are not a sufficient condition for achieving an ideal yeast biomass yield because adding low concentrations of $ZnSO_4$ (5 mg l^{-1}) promoted the yeast cell growth.

Colorimetric assay for zinc biosorption evaluation

The magnitude of biosorption was significantly different ($P<0.05$) in the presence of different concentrations of the initial $ZnSO_4$ in media (Figure 2). Except for $ZnSO_4$ concentrations exceeding 60 mg l^{-1}, higher $ZnSo_4$ supplementation resulted in enhanced zinc biosorption, so that the maximum Zn^{2+} biosorption was recorded at the initial zinc concentration of 30 mg l^{-1}

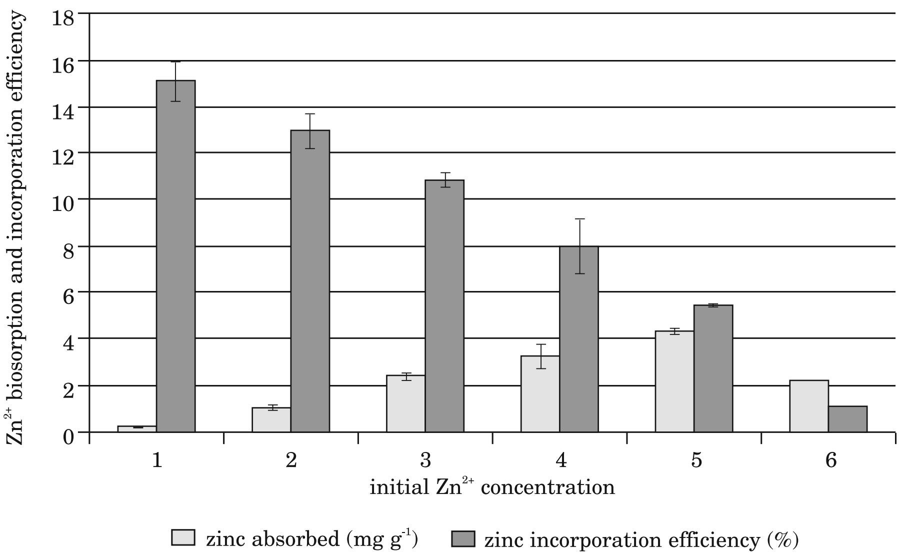

Fig. 2. Effect of initial Zn^{2+} concentration on zinc biosorption (mg g^{-1} of yeast) and Zn^{2+} incorporation efficiency (%)

Treatment: 1 – without the addition of zinc (control); 2–5; 3–10; 4–15; 5–30; 6–60 mg zinc l^{-1} of medium.

Note: Error bars denote standard deviation. Statistical significance by *t* test: $p<0.05$.

(4.33 mg g^{-1} of yeast) and the control culture contained the lowest amount of Zn (0.23 mg g^{-1} of yeast).

The introduction of Zn salts into culture medium results in the uptake of this mineral by yeast cells. The uptake of Zn^{2+} by *S. cerevisiae* requires the presence of functional groups such as the phosphomannan in their cell walls, the availability of free carboxyl, amine, phosphate, hydroxyl and hydrosulphide groups in the surface proteins and an intact membrane (BLACKWELL et al. 1995). As the amount of $ZnSO_4$ in the growth medium increased, the ability of yeast cells to adsorb it decreased due to the *saturation* of functional groups on the surface of the cellular walls and the *binding sites and* chelating agent within cells.

Moreover, the reduced Zn^{2+} adsorption due to the addition of 60 mg l^{-1} Zn sulfate to a medium may be explained by the fact that the Zn^{2+} uptake into cells is mediated by the plasma membrane ATPase activity via the transmembrane proton gradient (PONTA, BRODA 1970). Thus, the uptake of the metal usually takes place during the early stages of fermentation, when the availability of energy sources is the highest. Nevertheless, the cells of *S. cerevisiae* released some of the previously bound Zn^{2+} into the medium at the stationary growth stage to prevent the cell from Zn supersaturation and toxicity. It was reported that the mass of incorporated Zn ions was reduced towards the end of fermentation as cells released the metal ions due to the ageing process and reduction in the cell's charge (WALKER 2004).

The results indicated that the Zn^{2+} uptake capacity (q, μg g^{-1}) increased and the Zn^{2+} incorporation efficiency decreased when the initial external $ZnSO_4$ concentration in the nutrient medium was raised (Figure 2), although a 12-fold higher initial $ZnSO_4$ concentration in the yeast growth medium did not result in 12-fold higher total biosorption of Zn yeast cells after 72-h cultivation. This result might be explained by a smaller cell size due to a shorter exponential phase duration and hindered transport across the yeast cell membranes (ARSLAN 1987, HUGHES, POOLE 1991), since metal ions in the medium would not be adsorbed to the surface of the biomass, could not enter into intracellular organelles and be stored in vacuoles. The Zn^{2+} incorporation efficiency ranged from 1.19% to 15.01% when the initial supplemental $ZnSO_4$ concentration is 0-60 mg l^{-1}. The above percentages occurred in response to the highest initial $ZnSO_4$ concentration (60 mg Zn l^{-1}) and control culture, respectively.

ICP-MASS assay for Zn accumulation evaluation

Table 1 illustrates the quantity of total Zn accumulated by yeast cells. Except for the concentration of 60 mg l^{-1} $ZnSO_4$ added to the medium, the content of Zn accumulated by the yeast biomass was proportional to the $ZnSO_4$ concentration of the medium ($P<0.05$), namely after 72 h of incubation, the highest Zn bioaccumulation by yeast biomass was obtained in a medium supplemented with 30 mg l^{-1} $ZnSO_4$ ($P<0.05$), and the Zn content in that biomass reached 4132 μg g^{-1} of d.w. Also, the control culture had the lowest concentration of Zn in its biomass at 171 μg g^{-1}.

The direct relationship between the Zn content in cells and Zn supplemented in the culture medium indicates the cell's inability to curb zinc accumulation even at lethal doses. Nevertheless, this dependence was not linear (Table 1). Thus, under the increase in the exogenous $ZnSO_4$ concentration up to 30 mg l^{-1}, the Zn bioaccumulation increased. In response to higher $ZnSO_4$ concentrations supplemented, the concentration of accumulated Zn slightly decreased. Production of zinc-enriched yeast biomass has been reported by some other authors. STEHLIK-THOMAS et al. (2004) obtained the total concentration of Zn of 700 μg g^{-1} of dry biomass by the addition of 100 mg l^{-1} $ZnSO_4$ to the medium, while ŠILLEROVÁ et al. (2012) produced yeast biomass enriched with 3820 μg Zn g^{-1} of *S. cerevisiae* biomass through yeast cultivation in medium supplemented with 250 mg l^{-1} $ZnSO_4$.

Based on some previous study in hypertonic solutions, it can be concluded that microbial cells tend to shrink and reduce their volume. Depending on the osmotic stress, they may lose as much as 60% of their initial volume (GNIEWOSZ et al. 2006), and the maximum Zn bioaccumulation capacity by cells appeared to depend on cellular volumes (DE NICOLA et al. 2009*b*). On the other hand, the vacuole is the major site of metal storage in the *S. cerevisiae* cell, and Zn is translocated predominantly into the yeast vacuole (DE NICOLA et al. 2009 *a*).It is therefore conceivable that yeast with smaller cellular volumes and consequently smaller vacuoles are able to accumulate less Zn than larger cells. Also, the ability of cells to accumulate Zn at higher concentrations could depend on other factors, such as the presence of Zn-binding intra-vacuolar components, e.g. polyphosphate bodies (JONES, GADD 1990). Moreover, the size of the intracellular pool determines the activity of permease, a membrane transport protein that inhibits the uptake of a substance by trans-inhibition, so that at higher pool levels, more permease molecules are inactivated (FAILLA, WEINBERG 1977).

In addition, the production of Zn-enriched yeast depends not only on the Zn content in cells, but also on its amount in biomass. A high Zn content in cells was accompanied by a low biomass and vice versa. This result is in conformity with the report by DUSZKIEWICZ-REINHARD et al. (2005) on the binding capacity of magnesium in yeast.

Organically and inorganically bound zinc

Fractions of Zn accumulated in the biomass were also determined (Table 1). The amount of accumulated organically bound Zn was higher than inorganically bound Zn fractions in all thee treatments. Based on these results, the intracellular Zn content in yeast was in the range of 62% to 95% of the total Zn in cells. The control culture containing 171 μg Zn g^{-1} of biomass had 95% of organic bound zinc, which means that the yeast product was relatively free of inorganic Zn. On the other hand, T6 (60 mg l^{-1} $ZnSO_4$ - supplemented) contained 3895 μg Zn g^{-1} of yeast biomass, and 38% of the total Zn was present in the inorganic form. Moreover, the yeast cells accumulated up to a 20-fold higher amount of organically bound Zn with the 15 mg l^{-1} of $ZnSO_4$ in the medium compared to the control culture.

Organically bound Zn is the zinc fraction bound to organic molecules present in the cell. The higher $ZnSO_4$ concentrations resulted in enhanced intracellular Zn accumulation, evidently due to the Zn sorption by cell wall polysaccharides and intracellular organelles. Accumulation of a significant amount of Zn in extracellular polysaccharides has been observed by other researchers (Roepcke et al. 2011).

After absorbtion of Zn by yeast cells, metal ions are compartmentalized into different subcellular organelles (e.g. mitochondria and vacuoles), and this mineral combines with many intracellular organelles including cell wall, cell membrane, vacuoles and mitochondria. At excess Zn concentrations, vacuoles uptake and store high amounts of Zn to maintain the normal metabolism of cells (Daniel et al. 19990), bind this mineral with a chelating agent and accumulate them in the organic form.

As mentioned in the previous section, restriction of Zn-binding ligands within intracellular organelles including polyphosphate and organic anions, such as glutamate and citrate, may contribute to the reduction of Zn bioaccumulation capacity under high initial concentration of this salt (Kitamoto et al. 1988).

The results obtained from the ICP-MASS assay are partly consistent with measurements of the total Zn biosorption in yeast cell determined by colorimetry. However, the lack of strong similarity between these analytical methods is probably explained by the characteristics and limitations of colorimetric assays and weak surface binding of Zn in yeast cells.

CONCLUSION

The experimental results justify the conclusion that the concentration of Zn sulfate in the culture medium significantly affected the Zn content in yeast cell as well as the cell growth. Thus, the addition of Zn salts into a yeast (*S. cerevisiae*) medium enhances organic Zn production. The enhancement was higher under 30 mg l^{-1} $ZnSO_4$ levels and the highest level of zinc sulfate (60 mg l^{-1}) limited the yeast growth and Zn uptake. Therefore, addition of $ZnSO_4$ to a medium for yeast cultivation in a concentration higher than 30 mg l^{-1} is unreasonable because it inhibits the growth of yeast and causes accumulation of Zn in inorganic forms, which is unacceptable for production of a zinc enriched ingredient for food products. It seems that the former concentration of $ZnSO_4$, above which growth inhibitions occurs, is optimal for the production of Zn enriched yeast biomass. Moreover, the colorimetric assay of Zn was shown to be a fairly reliable and efficient tool for assessment of the Zn content in future studies.

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REFERENCES

ARSLAN P., BELTRAME M., TOMASI A. 1987. *Intracellular chromium reduction.* Biochim. Biophys. Acta., 931: 10-15.

BERG JM., TYMOCZKO JL., STRYER L. 2002. *Biochemistry.* New York, WH Free-man and Company, pp 270, 465, 687.

BLACKWELL KJ., SINGLETON I., TOBIN JM. 1995. *Metal cation uptake by yeast: a review.* Appl. Microbiol. Biotechnol., 43: 579-584.

BRADY D., DUNCAN JR. 1994. *Bioaccumulation of metal cations by Saccharomyces cerevisiae.* Appl. Microbiol. Biotechnol., 41: 149-154.

BROMBERG SK., BOWER PA., DUNCOMBE GR., FEHRING J., GERBER I., LAU VK., TATA M. 1997. *Requirements for zinc, manganese, calcium and magnesium in wort.* J. Am. Soc. Brew. Chem., 55: 123-128.

CASTRO CE., SEVALL JS. 1993. *Zinc deficiency, chromatin structure, and gene expression*. In: *Nutrient modulation of the immune response.* DEKKER M. (ed). New York, S. Cunningham-Rundles, pp 141-50.

COHEN A., NELSON H., NELSON N. 2000. *The family of SMF metal ion transporters in yeast cells.* J. Biol. Chem., 275: 33388–33394.

CORNELIS R., CREWS H., DONARD O., EBDON L., PITTS L., QUEVAUVILLE J. 2001. *Summary paper of the EC Network on trace element speciation for analysts, industry and regulators-what we have and what we need.* J. Environ. Monit., 3: 97-101.

DALGAARD P., KOUTSOUMANIS K. 2001. *Comparison of maximum specific growth rates and lag times estimated from absorbance and viable count data by different mathematical models. J. Microbiol. Methods, 43: 183-196.*

DANIEL JK., PAUL KH., SCOTT DE. 1990. *The fungal vacuoles composition, function and biogenesis.* Microbiol. Rev., 54: 266-292.

DARDENNE M., BACH JM. 1993. *Rationale for the mechanism of zinc interaction in the immune system*. In: *Nutrient modulation of the immune response.* DEKKER M. (ed). New York, S. Cunningham-Rundles, pp 501-509.

DE NICOLA R., HALL N., BOLLAG T., THERMOGIANNIS G., WALKER GM. 2009*a*. *Zinc accumulation and utilization by wine yeasts*. Int. J. Wine. Res., 1: 85-94.

DE NICOLA D., HALL N., MELVILLE SG., WALKER GM. 2009*b*. *Influence of zinc on distiller's yeast: cellular accumulation of zinc and impact on spirit congeners*. J. Inst. Brew., 15(3): 265-271.

DOBRZANSKI Z., JAMROZ D. 2003. *Bioavailability of selenium and zinc supplied to the feed for laying hens in organic and inorganic form*. EJPAU, 6: 1-8.

DUSZKIEWICZ-REINHARD W., GNIEWOSZ M., BŁAŻEJAK S., BAŃKOWSKI A. 2005. *Studies into Saccharomyces cerevisiae baker's yeast capacity for binding magnesium under batch conditions.* Pol. J. Food Nutr. Sci., 55: 249-255.

ECKER DJ., BUTT TR., STERNBERG EJ., NEEPER MP., DEBOUCK C., GORMAN JA., CROOK ST. 1986. *Yeast metallothionein functional in metal ion detoxification.* J. Biol. Chem., 261: 16895--16900.

EIDE D. 1997. *Molecular biology of iron and zinc uptake in eukaryotes*. Curr. Opin. Cell Biol., 9: 573-577.

FAILLA ML., WEINBERG ED. 1977. *Cyclic accumulation of zinc by candida utilis during growth in batch culture.* J. Gen. Microbiol., 99: 85-97.

GADD GM., MOWLL JL. 1983. *The relationship between cadmium uptake, potassium release and viability in Saccharomyces cerevisiae.* FEMS Microbiol. Let., 16: 45-48.

GAUDREAU H., CONWAY J., CHAMPAGNE CP. 2001. *Production of zinc-enriched extract*. J. Food. Sci. Technol. Mys., 38(4): 348-351.

GODLEWSKA-ZLKIEWICZ B. 2006. *Microorganisms in inorganic chemical analysis*. Anal. Bioanal. Chem., 38(4): 114-123.

GRECO MA., HRAB DJ., MAGNEV WDJ., KOSMAN J. 1990. *Cu, Zn superoxide dismutase and copper deprivation and toxicity in Saccharomyces cerevisiae.* J. Bacteriol., 172: 317-321.

HAMMOND J. 2004. *Yeast growth and nutrition*. In: *Brewing yeast fermentation performance.* SMART K. (ed). Oxford, Blackwell Sciences Plc, pp 77-85.

HUGHES MN., POOLE RK. 1991. *Metal speciation and microbial growth – the hard (and soft) facts.* J. Gen. Microbiol., 137: 725-734.

JONES RP., GADD GM. 1990. *Ionic nutrition of yeast: physiological mechanisms involved and implications for biotechnology.* Enzyme Microb. Technol., 12: 1-17.

JONES RP., GREENFIELD P. 1984. *A review of yeast ionic nutrition.* Process. Biochem., 19: 48-58.

KITAMOTO K., YOSHIZAWA K., OHSUMI Y., ANRAKU Y. 1988. *Dynamic aspects of vacuolar and cytosolic amino acid pools of Saccharomyces cerevisiae*. J. Bacteriol., 170: 2683-2686.

KONOPKA A., ZAKHAROVA T., BISCHOFF M., OLIVER L., NAKATSU C., TURCO RF. 1999. *Microbial biomass and activity in lead contaminated soil.* Appl. Environ. Microbiol., 65: 2256-2259.

LIU FX., SUPEK F., NELSON N., CULOTTA VC. 1997. *Negative control of heavy metal uptake by the Saccharomyces cerevisiae BSD2 gene.* J. Biol. Chem., 272(18): 11763-11769.

MACDIARMID C., MILANICK MA., EIDE DJ. 2002. *Biochemical properties of vacuolar zinc transport systems of Saccharomyces cerevisiae.* J. Biol. Chem., 277: 39187-39194.

GNIEWOSZ M., BŁAŻEJAK S., ROMAN J., DUSZKIEWICZ-REINHARD W. 2006. *A study on Saccharomyces cerevisiae and Candida utilis cell wall capacity for binding magnesium*. Eur. Food Res. Technol., 224: 49-54. DOI: 10.1007/s00217-006-287-z

MOWLL ML., GADD GM. 1983. *Zinc uptake and toxicity in the yeasts Sporobolomyces roseus and Saccharomyces cerevisiae.* J. Gen. Microbiol., 129: 3421-3425.

NELSON N. 1999. *Metal ion transporters and homeostasis*. EMBO J., 18(16): 4361-4371.

NORRIS PR., KELLY DP. 1977. *Accumulation of cadmium and cobalt by Saccharomyces cerevisiae.* J. Gen. Microbiol., 99: 317-324.

NOWAK D., KASIAK T., LEWICKI PP., DUSZKIEWICZ-REINHARD W. 2005. *Pilot-plant cultivation of brewery's yeast Saccharomyces cerevisiae enriched with magnesium*. Pol. J. Food Nutr. Sci., 14(2): 177-182.

PASSOW H., ROTHSTEINA A. 1960. *The binding of mercury by the yeast cell in relation to changes in permeability.* J. Gen. Physiol., 43: 621-633.

PONTA H., BRODA E. 1970. *Mechanism of zinc uptake by baker's yeast*. Planta, 95: 18-26.

REBAR EJ., MILLER JC. 2004. *Design and applications of engineered zinc finger proteins.* Bio. Tech. Int., 16(2): 20-24.

ROEPCKE CBS., VANDENBERGHE LPS., SOCCOL CR. 2011. *Optimized production of Pichia guilliermondii biomass with zinc accumulation by fermentation.* Anim. Feed. Sci. Technol., 163: 33-42.

SAS Institute. 1996. SAS/STAT User's Guide: Statistics. Release 6.11. SAS Institute Inc. Cary, NC.

ŠILLEROVÁ S., LAVOVÁ B., URMINSKÁ D., POLÁKOVÁ A., VOLLMANNOVÁ A., HARANGOZO Ľ. 2012. *Preparation of zinc enriched yeast (saccharomyces cerevisiae) by cultivation with different zinc salts.* JMBFS, 1: 689-695.

STEHLIK-THOMAS V., ZETIC VG., STANZER D., GRBA S., VAHCIC N. 2004. *Zinc, cooper and manganese enrichment in yeast Saccharomyces cerevisiae.* Food Technol. Biotechnol., 42(2): 115-120.

TEMPLETON DM., ARIESE F., CORNELIS R., DANIELSSON LG., MUNTAU H., VAN LEEUWEN HP., ŁOBIŃSKI R. 2000. *Guidelines for terms related to chemical speciation and fractionation of trace elements: definitions, structural aspects, and methodological approaches.* Pure Appl. Chem., 72 (8): 1453-1470.

VALEE B., FALCHUK KH. 1993. *The biochemical basis of zinc physiology.* Physiol. Rev., 78: 79-118.

VASANTHY M. 2004. *An investigation on removal of chromium (VI) using bacterial strains Asian.* J. Microbiol. Biotech. Env. Sci., 6(4): 583-586.

VIJVER MG., GESTEL CAMV., LANNO RP., VAN STRAALEN NM., PEIJNENBURG WJGM. 2004. *Internal metal sequestration and its ecotoxicological relevance: a review.* Environ. Sci. Technol., 38: 4705-4712.

VOLESKY B., MAY-PHILLIPS HA. 1995. *Biosorption of heavy metals by Saccharomyces cerevisiae.* Appl. Microbiol. Biotechnol., 42: 797-806.

WALKER MG. 2004. *Metals in yeast fermentation processes.* Adv. Appl. Microbiol., 54: 197-229.

WELCH JW., FOGEL S., CATHALA G., KARIN M. 1983. *Industrial yeasts display tandem gene iteration at the CUPI region.* Mol. Cell Biol., 3: 1353-1361.

WELCH RM. 1993. *Zinc concentrations and forms in plants for humans and animals*. In: *Zinc and soil and plants.* ROBSON A. (ed). Dordrecht, the Netherlands, Kluwer Academic Publishers, pp 183-195.

YANG X., CHENG X., JIN C. 2005. *Determination Cu, Pb, Zn, Fe and Mn in yeast by atomic absorption spectrometry.* Spectronic. Instrum. Anal., 4: 1-4.

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PRESSURE EXERTED BY ZINC ON THE NITRIFICATION PROCESS*

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Abstract

The objective of this study was to evaluate the nitrification rate in soil polluted with zinc. The experimental protocol was as follows: soil (sandy loam) collected from the 0-20 cm layer of a cropped field was passed through a 2 mm mesh sieve, placed in 150 cm^3 glass beakers, 100 g of soil in each, and polluted with the following doses of Zn^{2+} per 1 kg d.m. of soil: 0, 300, 600, 1 200 and 2 400. Zinc was applied in the form of $ZnCl_2$ aqueous solution. Afterwards, ammonia nitrogen as $(NH_4)_2SO_4$ was added to the soil material in two doses: 0 and 240 mg N kg^{-1} d.m. Once zinc chloride and ammonium sulphate had been thoroughly mixed with the soil, water was added until the soil moisture content reached 50% of capillary water holding capacity and then the beakers were placed in a laboratory incubator at 25°C. After 10, 20, 30 and 40 days, the incubated soil was tested to determine the content of N-NH_4 and N-NH_3. Additionally, after 10 and 40 days of incubation, the most probable counts of nitrifying bacteria involved in the first and second step of the nitrification process were determined. The experiment was run with three replicates for each day. Two determinations of each parameter were performed in the soil samples placed in beakers. In total, 6 results were obtained for each experimental variant. Based on the determinations, the amounts of nitrified and immobilized nitrogen were calculated and the resistance (RS) and resilience (RL) of the nitrification process and nitrifying bacteria to the contamination of soil with zinc were expressed.

It has been experimentally demonstrated that excess zinc in soil significantly disturbs the nitrification rate. As little as 300 mg Zn^{2+} kg^{-1} d.m. of soil significantly inhibits nitrification. Zinc contamination interferes with nitrification and other metabolic process which affect soil nitrogen, which is confirmed by depressed nitrogen immobilization at higher rates of soil contamination with this element.

The adverse effect of zinc on nitrification is primarily due to the negative impact of this element in the soil environment on nitrifying bacteria. Zinc more strongly inhibits the first than the second step nitrification bacteria, but ammonia-oxidizing bacteria recover more quickly than nitrate forms. The RS parameters for the nitrification process towards zinc pollution were on a low level and tended to decrease as the degree of zinc contamination rose. The resistance of nitrifying bacteria to zinc decreased parallel to the increasing amounts of zinc in soil.

Keywords: nitrification, zinc contamination, resistance of nitrification of contamination, nitrifying bacteria.

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PRESJA CYNKU NA PROCES NITRYFIKACJI

Abstrakt

Celem badań było określenie przebiegu procesu nitryfikacji w glebie zanieczyszczonej cynkiem. Procedura doświadczenia była następująca. Glebę (glinę piaszczystą) pobraną z użytku rolnego z warstwy od 0 do 20 cm przesiano przez sito o oczkach o średnicy 2 mm, następnie umieszczono po 100 g w szklanych zlewkach o pojemności 150 cm^3 i zanieczyszczono następującymi dawkami Zn^{2+} w przeliczeniu na 1 kg s.m.: 0, 300, 600, 1200 i 2400. Cynk stosowano w postaci wodnego roztworu $ZnCl_2$. Do tak przygotowanego materiału glebowego wprowadzono azot amonowy w postaci $(NH_4)_2SO_4$ w ilości 0 i 240 mg N kg^{-1} s.m. Po dokładnym wymieszaniu chlorku cynku oraz siarczanu amonu z glebą, glebę uwilgotniono do poziomu 50% kapilarnej pojemności wodnej, a następnie zlewki wstawiono do cieplarki o temp. 25°C. Po upływie 10, 20, 30 i 40 dni, w inkubowanej glebie oznaczono zawartość N-NH_4 i N-NO_3, i dodatkowo po 10 i 40 dniach najbardziej prawdopodobną liczbę bakterii nitryfikacyjnych (NPL) I i II fazy nitryfikacji. Doświadczenie wykonano w 3 powtórzeniach dla każdego terminu badań. W próbkach umieszczonych w zlewkach wykonano po 2 oznaczenia każdego parametru. Łącznie dla każdego obiektu uzyskano po 6 wyników. Na podstawie oznaczeń wyliczono ilość azotu znitryfikowanego oraz zimmobilizowanego, a także oporność (RS) procesu nitryfikacji i bakterii nitryfikacyjnych na zanieczyszczenie gleby cynkiem oraz zdolność powrotu tych cech do stanu równowagi (RL).

Stwierdzono, że nadmiar cynku w glebie powoduje istotne zaburzenia procesu nitryfikacji. Nawet 300 mg Zn^{2+} kg^{-1} s.m. gleby istotnie hamuje ten proces. Zanieczyszczenie cynkiem zakłóca nie tylko proces nitryfikacji, ale także inne procesy metabolizmu azotu glebowego, o czym świadczy zmniejszająca się immobilizacja azotu wraz ze zwiększeniem stopnia zanieczyszczenia gleby tym pierwiastkiem.

Niekorzystne oddziaływanie cynku na proces nitryfikacji wynika głównie z negatywnego oddziaływania nadmiaru tego pierwiastka w środowisku glebowym na bakterie nitryfikacyjne. Cynk w większym stopniu hamuje rozwój bakterii I fazy nitryfikacji niż II fazy, ale bakterie I fazy nitryfikacji szybciej powracają do stanu równowagi niż II fazy. Współczynniki oporności (RS) procesu nitryfikacji na zanieczyszczenie cynkiem kształtowały się na niskim poziomie i malały wraz z pogłębiającym się stopniem zanieczyszczenia. Oporność bakterii nitryfikacyjnych na działanie cynku była tym mniejsza, im większa jego ilość znajdowała się w glebie.

Słowa kluczowe: nitryfikacja, zanieczyszczenie cynkiem, oporność nitryfikacji na zanieczyszczenie, bakterie nitryfikacyjne.

INTRODUCTION

Nitrification is a key process in nitrogen cycling in the environment (Ros et al. 2011, Gómez-Rey et al. 2012, He et al. 2012). Without nitrification it would be impossible to remove excess amounts of nitrogen compounds from water and wastewater (Munz et al. 2012, Zeng et al. 2012) as it precedes the process of denitrification, which leads to reduction of nitrates to volatile nitrogen oxides and molecular nitrogen. On the other hand, nitrification in the soil environment creates a risk of nitrogen loss due to the leaching of NO_3^- (Abaas et al. 2012) outside the rhizosphere of plants and *via* nitrogen escape because nitrates are acceptors of electrons from the organic matter undergoing oxidation by denitrifying bacteria.

Both ammonium and nitrate nitrogen are easily absorbed by plants (Dzida et al. 2012) , although the ammonium cation is more stable in soil than the nitrate anion (Abaas et al. 2012). Beside, whenever nitrification becomes too intensive, for example due to over-fertilization, plants and especially nitrophilous species tend to accumulate excessive amounts of nitrates (Kucharski 1985, Smoleń et al. 2012). Nonetheless, the factors which favour an intensive course of nitrification in soil are also the ones which create optimal conditions for the growth and development of most crops. Soils in which nitrification proceeds undisturbed are well tilled for plant cultivation, their C:N ratio is close to 12-16, the soil reaction is neutral and the physicochemical properties are suitable for growing crops (Szukics et al. 2012). For this reason, the nitrification process in soil is often erroneously claimed to play a positive part in agricultural practice (Kucharski 1985). But the same characteristics make nitrification a good indicator of soil contamination, for example with herbicides (Kucharski, Wyszkowska 2008, Baćmaga et al. 2012), aromatic hydrocarbons (Kucharski et al. 2009, 2010, Wyszkowski, Sivitskaya 2012) and heavy metals (Donner et al. 2010, Ruyters et al. 2010, Kucharski et al. 2011, Trevisan et al. 2012). All the factors which retard the activity of soil enzymes also modify the enzymes responsible for oxidation of ammonia nitrogen (Trevisan et al. 2012). The same effect is produced by heavy metals (Mertens et al. 2010), although in this case their toxic effect on nitrifying bacteria plays a role as well (He et al. 2012).

Zinc is one of the heavy metals which can interfere with the metabolism of soil (Donner et al. 2010, Ryuters et al. 2010, Kucharski et al. 2011, Trevisan et al. 2012). Excessive amounts of zinc in soils are possible in industrial regions. Zinc may also occur as a point pollutant in soils lying in agricultural regions. The above considerations have encouraged us to undertake the present study with an aim of analyzing the course of nitrification in soil polluted with zinc.

MATERIAL AND METHODS

The study was conducted in laboratory conditions. Sandy loam, whose characteristics are specified in Table 1, was used as the test soil. The protocol of the experiment was as follows: soil sampled from the 0-20 cm layer of a cropped field was passed through a 2 mm mesh sieve and then placed in 150 cm^3 glass beakers, 100 g of soil in each, where it was polluted with the following doses of zinc per 1 kg d.m. of soil: 0, 300, 600, 1 200 and 2 400. Zinc was added in the form of $ZnCl_2$ aqueous solution. Afterwards, ammonia nitrogen as $(NH_4)_2SO_4$ was added to the soil material in the amounts of 0 and 240 mg N kg^{-1} d.m. Once zinc chloride and ammonia sulphide had been carefully mixed with the soil, the soil moisture content was raised to 50% of capillary water holding capacity and then the beakers were transfer-

Table 1

Some physicochemical properties of the soil

Soil texture (grain diameter in mm)			C_{org} (g kg^{-1})	Zn^{2+} (mg kg^{-1})	pH_{KCl}	Hh	S	T	V (%)
2 – 0.05	0.05–0.002	< 0.002							
Content (%)						(mmol$^{(+)}$ kg^{-1} d.m. soil)			
72	21	7	7.05	16.60	7.0	8.00	111.00	119.00	93.28

C_{org} – organic carbon content, Zn^{2+} – total zinc content, pH_{KCl} – pH in 1 M KCl, Hh – hydrolytic acidity, S – sum of exchangeable cations, T – total soil adsorption capacity, V – base saturation

red to a laboratory incubator set at a temperature of 25°C. After 10, 20, 30 and 40 days, the incubated soil was analyzed to determine the concentrations of N-NH_4 and N-NO_3. Additionally, after 10 and 40 days of incubation, the most probable number (MPN) of bacteria of the first and second step of nitrification were determined. The experiment was run with three replications for each of the four determination days. The samples kept in the beakers were submitted to determinations in two replicates. In total, 6 results were obtained for each experimental variant. A separate experimental series had been prepared for each determination day and – once the content of the nitrogen forms and counts of the bacteria were determined – it was disposed of.

Mineral nitrogen was extracted from soil with 1% aqueous solution of K_2SO_4. The soil to potassium sulphate ratio was 1 : 5. The extracts underwent determinations of the content of N-NH_4 with Nessler's reagent and N-NO_3 with phenoldisulphic acid (Kucharski et al. 2009). The most probable number of the first and second stage nitrification bacteria was determined according to Kucharski et al (2009).

The results of mineral nitrogen determinations were used to calculate amounts of nitrified nitrogen (N_{nit}) and immobilized nitrogen (N_{im}) from the following formulas:

$$N_{nit} = \frac{N_0}{N_d} \cdot 100,$$

where:

N_{nit} – nitrified nitrogen in %,

N_0 – content of N-NO_3 in the fertilized treatment minus the content N-NO_3 in the unfertilized treatment,

N_d – content of N-NH_4 and N-NO_3 in the fertilized treatment minus the content of N-NH_4 and N-NO_3 in the unfertilized treatment,

100 – conversion factor to %,

$$N_{im} = \frac{D - N_d}{D} \cdot 100,$$

N_{im} – immobilized nitrogen in %,
D – dose of N-NH_4,
N_d – content of N-NH_4 and N-NO_3 in the fertilized treatment minus the content of N-NO_3 in the unfertilized treatment,
100 – conversion factor to %.

Additionally, the resistance (RS) of the nitrification process and nitrifying bacteria to soil contamination with zinc and the resilience of nitrification and nitrifying bacteria in soils contaminated with zinc were calculated from the formulas proposed by Orwin and Wardle (2004):

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

$$RL = \frac{2|D_0|}{(|D_0| + |D_x|)} - 1,$$

D_0 – difference between the control (C_0) and contaminated soil (P_0) in time t_0,
D_x – difference between the control (C_x) and contaminated soil (P_x) in time t_x.

The results were submitted to three-factor analysis of variance (Anova), using the Duncan's test. All the calculations were aided with the Statistica version 10 software (StatSoft, Inc. 2011).

RESULTS AND DISCUSSION

The content of ammonia nitrogen (Table 2) and nitrate nitrogen (Table 3) in soil was a function of the soil contamination with zinc, ammonia sulphate fertilization and incubation time. In the unpolluted treatments, the amount of nitrogen increased significantly until day 20 of incubation, but began to decline on later days. In the nitrogen fertilized treatments, it decreased steadily as the incubation continued, so that on day 40 it was nearly 11-fold lower than on day 10. Zinc contamination of soil resulted in maintaining a high pool of ammonia nitrogen (Table 1) and a low amount of nitrate

Table 2

Content N-NH_4 in 1 kg d.m. soil (mg N)

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Dose N (mg kg^{-1})							
	0				240			
	days of soil incubation							
	10	20	30	40	10	20	30	40
0	19.094	58.862	40.618	4.950	142.062	66.199	48.950	13.341
300	16.871	57.857	39.504	2.552	164.528	165.849	154.744	119.540
600	23.997	56.789	41.039	25.453	171.899	216.431	250.632	203.198
1200	13.362	55.443	58.406	21.891	171.305	237.602	272.062	207.142
2400	9.503	46.032	74.558	18.903	184.466	241.782	284.254	208.343
r	-0.776	-0.971	0.972	0.543	0.843	0.755	0.783	0.710
LSD	*a* – 1.925; *b* – 1.722; *c* – 1.218; *ab* – 3.851; *ac* – 2.723; *bc* – 2.436. *abc* – 5.446							

LSD for *a* – zinc dose, *b* – incubation time, *c* – N dose, *r* – coefficient correlation

Table 3

Content of N-NO_3 in 1 kg d.m. soil (mg N)

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Dose N (mg kg^{-1})							
	0				240			
	days of soil incubation							
	10	20	30	40	10	20	30	40
0	27.281	49.549	40.443	40.482	60.674	208.722	229.711	221.440
300	7.948	27.095	32.999	27.065	21.983	85.624	109.346	104.225
600	6.612	8.893	10.162	10.812	18.123	27.566	25.795	29.384
1200	3.151	7.314	7.895	7.858	14.344	20.348	19.260	24.403
2400	1.228	6.116	7.722	7.723	11.364	18.312	18.612	19.259
r	-0.720	-0.735	-0.762	-0.761	-0.678	-0.694	-0.708	-0.714
LSD	*a* – 0.725; *b* – 0.648; *c* – 0.458; *ab* – 1.449; *ac* – 1.025; *bc* – 0.917. *abc* – 2.050							

Explanations see Table 1

nitrogen (Table 3) in soil. The content of nitrate nitrogen in both fertilized and unfertilized soil decreased as the degree of zinc pollution increased. The coefficients of the correlation between the content of N-NO_3 and dose of zinc were significantly negative and ranged from -0.720 to -0.762 in soil not fertilized with nitrogen and from -0.678 to -0.714 in nitrogen amended soil.

The intensity of nitrification in the analyzed soil was relatively high, as nearly 96% of ammonia nitrogen in the control treatment, with no zinc contamination, had been oxidized to nitrate nitrogen before day 20 of incubation (Table 4). Zinc, however, significantly inhibited the process. On day 40 of incubation, the dose of 300 mg Zn^{2+} kg^{-1} depressed the amount of nitrified nitrogen by 2.4-fold, whereas the highest dose, i.e. 2 400 mg Zn^{2+} kg^{-1}, reduced it by almost 17-fold. The coefficients of the correlation between the dose of zinc and the amount of nitrified nitrogen were significantly negative on each day of the determinations.

The values of the resistance index (RS) of the nitrification process to zinc pollution fluctuated on a low level (Table 5) and tended to decline under higher degrees of pollution. In the treatments polluted with 300 mg Zn^{2+} kg^{-1}, they varied from 0.171 to 0.689, and in the ones contaminated with 2 400 mg Zn^{2+} kg^{-1} they ranged from 0.023 and 0.106. The adverse effect of excess zinc in soil was persistent, which is confirmed by the negative values of the soil resilience index (Table 6). They varied from -0.181 to -0.179 in unfertilized soil and from -0.504 to -0.637 in soil fertilized with nitrogen.

The surplus zinc in soil also contributed to lower counts of nitrifying bacteria in soil (Tables 7, 8). The most probable count of the first step nitrification bacteria under the effect of 300 mg Zn^{2+} kg^{-1} declined by 3.5-fold, under 600 mg Zn^{2+} kg^{-1} it was 8.8-fold lower, the dose of 1 200 mg Zn^{2+} kg^{-1}

Table 4

Amounts of nitrified nitrogen (%)

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Days of soil incubation				Average
	10	20	30	40	
0	21.356	95.594	95.784	95.568	77.075
300	8.680	35.148	39.850	39.743	30.855
600	7.221	10.472	6.941	9.460	8.524
1200	6.618	6.677	5.050	8.199	6.636
2400	5.476	5.865	4.937	5.740	5.504
r	-0.662	-0.686	-0.691	-0.699	-0.692

Table 5

Resistance index (RS) values of nitrification to zinc contamination of soil

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Dose N (mg kg^{-1})							
	0				240			
	days of soil incubation							
	10	20	30	40	10	20	30	40
300	0.171	0.376	0.689	0.502	0.221	0.258	0.312	0.308
600	0.138	0.099	0.144	0.154	0.176	0.071	0.059	0.071
1200	0.061	0.080	0.108	0.107	0.134	0.051	0.044	0.058
2400	0.023	0.066	0.106	0.105	0.103	0.046	0.042	0.045
r	-0.946	-0.661	-0.630	-0.662	-0.933	-0.663	-0.629	-0.657

Table 6

Resilience index (RL) values of nitrification

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Dose N (mg kg^{-1})	
	0	240
300	0.181	-0.504
600	-0.179	-0.637
1200	-0.150	-0.619
2400	-0.114	-0.608
r	-0.457	-0.428

Table 7

First step nitrification bacteria (MPN 10^6 kg^{-1} d.m. soil)

Dose of Zn^{2+} ($mg\ kg^{-1}$ d.m. soil)	Dose N ($mg\ kg^{-1}$)					
	0			240		
	days of soil incubation					
	10	40	average	10	40	average
0	10.933	1.093	6.013	10.933	0.787	5.860
300	3.666	0.787	2.227	2.533	0.787	1.660
600	1.093	0.480	0.787	1.093	0.243	0.668
1200	1.093	0.243	0.668	0.361	0.098	0.230
2400	0.046	0.011	0.029	0.072	0.011	0.041
r	-0.711	-0.927	-0.736	-0.666	-0.846	-0.692
LSD	*a* – 0.184; *b* – 0.116; *c* – 0.116; *ab* – 0.260; *ac* – 0.260; *bc* – 0.164; *abc* – 0.367					

Table 8

Second step nitrification bacteria (MPN 10^6 kg^{-1} d.m. soil)

Dose of Zn^{2+} ($mg\ kg^{-1}$ d.m. soil)	Dose N ($mg\ kg^{-1}$)					
	0			240		
	days of soil incubation					
	10	40	average	10	40	average
0	36.667	25.333	31.000	48.000	22.267	35.133
300	25.333	3.667	14.500	25.333	2.533	13.933
600	25.333	2.533	13.933	25.333	2.147	13.740
1200	18.067	0.787	9.427	2.533	1.093	1.813
2400	14.000	0.243	7.121	1.093	0.787	0.940
r	-0.895	-0.625	-0.768	-0.865	-0.589	-0.805
LSD	*a* – 1.552; *b* – 0.982; *c* – 0.982; *ab* – 2.195; *ac* – 2.195; *bc* – 1.388; *abc* – 3.104					

depressed the count by more than 25-fold and the highest dose, 2 400 mg Zn^{2+} kg^{-1}, resulted in a nearly 143-fold lower count of bacteria. The same doses of zinc caused smaller changes in counts of nitrite-oxidizing bacteria than ammonia-oxidizing ones (Table 8). Thus, the lowest dose of zinc, i.e. 300 mg Zn^{2+} kg^{-1}, decreased the most probable count of the second step nitrification bacteria by 2.5-fold, the second dose – by 2.6-fold, the third dose – by 19.4-fold and the fourth dose – by 37.4-fold.

The resistance of nitrifying bacteria to zinc tended to weaken as the quantity of the pollutant in soil rose (Table 9). However, the resistance of ammonia-oxidizing bacteria was weaker on day 10 of incubation than on day 40, whereas that of nitrite-oxidizing bacteria was stronger on day 10 than on day 40. The first step nitrification bacteria recovered faster than the second step nitrification bacteria from the disorders caused by zinc (Table 10). The mean RL index, irrespective of the fertilization or zinc dose, was 0.888 for the first step nitrification bacteria and 0.004 for the second step nitrification bacteria. Such a low RL index for nitrate forming bacteria was due to the negative values of this parameter in the treatments not fertilized with nitrogen.

The intensity of nitrification is manifested by the correlations between the concentrations of ammonia nitrogen and nitrate nitrogen. In soil with optimal conditions for the development of nitrifying bacteria, N-NH_4 is relatively quickly oxidized to N-NO_3, meaning that as the amount of nitrate nitrogen increases, the content of ammonia nitrogen falls down (SZUKICS et al. 2012). This dependence could also be observed in treatments not polluted with zinc (Tables 2, 3), but excessive amounts of zinc introduced to soil interfered with its homeostasis, which was evidenced by the negative response of the first (Table 7) and second (Table 8) step nitrification bacteria to this contamination, as a result of which the rate of ammonia nitrogen oxidation was reduced (Table 4) or even almost completely halted when higher doses of zinc had been added to soil. The adverse effect of zinc on nitrification is most probably caused by both the direct toxic effect of excess zinc on nitrifying bacteria (RUYTERS et al. 2010) and its influence on the enzymes responsible for this process (TREVISAN et al. 2012). The negative influence of zinc on autochthonous soil microorganisms is well-documented in literature (KUCHARSKI et al. 2000, ZABOROWSKA et al. 2006, WYSZKOWSKA et al. 2007, 2008, RUYTERS et al. 2010) and proven by a rapidly depleting pool of immobilized

Table 9

Resistance index (RS) values of nitrifying bacteria to zinc contamination of soil

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Nitrification bacteria							
	first step				second step			
	dose N (mg kg^{-1})							
	0		240		0		240	
	days of soil incubation							
	10	40	10	40	10	40	10	40
300	0.201	0.563	0.131	1.000	0.528	0.078	0.358	0.060
600	0.053	0.281	0.053	0.182	0.528	0.053	0.358	0.051
1200	0.053	0.125	0.017	0.066	0.327	0.016	0.027	0.025
2400	0.002	0.005	0.003	0.007	0.236	0.005	0.012	0.018
r	-0.782	-0.888	-0.819	-0.702	-0.945	-0.902	-0.855	-0.915

Table 10

Resilience index (RL) values of nitrifying bacteria

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Nitrification bacteria			
	first step		second step	
	dose N (mg kg^{-1})			
	0	240	0	240
300	0.919	1.000	-0.313	0.069
600	0.883	0.895	-0.336	0.060
1200	0.841	0.878	-0.138	0.365
2400	0.819	0.867	-0.051	0.372
r	-0.924	-0.726	0.936	0.845

Table 11

Amounts of immobilized nitrogen (%)

Dose of Zn^{2+} (mg kg^{-1} d.m. soil)	Days of soil incubation				Average
	10	20	30	40	
0	34.850	30.620	17.667	21.105	26.060
300	32.628	30.617	20.172	19.105	25.630
600	33.578	25.702	6.156	18.201	20.909
1200	29.527	18.670	6.242	15.918	17.589
2400	22.875	13.356	8.089	16.260	15.145
r	-0.981	-0.969	-0.621	-0.834	-0.935

nitrogen under its effect (Table 11). The present study shows that the average immobilization of nitrogen in the control treatment was 26%, but in the one contaminated with 2 400 mg Zn^{2+} kg^{-1} it fell down to 15%. Irrespective of the dose of zinc, nitrogen immobilization oscillated from 35% to 8% on particular days of determinations.

CONCLUSIONS

1. Excess zinc in soil causes considerable disorders in the process of nitrification. Even the lowest tested dose, 300 mg Zn^{2+} kg^{-1} d.m. of soil, significantly inhibited nitrification.

2. Apart from interfering with nitrification, zinc contamination also disrupts other soil nitrogen metabolic processes, which is demonstrated by the decreased nitrogen immobilization under the effect of a higher degree of soil pollution with zinc.

3. The adverse influence of zinc on nitrification is mainly due to the negative effect of excess zinc in the soil environment on nitrifying bacteria.

4. Zinc more strongly inhibits the development of the first than the second step nitrification bacteria, but the former bacteria recover faster.

REFERENCES

Abaas E., Hill P.W., Roberts P., Murphy D.V., Jones D.L. 2012. *Microbial activity differentially regulates the vertical mobility on nitrogen compounds in soil*. Soil Biol. Biochem., 53: 120-123.

Baćmaga M., Boros E., Kucharski J., Wyszkowska J. 2012. *Enzymatic activity in soil contaminated with the Aurora 40 WG herbicyde*. Environ. Protec. Eng., 38(1): 91-102.

Donner E., Broos K., Heemsbergen D., Warne M.St.J., McLaughlin M.J., Hodson M.E., Nortcliff St. 2010. *Biological and chemical assessments of zinc ageing in field soils*. Environ. Pollut., 158: 339-345.

Dzida K., Jarosz Z., Michałojć Z. 2012. *Effect of nitrogen fertilization on the yield and nutritive value of Beta vulgaris L.* J. Elem., 17(1): 19-29. DOI:10.5601/jelem.2012.17.1.02.

Gómez-Rey M.X., Cauto-Vázquez A., González-Prieto S.J. 2012. *Nitrogen transformation rates* *and nutrient availability under conventional plough an conversation tillage*. Soil Till. Res., 124: 144-152.

HE JI-Z., HU H.W., ZHANG LI-M. 2012. *Current insights into the autotrophic thaumarchael ammonia oxidation in acids soils*. Soil Biol. Biochem., 55: 146-154.

KUCHARSKI J. WIECZOREK K., WYSZKOWSKA J. 2011. *Changes in the enzymatic activity in sandy loam soil exposed to zinc pressure*. J. Elem., 16(4): 577-589.

KUCHARSKI J., TOMKIEL M., BOROS E., WYSZKOWSKA J. 2010. *The effect of soil contamination with diesel oil and petrol on the nitrification process*. J. Elem., 15(1):111-118.

KUCHARSKI J., WYSZKOWSKA J. BOROWIK A. 2009. *Nitrification process in soil contaminated with benzene*. Ecol. Chem. Eng. A, 16 (8): 963-970.

KUCHARSKI J., WYSZKOWSKA J. 2008. *Biological properties of soil contaminated with the herbicyde Apyros 75 WG*. J. Elem., 13(3): 357-371. (in Polish)

KUCHARSKI J., WYSZKOWSKA J., NOWAK G., HARMS H. 2000. *Activity of enzymes in soils treated with sewage sludge*. Pol. J. Soil Sci., 32(2): 29-30.

KUCHARSKI J. 1985. *Studies on the usefulness of nitrification inhibitors in plant fertilization with nitrogen*. Acta Acad. Agricult. Tech. Olst., Agricult., Suppl. A, 41: 1-59. (in Polish)

MERTENS J., WAKELIN S.A., BROOS K., MCLAUGHLIN M.J., SMOLDERS E. 2010. *Extent of copper tolerance and consequences for functional stability of the ammonia-oxidizing community in long-term copper contaminated soils*. Environ. Toxic. Chem., 29: 27-37.

MUNZ G., SZOKE N., OLESZKIEWICZ J. A. 2012. *Effect of ammonia oxidizing bacteria (AOB) kinetics on bioaugmentation*. Biores. Technol., 125: 88-95.

ORWIN K.H., WARDLE D.A. 2004. *New indices for quantifying the resistance of soil biota to exogenous disturbances*. Soil Biol. Biochem., 36: 1907-1912.

ROS G.H., HANEGRAAF M.C., HOFFLAND E., VAN RIEMSDIJK W.H. 2011. *Predicting soil N mineralization: Relevance of organic matter fractions and soil properties*. Soil Biol. Biochem., 43: 1714-1722.

RUYTERS S., MERTENS J., SPRINGAEL D., SMOLDERS E. 2010. *Stimulated activity of the soil nitrifying community accelerates community adaptation to Zn stress*. Soil Biol. Biochem., 42: 766-772.

SMOLEŃ S., SADY W., WIERZBIŃSKA J. 2012. *The influence of nitrogen fertilization with ENTEC-26 and ammonium nitrate on the concentration of thirty-one elements in carrot (Daucus carota* L.*) storage roots.* J. Elem., 17(1): 115-137. DOI:10.5601/jelem.2012.17.1.11.

StatSoft, INC. 2011. Statistica (data analysis software system), version 10. www. statsoft.com.

SZUKICS U., HACKL E., ZECHMEISTER-BOLTENSTERN S., SESSITCH A. 2012. *Rapid and dissimilar response of ammonia oxidizing archea and bacteria to nitrogen and water amendment in two temperatre forest soils*. Micobiol. Res., 167: 103-109.

TREVISAN M., COPPOLECCHIA D., HAMON R., PUGLISI E. 2012. *Potential nitrification, nitrate reductase, and β-galactosidase activities as indicators of restoration of ecological functions in a Zn-contaminated soil*. Biol. Fertil. Soils., 48: 923-931.

WYSZKOWSKA J., KUCHARSKI J., BOROWIK A. BOROS E. 2008. *Response of bacteria to soil contamination with heavy metals*. J. Elem., 13(3): 443-453.

WYSZKOWSKA J., BOROS E., KUCHARSKI J. 2007. *Effect of interactions between nickel and other heavy metals on the soil microbiological properties*. Plant Soil Environ., 53(12): 544-552.

WYSZKOWSKI M., SIVITSKAYA V. 2012. *Changes in the content of organic carbon and available forms of macronutrients in soil under the influence of soil contamination with fuel oil and application of different substances.* J. Elem., 17(1): 139-148.

ZABOROWSKA M., WYSZKOWSKA J., KUCHARSKI J. 2006. *Microbial activity in zinc contaminated soil of different pH*. Pol. J. Environ. Stud., 15(2a): 569-574.

ZENG Y., GUARDIA A., DAUMOIN M., BENOIST J-C. 2012. *Characterizing the transformation and transfer of nitrogen during the aerobic treatment of organic wastes and digestates*. Waste Manage., 32: 2239-2247.

J. Elem. s. 339–350 DOI: 10.5601/jelem.2014.19.2.663

FIELD-SCALE SPATIAL AUTOCORRELATION OF SOME SODIUM AND POTASSIUM FORMS IN A LUVISOL HUMIC HORIZON

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Abstract

Knowledge of the spatial variability of the content and transformations of soil nutrients is important for precision agriculture and environmental protection. Spatial patterns of exchangeable (Na-Exch, K-Exch) and water soluble (Na-WS, K-WS) forms of sodium and potassium were examined in Luvisol soil lying in the region of Cuiavia and Pomerania, northwest Poland, so as to identify their spatial distribution for the implementation of site-specific management. In April 2007, soil samples were collected in a system of 10 x 10 m grids (n = 50) from an area of 0.5 ha located in an intensively used arable field. Water soluble forms of Na and K were determined after extraction with distilled water in a 1:5 soil to water ratio, while the exchangeable forms of these elements were assayed in 0.1 M $BaCl_2$. The data were analyzed both statistically and geostatistically from semivariograms and their modelling. The spatial autocorrelation of a data set is described with the Moran's I correlograms, hence adequate correlograms were drawn. Among all the properties determined, it was only water soluble K that showed significant spatial autocorrelation. Other soil properties (Na-WS, Na-Exch, K-Exch) did not demonstrate any spatial autocorrelation (the Moran's I values were close to zero at $p < 0.05$), which indicated their random spatial variability. In order to assess the spatial variability of K-WS, a spherical model with the nugget effect was fitted into the calculated semivariogram. The results were assigned to the moderate variability class (the nugget effect 52%), and the range of the spatial impact stretched for 38 m.

Keywords: Luvisol, exchangeable and water soluble forms of K and Na, Moran's I, spatial variability, geostatistics.

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KORELACJA PRZESTRZENNA WYBRANYCH FORM SODU I POTASU W POZIOMIE ORNO-PRÓCHNICZNYM GLEBY PŁOWEJ W SKALI POLA UPRAWNEGO

Abstrakt

Znajomość zmienności przestrzennej zawartości i przemian składników odżywczych w glebie ważna jest zarówno z punktu widzenia rolnictwa precyzyjnego, jak i ochrony środowiska. Zmienność przestrzenną wymiennych (Na-Exch, K-Exch) oraz wodnorozpuszczalnych (Na-WS, K-WS) form sodu i potasu badano w glebie płowej regionu Pomorza i Kujaw (północno-wschodnia Polska). Próbki glebowe do badań pobierano w siatce kwadratów o boku 10 m (n = 50) w kwietniu 2007 r. z obszaru o powierzchni 0,5 ha znajdującego się w obrębie pola uprawnego. Formy wodno-rozpuszczalne Na i K oznaczono po ekstrakcji z wodą destylowaną w stosunku 1 : 5, natomiast formy wymienne analizowano wg metody z 0,1 M $BaCl_2$. Wyniki poddano analizie statystycznej i geostatystycznej opartej na wykreśleniu semiwariogramów i ich modelowaniu. W celu określenia korelacji przestrzennej wyliczono indeks Morana oraz wykreślono odpowiednie korelogramy. Spośród badanych zmiennych tylko zawartość K-WS wykazywała istotną autokorelację przestrzenną. Pozostałe badane zmienne (Na-WS, Na-Exch, K-Exch) nie wykazywały podobnej zmienności (wartości indeksu Morana I zbliżone były do zera i wartości p były większe od 0.05), co wskazuje na losowe rozmieszczenie ich wartości w przestrzeni. W celu scharakteryzowania zmienności przestrzennej wartości K-WS, do teoretycznego semiwariogramu dopasowano model sferyczny z efektem samorodka. Wartości badanej zmiennej mieściły się w umiarkowanej klasie zmienności, z efektem samorodka wynoszącym 52,0%, a zakres oddziaływania przestrzennego wynosił 38 m.

Słowa kluczowe: Luvisol, wymienne i wodnoropuszczalne formy K i Na, indeks Morana, zmienność przestrzenna, geostatystyka.

INTRODUCTION

Spatial heterogeneity is one of the most serious obstacles to successful monitoring and modeling of nutrient transformations in soil, which is extremely important for good plant nutrition as well as environmental control and protection. Soil fertility management and adequate application of fertilizers and other chemicals must be based on our understanding of the distribution of exchangeable and water soluble forms of nutrients. Traditionally, soil management used to rely on the concept that fields were homogenous areas and all field operations should therefore be planned for a whole field (Patil et al. 2011). However, at least 70 years ago it was concluded that fields were not homogenous and specific sampling techniques were recommended to describe spatial variability (Flower et al. 2005, Santra et al. 2008). Recently, geostatical methods have been employed to testing the spatial variability of soil properties. Geostatistics, an increasingly popular solution in soil science, helps to predict the spatial distribution of spatially dependent soil properties in a field, according to several soil samples (McBratney, Webster 1983, Kerry, Oliver 2004, Aşkin, Kizilkaya 2006, Aşkin et al. 2012). Semivariograms and autocorrelograms are typically used to study the spatial structure of soil properties.

Like nitrogen and phosphorus, potassium is a basic element that determines the soil's production potential. In plants, potassium affects the water management and enzymatic activity (Randame-Malvi 2011). Potassium is considered to be a highly mobile element in soil, although it is easily absorbed as a cation (Burzyńska, Pietrzak 2010), leading to a potassium deficit and worse crop yields (Ragály, Kádár 2005). Although sodium is an antagonist of potassium, it may alleviate the effects of a minor deficit of potassium. Sodium may stimulate the sugar beet yield and the content of sucrose (Prośba-Białczyk, Mydlarski 2002, Szulc et al. 2008). However, a high sodium content can trigger a series of negative changes in soil properties, such as increased salinity, worse soil structure and impeded uptake of potassium by plants. The colloidal system of soil tends to disperse when the sodium content at exchangeable sites increases. Excessive amounts of salts cause high osmotic pressure, which adversely affects the water uptake by plants. In general, high soil pH values are associated with high sodium concentrations (Ardahanlioglu et al. 2003). Therefore, it is very important, especially in precision agriculture, to monitor the content of available forms of Na and K (both water soluble and exchangeable forms) and to assess their spatial variability in cultivated soils (Franzen 2011).

The objective of the present study has been to assess the spatial autocorrelation and variability of soil water soluble and exchangeable forms of Na and K in a field-scale study using geostatistical techniques.

MATERIAL AND METHODS

Study site and soil sampling

In the present research, the spatial variability of soil exchangeable and water soluble forms of K and Na have been determined on an area of 0.5 ha of Luviosol soil, in a field located at the village of Orlinek near Mrocza, in the region of Cuiavia and Pomerania (53°15′31″N, 17°32′43″E), in northwest Poland. Soil samples were taken in April 2007, in a field cropped with winter wheat which followed winter oilseed rape. The apparently homogenous area of 0.5 ha was divided into 10 x 10 m grids for sampling. Samples were collected from the 0-20 cm top layer across the field. There were 50 sampling points. Each soil sample represented a mean value of 10 individual samples. The samples were air dried and ground to pass a 2 mm sieve for chemical analyses.

Soil analyses

Exchangeable K and Na (K-Exch, Na-Exch) were determined in 0.1 M $BaCl_2$ (according to ISO 11260), while the water-soluble forms of both elements (K-WS, Na-WS) were assayed after extraction in distilled water (1 : 5

soil to water ratio). The content of K^+ and Na^+ in the extracts was determined by atomic absorption spectroscopy (AAS) on a Philips PU 9100X spectrometer. Basic chemical parameters were determined as follows: pH in 1 M KCl by the potentiometric method (PN-ISO 1039) and organic carbon and total nitrogen content in a dry combustion CN analyzer (Vario Max CN). The verification of the results was done with the TILL 3 certificate.

Statistical analysis

The intra-population variability was analyzed by classical statistics (mean, maximum, minimum, standard deviation, skewness and coefficient of variation). Normality of all the properties analyzed was tested by the Shapiro-Wilk test (*p*-value). The spatial autocorrelation of the sampling variables was measured with the Moran's *I* autocorrelation coefficient (Moran 1948). The Moran's *I* was determined using a 50-m active lag distance and a 10-m lag interval (ArcGIS 9.3). Properties with the highest Moran's *I* index were chosen to be modelled. Autocorrelograms and semivariograms were drawn with the Isatis software (Geovariance Co.) and the models were verified with the cross-validation method. Empirical correlograms and semivariograms were drawn at the difference lag intervals. The geostatistical techniques including the semivariogram analysis and kriging were used to model the spatial variability and interpolation of data values at unsampled locations, and for mapping in the district. Semivariance γ(h) is defined by the following equation:

$$\gamma(h) = \frac{1}{2N(h)} \Sigma \left[Z(x_i) - Z(x_i + h) \right]^2 ,$$

where, N(h) is the number of sample pairs at each distance interval h, and Z (Xi,) and Z (Xi + h) are the values of a variable at any two places separated by the distance h. A semivariogram is drawn by plotting semivariance against the distance. Its shape indicates whether the variable is spatially dependent. The experimental semivariograms were fitted into theoretical models that had well-known parameters, such as the nugget (CO), sill (CO + C) and range (AO) of spatial dependence (Cambardella et al. 1994). A suitable model of variogram was based on the minimum residual sum of a square. The nugget semivariance is the variance at zero distance; the sill is the lag distance between measurements at which one value for a variable does not influence neighboring values; and the range is the distance at which values of one variable become spatially independent from values of other variables. In our analysis, two indices of spatial dependence were determined. One is the nugget variance, which was expressed as the percentage of total semivariance used to define spatial dependence of soil variables. In order to define different classes of spatial dependence for the soil variables, the ratio between the nugget semivariance and the total semivariance or the sill was used (Cambardella et al. 1994). If the ratio was ≤ 25%, the variable was considered to be strongly spatially dependent, or strongly distributed; if the ratio was between 26 and 75%, the soil variable was considered to be moderately spatially dependent; if the ratio was greater than 75%, the soil variable was considered to be weakly spatially dependent. The other index is the range, which indicates the limit of spatial dependence.

RESULTS AND DISCUSSION

The reaction of the analyzed soil samples ranged from acidic to neutral (pH from 4.8 to 6.8). The organic carbon content was 5.5 - 9.0 g kg^{-1} (on average 7.3 g kg^{-1}) and the total nitrogen content varied from 0.68 to 0.98 g kg^{-1} (on average 0.80 g kg^{-1}) More detailed data describing the basic physicochemical properties of the soil were presented earlier (Piotrowska, Długosz, 2012).

The content of K-Exch in the studied area was 3.2-6.2 mmol kg^{-1} (on average 4.3 mmol kg^{-1}) – Table 1. Its contribution to the CEC equalled 3.6-8.1% (on average 5.7%), which was slightly above the acceptable share of this ion in the CEC, such as 5%. The limit was exceeded in 76% of the soil samples. The content of Na-Exch ranged from 0.4 to 2.8 mmol kg^{-1} (on average 2 mmol kg^{-1}) – Table 1, and its contribution to the CEC was between 0.5 and 3.8% (on average 1.6%), i.e. below the limit value, which is set at 5%. The content of water soluble forms of the two nutrients was relatively low, especially that of Na-WS (Table 1). Less K-WS was determined in surface horizons of Luvisols in the Czech Republic (Škarpa, Hlušek 2012).

Mean and median values served as primary estimates of the central tendency, while standard deviation (SD), coefficient of variation (CV), skewness and kurtosis were used as estimates of variability (Table 1). The mean and median values of the soil properties were similar, indicating that the outliers did not dominate the measure of the central tendency and could be used for exploratory data analysis. The coefficients of variation (CV%) of the

Table 1

Statistics of soil properties (n = 50)

Property	Min	Max	Mean	Geometric mean	Median	SD	Skewness	Kurtosis	CV
	(mg kg^{-1})								(%)
K-WS	40.7	118.4	65.3	62.6	59.5	19.9	1.05	0.25	30.6
Na-WS	4.1	11.8	7.4	7.3	7.1	1.4	0.68	1.31	19.2
	(mmol kg^{-1})								(%)
K-Exch	3.2	6.2	4.3	4.3	4.2	0.64	0.87	1.30	14.9
Na-Exch	0.4	2.8	1.2	1.1	1.2	0.6	0.96	0.72	46.0

CV – coefficient of variation, SD – standard deviation soil properties were divisible into three classes: the least (<15%), moderately (15% - 35%), and the most (>35%) variable ones (WILDING 1986). The results shown in Table 1 prove that only one of the four measured properties, namely K-Exch, belonged to the least variable class (coefficient of variation less than 15%), whereas Na-WS and K-WS were moderately variable (CV > 15 ≤ 35%), and Na-Exch was highly variable (CV > 35%).

Skewness and kurtosis coefficients have been used to verify the statistical distribution of parameters (CERRI et al. 2004). It is known that if skewness is close to zero the parameters it describes represent the classical, normal distribution, but positive skewness is the sing of a long tail of high values (to the right) in the data distribution, making the median less than the mean (LI et al., 2012). In this study, all variables presented positive skewness values ranging from 0.68 to 1.05. Kurtosis is a parameter that describes the shape of a random variable probability density function. Kurtosis greater than one shows that a given random variable is leptokurtic but kurtosis less than one indicates a variable that is platykurtic. The kurtosis of the studied variables fluctuated between 0.25 and 1.31.

Spatial autocorrelation can be used to describe and compare the spatial structure of data. Significant spatial autocorrelation (<0.05) was found only for K-WS (Table 2). The autocorrelogram (Figure 1a) for K-WS was characterized by a positive MC (Moran's coefficient) for separation distances generally < 20 m and a negative MC at > 20 m. The highest K-WS spatial autocorrelation was at 10 m, decreasing gradually at further distances and approaching zero at a 23 m separation distance. Over that distance, the spatial autocorrelation of this property continued to decrease, falling down to negative values and finally approaching zero again (at a distance of 57 m).

Table 2

Moran's *I* for soil properties

Parameter	K		Na	
	Exch	WS	Exch	WS
Moran's index	-0.0322	0.1100	0.0250	0.0010
Z Score	-0.2470	2.7157	0.9499	0.4503
p-value	0.8049	0.0066	0.3421	0.6525

Other soil properties (Na-WS, Na-Exch, K-Exch) did not show spatial autocorrelation, which was confirmed by the Moran's *I* values close to zero and the *p* values higher than 0.05 (Table 1). This indicated the random spatial variability of these properties, which was confirmed by the impossibility of drawing up empirical semivariograms and adjusting relevant models, despite using different lag intervals. The models achieved did not satisfy the requirements of well-fitted models (high values of thee standard error variance). Additionally, the verification of a pure nugget model in the semivariogram of K-Exch did not confirm its presence (Figure 2a).

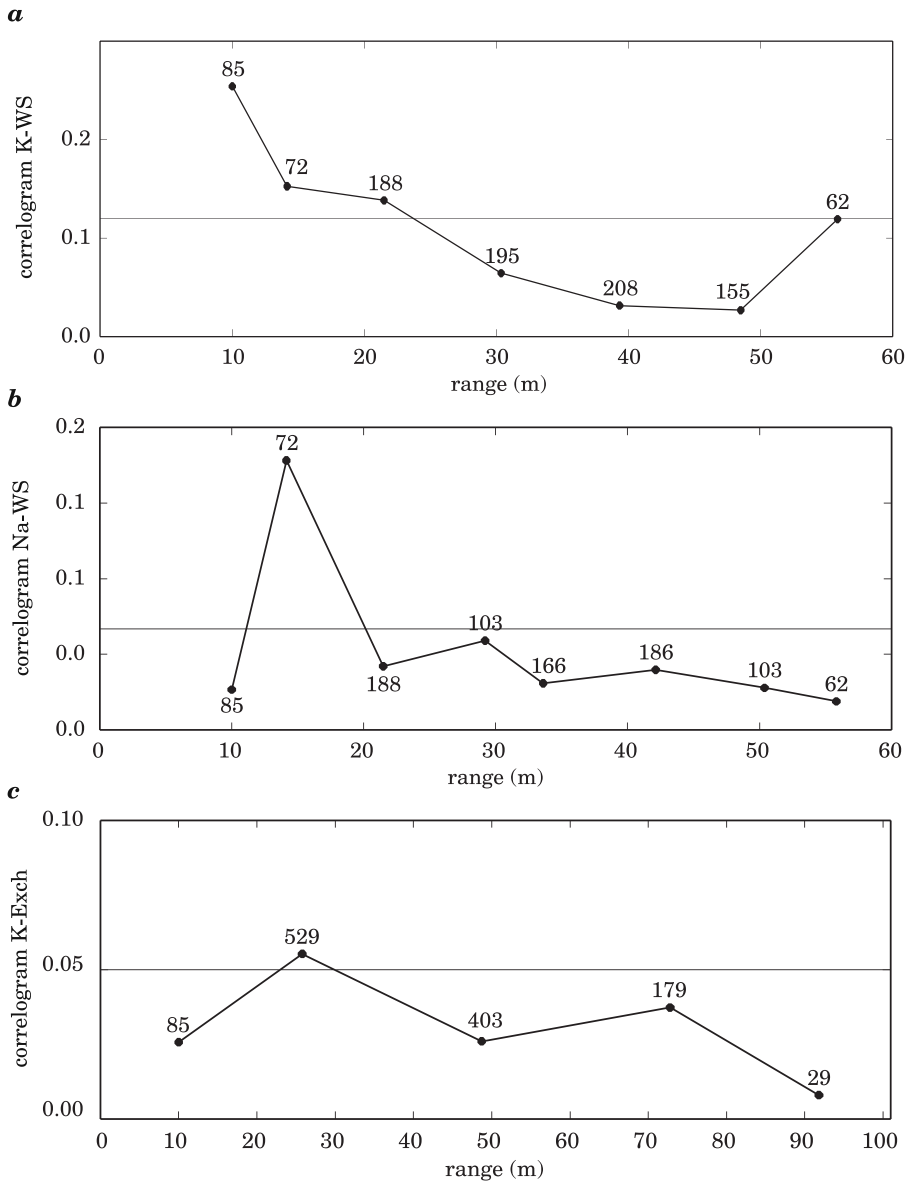

Fig. 1. Correlograms of (*a*) K-WS, (*b*) Na-WS, (*c*) K-Exch

In order to characterize the spatial variability of the K-WS spherical model with the nugget effect were fitted to calculated semivariogram (Figure 2b). The same model was adjusted to describe the spatial variability of the water soluble Mg form in the same research area (KOBIERSKI et al. 2011). The parameters of this model were as follows: the nugget (Co) 0.143, the sill (Co + C) 0.253, and the nugget effect 52.0%, which indicated that the data

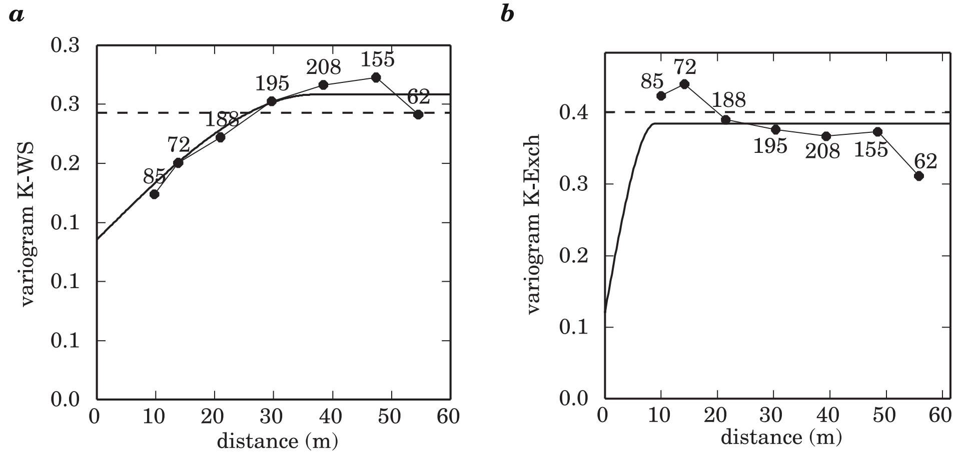

Fig. 2. Experimental semivariograms of (a) K-Exch, and (b) K-WS

were moderately spatially dependent; the range was 38 m and the standard error equaled -0.0127. The adjustment of the model to the empirical variogram was confirmed by the value of the standard error variance: 1.0008. The spatial variability of K-WS based on the percentage of total variance (*sill*) presented as random variance [(Co/Co+C),%] was considered to be moderately spatially dependent (52%). It has been found that the nugget effect reflects the variability unexplained in terms of distance for a sample used, such as local variations, errors in analysis, sampling and others. Since it is impossible to quantify the individual contribution to these errors, the nugget effect is expressed as a percentage of the level, thus facilitating the comparison of the extent to which analyzed variables are spatially dependent (VIEIRA 2000). Moderate spatial dependence has been attributed to both intrinsic (texture and mineralogy) and extrinsic variation (soil management, e.g. fertilizer application, tillage and land use) (CAMBARDELLA et al. 1994). The results indicated that 52% of the total variance of an analyzed property was due to random variability. That observation suggested that extrinsic factors such as fertilization, plowing and other soil management practices weakened their spatial correlation after a long history of cultivation. Other properties did not show a regular spatial structure, which was confirmed by the lack of autocorrelation. Similarly, a weak spatial structure for the K and Na forms was noted by YANAI et al. (2003), who reported a nugget/sill ratio of 95% for exchangeable K and 78% for exchangeable N, while moderate spatial dependence was found for exchangeable K by TOBI and OGUNKUNLE (2007) in the 0-15 cm soil horizon of Alfisols and by AISHAH et al. (2010) for paddy soil, where a nugget/sill ratio equalled 70% for K and 50% for N.

The spatial autocorrelation of the K-WS results is confirmed by the range of influence reaching 38 m. Since the range is the maximum distance over which the results are correlated (BERGSTROM et al. 1998), the sampling scheme for the analyzed properties (10 m ×10 m rgids) was suitable only for water soluble K. Other properties did not show the ranges of autocorrelation, which indicated that a lag distance should be less than 10 m, and that 50 samples were an insufficient number to describe their true characteristics in the analyzed area.

The main application of geostatistics to soil science is to estimate and map soil properties in unsampled areas. Since spatial autocorrelation is a necessary condition for the spatial prediction of soil properties, only one krigged map of spatial variability was presented for K-WS (Figure 3). An area with the highest values of the property occurred in the south-western triangle of the field (at 0-20 m of the length and 0-20 m of the width), running vertically from the centre to the south-eastern part of the area.

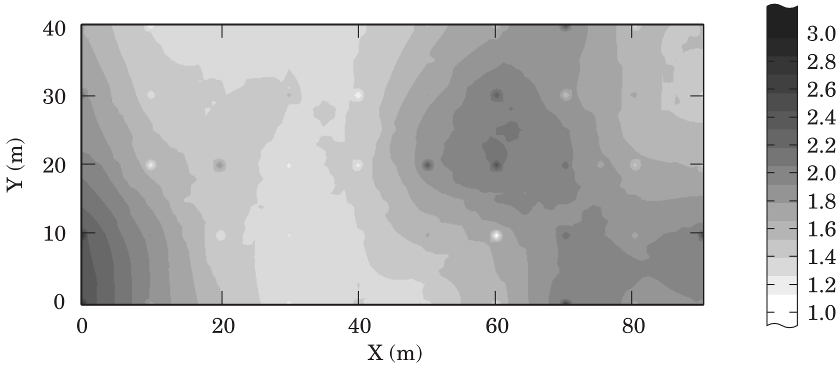

Fig. 3. Spatial distribution of K-WS

CONCLUSIONS

1. Significant positive spatial autocorrelation, indicating similar values of a given property in nearby places, was found only for water soluble K, suggesting that observations made at different locations were dependent on each other across the determined space and that they showed spatial dependence.

2. The spatial range values indicated that the sampling interval established in the study (10 m) was proper only for water soluble K. For the other properties, more samples should be collected over the analyzed area in the future, and the sampling should be done at different lag intervals to obtain the spatial autocorrelation of the data.

3. The nugget-to-sill ratios calculated to determine the level of spatial dependence of all the analyzed properties showed a moderate contribution of random variance to the total variability only in the case of K-WS.

REFERENCES

Aishah A.W., Zauyah S., Anuar A.R., Fauziah C.I. 2010. *Spatial variability of selected chemical characteristics of paddy soils in sawah sempadan, Selangor, Malaysia.* Malaysian J. Soil Sci., 14: 27-39.

Ardahanlioglu O., Oztasw T., Salih E.S., Yilmaz H., Yildirim Z. N. 2003. *Spatial variability of exchangeable sodium, electrical conductivity, soil pH and boron content in salt- and sodium-affected areas of the Igdir Plain (Turkey).* J. Arid Environ., 54: 495-503. DOI: 10.1006/jare.2002.1073

Aşkin T., Kizilkaya R. 2006. *Assessing spatial variability of soil enzyme activities in pasture topsoils using geostatistics.* Eur. J. Soil Biol. 42(1): 230-237.

Aşkin T., Kizilkaya R., Yilmaz R., Olekhov V., Mudrykh N., Samofalova I. 2012. *Soil exchangeable cations: A geostatistical study from Russia.* Eurasian J. Soil Sci., 1: 34-39.

Bergstrom D.W., Monreal C.M., Millette J.A., King D.J. 1998. *Spatial dependence of soil enzyme activities along a slope.* Soil Sci. Soc. Am. J., 6: 1302-1308.

Burzyńska I., Pietrzak S. 2010. *The content of soluble forms of potassium and doc in the soil layer from under long-term manure storage place.* Water-Environment-Rural Areas, 10(4): 23-32. (in Polish)

Cambardella C.A., Moorman T.B., Novak J.M., Parkin T.B., Karlen D.L., Turco R.F., Konopka A.E. 1994. *Field-scale variability of soil properties in Central Iowa soils.* Soil Sci. Soc. Am. J., 58: 1501-1511.

Cerri C.E.P., Bernoux M., Chaplot V., Volkoff B., Victoria R.L., Melillo J.M., Paustian K., Cerri C.C. 2004. *Assessment of soil property spatial variation in an Amazon pasture: basis for selecting an agronomic experimental area.* Geoderma, 123: 51-68. DOI:10.1016/j.geoderma.2004.01.027.

Flower M., Weisz R., White G. 2005. *Yield-based management zones and grid sampling strategies: Describing soil test and nutrient variability.* Agron. J., 97: 968-982.

Franzen D.W. 2011. *Collecting and analyzing soil spatial information using kriging and inverse distance.* In: *GIS applications in agriculture: nutrient management for energy efficiency*. Clay D.E., Shanahan J.F. (Eds.). Taylor and Francis, 61-80.

Kerry R., Oliver M.A. 2004. *Average variograms to guide soil sampling.* Int. J. Appl. Earth Obs. Geoinf., 5: 307-325.

Kobierski M., Długosz J., Piotrowska A. 2011. *Spatial variability of different magnesium forms in luvisols formed from glacial till*. J. Elem., 16(2): 205-214. DOI: 10.5601/jelem.2011.16.2.01.

Li Y., Qin J., Guo Z., Wang Z., Ao Y. 2012. *Spatial variability of soil quality and asparagus spear yield in an area of plastic-greenhouse cultivation on Chongming Island, China.* Afr. J. Agric. Res., 7(15): 2262-2272. DOI: 10.5897/AJAR10.052

McBratney, A.B., Webster R. 1983. *Optimal interpolation and isarithm mapping of soil properties:* V. *Coregionalization and multiple sampling strategy.* J. Soil Sci., 34: 137-162.

Moran P. 1948. *The interpretation of statistical maps.* J. Royal Stat. Soc., Ser. B, 10: 243–251.

Patil S.S., Patil V.C. Al-Gaadi K.A. 2011. *Spatial variability in fertility status of surface soils.* World Appl. Sci. J., 14(7): 1020-1024.

Piotrowska A., Długosz J. 2012. *Spatio–temporal variability of microbial biomass content and activities related to some physicochemical properties of Luvisols.* Geoderma, 173-174: 199-208. DOI: 10.1016/j.geoderma.2011.12.014.

Prośba-Białczyk U., Mydlarski M. 2002. *The effect of sodium chloride sprayed on leaves on productivity and technological value of sugar beet.* Bull. Plant Breed. Acclimat. Inst., 222: 215-222. (in Polish)

Ragály P., Kádár I. 2005. *Long term effects of mineral nutrition on the yield and element content in grass.* Fertilizers Fertilization, 5: 395-400.

RANDAME-MALVI U. 2011. *Interaction of micronutrients with major nutrients with special references to potassium.* Karnataka J. Agric. Sci., 24(1): 106-109.

SANTRA P., CHROPRA V.K., CHAKRABORTY D. 2008. *Spatial variability of soil properties and its application in predicting surface map of hydraulic parameters in an agricultural farm.* Curr. Sci., 95(7): 937-945.2008.

ŠKARPA P., HLUŠEK J. 2012. *Effect of years, fertilization and growing regions on the content and forms of potassium in soil.* J. Elem., 17(2): 305-315. DOI: 10.5601/jelem.2012.17.2.12

SZULC M.P., KOBIERSKI M., NOWAKOWSKI M., KUBICKI K. 2008. *The effect of sodium fertilization on the yield and quality parameters of sugar beet roots.* Bull. Plant Breed. Acclimat. Inst., (Bull. IHAR), 248: 77-85. (in Polish)

TABI F.O., OGUNKUNLE A.O. 2007. *Spatial variation of some soil physico-chemical properties of an Alfisol in Southwestern Nigeria.* Nig. J. Soil Environ. Res., 7: 82-91.

WILDING L.P. 1985. *Spatial variability: its documentation, accommodation, and implication to soil surveys,* In: NIELSEN D.R., BOUMA J. (Eds.). *Soil spatial variability*. Pudoc, Wageningen, pp. 166-194.

VIEIRA, S.R. 2000. *Geostatistical study of spatial variability of soil*, In: Novaris, *Topics in soil science*. R.F. ALVAREZ, V.V.H. SCHAEFER, C.E.G.R. (Eds.). Brazilian Society of Soil Science, Campinas, SP, Brazil (in Portuguese), pp. 1-54.

YANAI J., SAWAMOTO T., OE T., KUSA K., YAMAKAWA K., SAKAMOTO K., NAGANAWA T., INUBUSHI K., HATANO R., KOSAKI T. 2003. *Spatial variability of nitrous oxide emissions and their soil-related determining factors in an agricultural field.* J. Environ. Qual., 32(6): 1965-1977.

J. Elem. s. 351–360

DOI: 10.5601/jelem.2014.19.1.638

EFFECT OF FLAT COVERS ON MACRONUTRIENT CONCENTRATIONS IN ARUGULA LEAVES

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Abstract

Arugula is a common name for several leafy vegetable species of the family Brassicaceae, characterized by a strong aroma and pungent peppery flavor. The perennial wall rocket *Diplotaxis tenuifolia* (L.) D.C. and the annual salad rocket *Eruca sativa* Mill. are grown commercially for human consumption. The objective of this study was to determine the effect of two types of flat covers, perforated PE film and non-woven PP fabric, on macroelement concentrations in arugula leaves. A two-factorial experiment was conducted in 2006 - 2008 in the Experimental Garden of the University of Warmia and Mazury in Olsztyn. The experimental factors were: (1) plant species *Diplotaxis tenuifolia* (L.) D.C. and *Eruca sativa* Mill., (2) the type of plant cover perforated PE film with 100 openings per m^2 and non-woven PP fabric with surface density of 17 g m^{-2}; plants grown without protective covers served as control. Arugula was grown on proper black earth soil of quality class IIIb and cereal-fodder strong complex. Each year, seeds were sown in the middle of April. After planting out in the field, the seedlings were covered with PE film or non-woven PP fabric. The covers were removed after approximately five weeks. Leaves were harvested gradually over the growing season, one to three times from each treatment. The concentrations of mineral compounds were determined in dried plant material from the first harvest, as follows: total nitrogen – by the Kjeldahl method, phosphorus – by the vanadium molybdate method, potassium and calcium – by emission flame photometry (EFP), magnesium – by atomic absorption spectrometry (AAS). The concentrations of all analyzed macroelements in arugula leaves were significantly affected by both plant species and the type of cover. Leaves of *Diplotaxis tenuifolia* (L.) D.C. had a higher content of phosphorus, potassium and calcium, whereas leaves of *Eruca sativa* Mill. accumulated more total nitrogen and magnesium. Leaves of arugula plants covered with non-woven PP fabric contained the highest concentrations of phosphorus, potassium, calcium and magnesium, while plants covered with PE film had the highest total nitrogen content. The Ca:P, Ca:Mg and K:Mg ratios were wide in all treatments. The K:(Ca+Mg) and K:Ca ratios were relatively narrow, but they remained within the normal range.

Keywords: *Diplotaxis tenuifolia* (L.) D.C., *Eruca sativa* Mill., perforated PE film, non-woven PP fabric.

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ZAWARTOŚĆ MAKROELEMENTÓW W LIŚCIACH RUKOLI W ZALEŻNOŚCI OD RODZAJU ZASTOSOWANYCH OSŁON PŁASKICH

Abstrakt

Rukola to wspólna handlowa nazwa kilku gatunków warzyw należących do rodziny Brassicaceae o jadalnych liściach. Charakteryzują się one wyrazistym aromatem i ostrym gorzkawym smakiem. Największe znaczenie gospodarcze mają dwa z nich – bylina *Diplotaxis tenuifolia* (L.) D.C. (dwurząd wąskolistny) oraz gatunek jednoroczny *Eruca sativa* Mill. (rokietta siewna). Celem badań była ocena wpływu płaskiego osłaniania folią perforowaną i włókniną polipropylenową na zawartość makroelementów w liściach rukoli. Dwuczynnikowe doświadczenie przeprowadzono w latach 2006-2008 na polu Ogrodu Doświadczalnego Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. Badanymi czynnikami były: gatunek rośliny – *Diplotaxis tenuifolia* (L.) D.C. i *Eruca sativa* Mill. oraz rodzaj stosowanych osłon – folia perforowana o 100 otworach na 1 m^2, włóknina polipropylenowa o masie 17 g m^{-2}; rośliny uprawiane bez osłon stanowiły obiekt kontrolny. Rukolę uprawiano na glebie typu czarna ziemia właściwa, zaliczonej do klasy bonitacyjnej IIIb, należącej do kompleksu zbożowo-pastewnego mocnego. Nasiona każdego roku wysiewano w połowie kwietnia. Bezpośrednio po siewie poletka przykrywano osłonami, które zdejmowano po ok. 5 tygodniach. Liście zbierano w miarę ich dorastania, od 1 do 3 razy z każdego wariantu doświadczenia. Analizy na zawartość składników mineralnych wykonywano w materiale suchym z pierwszych zbiorów. Oznaczenia zawartości azotu ogółem dokonano metodą Kjeldahla, fosforu – metodą wanadowo-molibdenową, potasu i wapnia – metodą emisyjnej spektrometrii fotometrii płomieniowej (ESA), natomiast magnezu – metodą absorpcyjnej spektrometrii atomowej (ASA). Na zawartość wszystkich makroelementów w liściach rukoli istotny wpływ wywarły zarówno cechy gatunku, jak i rodzaj osłony. Liście *Diplotaxis tenuifolia* (L.) D.C. zawierały więcej fosforu, potasu i wapnia, natomiast liście *Eruca sativa* Mill. więcej azotu ogółem i magnezu. Najwięcej fosforu, potasu, wapnia i magnezu oznaczono w liściach roślin osłanianych włókniną, natomiast azotu ogółem – folią perforowaną. Proporcje Ca:P, Ca:Mg oraz K:Mg we wszystkich wariantach doświadczenia były szerokie, natomiast K:(Ca+Mg) oraz K:Ca były zawężone, niemniej jednak mieściły się w przedziale wartości, które są uznawane za prawidłowe.

Słowa kluczowe: *Diplotaxis tenuifolia* (L.) D.C., *Eruca sativa* Mill., folia perforowana, włóknina polipropylenowa.

INTRODUCTION

The structure of vegetable consumption in Poland shows that only a few vegetable species are consumed in larger amounts. Despite their high nutritional value, leafy vegetables are not fully appreciated in our country. In modern culture, leafy vegetables are a vital component of a healthy and balanced diet. The total area under vegetable cultivation and the consumption levels of vegetable species, including less popular ones, have been steadily increasing (Adamczyk 2002, Kawashima, Valente-Soares 2003, Dyduch, Najda 2005).

Arugula is a common name for several leafy vegetable species of the family *Brassicaceae*, characterized by a peppery taste and pungent flavor. The perennial *Diplotaxis tenuifolia* (L.) D.C., known by the common name of wall rocket, and the annual *Eruca sativa*, commonly known as salad rocket, are grown commercially for human consumption. The above species differ with respect to biological characteristics and structure, yet their properties and uses as well as cultivation methods are identical. Arugula has been grown since the Roman times. Once forgotten, it has only recently been rediscovered. Today, arugula is grown as a leafy vegetable and a spice plant mainly in the Mediterranean region, Central Asia and the USA. Arugula leaves can be eaten raw in a salad, added to sandwiches or mixed with cottage cheese. Because of its exceptionally strong flavor, arugula is often used in mixed salads, including the Mesclun salad mix, pizzas and spaghetti. Arugula can be served stewed, cooked and fried (MORALES, JANICK 2002, MORALES et al. 2006, HALL et al. 2012). Apart from its culinary uses, arugula is highly valued for its medicinal and therapeutic properties (stimulating, anti-scurvy, diuretic, promoting the passage of foods through the digestive tract) (PIGNONE 1997). Arugula leaves contain healthy compounds, which play an important role in the prevention of neoplastic diseases, in particular the colorectal cancer (NITZ, SCHNITZLER 2002, BARILLARI et al. 2005, MELCHINI et al. 2009, D'ANTUONO et al. 2008).

In both animals and plants, macroelements have an important body building function. Compared with other edible plants, vegetables are a rich source of calcium and potassium, but their magnesium content is relatively low. Macronutrient deficiencies can have serious health consequences in humans and animals. Magnesium and calcium shortages are among the most common mineral deficiencies, since their dietary intake levels are often inadequate and insufficient to meet the requirements of healthy individuals (JĘDRZEJCZAK et al. 1999).

According to SIWEK and LIBIK (2005), as well as MAJKOWSKA-GADOMSKA (2010), plastic covers improve thermal conditions in the formative region of a plant. Higher temperatures stimulate the plant growth, development of roots and mineral uptake (SZULC, KRUCZEK 2008).

The aim of this study was to determine the effect of two types of flat covers, perforated PE film and non-woven PP fabric, on macronutrient concentrations in arugula leaves.

MATERIAL AND METHODS

A field experiment in a split-plot design with three replications was conducted in the Garden of the Agricultural Research and Experimental Station of the University of Warmia and Mazury in Olsztyn (20°29´E, 53°45´N; 125 m a.s.l.) in 2006-2008. The experimental factors were as follows:

- plant species: *Diplotaxis tenuifolia* (L.) D.C. (wall rocket) supplied by Enza Zaden, and *Eruca sativa* Mill. (salad rocket) from Hortag Seed;
- type of plant cover: perforated PE film with 100 openings per m^2 and

non-woven PP fabric with the surface density of 17 g m^{-2}; plants grown without protective covers served as the control.

All plants were grown on typical black earth soil of quality class IIIb and cereal-fodder strong complex (*Classification* ... 1989). Our analysis of the chemical composition of soil prior to the experiment revealed the following levels of mineral nutrients: N-NO_3 – 32, P – 121, K – 97, Ca – 2340, Mg – 166 and Cl – 11 (mg dm^{-3}). Based on the soil nutrient analysis, 90 kg N ha^{-1} (ammonium nitrate) and 50 kg K ha^{-1} (60% KCl, because of a relatively high potassium content) were applied before sowing the seeds. Phosphates were not used. The recommended tillage treatments were applied.

Each year, seeds were sown in mid-April. The plot surface area was 1.0 m^2 and the row spacing was 20 cm. Immediately after sowing the seeds, the plots were covered with PE film or non-woven PP fabric. The covers were removed after approximately five weeks. The plots were weeded by hand whenever needed. Leaves were harvested progressively over the growing season, one to three times from each treatment. The mineral content was determined in dry plant material collected during the first harvest season, in three replications. Averaged leaf samples from each treatment were dried to constant mass at 65°C and then ground. The plant material was wet mineralized in H_2SO_4+H_2O_2, and analyzed to determine the content of total nitrogen by the Kjeldahl method, phosphorus by the vanadium-molybdate method, potassium and calcium by atomic emission spectroscopy (AES), and magnesium by atomic absorption spectrometry (AAS). The following mineral ratios were also calculated: Ca:P, Ca:Mg, K:Mg, K:(Ca+Mg), and K:Ca.

The results were processed statistically by Anova, using the Statistica 10 software. The significance of differences between means was evaluated by constructing the Tukey's confidence intervals at α = 0.05.

RESULTS AND DISCUSSION

Both experimental factors had a significant effect on the macronutrient content of arugula leaves (Table 1). The macronutrient levels in the analyzed plant material were similar to those determined in leaves of *Eruca sativa* Mill. and *Diplotaxis tenuifolia* (L.) D.C. by Cavarianni et al. (2008) and Bozokalfa et al. (2009), and in leaves of *Eruca sativa* Mill. reported by Acikgoz (2011), Esiyok et al. (1999), Nurzyńska-Wierdak (2009), Barlas et al. (2011), Kawashima and Valente-Soares (2003). The total nitrogen content of arugula leaves ranged from 25.71 (plots without covers) to 36.52 g kg^{-1} d.m. (*Eruca sativa* Mill. covered with perforated PE film). Average total nitrogen concentrations were higher in *Eruca sativa* Mill. than in *Diplotaxis tenuifolia* (L.) D.C. A slightly higher nitrogen content was determined in *D. tenuifolia* (L.) D.C. by Santamaria et al. (2002), but the difference was sta-

Table 1

Concentrations of selected macronutrients in arugula leaves depending on the species and type of plant cover

Species	Type of plant cover	(g kg^{-1} d.m.)				
		N_{total}	P	K	Mg	Ca
Diplotaxis tenuifolia (L.) D.C.	PE film non-woven PP fabric without cover	35.08 32.20 25.71	7.45 8.28 6.91	29.33 30.38 26.01	3.03 3.53 2.29	32.52 39.41 28.34
Mean		31.00	7.55	28.57	2.95	33.43
Eruca sativa Mill.	PE film non-woven PP fabric without cover	36.53 31.48 29.32	6.82 7.37 7.91	28.03 27.77 27.78	3.45 3.79 4.17	28.59 31.79 34.74
Mean		32.44	7.37	27.86	3.80	31.70
Mean for type plant cover	PE film non-woven PP fabric without cover	35.81 31.84 27.51	7.13 7.83 7.44	28.68 29.08 26.89	3.24 3.66 3.23	30.56 35.60 31.54
$LSD_{0.05}$ species type of cover interaction		 0.74 0.91 1.28	 0.11 0.13 0.18	 0.26 0.32 0.45	 0.05 0.06 0.08	 0.45 0.55 0.78

tistically insignificant. The leaves of arugula plants grown in covered plots had a higher total nitrogen content, irrespective of the cover type. The same trend was observed by Orłowski et al. (2005) in bunch harvested shallots. In a study by Błażewicz-Woźniak (2010), leaves of fennel plants grown in plots covered with black PF film had a lower total nitrogen content, whereas leaves of plants covered with black PP non-woven fabric had a higher nitrogen content than leaves of fennel plants grown without covers. Contrary results were reported by Jadczak et al. (2006), who found that basil plants in control plots accumulated the largest amounts of total nitrogen.

The phosphorus concentrations in arugula leaves were determined in the range of 6.82 to 8.28 g kg^{-1} d.m., in the following order: *D. tenuifolia* (L.) D.C. covered with non-woven PP fabric > *E. sativa* Mill. without covers > *D. tenuifolia* (L.) D.C. covered with PE film > *E. sativa* Mill. covered with non-woven PP fabric > *D. tenuifolia* (L.) D.C. without covers > *E. sativa* Mill. covered with PE film. Unlike in a study by Cavarianni et al. (2008), *Diplotaxis tenuifolia* (L.) D.C. accumulated larger amounts of phosphorus. Bozokalfa et al. (2009) reported no differences in the phosphorus content between the analyzed species. PE film contributed to a decrease, and non-woven PP fabric to an increase in phosphorus concentrations in arugula leaves, as compared with plants grown in plots without covers. Błażewicz-Woźniak (2010) demonstrated that the use of covers decreased the phosphorus content of the edible parts of fennel. Orłowski et al. (2005), and Biesiada and Kędra (2012) reported that flat covers contributed to an increase in phosphorus levels in shallots and dill, respectively. Protective covers had no effect on the phosphorus content of basil leaves (JADCZAK et al. 2006).

The potassium content of arugula leaves ranged from 26.01 to 30.38 g kg^{-1} d.m. In the current study, unlike in experiments carried out by CAVARIANNI et al. (2008) and BOZOKALFA et al. (2009), the leaves of *Diplotaxis tenuifolia* (L.) D.C had a higher potassium content. The highest potassium concentrations were found in plant samples collected from plots covered with non-woven PP fabric, which is consistent with the findings of BŁAŻEWICZ-WOŹNIAK (2010) who studied fennel bulbs, and of BIESIADA and KĘDRA (2012) who analyzed dill leaves. Different results were reported by JADCZAK et al. (2006) and ORŁOWSKI et al. (2005), who noted the lowest potassium levels in the edible parts of basil and shallot plants covered with non-woven PP fabric, respectively.

Magnesium concentrations in arugula leaves were determined in a relatively wide range of 2.29 to 4.17 g kg^{-1} d.m., and *Eruca sativa* Mill. grown in control plots had the highest magnesium content. Magnesium levels were considerably affected by the plant species. In the present study, the magnesium content was on average nearly 29% higher in the leaves of *Eruca sativa* Mill., which corroborates the findings of CAVARIANNI et al. (2008). Non-woven PP fabric had a positive effect on magnesium accumulation in arugula leaves. ORŁOWSKI et al. (2005), JADCZAK et al. (2006), and BIESIADA and KĘDRA (2012) demonstrated that flat covers had no influence on magnesium levels in the edible parts of the analyzed vegetable species.

The calcium concentrations in arugula leaves ranged from 28.34 to 39.41 g kg^{-1} d.m. The highest calcium content was determined in plant samples collected from plots covered with non-woven PP fabric, where it was by 16% and nearly 9% higher than in plots covered with PE film and in plots without covers, respectively; the noted differences were significant. BŁAŻEWICZ-WOŹNIAK (2010) in a study of fennel bulbs and ORŁOWSKI et al. (2005) in a study of shallots reported that plants covered with PE film had the lowest calcium content. However, JADCZAK et al. (2006) demonstrated that basil plants grown under PE film contained the highest calcium concentrations. In the current experiment, the leaves of *D. tenuifolia* (L.) D.C. were higher in calcium, which is in agreement with the findings of CAVARIANNI et al. (2008). According to BOZOKALFA et al. (2009), calcium levels are similar in both species.

The use of perforated PE film and non-woven PP fabric caused an increase in soil temperature. The highest temperature rise was observed in plots covered with PE film, compared with the control treatment. Soil temperature increased from 1.7 to 3.6°C at a depth of 5 cm, and from 1.2 to 2.8°C at a depth of 10 cm (FRANCKE 2011). Based on long-term observations, SZULC and KRUCZEK (2008) concluded that temperature is one of the key factors that determined the mineral uptake. In the present study, arugula plants grown under flat covers contained more total nitrogen, potassium and magnesium than plants harvested from control plots without covers, regardless of a cover type. The edible parts of arugula plants covered with non-woven PP fabric had the highest phosphorus and magnesium levels. Phosphorus and magnesium concentrations in the leaves of plants grown under PE film were lower, compared with plots covered with non-woven PP fabric and control plots.

The nutritional value of edible plant parts is determined by their mineral content and nutrient ratios (Kotowska, Wybieralski 1999). According to Czapla and Nowak (1995) and Radkowski et al. (2005), the optimal macronutrient ratios in edible plants are as follows: Ca:P – 2, Ca:Mg – 3, K:(Ca+Mg) – 1.6 – 2.2, K:Mg – 6, and K:Ca – 2. The above ratios may vary widely depending on various factors such as a plant species, the part(s) sampled, degree of ripeness, planting and harvesting times and fertilization levels (Wróbel, Marska 1998, Kotowska, Wybieralski 1999, Matraszek et al. 2002, Michałojć, Buczkowska 2009, Francke 2010*a*,*b*). Wider than optimal Ca:Mg and Ca:P ratios are indicative of nutritional magnesium and/or phosphorus deficiency. Irrespective of the species and cover type, the Ca:P ratios in arugula leaves remained on a similar level, ranging between 4.2 and 4.8, thus being wider than optimal. The Ca:Mg ratios in the analyzed plant material were also broadened in all the treatments (8.3-12.4). More desirable values of the above ratios were noted in *Eruca sativa* Mill. The use of flat covers, regardless of their type, improved the proportions between the analyzed macronutrients (Table 2). Widely varying Ca: Mg ratios were also noted in tree onions by Jadczak (2005). Quantitative K:Mg ratios in arugula leaves were wider than optimal, ranging from 6.7 to 11.4, and the most desirable values were observed in *E. sativa* Mill. grown

Table 2

Ratios between macronutrients in arugula leaves depending on the species and type of plant cover

Species	Type of plant cover	K:Ca	K:Mg	Ca:P	Ca:Mg	K:(Ca+Mg)
Diplotaxis tenuifolia (L.) D.C.	PE film	0.9	9.7	4.4	10.7	0.8
	non-woven PP fabric	0.8	8.6	4.8	11.2	0.7
	without cover	0.9	11.4	4.1	12.4	0.8
Mean		0.9	9.9	4.4	11.4	0.8
Eruca sativa Mill.	PE film	1.0	8.1	4.2	8.3	0.9
	non-woven PP fabric	0.9	7.3	4.3	8.4	0.8
	without cover	0.8	6.7	4.4	8.3	0.7
Mean		0.9	7.4	4.3	8.3	0.8
Mean for type plant cover	PE film	1.0	8.9	4.3	9.5	0.9
	non-woven PP fabric	0.9	8.0	4.6	9.8	0.8
	without cover	0.9	9.1	4.3	10.4	0.8
Mean		0.9	8.7	4.4	9.9	0.8

without covers. The K:Mg ratio was more favorable in *Eruca sativa* Mill. (7.4 on average). The use of non-woven PP fabric improved the values of the above ratio. The K:Ca ratios in arugula leaves were comparable in all treatments, at 0.8-1.0, and never exceeded the optimal level. The edible parts of *D. tenuifolia* (L.) D.C. and *E. sativa* Mill. were characterized by identical K:Ca ratios. The use of PE film had a positive albeit small effect on the proportions between the above macronutrients. Analogous relationships were observed for the K:(Ca + Mg) ratios, which were similar in the studied species. The use of PE film insignificantly improved their values. In a study by Jadczak (2005), the K:(Ca + Mg) ratio varied depending on the age and organ(s) of tree onions. The most desirable values of the above ratio were noted in onion leaves, where they remained within the normal limits, similarly to arugula leaves in the present experiment. The K:(Ca + Mg) ratios in other edible parts of tree onions were substantially wider.

CONCLUSIONS

1. Macronutrient concentrations in arugula leaves were significantly affected by both experimental factors, i.e. the plant species and cover type.

2. The leaves of *Diplotaxis tenuifolia* (L.) D.C. had higher concentrations of phosphorus, potassium and calcium, whereas the leaves of *Eruca sativa* Mill. had a higher content of total nitrogen and magnesium.

3. The use of flat covers increased the accumulation of macronutrients in the edible parts of arugula plants. The leaves of plants covered with non -woven PP fabric had the highest concentrations of phosphorus, potassium, calcium and magnesium, and plants protected with PE film had the highest total nitrogen content.

4. The Ca:P, Ca:Mg and K:Mg ratios were broader in all the treatments. The K:(Ca+Mg) and K:Ca ratios remained within the normal ranges.

REFERENCES

Acikgoz F. E. 2011. *The effects of different sowing time practices on Vitamin C and mineral material content for rocket (Eruca vesicaria subsp. sativa (*Mill*)).* Sci. Res. Essays, 6(15): 3127-3131.

Adamczyk G. 2002. *Selected aspects of consumer behaviour and dietary patterns in Polish households in the 1990s*. Rocz. AR Poznań CCCXLIII, Ekon., 1: 31-41. (in Polish)

Barillari J., Canistro D., Paolini M., Ferroni F., Pedulli G.F., Iori R., Valgimigli L. 2005. *Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (Eruca sativa* Mill.*) seeds and sprouts.* J. Agric. Food Chem., 53: 2475-2482.

Barlas N. T., Irget M. E., Tepecik M. 2011. *Mineral content of the rocket plant (Eruca sativa).* Afr. J. Biotechnol., 10(64): 14080-14082.

Biesiada A., Kędra K. 2012. *The effect of emergence-improving treatments on the growth, yield* *and content of macroelements in leaves of garden dill (Anethum graveolens* L.*) cultivated for early crop*. Acta Sci. Pol., Hort. Cult., 11(4): 89-100.

Błażewicz-Woźniak M. 2010. *Effect of soil and plant covering as well as sowing term upon fennel bulb nutritional value.* Acta Sci. Pol., Hort. Cult., 9(1): 3-12.

Bozokalfa M. K., Yagmur B., Ilbi H., Esiyok D. Kavak S. 2009. *Genetic variability for mineral concentration of Eruca sativa L. and Diplotaxis tenuifolia L. accessions.* Crop Breed. Appl. Biotechnol., 9: 372-381.

Cavarianni R.L., Filho A.B.C., Cazetta J.O., May A., Corradi M.M. 2008. *Nutrient contents and production of rocket as affected by nitrogen concentrations in the nutritive solution.* Sci. Agric., 65(6): 652-658.

Czapla J., Nowak G. 1995. *Yields and yield quality at differentiated potassium, sodium, calcium and magnesium nutrition.* Part II. *Oat*. Acta Acad. Tech. Olst. Agric., 61: 101-107. (in Polish)

D'Antuono L.F., Elementi S., Neri R. 2008. *Glucosinolates in Diplotaxis and Eruca leaves: Diversity, taxonomic relations and applied aspects.* Phytochemistry, 69: 187-199.

Dyduch J., Najda A. 2005: *Changes in the content of dry matter and L-ascorbic in leaves of two cultivars of stalk celery (Apium graveolens* L. *var. dulce* Mill/Pers.*) depending on the age of harvested plants and soil litter cover.* Zesz. Nauk AR Wrocław, Rol., 86(515): 111-119. (in Polish)

Eşiyok D., Okur B., Delibacak S., Duman I. 1999. *Effect of manure doses and growth media on the productivity and mineral composition of rocket leaves (Eruca sativa).* Develop. Plant Soil Sci., 86: 237-240.

Francke A. 2010*a*. *The effect of magnesium fertilization on the macronutrient content of pepino dulce (Solanum muricatum Ait.) fruit*. J. Elem., 15(3): 467-475.

Francke A. 2010*b*. *The effect of potassium fertilization on the macronutrient content of pepino dulce (Solanum muricatum Ait.) fruit.* Acta Sci. Pol., Hort. Cult., 9(3): 51-57.

Francke A. 2011. *The effect of flat covers on the quantity and quality of arugula yield.* Acta Sci. Pol., Hort. Cult., 10(4): 3-14.

Hall M. K.D., Jobling J. J., Rogers G. S. 2012. *Some perspectives on rocket as a vegetable crop: A review.* Veg. Crops Res. Bull., 76: 21-41.

Jadczak D. 2005. *Effect of some agronomic factors on yields and nutritional value of tree onion.* Wyd. AR w Szczecinie, Rozprawy, 228. (in Polish)

Jadczak D., Błaszczuk A., Rekowska E. 2006. *Effect of covering on the content of macroelements in yield of basil (Ocimum basilicum* L.*) cultivated for a bunch harvest*. J. Elementol., 11(2): 135-141.

Jędrzejczak R., Ręczajska W., Szteke B. 1999. *Magnesium and other macronutrients in edible plant produce.* Biul. Magnezol., 4(1): 72-76. (in Polish)

Kawashima L.M., Valente-Soares L.M. 2003. *Mineral profile of raw and cooked leafy vegetables consumed in Southern Brazil.* J. Food Comp. Anal., 16(5): 605-611.

Kotowska J., Wybieralski J. 1999. *The shaping of quantitative ratios between K, Ca and Mg in soil and in plants.* Biul. Magnezol., 4(1): 104-110. (in Polish)

Majkowska-Gadomska J. 2010. *Studies on the direct effect of plant covers and soil liter cover on the growth, developent and yields of melonds (Cucumis melo* L.*).* Wyd. UWM Olsztyn, Rozpr. i Monogr., 159. (in Polish)

Matraszek R., Szymańska M., Wróblewska M. 2002. *Effect of nickel on yielding and mineral composition of the selected vegetables*. Acta Sci. Pol., Hort. Cult., 1(1): 13-22.

Melchini A., Costa C., Traka M., Miceli N., Mithen R., De Pasquale R., Trovato A. 2009. *Erucin, a new promising cancer chemopreventive agent from rocket salads, shows antiproliferative activity on human lung carcinoma A549 cells.* Food Chem. Toxicol., 47: 1430-1436.

MICHAŁOJĆ Z., BUCZKOWSKA H. 2009. *Content of macroelements in eggplant fruits depending on varied potassium fertilization*. J. Elem., 14(1): 111-118.

MORALES M., JANICK J. 2002. *Arugula: A promising specialty leaf vegetable*. In: *Trends in new crops and new uses*. JANICK J., WHIPKEY A. (eds.) ASHS Press, Alexandria, VA, 418-423.

MORALES M., MAYNARD E., JANICK J. 2006. *'Adagio': A slow-bolting arugula.* Hort. Sci., 41(6): 1506-1507.

NITZ G.M., SCHNITZLER W.H. 2002. *Variation der glucosinolat gehalte bei den rucola arten Eruca sativa und Diplotaxis tenuifolia in abhängigkeit des erntesschnittes*. J. Appl. Bot., 76(3/4): 82-86.

NURZYŃSKA-WIERDAK R. 2009. *Growth and yield of garden rocket (Eruca sativa* Mill.*) affected by nitrogen and potassium fertilization*. Acta Sci. Pol., Hort. Cult., 8(4): 23-33.

ORŁOWSKI M., JADCZAK D., ŻURAWIK A. 2005. *Effect of covers and diameter of planted bulbs on the volume and quality of bunch harvested shallots*. Zesz. Nauk. AR we Wrocławiu, Rol., 515(86): 387-393. (in Polish)

PIGNONE D. 1997. *Present status of rocket genetic resources and conservation activities.* In: *Rocket: A Mediterranean crop for the world*. Conf. IPGRI Legnaro (Italy), 13-14 December 1996, 2-12 pp.

RADKOWSKI A., GRYGIERZEC B., SOŁEK-PODWIKA K. 2005. *Content of mineral nutrienst in some grass species and varieties*. J. Elem., 10(1): 121-128. (in Polish)

SANTAMARIA P., ELIA A., SERIO F. 2002. *Effect of solution nitrogen concentration on yield, leaf element content, and water and nitrogen use efficiency of three hydroponically-grown rocket salad genotypes*. J. Plant Nutr., 25(2): 245-258.

SIWEK P., LIBIK A. 2005. *Effect of film and fibre covers used in growing early stalk celery on the volume and quality of yield.* Zesz. Nauk. AR we Wrocławiu, Rol. LXXXVI, 515: 483-490. (in Polish)

Classification of Polish Soils. 1989. Rocz. Gleb., 40(3/4): 7-103. (in Polish)

SZULC P., KRUCZEK A. 2008. *Effect of precipitation and temperaturę on accumulation of dry matter and mineral uptake by maize in the early development phase, depending on fertilization.* Acta Agroph., 11(3): 753-766. (in Polish)

WRÓBEL J., MARSKA E. 1998. *Effect of dolomite fertilizer on concentration of calcium, magnesium and potassium in leaves and pods of two cultivares of runner bean as indicators of its biological value*. Biul. Magnezol., 3(4): 199-205. (in Polish)

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VARIABILITY OF ZINC CONTENT IN SOILS IN A POSTGLACIAL RIVER VALLEY – A GEOCHEMICAL LANDSCAPE APPROACH*

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Abstract

The paper presents the research results on the relation between the contents of total zinc and its bioavailable form (Zn_a) and physicochemical properties of soil carried out along three catenas in the postglacial valley of the middle Łyna River, in NE Poland. We focused on topographical factors to determine the amount of Zn in the soil in relation to specific geochemical landscape types. The analyzed soil showed a relatively low level of soil pollution with Zn and did not exceed the threshold values for soil contamination with Zn. The average Zn content amounted to 45.75 mg kg^{-1} d.m. and ranged from 8.80 to 176.26 mg kg^{-1} d.m. The heavy metal content in the soil was related to organic matter and clay fraction, while it was inversely proportional to the share of sandy fraction. Distribution of zinc showed variability due to factors derived from topography, soil heterogeneity in the river valley as well as fluvial processes taking place within the floodplain. Different geochemical landscapes showed depressive trends in both Zn and Zn_a contents along the catenas. It diminished from eluvial to transeluvial landscapes and increased again to superaqual landscape. Depressions after former river channel were favorable for the Zn_a accumulation. The most abundant in Zn_a were upper horizons of Fluvisols in superaqual landscape (45.12 mg kg^{-1}) filling overgrown and terrestialized floodplain lakes. The share of Zn_a was the highest in organic horizons of Fluvisols and achieved 51.4% of total Zn**.** The nature and power of functional links between the heavy metal mobility and the soil properties were determined with multivariate statistics and GAM models. Applied ordination statistics confirmed its usefulness in soil factor analyses.

Keywords: zinc, geochemical background, soil, river valley, geochemical landscape.

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ZMIENNOŚĆ ZAWARTOŚCI CYNKU W GLEBACH MŁODOGLACJALNEJ DOLINY RZECZNEJ W ASPEKCIE KRAJOBRAZÓW GEOCHEMICZNYCH

Abstrakt

W pracy przedstawiono wyniki badań dotyczących związku między całkowitą zawartością cynku i jego formą biodostępną (Zn_a) a fizykochemicznymi właściwościami gleb. Badania prowadzono w trzech katenach glebowych w środkowym odcinku młodoglacjalnej doliny Łyny w NE Polsce. Skupiono się na czynnikach topograficznych determinujących ilość cynku w glebach w odniesieniu do typologii krajobrazów geochemicznych. Stwierdzono stosunkowo niski poziom zanieczyszczenia badanych gleb cynkiem, którego zawartość nie przekraczała wartości progowych. Zawartość cynku wahała się od 8,80 do 176,26 mg kg^{-1} s.m. (średnio 45,75 mg kg^{-1} s.m.). Zawartość tego mikroelementu była wprost proporcjonalna do zawartości materii organicznej i frakcji ilastej, natomiast odwrotnie proporcjonalna do frakcji piaszczystej. Rozmieszczenie cynku wykazywało zmienność ze względu na czynniki topograficzne, sekwencję gleb w dolinie rzecznej, a także procesy fluwialne w obrębie terenów zalewowych. W sekwencji krajobrazów geochemicznych, tj. wzdłuż katen, obydwie formy cynku wykazywały trend depresyjny: ich zawartość zmniejszała się od krajobrazu eluwialnego do transeluwialnego i ponownie wzrastała w krajobrazie superakwalnym. Akumulacji Zn_a sprzyjały obniżenia po byłym korycie rzeki. Najbardziej zasobne w Zn_a były powierzchniowe poziomy mad rzecznych w krajobrazie superakwalnym (42,12 mg kg^{-1}). Udział Zn_a w stosunku do całkowitej formy tego mikropierwiastka był najwyższy w powierzchniwych poziomach gleb limnowo-saprowych (51,4%). Charakter i siłę powiązań między mobilnością cynku i właściwościami gleb ustalono na podstawie statystyki wielowariantowej wymiarowej PCA i modelu GAM. Zastosowane techniki ordynacyjne potwierdziły ich przydatność w analizach czynników glebowych.

Słowa kluczowe: cynk, tło geochemiczne, gleba, dolina rzeczna, krajobraz geochemiczny.

INTRODUCTION

Zinc belongs to the natural components of soil, and its content depends primarily on a type of parent material and soil-forming processes. Zinc, like other heavy metals in soil, can occur in the form of free metal ions, metal adsorbed onto organic and inorganic complexes, and metal bound to organic and inorganic particulate matter (Alloway 2005). The mean total Zn content in the lithosphere is estimated to be 80 mg kg^{-1} d.m. and most of its surface soil is characterized by Zn levels within the range 17-125 mg kg^{-1} d.m., mean 64 mg kg^{-1} d.m. (Alloway 2005). According to IUNG (Kabata-Pendias et al. 1993) the natural content of Zn in Polish soils, amounts to *ca.* 32.7 mg kg^{-1} d.m. and shows a very high degree of purity (98.5%). In north-eastern Poland, an average Zn content in soils has been reported from 29.4 (Terelak 2001) to 48.8 mg kg^{-1} d.m. (Niesiobędzka 2001). Soil derived from sands do not usually contain more than 30 mg of Zn kg^{-1} of d.m., from sandy loams about 60 mg of Zn kg^{-1}, and from clay loams or clay more than 80 mg of Zn kg^{-1}.

As it is widely reported (Foster, Charlesworth 1996, Domańska 2009, Du Laing et al. 2009, Diatta 2013), heavy metals, including Zn, show decreased or increased mobility in soil in relation to the geological background of a given area. The geochemical variability of soil within river valleys results from topographical and hydrological factors. The lateral migration pattern of elements in soil led to the geochemical landscape classifications developed in the mid sixties of XX century by Russian pedologists such as Glazovskaya and Perel'man (FOSTERSQUE 1980, WICIK OSTASZEWSKA 2012). Thus, considerable differences in the share of Zn and soil parameters, e.g. particle size in particular soil genetic levels are anticipated between eluvial and/or transeluvial landscapes representing upper parts of valley slopes with a deep groundwater table and superaqual and/or subaqual landscapes associated with alluvial floodplains (NIESIOBĘDZKA 2001, DIATTA 2013). Factors controlling the potential availability of zinc are also complex. The biologically available form of Zn (HCl-extracted) can be site-specific, related to particular physicochemical characteristics of the soil or specific mixed contaminants (FOSTER, CHARLESWORTH 1996, BIRCH et al.1999).

The nature of the spatial variability of heavy metal accumulation in alluvial soil is under a direct influence of river activity and diversified by conditions of sedimentation (CZARNOWSKA et al. 1995, MIDDELKOOP 2000, WALLING et al. 2003, CISZEWSKI et al. 2004, OBOLEWSKI, GLIŃSKA-LEWCZUK 2013), particularly during flooding periods (ZHAO et al. 1999). Results from the research on the distribution of heavy metals across floodplains indicates a decrease in Zn concentrations in soils with an increasing distance from the active river channel (MIDDELKOOP 2000, WALLING et al. 2003, CISZEWSKI et al. 2004). In lowland meandering river valleys that distribution may be distorted due to other various water bodies (old -river channels, floodplain lakes) playing the role of sinks in the river landscape (GLIŃSKA-LEWCZUK et al. 2009, OBOLEWSKI, GLIŃSKA-LEWCZUK 2013).

The objective of the present study is the identification of spatial differences in zinc content along soil catenas in the postglacial river valley, which would indicate landscapes where the metal levels were naturally higher than in the others. The investigation on the factors limiting total Zn and its available form have been recognized in the valley of the middle Łyna River in north-eastern Poland.

MATERIAL AND METHODS

Study site

The present study was located in the free-flowing section of the Łyna River in north-eastern Poland, 25 km north of Olsztyn - the largest city in the Warmia and Mazury region (Figure 1). It flows northward to the Pregoła River in the Kaliningrad District (Russia).

Contemporary land relief of the Łyna River catchment shows a young-glacial character, formed as a result of melted glacial waters after the Pomeranian stage of Würm glaciation (Pleistocene). Absolute altitudes come to 75-90 m a.s.l. with relative excess about 20-25 m above the river water. The

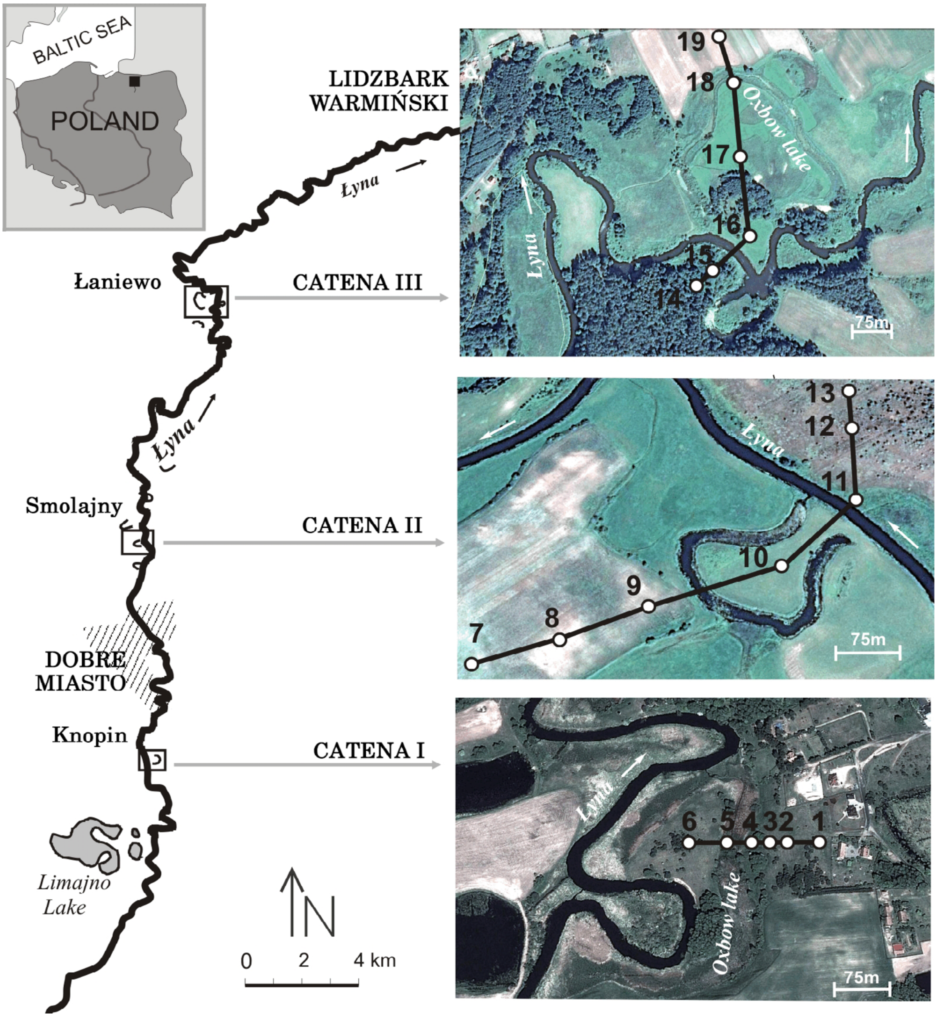

Fig. 1. Location of the study area and catenas with numbers of soil profiles

middle part of its catchment covers a mosaic of soils with features characteristic of morainic hills used by agriculture (53%), forests (29%), lakes (7%) and built-up areas (11%).

At the village of Smolajny, the River Łyna drains a 2290 km^2 area. The average river discharge is 14 m^{3}s^{-1}, ranging from 7 m^3 s^{-1} to 35 m^3 s^{-1} (GLIŃSKA-LEWCZUK 2005). An integral part of the valley is a diverse hydrographic system rich in numerous floodplain lakes, waterlogged depressions, near-shore sandbanks, and flood and over-flood terraces. The common feature of those ecosystems is a strong dependence on the river's activity, which water level fluctuations do not exceed 2 m. Flat floodplain areas are covered by short plants (meadows, pastures) that favor the lateral erosion.

Study sites were located in a distance from possible obvious sources of heavy metals (e.g. major roads, industrial or urban areas). Soil used in this study was taken from three catenas located across the Łyna river valley (Figure 1): at the villages of Knopin (catena I – profiles 1-6), Smolajny (catena II – profiles 7-13) and Łaniewo (catena III – profiles 14-19). The distance between external catenas is *ca.* 20 km. The distance between single soil profiles ranges from 30 m to 70 m, depending on the valley width and its internal structure (floodplain width, levees, oxbow lakes, slopes etc.) and a soil type. The catenas, perpendicular to the valley axis, reflect conditions of potential migration of Zn within the hypergenesis zone. According to the Typology of Geochemical Integration by Glazovskaya (FORTESQUE 1980) four types of geochemical landscapes have been distinguished: (1) eluvial or transeluvial, (2) eluvial accumulative, (3) trans-superaqual and (4) superaqual. Eluvial landcapes have a good natural drainage, e.g. at flat tops of morainic hills where the fluctuations in groundwater level impose no effect on soil parameters. Trans-eluvial forms, represented by steeper slopes, are susceptible to erosion and transport of matter. The eluvial-accumulative landscape is associated to foot-slopes, where a deluvial cover develops. The superaqual landscapes are related to concave forms in the landscape, where capillary rise supplies the root zone in water. In the conditions of active water exchange (hyporheic zone) soil is rich in immobilized forms of chemical elements (heavy metals) what is typical of trans-superaqual landscapes (WICIK, OSTASZEWSKA 2012)

Soil sampling and analytical procedures

In the analyses, 57 soil samples taken from characteristic genetic horizons from 19 soil profiles have been used. The samples were transferred to the laboratory in separate bags and then air-dried for further physical and chemical analyses. Particle size distribution was determined aerometrically following the Casagrande's method modified by Prószyński. Texture classes were determined according to Polish Society of Soil Science using USDA standards (PTG 2009). To obtain the content of organic matter, all samples were combusted at 550°C. Soil pH was measured in KCl with a potentiometer. Calcium carbonate was determined with the gasometrical method using the Scheibler's device.

The content of total Zn in all of the soil samples was determined after digestion of the 3:1 solution of nitric and hydrochloric acids (aqua regia acid) at 150°C (OSTROWSKA et al. 1991). To determine the available form of Zn (Zn_a) soil was extracted in 1M HNO_3 (mineral samples) or 0.5 M HNO_3 (organic samples). In every extract the concentrations of Zn or Zn_a were determined in triplicate using AAS technique in a certified laboratory. The detection limits for Zn were 1 ppm. All the metal contents are given in mg kg^{-1} of dry mass. In order to interpret the results of soil tests in terms of Zn contamination in the middle Łyna River valley, natural metal levels in the soil have been determined. Geochemical background of Zn in soil was calculated based on its average content in parent material from depths > 90 cm and determined according to the Czarnowska method (CZARNOWSKA 1996).

Statistical procedure

To assess the general differences among groups of soils texture classes, pH_{KCL}, organic matter were subjected to non-parametric analysis of variance with the use of Kruskal-Wallis test (K-W; $P \leq 0.05$). The precise statistical significance of differences in analyzed Zn and Zn_a among the studied objects was determined with the Dunn's test ($P < 0.05$). Except of the Dunn's test, statistical analyses were performed using the software package Statistica 10.0 PL for Windows.

In order to identify the primary environmental gradients affecting Zn contents in soil a multivariate statistical analyses involving a linear indirect method of Principal Component Analysis (PCA) was performed. The data was transformed to logarithms log(n+1) to satisfy conditions of normality. For the ordination analysis Canoco 4.5 software was used (TER BRAAK, ŠMILAUER 2002). The generalized additive model (GAM, $P \leq 0.001$) has been provided towards correct interpretation of ordination diagram computed for the Zn contents in relation to physical properties of soil. Variables that were not significant ($P \leq 0.05$) were dropped from the model. The Akaike Information Criterion (AIC) was given in the model, as well. The GAM built here was useful to model Zn content in soil and to predict its spatial variations in the river valley.

RESULTS AND DISCUSSION

Soil characteristics

In the middle Łyna river valley, according to the Polish Soil Classification system (*Polish soil* ... 2011), alluvial soil derived from sands and silts as well as peat-mud soils prevails. In the areas adjacent to the bottom of the valley one may found luvisols and deluvial soil derived from loams, silts and clays. The slope of the valley is also covered by rusty soil, arenosols and deluvial soils derived from sands. According to the WRB classification of soils (IUSS Working Group WRB 2006), the Łyna River valley is covered by Mollic Fluvisoils, Haplic Fluvisols and Limnic Sapric Histosols, whereas slopes of the valley by Haplic Luvisols, Gleyic Luvisols, Cumulinovic Arenosols, Mollic Gleysols (Colluvic), Brunic Arenosols (Distric) and Haplic Arenosols (Table 1).

Changes in soil properties along the soil profile in a variety of locations within toposequences and their accumulation series indicate relations involving matter transport and accumulation. In most soil types in the elu-

Table 1

Chosen properties of soil types in relation to the Zn content and its availability on the background of geochemical landscape units

Geochemical landscape unit*	Profile No	Soil type	Hori-zon	Depth (cm)	Texture class	pH_{KCl}	OM	$CaCO_3$	% fraction of diameter (mm)				Zn_t average	Zn_a average	Availability of Zn (%)
							(%)		>2	2-0.05	0.05-0.002	<0.002	(mg kg^{-1})		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Eluvial/ transeluvial	1	Brunic Arenosol (Distric)	A	0-26	S	4.1	1.24	0.13	0	89	9	2	19.27	5.11	26.5
			Bv	26-120	S	5.4		1.15	0	88	11	1	20.56	2.54	12.4
			C	120-150	S	7.6		0.17	0	96	3	1	21.80	1.32	6.1
	19		Ap	0-26	S	4.4	1.53	0.09	0	84	16	0	37.67	4.90	13.0
			Bv	26-80	S	5.6		0.09	0	95	3	2	12.80	2.72	21.3
			C	80-150	S	7.1		0.0	0	92	8	0	14.52	2.96	20.4
	2	Haplic Arenosol	A	0-28	S	4.3	1.12	0.17	0	89	9	2	117.47	4.79	4.1
			C	28-150	S	8.3		6.13	0	100	0	0	63.20	2.17	3.4
	13		A	0-22	S	4.4	1.03	0.0	5	95	5	0	19.53	3.82	19.6
			C	22-150	S	4.5		0.0	0	99	1	0	19.87	4.06	20.4
	7	Haplic Luvisol	Ap	0-31	L	6.9	4.02	0.0	0	47	42	11	102.33	28.99	28.3
			Et	31-56	SiL	6.2	-	0.0	0	12	62	26	55.67	8.44	15.2
			Bt	56-102	SiC	6.3	-	0.0	0	7	43	50	48.73	18.98	38.9
			C	102-150	SiCL	6.4	-	0.0	0	8	52	40	154.80	16.56	10.7
	8	Gleyic Luvisol	Ap	0-33	SL	4.4	2.23	0.0	0	71	23	6	47.33	17.52	37.0
			Et	33-58	SiL	5.1	-	0.0	0	40	53	7	24.47	4.11	16.8
			Bt	58-90	C	5.1	-	0.0	0	16	29	55	44.47	17.62	39.6
			Cg	90-150	HC	6.1	-	0.0	0	10	24	66	42.20	24.41	57.8
Aluvial accumulative	3	Mollic Gleysol (Colluvic)	A	0-68	LS	5.6	2.74	0.13	0	75	21	4	161.87	5.50	3.4
			C	68-150	SiCL	5.7		1.28	0	14	54	32	53.73	12.25	22.8
	9		Ap	0-30	SL	5.1	2.85	0.0	0	71	23	6	15.67	6.08	38.8
			A2	30-56	SiL	5.1	3.69	0.0	0	31	52	17	29.60	7.15	24.2
			A3	56-107	SiL	5.0	1.61	0.0	0	27	53	20	39.80	10.72	26.9
			G	107-150	S	5.8		0.0	45	87	6	7	20.80	5.72	27.5
	6	Cumulinovic Arenosol	A1	0-30	S	7.0	3.12	0.26	0	88	12	0	22.33	3.85	17.2
			A2	30-110	S	7.1	2.79	0.17	0	92	8	0	65.53	5.26	8.0
			2C	110-150	SiCL	7.3		17.45	0	12	59	29	176.26	8.78	5.0
	12		A1	0-30	S	7.3	2.84	0.10	13	91	9	0	22.07	8.24	37.3
			A2	30-110	S	7.2	1.67	0.19	12	91	8	1	33.93	9.31	27.4
			A3	110-150	S	7.0	1.01	0.0	9	90	9	1	24.47	2.37	9.7

cont. Table 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Trans-superaqual	14	Haplic Fluvisol	A	0-32	SL	6.5	2.31	0.17	0	55	26	19	85.47	43.89	51.4
			C1	32-90	SL	7.2		1.36	0	57	33	10	27.73	8.61	37.9
			C2	90-150	SL	7.1		1.88	0	67	32	3	33.87	7.38	21.8
	16	Haplic Fluvisol	A	0-18	SL	6.7	2.77	0.17	0	59	41	0	33.33	16.67	50.9
			C1	18-60	SL	7.6		1.11	0	69	29	2	21.00	4.03	19.2
			C2	60-150	S	8.0		1.45	0	89	9	2	14.73	3.03	20.6
	17		A	0-16	SL	6.7	2.52	0.21	0	57	41	2	33.33	13.15	39.5
			C1	16-50	L	7.0		0.26	0	50	41	9	25.93	5.71	22.0
			C2	50-150	SL	7.7		0.85	0	71	27	2	14.00	6.07	43.4
Super-aqual	10	Mollic Fluvisol	A1	0-23	SL	6.9	9.37	0.0	0	55	37	8	54.13	21.29	39.3
			C1	23-90	SiL	6.9	9.91	0.0	0	27	57	16	65.13	17.29	26.5
			AC	90-150	SL	7.5	2.55	1.30	0	66	26	8	25.67	10.02	39.0
	11		A1	0-26	SiL	5.9	18.71	0.0	0	32	53	15	127.13	35.20	27.7
			A2	26-73	SiL	6.1	12.45	0.0	0	24	56	20	96.47	19.54	20.3
			A3	73-100	L	6.4	4.88	0.0	0	45	40	15	36.27	11.23	31.0
			AC	100-150	LS	6.5	2.74	0.0	0	74	22	4	25.80	9.75	37.8
	15		A	0-32	SL	6.5	3.58	0.54	0	56	35	9	93.67	45.12	48.2
			C1	32-90	SL	7.5		1.96	0	62	35	3	32.27	7.27	22.5
			C2	90-150	LS	7.8		1.45	0	83	15	2	9.27	3.59	38.7
	4	Limnic Sapric Histosol	Lc	0-50	L	7.0	10.57	0.00	0	79	21	0	25.73	9.65	37.5
			Oa	50-120	fen peat	6.5	19.73	0.73	-	-	-	-	7.56	3.87	51.2
			C	120-150	LS	7.1		0.85	0	48	40	12	37.93	9.31	24.5
	5		Lc	0-90	SL	6.8	17.52	0.0	0	55	41	4	49.67	17.01	34.2
			Oa	90-117	fen peat	6.5	53.74	0.68	-	-	-	-	29.67	9.92	33.4
			Lcm	117-150	gyttja	6.6		2.83	-	-	-	-	55.00	15.76	28.7
	18		Lc	0-60	SiL	7.2	16.64	1.05	0	33	52	15	68.53	32.85	47.9
			C	60-150	S	7.7		1.28	0	92	8	0	8.80	4.06	46.1

*According to FORTESQUE (1980) after GLAZOVSKAYA (1963) vial landscape (except for Haplic Luvisols), influences horizon A shows the highest acidity, with significantly lower pH (<4.5) and the lowest organic matter content when compared to other landscape units. Despite differences between individual catenas, changes in Zn and Zn_a concentrations are regular. In general, two change patterns occur. The first characterizes catenas, where Zn concentrations are the lowest in autonomous locations, growing steadily downhill all the way to the floodplain. In the second pattern, the fluctuations of concentrations occur involving a clear decrease in Zn contents from the top to the middle slope zones, with a relatively fast increase in its concentrations at the foot-slope and in the floodplain.

Zinc content and natural background values in soil

The average content of Zn in the investigated soil amounted to 47.04 mg kg^{-1} d.m. The average Zn content in soil types distinguished from 19 soil profiles, ranged from 8.80 to 176.26 mg kg^{-1}d.m. (Table 1). Maximal Zn content was noted for Cumulinovic Arenosol at the horizon 2C in profile 6 at Knopin. According to the regulation of the Ministry of Environment in Poland dated of 4 Oct. 2002 on soil quality standards, the soils do not pose a threat to people's health or the environment in terms of zinc content.

The range of Zn_a content amounted from 1.32 to 45.12 mg kg^{-1} d.m. (Table 1). The highest values of Zn_a characterized upper (A) horizons of Haplic Fluvisols and Mollic Fluvisols, in which their availability achieved *ca.* 50% of total Zn. Although a significant correlation between the two forms of Zn was stated (r = 0.571 at $P \leq 0.001$), a significant variability of both forms were found in terms of soil morphological properties (Figure 2).

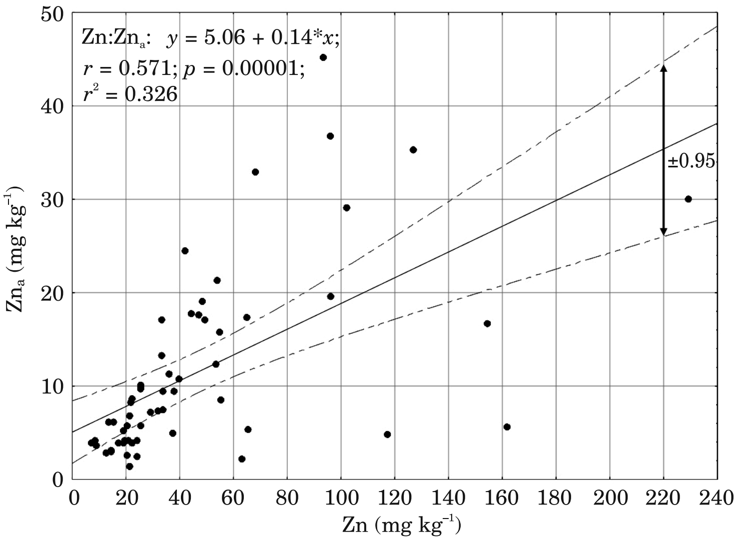

Fig. 2. Relationship between total Zn content and extractable Zn_a in soils of the middle Łyna River valley

Table 2

Zn content in sedimentary rocks at depths >90 cm in the middle Łyna River valley as reference background levels

Parent rock and a textural group	Number of samples	Average background level (mg kg^{-1} d.m.)
Quarternary clays	3	83.6
Fluvioglacial sands including: loamy sands sands	13 4 9	22.3 26.8 22.1
Average for all studied samples of parent materials	16	33.8

In order to differentiate between natural and anthropogenic origins of Zn in the valley of the Łyna River the geochemical background of parent material was determined. The average background value for Zn amounted to 33.8 mg kg^{-1}, but some distinct differences among the investigated parent material were noticed (Table 2).

The background value presented for sands was similar to those reported by CZARNOWSKA (1996) and amounted to 22.3 mg kg^{-1}. However, our investigation displayed more than 1.5 times higher background level of Zn for clays (83.6 mg kg^{-1}) in comparison to the values obtained by CZARNOWSKA (49.0 mg kg^{-1}). Nevertheless, our results confirm the data of other researchers (KABATA-PENDIAS et al. 1993, TERELAK 2001).

Zn distribution and soil properties

Both total and available forms of Zn showed somehow sensitivity to many physical and chemical properties of the soil in the investigated section of the river valley (Table 3, Figure 3).

Table 3

Correlation coefficients between physical-chemical properties of soil and the contents of Zn and available Zn_a in investigated soils

Property of soil	Zn	Zn_a
Organic matter	0.430*	0.525*
$CaCO_3$	-0.181	-0.230
pH_{KCl}	-0.199	-0.112
Depth of the soil uptake	0.161	-0.291
Fractions ∅ (mm):		
sand 2.0-0.05	-0.449*	-0.512*
silt 0.05-0.002	0.428*	0.464*
clay <0.002	0.341*	0.419*

The investigated soil properties that significantly influence Zn and Zn_a contents may be put in the following decreasing order: Zn or Zn_a**:** organic matter > silt > clay. Soil organic matter is the main component of the soil solid fraction and a key factor in Zn accumulation. In general, the higher the organic matter content the greater the ability to retain the heavy metal.

Organic horizons of Histosols stated in catena I at Knopin contained from 26 to 55 mg of Zn kg^{-1} d.m. and from 10 to 17 mg of Zn_a kg^{-1} d.m. The content of total Zn in the upper soil horizons was by 30% higher in comparison to the Zn content in mineral subsoil. Available form of Zn in organic horizons dominated more than 3 times over mineral substratum.

A distinct tendency to the zinc decrease down the profiles in Mollic Fluvisols derived from silt (catena II) was observed. Surface horizons contained from 54 to 127 mg of Zn kg^{-1} d.m., whereas parent material *ca.* 25 mg Zn kg^{-1} d.m. (profiles 10 and 11; Figure 1 and Table 1). The share of Zn_a amounted to 20-39% of total Zn and was proportionally distributed in relation to Zn.

Widespread Fluvisols (profiles 16, 17), derived from well-sorted sands in the valley of the Łyna River at Łaniewo were found to have relatively low Zn contents. At soil surface, Zn values achieved 33 mg kg^{-1} d.m., whereas subsurface horizons showed Zn content below 14 mg kg^{-1} d.m. Zn_a content displayed similar values in analogous soils in the catena II at Smolajny. The finest fractions in the catenas have also a distinctly pronounced Zn difference between the silt (r = 0.428) and sand fractions (r = -0.449), what is the evidence of intensive adsorption of metals to fine particles. The clay minerals have a much greater cation exchange capacity, and thus they have a much greater tendency for immobilizing metal ions such as Zn. The available form of Zn was also related to the clay fraction, however to less extent than the total form of the metal. The fraction of clay was also related to the Zn_a content (r = 0.419; $P \leq 0.05$).

The soil's ability to immobilize heavy metals increases with rising pH and peaks under mildly alkaline conditions. The relatively high mobility of Zn was observed in the eluvial unit in acid soils (pH 4.2-6.6), while in alkaline soils (pH 6.7-7.8) Zn became moderately mobile or even immobile. Researchers have reported a statistically significant correlation between soil pH and Zn content but our results do not support this and show no statistically significant correlation (Kabata-Pendias et al. 1993, Terelak 2001).

To assess the relation between Zn content, $CaCO_3$, pH, organic matter and the share of soil fractions, a multivatiate method of PCA was applied (Figure 3). The relationship explained 60.9% of total variation. The first axis explained almost 56.1% of the variance of species-environment relationship, this axis is mainly negatively correlated to gravel, and then to $CaCO_3$, while it is positively correlated mainly with organic matter, and subsequently to Zn and Zn_a. This means that sampling sites situated on the right side of the first axis are characterized by low ability to bound or absorb zinc. On the

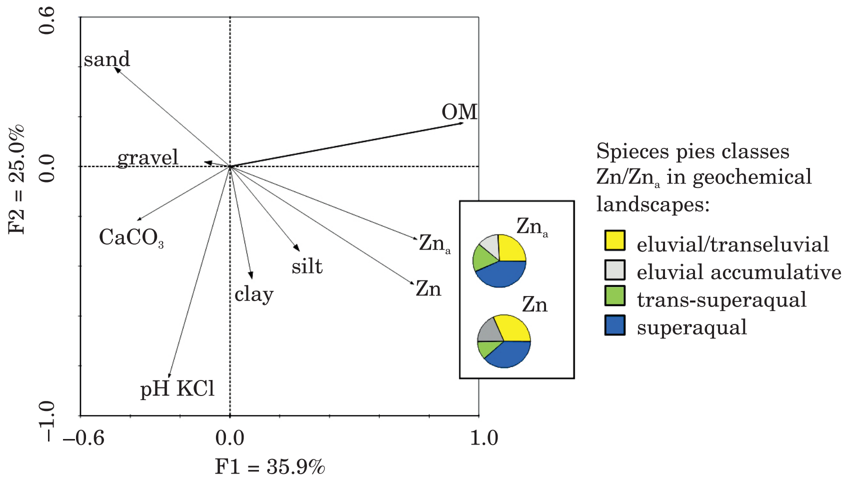

Fig. 3 Biplot of PCA computed for the zinc (Zn and Zn_a) and soil properties. Pies classes denote the shares of Zn and Zn_a in the landscape units defined for the Łyna River valley

left side of this axis sampling sites with higher share of OM are shown. This axis could be interpreted as organic matter environmental gradient. The second axis with 16.8% of the variance of species-environment relationship explained is negatively correlated with pH_{KCl}, and then fine fractions of silt and clay and positively correlated mainly with sand fraction.

To show the share of Zn and Zn_a in the geochemical landscape units a pies classes graphs have been inserted into Figure 3, where the role of superaqual landscape in Zn immobilization can be seen.

Zn in soils of geochemical landscapes

Figure 4 is shows the distribution of soil samples with sizes proportional to the Zn content based on the PCA standard analysis. Each of them represents one of four geochemical landscape types distinguished for the Łyna River valley. Eluvial and transeluvial (Arenosols, Luvisols) as well as trans-superaqual sites (Haplic Fluvisols) rich in Zn are grouped in the diagram (Figure 4) in the neighborhood of clay and silt, whereas eluvial accumulative and superaqual (Fluvisols) are located near OM. Based on the Zn concentrations and soil properties, a GAM model has been built ($P \leq 0.001$) to present isolines of Zn distribution in relation to soil properties.

Among soils in eluvial and transeluvial landscapes, the metal content was conditioned by physical properties of soil, mainly fine particles. The highest concentrations of Zn were stated in soils derived from clay (profile 7), namely Haplic Luvisol. In the uppermost layer, to a depth of 30 cm, its content amounted to 102 mg kg^{-1} d.m. Beneath the layer, zinc content decreased by 50%. Such a distribution is an effect of a very high content (ca. 50%) of the fraction <0.002 mm. Among soils in the eluvial landscape unit (profiles

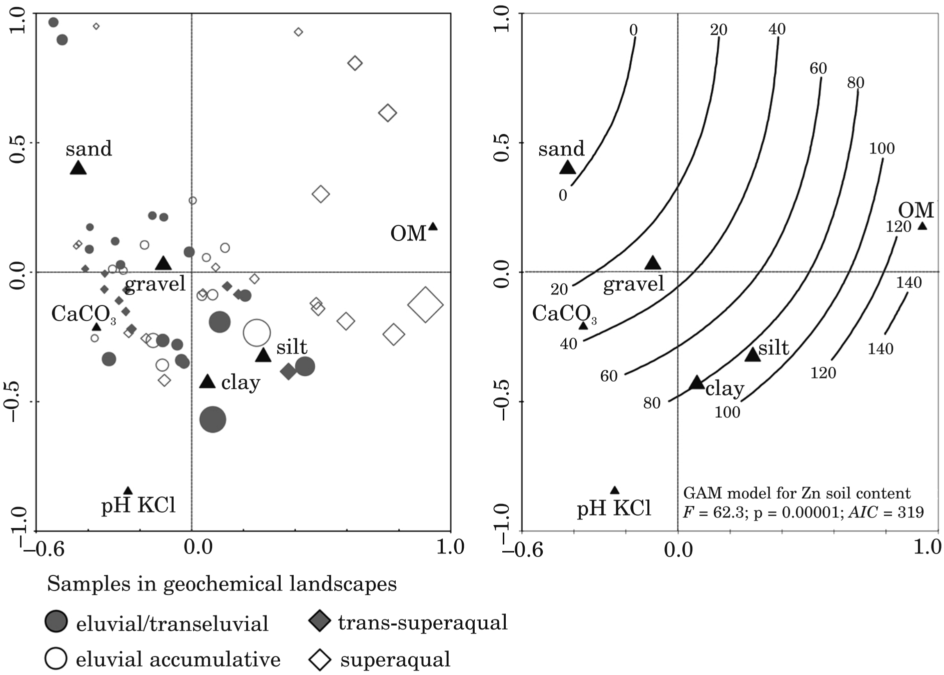

Fig. 4. Variability of Zinc content in soil samples taken from profiles from various geochemical landscape units within the Łyna River valley (left graph). The symbol size is relative to the Zn content in soil. Generalized Additive Model (GAM) computed for the Zn content in relation to physical properties of soil (right graph)

1, 19), rusty soils showed the lowest values of Zn. The fluvioglacial sands are characterized by relatively low contents of Zn (35 mg kg^{-1}) in comparison to the clay of the Quaternary origin (75 mg kg^{-1}). In soils derived from these sedimentary rocks, a correlation between clay fraction and the amount of Zn is positive and statistically significant (r = 0.54). Other researchers have reported the same relationship (FOSTER, CHARLESWORTH 1996, DUBE et al. 2001).

These depressions, in the form of overgrowing floodplain lakes, are common in the study reach (GLIŃSKA-LEWCZUK 2005). Due to frequent inundations, they receive regular inputs of metals, as well. Existing, filled with alluvium, old-river beds in the catenas I and III accumulated Zn mainly at the upper soil layer (0-30 cm) built of a significant amount of clay (20%) and organic matter (e.g. profile 14, see Figure 1). Within these depressions, Zn showed the highest values (60-80 mg kg^{-1}) among all of the profiles across the floodplain. Available fraction of Zn was also the highest in those depressions and amounted to 35-42 mg kg^{-1}. Accumulation of Zn is indicative then, for any storage properties of e.g. oxbow lakes playing a role as a sink in a river valley. Zn content in their bottom sediments has been reported at a level of 65.7 mg kg^{-1} d.m (GLIŃSKA-LEWCZUK et al. 2009).

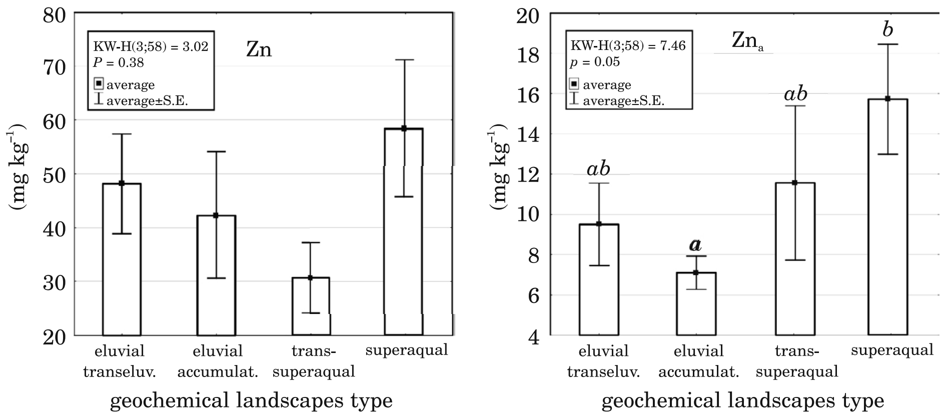

Fig. 5. The distributions of Zn and available Zn form (Zn_a) across the middle Łyna River valley Vertical bars denote standard error of mean (±SE). Statistically different groups of soils classified to a geochemical landscape type in the Dunn's test is denoted with different letters ($P \leq 0.05$)

Different geochemical landscapes showed depressive trends (see FORTESCUE 1980), for both Zn and Zn_a contents along three studied catenas (Figure 5). Values of Zn diminished from eluvial/transeluvial landscapes (48 mg kg^{-1}) to trans-superaqual (31 mg kg^{-1}) and increased again in superaqual landscapes (58 mg kg^{-1}). The superaqual landscape was favorable for the Zn_a immobilization (15 mg kg^{-1}), while eluvial accumulative landscape was characterized by the higher Zn mobility (7 mg kg^{-1}). Soil profiles (Fluvisols) adjacent to the river channel, and within the reach of the river flooding, showed stabile Zn concentrations (38 mg kg^{-1}). Evenly distributed Zn contents within the floodplain, indicate no significant metal contamination by the river activity in spite of possible influence of the towns located upstream the Łyna river, which are potential sources of soil contamination e.g. with municipal sewage.

CONCLUSIONS

The distribution of Zn in the middle Łyna river valley shows variability due to many factors derived from the topographical location of soil, soil heterogeneity within the river valley as well as fluvial processes taking place within the floodplain. An analysis of the geochemical landscape types applied to the migration of Zn in conditions of postglacial area enabled the recognition of specific variability of landscape structure and functioning eluvial, illuvial and deluvial processes within a single catena. Therefore, upland units are characterized by matter outflow, transitional slopes by matter transportation, with local accumulation or denudation, while spots located at foot-slopes, on the valley bottom and in subordinate depressions, are characterized by accumulation. A geochemical gradient found on the base of Zn in a postglacial river valley appears to have a depressive trend in terms of Zn and Zn_a contents along the catenas. It diminished from eluvial to transeluvial landscapes and increased again in the superaqual landscape. The nature and power of functional links between the metal mobility and the soil properties can be analyzed with multivariate statistics and supported by GAM models.

REFERENCES

Alloway B.J. 2005. *Bioavailability of elements in soil*. In: *Essentials of medical geology*. Eds. Selinus O., Alloway B.J. Impacts of the Natural Environment on Public Health. Elsevier, Academic Press, pp. 812.

Birch G.F. Robertson E., Taylor S.E., Mcconchie D.M. 2000.*The use of sediments to detect human impact on the fluvial system*. Environ. Geol., 39(9): 1015-1028.

Ciszewski D., Malik I., Szwarczewski P. 2004. *Pollution of the Mała Panew River sediments by heavy metals.* Part II. *Effect of changes in river valley morphology*. Pol. J. Environ. Stud., 13(6): 597-605.

Dube A., Zbytniewski R., Kowalkowski T., Cukrowska E., Buszewski B. 2001. *Adsorption and migration of heavy metals in soil.* Pol. J. Environ. Stud., 10(1): 1-10.

Czarnowska K. 1996. *Total content of heavy metals in parent rocks as the reference background levels of soils*. Rocz. Glebozn., 47(supl.): 43-50. (in Polish with English summary)

Czarnowska K., Broda D., Chojnicki J., Turemka E. 1995. *Heavy metals in the alluvial soils of the Vistula River Valley.* Rocz. Glebozn., 46 (3/4), 5: 5-18.

Diatta J.B. 2013. *Geoavailability and phytoconcentration of Zn: facing the critical value challenge (Poland)*. J. Elem., 18(4): 589-604. DOI: 10.5601/jelem.2013.18.4.363.

Domańska J. 2009. *Soluble forms of zinc in profiles of selected types of arable soils.* J. Elem., 14(1): 55-62.

Du Laing G., Rinklebe J., Vandecasteele B., Meers E., Tack F. M. G. 2009. *Trace metal behaviour in estuarine and riverine floodplain soils and sediments: a review.* Sci. Total Environ., 407(13):3972-3985. DOI:10.1016/j.scitotenv.2008.07.025.

Foster I.D.L., Charlesworth S.M. 1996.*Heavy metals in the hydrological cycle: trends and explanation.* Hydrological Processes, 10: 227-261.

Fortescue J.A.C. 1980. *Environmental geochemistry*. Springer-Verlag New York Inc.

Glińska-Lewczuk K. 2005. *Oxbow lakes as biogeochemical barriers for nutrient outflow from agricultural areas.* In: *Dynamics and biogeochemistry of river corridors and wetlands.* Eds: L. Heathwaite, B. Webb, D. Rosenberry, D. Weaver, M. Hayashi. IAHS Publ., 294: 55-68.

Glińska-Lewczuk K., Skwierawski A., Kobus S., Krzyżaniak M. 2009. *Distribution of selected heavy metals in bottom sediments of the Łyna River oxbows differed by hydrological connectivity*. Fresen. Environ. Bull., 18(6): 562-569.

IUSS WORKING GROUP WRB 2006. *World reference base for soil resources*. 2nd edition. World Soil Resources Reports No. 103. FAO, Rome, 132 pp.

Kabata-Pendias A., Motowicka-Terelak T., Piotrowska M., Terelak H., Witek T. 1993. *The assessment of a sulphur and heavy metals pollution. Framework guidelines for agriculture.* Wyd. IUNG Puławy, Ser. P(53), pp 20.

Middelkoop H. 2000. *Heavy metal pollution of the River Rhine and Meuse floodplains in the Netherlands.* Neth. J. Geosc., 79: 411-428.

NIESIOBĘDZKA K. 2001. *Speciation of heavy metals in light of soil properties.* In: *Cykling of elements in nature. Bioaccumulation. Toxicity. Effects*. Eds. B. GWOREK and A. MOCEK. T.I., IOŚ Warszawa, pp 456. (in Polish)

OBOLEWSKI K., GLIŃSKA-LEWCZUK K. 2013. *Distribution of heavy metals in bottom sediments of floodplain lakes and their parent river – a case study of the Słupia.* J. Elem., 18(4): 673-682, DOI: 10.5601/jelem.2013.18.4.435.

ORZECHOWSKI M., SMÓLCZYŃSKI S. 2010. *Content of Ca, Mg, Na, K, P, Fe, Mn, Zn, Cu in soils developed from the Holocene deposits in north-eastern Poland*. J. Elem. 15(1): 149-159.

OSTROWSKA A., GAWLIŃSKI S., SZCZUBIAŁKA Z. 1991. *Methods for analysis and assessment of Polish soil and plant properties.* Wyd. Inst. Ochr. Środ., Warszawa, pp. 334.

Polish soil classification. 2011. 5th edition. Rocz. Glebozn., 62(3): 193. (in Polish with English summary)

PTG 2009. *Particle size distribution and textural classes of soils and mineral materials – classification of Polish Society of Soil Science 2008*. Rocz. Glebozn., 60(2): 5-16. (in Polish with English summary)

SMÓLCZYŃSKI S., ORZECHOWSKI M. 2010. *Distribution of elements in soils of moraine landscape in Masurian Lakeland*. J. Elem., 15(1): 177-188.

TER BRAAK C.J.F., ŠMILAUER P. 2002.CANOCO. *Reference manual and CanoDraw for Windows user's guide: Software for Canonical Community Ordination* (version 4.5) – Microcomputer Power (Ithaca, NY, USA), 500 pp.

TERELAK H., TUJAKA A., MOTOWICKA-TERELAK T. 2001. *The content of trace elements and sulphur in cultivated soils in the warminsko-mazurskie voivodship.* Zesz. Probl. Post. Nauk Rol., 476: 327-334. (in Polish)

WALLING D.E., OWENS P.N., CARTER J., LEEKS G.J.L., LEWIS S., MEHARG A.A., WRIGHT J. 2003. *Storage of sediment-associated nutrients and contaminants in river channel and floodplain systems*. Appl. Geochem., 18: 195-220.

WICIK B., OSTASZEWSKA K. 2012. *Classification of geochemical landscapes*. In: *Landscape geochemistry*. Eds. POKOJSKA U., BEDNAREK R. Wyd. Nauk. UMK, Toruń. (in Polish)

ZHAO Y., MARRIOTT S., ROGERS J., IWUGO K. 1999. *A preliminary study of heavy metal distribution on the floodplain of the River Severn, UK by a single flood event*. Sci. Total Environ., 243/244: 219-231.

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CHEMICAL AND BIOLOGICAL PROPERTIES OF COMPOSTS PRODUCED FROM ORGANIC WASTE

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Abstract

The aim of the investigations was to determine the effect of composting municipal waste with various added substances (starch, edible oil or urea) on the content of selected forms of zinc, cadmium, copper and lead, the quality of organic matter and counts of some groups of physiological microorganisms. The above properties of compost may provide the basis for assessment of the composting process efficiency. The research object was biomass prepared from plant and other biodegradable waste generated in the area of Krakow. The biomass for composting was prepared from the following organic waste: deciduous tree chips, chicory coffee production waste, grass and tobacco waste. There were two stages of the composting process: I – lasting for 14 days, to obtain "heated" compost, and II – lasting for 210 days, when starch, edible oil or urea was added to the composted biomass. The total content of Zn, Cd, Cu and Pb determined in the analyzed composts does not pose a threat to the purity of the soil environment. The content of water-soluble forms of trace elements and forms bound to organic matter was affected by the loss of organic matter, chemical properties of a given element and the addition of supplement, mainly urea, to the composted biomass. Analysis of the fractional composition of humic compounds revealed higher values of the Cha:Cfa ratio in the composts with added edible oil or urea than in the other composts, which may indicate a much more advanced decomposition process of the material subjected to composting. Among the analyzed microorganisms, bacteria were most numerous in the composts. The introduction of urea to the composted biomass reduced microbial activity. Adding starch or oil stimulated microbial development and may have stimulated the composting process.

Keywords: municipal waste, composts, trace elements, organic matter, microorganisms.

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WŁAŚCIWOŚCI CHEMICZNE I BIOLOGICZNE KOMPOSTÓW WYTWORZONYCH Z ODPADÓW ORGANICZNYCH

Abstrakt

Celem badań było określenie wpływu procesu kompostowania odpadów komunalnych z różnymi dodatkami (skrobią, olejem jadalnym, mocznikiem) na zawartość wybranych form cynku, kadmu, miedzi i ołowiu, jakość materii organicznej oraz liczebność niektórych grup fizjologicznych mikroorganizmów. Wymienione właściwości kompostów mogą stanowić podstawę oceny efektywności procesu kompostowania. Obiekt badań stanowiła biomasa przygotowana na bazie odpadów roślinnych i innych biodegradowalnych powstających na terenie miasta Krakowa. Biomasę do kompostowania przygotowano z organicznych materiałów odpadowych, takich jak: zrębki z drzew liściastych, odpad z produkcji kawy zbożowej, trawa oraz odpad tytoniowy. W procesie kompostowania wyróżniono dwa etapy: I – trwający 14 dni, którego celem było uzyskanie „kompostu grzejnego"; II – trwający 210 dni, w którym do kompostowanej biomasy wprowadzono dodatek skrobi, oleju jadalnego i mocznika. Oznaczona ogólna zawartość Zn, Cd, Cu i Pb w badanych kompostach nie stanowi zagrożenia dla czystości środowiska glebowego. Na zawartość wodnorozpuszczalnych form pierwiastków śladowych oraz form związanych z materią organiczną miały wpływ: ubytek substancji organicznej, właściwości chemiczne pierwiastka oraz dodatek do kompostowanej biomasy, głównie mocznika. Analiza składu frakcyjnego związków próchnicznych kompostów wykazała wyraźnie większe wartości stosunku Ckh do Ckf w kompostach z dodatkiem oleju jadalnego i mocznika, niż w pozostałych kompostach, co może wskazywać na bardziej zaawansowany proces dekompozycji materiałów poddanych kompostowaniu. Spośród badanych drobnoustrojów najliczniejszą grupę w kompostach stanowiły bakterie. Wprowadzenie do kompostowanej biomasy mocznika spowodowało zmniejszene aktywności mikrobiologicznej. Dodatek skrobi i oleju stymulował rozwój drobnoustrojów i mógł sprzyjać intensyfikacji procesu kompostowania.

Słowa kluczowe: odpady komunalne, komposty, pierwiastki śladowe, materia organiczna, mikroorganizmy.

INTRODUCTION

In the European Union, composting is one of the most widely promoted methods of the biological processing of biodegradable waste, which is arduous waste due to its high water content, which makes storage difficult. Composting may be defined as controlled, biological decomposition and stabilization of organic substrates, carried out under aerobic conditions to an adequate moisture content, during which process the temperature of the composted material rises to the thermophilic range (Horiuchi et al. 2003, Ishii, Taki 2003, Wang et al. 2004). The final product of well-conducted composting should be sanitary safe material, abundant in humus and biogenic compounds (Himanen, Hanninen 2011).

A high content of organic substance and fertilizer components in some biodegradable waste makes it good raw material for composting. The factors which restrict later use of ready compost may be its immaturity or excess pollutants, including trace elements (Garcia et al. 1995, Bowszys et al. 2009, Sądej, Namiotko 2010).

The variety of functions which organic matter performs in the environment means that both its quantity and quality in processed organic waste, including composts, should be taken into consideration while assessing the value of biologically transformed products (HUANG et al. 2006). In general, composting should improve the quality of organic matter, e.g. by increasing the share of its stabile forms in mature composts (HUANG et al. 2006). Inadequate conditions in which composting is conducted, e.g. insufficient aeration or wrong biomass composition, lead to quantitative and qualitative changes in microbial populations, which result in disadvantageous modifications of the quantitative and qualitative composition of organic matter in compost (KULCU, YALDIZ 2004).

The biochemical processes which occur during composting may activate initially inert trace elements (HSU, LO 2001), which, depending on how given compost is intended to be used, may create undesirable conditions for the plant growth and development. Thus, it is justifiable to assess not only the total content of trace elements in composts but also their mobile forms and those bound to organic matter. These forms usually constitute a considerable proportion of the total content and, under suitable soil conditions (GONDEK 2007, KANG et al. 2011, JAKUBUS 2012), may undergo fast degradation.

The organic fraction of composted waste, particularly the final product, contains a considerable amount of microbial biomass. Two groups dominate during the composting process: bacteria, including actinomycetes, and fungi. Changes in the composition of the microbial population depend mainly on the temperature maintained during the process, biomass aeration and the content of readily available forms of organic matter in the composted material. The temperature induces quantitative and qualitative changes of the microflora and causes sterilization of the product, i.e. compost (TIQUIA et al. 1996, HUANG et al. 2010, ZHANG et al. 2011).

The aim of the conducted investigations was to determine the effect of composting, mainly of municipal waste, with various added substances (starch, edible oil or urea) on the content of selected forms of trace elements, quality of organic matter and counts of some groups of physiological microorganisms, which may provide the basis for the assessment of composting process efficiency. It was assumed that the amount and composition of added substances would make composting easier.

MATERIAL AND METHODS

The object of the investigations was the biomass prepared from plant waste and other biodegradable waste generated in the urban area of Krakow. The biomass for composting was prepared from the following organic waste materials (in the waste catalogue marked by 20 02 code: waste from gardens and parks): deciduous wood chips (43.87% share in the biomass), grass (23.13% share in the biomass), waste (02 03 03) from chicory coffee production (21.94% share in the biomass) and plant waste (02 03 82) from the tobacco industry (10.96% share in the biomass). The moisture content of the mixed biomass was 45.30%, and the C:N ratio was 1:20. A 0.5 x 0.4 x 0.3 m (height x length x width) bioreactor was equipped with an aerating system and a system of post-process water draining. During the experiment, the biomass temperature and moisture content were controlled in order to maintain optimal conditions for the process. Biomass in the bioreactors was aerated in a 0.01 $m^3 \cdot min^{-1}$ system four times in 24 hours. For better aeration and homogenization, the composted biomass was taken out of the bioreactors once a week and mixed manually. Two stages were identified in the composting process: I – lasting for 14 days, aiming to obtain "heated" compost, and II – lasting for 210 days, when different substances were added (5% of the fresh mass of "heated" compost) to composted biomass. Selected properties of "heated" compost are presented in Table 1.

Table 1

Selected properties of raw "heated" compost after removal from the bioreactor

Determination	Value ± SD
Dry matter (g kg^{-1})	549.8 ± 2.20
pH H_2O	6.52 ± < 0.01
Organic C (g kg^{-1} d.m.)	320.1 ± 7.16
Electrolytic conductivity (mS cm^{-1})	5.58 ± 0.07
Total forms (mg kg^{-1} d.m.)	
Zn	177.8 ± 3.3
Cd	0.67 ± 0.01
Cu	27.2 ± 0.5
Pb	11.52 ± 0.22

± standard deviation

The experiment comprised the following variants in two replications: C1 – raw compost without additions (control); C2 – raw compost + starch (edible product); C3 – raw compost + edible oil; C4 – raw compost + urea (chemically pure). After composting, all the materials were thoroughly mixed and then sifted through a 1 cm mesh, and 3 kg samples were collected for chemical and microbiological analyses.

The following were determined in the initial and composted material: dry matter content at 105°C (the process lasted for 12 hours); pH by a potentiometer in an aqueous suspension of compost at the 1 : 10 dry matter to water ratio, electrolytic conductivity by a conductometer, the total content of zinc, copper, cadmium and lead determined after sample mineralization in a muffle furnace (450°C for 5 hours) and dissolution in diluted (1:2) nitric acid. The determinations were made using the methods suggested by Baran and Turski (1996). The water soluble forms of these elements and their forms bound to organic matter were extracted using redistilled water and 0.1 mol dm^{-3} $K_4P_2O_7$ solution, respectively. The trace element content in the solutions and extracts was determined with the ICP-AES method on a JY 238 Ultrace apparatus (Jobin Yvon).

The total content of organic carbon was determined with the oxidation--titration method following sample mineralization in potassium dichromate. The humic compounds were extracted from the composts with distilled water for 24 hours (extracted C). In order to cause the coagulation of humic acids, some of the obtained extract was acidified with sulfuric acid to reach pH 2. After filtering, the precipitate of humic acids was dissolved in hot NaOH of 0.05 mol dm^{-3} concentration. The organic carbon content was determined in the extracts with the oxidation-titration method after sample mineralization in potassium dichromate. Fulvic acid carbon was calculated as the difference between the content of C extracted with water and the humic acid carbon content.

Microbiological quantitative analyses of dormant and vegetative forms of mesophilic bacteria and microbiological quantitative analyses of fungi, actinomycetes, and amylolitic, proteolytic and lipolytic bacteria were conducted using the serial dilution method. The analyzed material was cultured on general purpose and selective culture media, and after an adequate period of incubation colony-forming units were counted. The results were converted into 1 g of sample dry matter (Atlas, Parks 1997).

The analytical results are arithmetic means of four replications. The standard deviation was calculated for the mean values presented in the tables. Average pH values of the composts and standard deviation values were computed after converting pH to the hydrogen ion content.

RESULTS AND DISCUSSION

The dry matter content in the analyzed samples was differentiated by the composting process depending on the kind of an added component (Table 2). In comparison with the control compost (C1), the dry matter content increased in the biomass of composts made with starch (C2) and urea (C4).

Soil reaction is one of the most important physicochemical properties of waste substances and also a good indicator of the course of compost maturing. Based on the conducted experiments, a marked increase in the pH value was observed in the analyzed composts in comparison with the initial material ("heated" compost) – Tables 1, 2. Iglesias-Jimenez and Perez-Garcia

Table 2

Selected properties of organic materials after composting

Determination	Type of compost			
	C1	C2	C3	C4
Dry matter (g kg^{-1})	515.0 ± 20.2	645.9 ± 28.6	512.7 ± 21.7	544.3 ± 24.7
pH	7.85 ± < 0.01	7.84 ± 0.03	7.74 ± 0.02	7.58 ± 0.01
Electrolytic conductivity (mS cm^{-1})	6.31 ± 0.07	5.98 ± 0.06	5.90 ± 0.07	5.76 ± 0.27

± standard deviation,
C1 – raw compost without addition (control), C2 – raw compost + starch (food product),
C3 – raw compost + edible oil, C4 – raw compost + urea (chemically pure);

(1991) also demonstrated an increase in the pH value of composted waste. An increase in the pH value of composted biomass may be caused by an the content of soluble forms of alkaline elements, such as magnesium or calcium, increasing with time, as demonstrated by DROZD et al. (1996). Moreover, it may be the result of the degradation of organic compounds, mostly proteins, leading to the release of ammonia.

Excess soluble salts in composts applied to soil may distort the balance in the soil solution, retarding the plant growth. A good indicator of compost salinity is electrolytic conductivity. The electrolytic conductivity of water solutions depends on the kind of soluble substances, their concentration and temperature. The value of electrolytic conductivity in the analyzed composts, irrespectively of the added substances, was higher than in "heated" compost (Tables 1, 2). The elevated value of electrolytic conductivity in the composts at the termination of composting resulted from the progressing mineralization of organic matter, which causes the release of mineral components, responsible for a higher value of the electrolytic conductivity of the analyzed materials. In the previous investigations conducted by GONDEK (2006) and DIMAMBRO et al. (2007), much lower values of electrolytic conductivity were determined both in composts based on non-segregated municipal waste and in ones produced from plant and other biodegradable waste.

The total cadmium content was on a similar level, regardless of the compost (Table 3). The content of cadmium extracted with water (Cd H_2O) was between 5 and 10-fold higher in the compost with urea than in the other ones.

The highest cadmium content was determined in organic matter compounds. The share of this Cd form in the total content was between 15% in the compost with starch (C2) and 24% in the compost without additions (C1).

The total zinc content was the highest in the compost with no additions (Table 3). According to the limits set for heavy metals in composts by the European Commission, the analyzed materials (irrespectively of the added substance) belong to the second class in respect of the content of zinc

Table 3

Content of trace elements in organic materials after composting

Determination	Type of compost*			
	C1	C2	C3	C4
	(mg kg^{-1} d.m.)			
Cd H_2O	0.060 ± < 0.01	0.051 ± < 0.01	0.032 ± < 0.01	0.350 ± 0.10
Cd $K_4P_2O_7$	0.420 ± 0.01	0.357 ±0.01	0.329 ± 0.01	0.253 ± 0.01
Total Cd	0.86 ± 0.02	0.81 ± 0.05	0.81 ± 0.01	0.91 ± 0.09±
Zn H_2O	11.69 ± 0.10	9.51 ± 0.27	9.19 ± 0.20	64.20 ±0.79
Zn $K_4P_2O_7$	193.4 ± 5.1	165.3 ± 3.7	181.8 ± 5.9	125.7 ± 8.0
Total Zn	273.1 ± 1.2	251.8 ± 1.7	255.2 ± 1.0	245.8 ± 1.0
Cu H_2O	6.64 ± 0.24	5.74 ± 0.20	5.83 ± 0.70	26.43 ± 0.73
Cu $K_4P_2O_7$	4.63 ± 0.88	4.90 ± 0.70	6.02 ± 0.16	2.90 ± 0.18
Total Cu	37.96 ± 0.51	36.78 ± 0.46	38.17 ± 0.40	44.50 ± 1.52
Pb H_2O	0.49 ± 0.07	0.41 ± 0.07	0.33 ± 0.03	2.30 ± 0.10
Pb $K_4P_2O_7$	5.27 ± 0.72	5.28 ± 0.76	5.29 ± 0.33	4.63 ± 0.16
Total Pb	14.87 ± 1.00	15.36 ± 1.09	15.62 ± 0.40	15.49 ± 2.36

± standard deviation
* explanation as in Table 2

(Wasiak, Madej 2009). The content of water-extracted zinc was the highest in the compost with urea. The share of this zinc form in the total content in the composts with starch, edible oil or without additives did not exceed 5%. In the compost with urea, the share was on average over 6-fold higher. The highest amounts of zinc in the composts were determined in forms bound to organic matter. On average, the share of organically bound zinc with reference to the total zinc content in the composts with starch, edible oil or without additives reached almost 70%, being almost 20% lower in the compost with urea. The content of various zinc forms change dynamically during waste composting. Drozd and Licznar (2004*b*) showed that the content of soluble zinc forms decreased in the first weeks of composting. The moisture content of the composted waste may have significantly affected the content of mobile forms of this element. According to Drozd and Licznar (2004*b*), a higher moisture content of composted waste decreases the content of water soluble forms of this element. In the authors' own investigations, adding urea to composted waste caused an increase in the content of water extracted zinc. Drozd and Licznar (2004*b*) also point to the increased solubility of zinc compounds in composted waste depending on added nitrogen. According to Rosik-Dulewska and Mikszt (2004), the content of zinc bound to organic matter in composts from municipal waste did not exceed 30% of the total content of this element. In the authors' own research, the share was twice as high.

The total copper content in the composts with the addition of starch (C2), edible oil (C3) and in the control compost (C1) was on a similar level, i.e.

37.63 mg kg^{-1} d.m. on average (Table 3). About 7 mg kg^{-1} d.m. of the total copper forms was determined in the compost with added urea (C4). The total Cu content determined in the analyzed materials was much lower than the limit for copper in composts suggested by the European Commission (WASIAK, MADEJ 2009). The content of water-extracted Cu was the highest in compost with urea (Table 3). In comparison, the content of this copper form in the other composts was 20 mg lower. This copper form constituted over 30% of the total Cu content in the compost with urea, and slightly over 15% in the other composts. Irrespectively of the applieds ubstance, a relatively low share of copper bound to organic matter was detected in the total content of this element. According to ROSIK-DULEWSKA and MIKSZT (2004), the highest amount of copper in composted municipal waste, up to about 60% in the total content, is accumulated in the fraction bound to organic matter. The results of the authors' investigations conducted on municipal plant waste indicate that copper in the organic fraction did not exceed 20% of the total content of this element, which resulted from a considerable share of water-soluble forms. WONG and SELVAM (2006) also showed that composting caused transformation of the copper residual form into mobile forms.

The total lead content in the composts was comparable and ranged between 14.87 mg and 15.62 mg kg^{-1} d.m. (Table 3). As in the case of cadmium, copper and zinc, the highest amount of lead forms was extracted with water from the compost with urea. The lead content in the forms bound to organic matter was much higher than the content of water-extracted forms. The content of water-soluble forms of lead in the analyzed composts was low, except for the compost with urea addition (C4).

The total content of trace elements in the composts depends above all on their content in the material from which the biomass for composting was prepared. Changes in the organic matter content, and especially its loss, are not without importance for the content and availability of trace elements. The authors' investigations indicate that the composting of biodegradable materials with various added substances causes an increase in the total content of trace elements, as confirmed by results of other authors (LAZZARI et al. 2000, NOMEDA et al. 2008). CZEKAŁA (2006) went further, suggesting that changes in the trace element availability in composted waste were connected with changes in the content of soluble forms of organic matter.

The organic carbon content decreased in all the composts in comparison with the content determined in "heated" compost collected after removal from the bioreactor (Tables 1, 4). ZORPAS et al. (2003) and LIU et al. (2007) stated that the loss of organic carbon concerned mainly easily degradable organic compounds of composted biomass. Moreover, DROZD and LICZNAR (2004*a*) showed that the loss of organic carbon in composted waste depended on the biomass moisture content during the process. At a higher moisture content, the temperature of the composted mass was observed to be lower, especially during the thermophilic phase of the process.

Table 4

Fractional composition of humus in organic materials after composting

Determination	Type of compost*			
	C1	C2	C3	C4
	(g kg^{-1} d.m.)			
Organic C	309 ± 4.1	312 ± 37	302 ± 7.7	318 ± 60
Extracted C	23.17 ± 0.4	18.97 ± 0.10	17.61 ± 0.59	58.10 ± 2.71
C humic acids	9.89 ± 0.2	9.20 ± 0.20	14.53 ± 1.19	39.47 ± 1.94
C fulvic acids	13.28 ± 0.60	9.76 ± 0.20	5.27 ± 0.59	18.64 ± 0.78
Cha:Cfa	0.74 ± 0.05	0.94 ± 0.04	2.75 ± 0.01	2.11 ± 0.02

± standard deviation
* explanation as in Table 2

The analysis of the content of humic compounds revealed considerable diversity of both the content of extracted carbon and the content of humic acids (Table 4). Most carbon was extracted from the compost with urea (C4). The content of carbon extracted from the composts with starch (C2) and edible oil (C3) did not differ. The humic acid carbon content in the analyzed composts ranged widely, from 9.20 g kg^{-1} to 39.47 g kg^{-1} d.m. The highest amount of humic acid carbon was determined in the compost with urea (C4).

The fulvic acid carbon content ranged more narrowly, from 5.27 g kg^{-1} to 18.64 g kg^{-1} d.m. (Table 4). As in the case of humic acid carbon, the biggest amount of this fraction of humic compounds was determined in the compost with urea (C4).

The varied content of Cha and Cfa was reflected in the ratio of both fractions. The composts with edible oil (C3) and urea (C4) were characterized by markedly higher values of the Cha:Cfa ratio: 2.75 and 2.11, respectively (Table 4). Much lower values (less than one) of that ratio were found in the control compost (C1) and in the compost with starch (C2). The results of the research conducted by Raj and Antil (2011) on the maturity and stability of composts manufactured from agri-industrial waste indicate that the process led to the attainment of an optimal value of the Cha:Cfa ratio, i.e. 1.9. However, Inbar et al. (1990) stated that 1.5 was the optimal value of the above ratio. In practice, a relatively small difference between these two values of the Cha:Cfa ratio may mean significant changes in the level of mineralization of composted materials, which might not necessarily be favorable for properties of the final product.

The most numerous group of microorganisms in the analyzed composts were bacteria, in contrast to fungi, which were the least numerous (Table 5). In the compost with starch, the number of vegetative forms of bacteria was almost three times as high as in the compost with oil and 9-fold higher than in the control compost. In the compost with urea, the number of dormant forms of bacteria was higher than the numerical strength of this physiological

Table 5

Counts of microorganisms in organic material after composting

Microorganisms	Type of compost*			
	C1	C2	C3	C4
	(jtk g^{-1} d.m.)			
Vegetative bacteria	23 296 · 10^3	211 200 · 10^3	64 670 · 10^3	12 772 · 10^3
Endospore-forming bacteria	1 792 · 10^3	105 60 · 10^3	14 049 · 10^3	3 090 · 10^3
Fungi	426 048	1 003 200	1 583 300	412 103
Actinomycetes	11 200 · 10^3	19 712 · 10^3	23 192 · 10^3	824 · 10^2
Proteolytic bacteria	873 600	17 600	66 900	1648
Amylolytic bacteria	5 376 · 10^3	6 512 · 10^3	4 683 · 10^3	6 592 · 10^2
Lipolytic bacteria	269	229	11 596	82

± standard deviation
* explanation as in Table 2

group of microorganisms in the control compost (Table 5). The compost with urea contained over 8-fold fewer amylolytic bacteria compared to the numerical strength of this group of microorganisms found in the control compost (C1). The highest number of amylolytic bacteria (which decompose starch) was determined in the compost with starch addition.

Fungi and actinomycetes thrived best in the compost with oil, which was a processed source of carbon (Table 5). Fungi were the least numerous group of microorganisms in the compost. Adding urea to composted biomass considerably decreased the number of all groups of microorganisms. The highest reduction in the number of microorganisms was observed in proteolytic bacteria (*ca* 530-fold fewer than in the control) and actinomycetes (whose count decrased by about 136-fold) According to Ma et al. (2003), the efficiency of composting process depends on the composition of biomass subjected to biological transformation and on the metabolic activity of microorganisms. Among the physical parameters, an adequate reduction of the composted biomass volume and its aeration are essential for the right course of composting. According to Piotrowska-Cyplik et al. (2008), a better indicator in an assessment of the biological activity of composted material is their enzymatic activity. These authors claim that the above measure allows for the explanation of the mechanisms and dynamics of the composting process.

CONCLUSIONS

1. The total content of cadmium, zinc, copper and lead determined in the analyzed composts does not pose a threat to the purity of the soil environment. The loss of organic matter, the chemical properties of elements

and the type of the added substances, mainly urea, affected the content of their water-soluble forms and forms bound to organic matter.

2. The analysis of the fractional composition of humic compounds of the composts revealed varied effects of the added substances on the content of extracted carbon and on the value of humic acid to fulvic acid carbon ratio. Markedly higher values were determined for the Ckh:Ckf ratio in the composts with edible oil and urea, which may indicate a more advanced decomposition of the composted material.

3. Out of the the analyzed microorganisms, bacteria were the most numerous in the composts. The added substances (oil, starch and urea) modified the quantitative composition of the microflora in the composts.

4. By adding 5% urea to biomass in order to improve the C:N ratio, mineralization is accelerated, which in turn disturbs the microbiological process, causing undesirable changes in quality of the composts.

REFERENCES

Atlas R.M., Parks L.C. 1997. *Handbook of microbiological media*. CRC Press, Boca Raton.

Baran S., Turski R. 1996. *Specialist exercises on waste and sewage disposal*. Wyd. AR w Lublinie. (in Polish)

Bowszys T., Wierzbowska J., Bowszys J., Bieniek A. 2009. *Modification in the content of available zinc and copper in soil fertilized with bio-waste compost.* J. Elem., 14(1), 33-42.

Czekała J. 2006. *Copper and manganese chemical bounds in a sewage sludge composted with straw in a bioreactor under conditions of differentiated air supply.* Pol. J. Environ. Stud., 15(a): 30-35.

Dimambro M.E., Lillywhite R.D., Ran C.R. 2007. *The physical, chemical and microbial characteristics of biodegradable municipal waste derived composts*. Compost Sci. Utilizat. 15(4): 243-252.

Drozd J., Licznar M. 2004*a*. *Transformation of organic matter during composting of municipal waste in different humidity conditions and at different urea addition.* In: *Composts from municipal waste production, utilization and impact on the environment.* Drozd J. (Ed.). Wyd. PTSH, 130-140. (in Polish)

Drozd J., Licznar M. 2004*b*. *Changes in macro-and micronutrients during the composting of municipal waste in different humidity conditions and at different urea addition.* In: *Composts from municipal waste production, utilization and impact on the environment*. Drozd J. (Ed.). Wyd. PTSH, 151-170. (in Polish)

Drozd J., Licznar M., Patorczyk-Pytlik B., Rabikowska B. 1996. *Changes in chemical composition of municipal waste composts during the composting.* Zesz. Probl. Post. Nauk Rol., 437: 131-138. (in Polish)

Garcia C., Moreno L.J., Hernandez T., Costa F., Polo A. 1995. *Effect of composting on sewage sludges contaminated with heavy metals.* Biores. Technol., 53(1): 13-19.

Gondek K. 2006. *The content of various forms of heavy metals in sewage sludge and composts.* Acta Agrophys., 8(4), 825-838.

Gondek K. 2007. *Content of carbon, nitrogen and selected heavy metals in compost*. J. Elem., 12(1): 13-23.

Himanen M., Hanninen K. 2011. *Composting of bio-waste, aerobic and anaerobic sludges – Effect of feedstock on the process and quality of compost*. Biores. Technol., 102(3): 2842-2852.

Horiuchi J.I., Ebie K., Tada K., Kobayashi M., Kanno T. 2003. *Simplifield method for estimation of microbial activity in compost by ATP analysis*. Biores. Technol., 86: 95-98.

Hsu J.H., Lo S.L. 2001. *Effect of composting on characterization and leaching of copper, manganese and zinc from swine manure.* Environ. Pollut., 114(1): 119-127.

Huang D., Zeng G., Feng Ch., Hu S., Lai C., Zhao M., Su F., Tang L., Liu H. 2010. C*hanges of microbial population structure related to lignin degradation during lignocellulosic waste composting.* Biores. Technol., 101(11): 4062-4067.

Huang G.F., Wu T.Q., Wong J.W.C., Nagar B.B. 2006. *Transformation of organic matter during co-composting of pig manure with sawdust*. Biores. Technol., 97(15): 1834-1842.

Iglesias-Jimenez E., Perez-Garcia V. 1991. *Composting of domestic refuse and sewage sludge. Part. 1. Evaluation of temperature, pH, C/N ratio and cation exchange capacity*. Res. Conserv. Rec., 6: 45-60.

Inbar Y., Chen Y., Hadar Y. 1990. *Humic substances formed during the composting of organic matter.* Soil Sci. Soc. Am. J., 54: 1316-1323.

Ishii K., Takii S. 2003. *Comparison of microbial communities in four different composting processes as evaluated by denaturing gradient gel electrophoresis analysis*. J. Appl. Microbiol., 95: 109-119.

Jakubus M. 2012. *Phytotoxicity and speciation of copper and nickel in composted sewage sludge*. J. Elem., 17(1): 43-56,. DOI: 10.5601/jelem.2012.17.1.04

Kang J., Zhang Z., Wang J.J. 2011. *Influence of humic substances on bioavailability of Cu and Zn during sewage sludge composting*. Biores. Technol., 102 (17): 8022-8026.

Kulcu R., Yaldiz O. 2004. *Determination of aeration and kinetics of composting some agricultural waste.* Biores. Technol., 93(1): 49-57.

Lazzari L., Sperni L., Berlin P., Pavoni B. 2000. *Correlation between inorganic (heavy metals) and organic (PCBs and PAHs) micropollutant concentrations during sewage sludge composting processes.* Chemosphere, 41: 427-435.

Liu Y., Ma L., Li Y., Zheng L. 2007. *Evolution of heavy metal speciation during the aerobic composting process of sewage sludge.* Chemosphere, 67: 1025-1032.

Ma Y., Hang J.Y., Wong M.H. 2003. *Microbial activity during composting of anthracene contaminated soil.* Chemosphere, 52: 1505-1513.

Nomeda S., Valdas P., Chen S.Y., Lin J.G. 2008. *Variations of metal distribution in sewage sludge composting*. Waste Manage., 28: 1637-1644.

Piotrowska-Cyplik A., Cyplik P., Białas W., Czarnecki Z. 2008. *Wpływ sposobu kompostowania odpadów z przemysłu tytoniowego na wybrane parametry fizyko-chemiczne i enzymatyczne*. Acta Agrophys., 12(2): 487-498.

Raj D., Antil R.S. 2011. *Evaluation of maturity and stability parameters of composts prepared from agro-industrial wastes.* Biores. Technol., 102(3): 2868-2873.

Rosik-Dulewska Cz., Mikszta M. 2004. *Forms of selected heavy metals in composts from municipal waste as an indicator of environmental impact.* In: *Composts from municipal waste production, utilization and impact on the environment.* Drozd J. (Ed.). Wyd. PTSH, 171-185. (in Polish)

Sądej W., Namiotko A. 2010. *Content of zinc in plants fertilized with municipal solid waste and urban green waste composts.* J. Elem., 15(4): 679-692.

Tiquia S.M., Tam N.F.Y., Hodgkiss I.J. 1996. *Microbial activities during composting of spent pig – manure sawdust litter at different moisture contents.* Biores. Technol., 55(3): 201-206.

Wang P., Changa C.M., Watson M.E., Dick W.A., Chen Y., Hoitink H.A.J. 2004. *Maturity indices for composted dairy and pig manures.* Soil Biol. Biochem., 36(5): 767-776.

Wasiak G., Madej M. 2009. *Quality of Polish composts in the light of the criteria of the*

European Union and other countries. Eko Problemy. http://odpady.org.pl/plugins/content/content.php?content.2833 (in Polish)

WONG J.W., SELVAM A. 2006. *Speciation of heavy metals during co-composting of sewage sludge with lime.* Chemosphere, 63(6): 980-986.

ZHANG J., ZENG G., CHEN Y., YU M., YU Z., LI H., YU Y., HUANG H. 2011. *Effect of physico-chemical parameters on the bacterial and fungal communities during agricultural waste composting*. Biores. Technol., 102(3): 2950-2956.

ZORPAS A.A., ARAPOGLOU D., KARLIS P. 2003. *Waste paper and clinoptilolite as a bulking material with dewatered anaerobically stabilized primary sewage sludge (DASPSS) for compost production.* Waste Manage., 23, 27-35.

J. Elem. s. 391–400 DOI: 10.5601/jelem.2014.19.1.637

EFFECT OF SOME METEOROLOGICAL PARAMETERS ON YIELDS AND THE CONTENT OF MACROELEMENTS IN WINTER WHEAT GRAIN

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Abstract

The volume and quality of cereal yields are largely determined by the course of climatic conditions, mainly air temperature and precipitation. The objective of this paper was to determine the impact of meteorological conditions during the spring and summer growing season of winter wheat on grain yields (in 1993-2012) and on the content of nutrients in grain (in 1994-2009). The study was based on a field experiment conducted at the Research Station located in Tomaszkowo near Olsztyn, which belongs to the University of Warmia and Mazury in Olsztyn. The impact of meteorological parameters during the spring and summer season on winter wheat yields and the content of elements was investigated by correlation analysis and multiple regression analysis. The examined weather conditions in the spring and summer did not produce any significant impact on the yields. However, some tendencies were noted, indicating that the average minimum temperature had the highest negative impact and the number of days with precipitation produced the highest positive impact on winter wheat yielding. Among the tested macroelements (N, P, K, Mg, Ca), the analyzed meteorological parameters significantly affected the content of nitrogen and phosphorus in winter wheat grain. The accumulation of nitrogen was positively influenced by precipitation, yet significantly negatively affected by the minimum temperature during the spring and summer growing season. The mean daily air temperature during that time had a significant negative impact on the concentration of phosphorus in wheat grain, which was significantly positively raised by the average minimum daily air temperature during the growing season.

Keywords: temperature, precipitation, winter wheat, yielding, macroelements.

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WPŁYW WYBRANYCH ELEMENTÓW METEOROLOGICZNYCH NA PLONOWANIE I ZAWARTOŚĆ MAKROELEMENTÓW W ZIARNIE PSZENICY OZIMEJ

Abstrakt

Wielkość i jakość plonu zbóż w znacznym stopniu zależą od przebiegu warunków pogodowych, głównie temperatury powietrza i opadów. Celem pracy było określenie wpływu warunków meteorologicznych w okresie wiosenno-letniej wegetacji pszenicy ozimej na plonowanie (1993-2012) i zawartość składników pokarmowych w ziarnie (1994-2009). W pracy wykorzystano wyniki badań polowych w Ośrodku Dydaktyczno-Doświadczalnym Uniwersytetu Warmińsko-Mazurskiego w Olsztynie, położonym w Tomaszkowie k. Olsztyna. Wpływ elementów meteorologicznych na plonowanie pszenicy ozimej i zawartość pierwiastków w okresie wegetacji wiosenno-letniej wyjaśniano, stosując analizę korelacji i regresji wielokrotnej. Badane elementy meteorologiczne w okresie wiosenno-letniej wegetacji pszenicy ozimej nie wywierały istotnego wpływu na plonowanie. Niemniej jednak zarysowały się pewne tendencje, wskazujące, iż średnia temperatura minimalna miała największy ujemny wpływ na wydajność pszenicy ozimej, a liczba dni z opadem największy wpływ dodatni. Spośród badanych makroelementów (N, P, K, Mg, Ca) w ziarnie pszenicy ozimej, analizowane elementy meteorologiczne wywierały istotny wpływ jedynie na zawartość azotu i fosforu. Na gromadzenie azotu istotnie dodatnio oddziaływała suma opadów, a istotnie ujemny wpływ miała temperatura minimalna w okresie wegetacji wiosenno-letniej. Na zawartość fosforu w ziarnie pszenicy istotnie ujemny wpływ miała średnia dobowa temperatura powietrza w okresie wegetacji wiosenno letniej, a istotnie dodatni – średnia dobowa temperatura minimalna powietrza w okresie wegetacji.

Słowa kluczowe: temperatura, opady, pszenica ozima, plony, makroelementy.

INTRODUCTION

The climatic conditions in Poland are a relatively unstable factor in cereal cultivation and weather anomalies limit the volume and quality of yielding (PISULEWSKA et al. 1998, JACZEWSKA-KALICKA 2008, KRASKA, PAŁYS 2009, ORZECH et al. 2009). The deficiency or excess of water modifies the growth of plants and the development of yield components, which directly determines the volume of yields (JACZEWSKA-KALICKA 2008). It is claimed that winter wheat yields depend strongly on meteorological conditions, mainly available water. This cereal is extremely sensitive to water shortage from the shooting till flowering (the so-called “critical period”). The role of air temperature during the spring development of what is less important (ORZECH et al. 2009). Some authors believe that in June and in the first half of July relatively cold weather with sufficient precipitation are beneficial (JACZEWSKA-KALICKA 2008). There are reports on an ambiguous impact of temperature, especially higher temperatures from the head emergence to hard dough stage (DEPUTAT, MARCINKOWSKA 1999, JACZEWSKA-KALICKA 2008, RYMUZA et al. 2007, ORZECH et al. 2009). Economically speaking, yield volume is important but other factors, like yield quality, including the content of minerals in grain, must also be considered. In an animal body, minerals ensure a proper course of different metabolic processes, and their role gains importance when mineral deficiencies occur. Feeds rich in minerals are the simplest way to replenish lacking minerals (STANKIEWICZ et al. 2003). Wheat grain may serve as a natural source of mineral compounds, whose concentrations are determined by numerous factors, such as a plant species, cultivar, soil and weather conditions and agricultural procedures, including fertilization and herbicide application, which in extreme cases may deteriorate the nutritional value of yields (DUCSAY, LOŽEK 2004, MAKARSKA et al. 2010). The content in minerals in grain and investigations into this subject are therefore essential. Presumably, the meteorological conditions have a strong impact on the volume and quality of winter wheat yields. This study has been carried out to verify such assumptions, and to determine the impact of the weather during the spring and summer growing season of winter wheat on the yields and content of nutrients in grain.

MATERIAL AND METHODS

This paper is based on the results of field studies on winter wheat carried out in 1993-2012, at the Research Station in Tomaszkowo near Olsztyn, which belongs to the University of Warmia and Mazury in Olsztyn. They were controlled field experiments set up on medium and heavy typical brown soil classified as good wheat complex. A series of multiannual experiments of with fertilization as well as fertilization and plant protection treatments were carried out in a randomized sub-block design with 4 replications, including dynamic arrangement and change of cultivars, which took place every few years by selecting the then recommended varieties for cultivation in Warmia and Mazury, north-eastern Poland (Almari 1993-1999, Elena 2000-2003, Rysa 2004-2006, Bogatka 2007-2009, Türkis 2010-2012). This paper reports on the grain yields from standard objects with medium nitrogen (app. 130 kg N ha^{-1}), phosphorus (P – app. 35 kg ha^{-1}) and potassium (K – app. 90 kg ha^{-1}) fertilization and application of herbicides against dicotyledonous weeds and fungicides. During the combine harvesting, samples of grain were collected for analyses of macroelements, such as N, P, K, Mg and Ca. The analyses were performed at the Chemical and Agricultural Station in Olsztyn. The following methods were applied: potentiometry (N), vanadium-molybdenum method (P), atomic absorption spectrometry (Mg) and flame photometry (K and Ca).

The spring growth was assumed to begin after the average daily temperature reached ≥5.0°C on five consecutive days. The beginning of this thermal period overlaps with the commencement of the wheat physiological growth. The following independent variables were used to determine the impact of meteorological conditions during the spring and summer growing season of winter wheat on the volume of yields (Y): x_1 – number of vegetative days, x_2 – average daily temperature, x_3 – average minimum daily temperature,

x_4 – volume of precipitation, and x_5 – number of days with precipitation. The values of these meteorological parameters were also used to investigate their impact on the content of N, P, K, Mg, and Ca in grain as Y_1, Y_2, Y_3, Y_4, and Y_5 in 1994-2009, respectively.

The results were evaluated with correlation and linear regression analyses. The calculations were performed with the help of a Statistica software package.

RESULTS AND DISCUSSION

During the twenty years of the field experiment, the weather conditions were highly variable (Table 1). Likewise, the wheat yields harvested in

Table 1

Selected meteorological parameters in the spring and summer growing season of winter wheat

Year	Number of vegetation days	Average daily temperature (°C)	Average minimum daily temperature (°C)	Volume of precipitation (mm)	Number of days with precipitation
	x_1	x_2	x_3	x_4	x_5
1993	99	15.4	9.0	245	43
1994	114	14.2	7.5	117	31
1995	97	14.9	9.0	202	44
1996	124	13.5	7.6	204	54
1997	104	15.2	1.,0	333	50
1998	126	14.2	9.1	283	62
1999	117	13.4	7.6	358	55
2000	106	14.8	8.1	188	49
2001	120	13.6	9.1	114	48
2002	113	14.6	8.8	205	52
2003	107	15.6	9.3	207	33
2004	140	11.8	7.5	356	75
2005	113	13.2	7.2	140	36
2006	114	13.4	8.1	207	38
2007	129	13.7	8.3	375	54
2008	119	15.2	8.4	159	49
2009	115	14.1	8.1	243	40
2010	131	14.3	9.2	319	59
2011	118	14.2	9.5	342	49
2012	96	15.2	10.5	294	48
Average	115	14.2	8.6	245	48

Table 2

Yields and the content of selected macroelements in winter wheat grain

Year	Yield ($t\ ha^{-1}$)	Macroelements ($g\ kg^{-1}$ d.m.)				
		N	P	K	Mg	Ca
1993	5.45	-	-	-	-	-
1994	5.85	20.6	3.9	4.3	1.1	0.5
1995	6.38	18.6	3.6	4.4	1.1	0.4
1996	7.34	16.9	4.1	4.6	1.1	0.4
1997	5.61	19.1	3.8	3.7	1.3	0.4
1998	6.33	17.7	4.2	3.6	1.0	0.4
1999	5.65	22.4	4.8	5.3	1.5	0.9
2000	6.40	20.1	4.0	5.5	1.4	0.6
2001	4.11	16.5	3.6	4.7	1.1	0.4
2002	7.39	19.5	3.8	4.2	1.1	0.4
2003	7.27	17.8	3.4	4.4	1.2	0.7
2004	9.04	18.2	4.2	4.0	1.3	0.6
2005	6.13	17.7	3.8	4.2	1.3	0.4
2006	6.84	19.6	4.1	4.6	1.3	0.5
2007	5.68	20.7	4.1	4.5	1.3	0.5
2008	7.42	17.8	3.7	3.8	1.3	0.6
2009	7.68	20.7	4.1	3.8	1.3	0.4
2010	6.80	-	-	-	-	-
2011	5.49	-	-	-	-	-
2012	6.56	-	-	-	-	-
Average	6.47	19.0	4.0	4.4	1.2	0.5

this period varied from year to year (Table 2). The average grain yield was 6.47 $t\ ha^{-1}$, ranging from 4.11 $t\ ha^{-1}$ in 2001 to 9.04 $t\ ha^{-1}$ in 2004. In 2004, the spring and summer growing season was the longest (140 days), the precipitation was high (356 mm) and frequent (75 days with precipitation, without torrential rains) and the average daily temperature was relatively low (11.8°C). The lowest yield (4.11 $t\ ha^{-1}$) was recorded in 2001, a year with a relatively high average daily temperature (13.6°C) and low precipitation (114 mm) occurring in just 48 days.

The average content of macroelements in wheat grain ($g\ kg^{-1}$ d.m.) was as follows: nitrogen 19.0, phosphorus 4.0, potassium 4.4, magnesium 1.2 and calcium 0.5 (Table 2). The grain harvested in 1999 was the richest in mineral compounds (the average yield of 5.65 $t\ ha^{-1}$), containing nitrogen at 22.4 $g\ kg^{-1}$ d.m., phosphorus at 4.8 $g\ kg^{-1}$ d.m., potassium at 5.3 $g\ kg^{-1}$ d.m., magnesium at 1.5 $g\ kg^{-1}$ d.m., and calcium at 0.9 $g\ kg^{-1}$ d.m.

The correlation and linear regression analyses indicate absence of any significant impact of the examined meteorological parameters on wheat

Table 3

Linear correlation coefficients (r) between the selected meteorological parameters (x_1, x_2, x_3, x_4, x_5) and grain yield and content of macroelements in winter wheat grain

Parameter	Number of vegetation days	Average daily temperature (°C)	Average minimum daily temperature (°C)	Amount of precipitation (mm)	Number of days with precipitation
	x_1	x_2	x_3	x_4	x_5
Grain yield	0.303	-0.212	-0.280	0.103	0.296
N	-0.136	-0.010	-0.260	0.419	-0.102
P	0.461*	-0.564**	0.510**	0.603**	0.477^{x}
K	-0.183	-0.109	-0.314	-0.082	-0.095
Mg	-0.017	-0.166	-0.334	0.402	0.050
Ca	0.084	-0.038	-0.249	0.321	0.102

* $p< 0.10$, ** $p<0.05$

yields (Tables 3, 4). However, some tendencies were observed, such as the highest negative impact of the average minimum daily temperature (b = -0.475) and the highest positive impact of the number of days with precipitation (b = 0.380) during the spring and summer plant growing season.

The effect of the analyzed random variables (x_1, x_2, x_3, x_4, x_5) which define the relationships between the examined meteorological parameters and the content of macroelements (N, P, K, Mg, Ca) in wheat grain is described by linear correlation coefficients and multiple regression equations (Tables 3, 4). The equations demonstrate that a significant degree of the explanation of these regression models was achieved for nitrogen and phosphorus. Among the examined meteorological parameters, standardized regression coefficients indicate that the volume of precipitation (x_4) had a significant positive impact while the average minimum daily temperature in the growing season (x_3) had a significant negative impact on the concentration of nitrogen in wheat grain dry matter. Regarding phosphorus, its accumulation in grain was significantly negatively influenced by the average daily temperature in the spring and summer x_2 (b'= -0.596**, r = -0.564**), and significantly positively affected by the average minimum daily temperature x_3 (b' = 0.592**, r = 0.510**). Moreover, the concentration of phosphorus in wheat grain correlated significantly and positively with the amount of precipitation in the spring and summer growing season (x_4), with the number of vegetative days (x_1) and number of days with precipitation (x_5). It means that as the values of these parameters went up, so did the concentration of phosphorus in grain dry matter. The estimated linear regression equations for phosphorus (Y_2) are as follows: $Y_2 = 0.0144x_1 + 2.2727$; $Y_2 = -0.1926x_2 + 6.6627$; $Y_2 = -0.2109x_3 + 5.7123$; $Y_2 = 0.0023x_4 + 3.4185$; $Y_2 = 0.0139x_5 + 3.2808$.

The calculated multiple regression equations for the other macroelements (potassium, magnesium and calcium) indicate insignificant effects of the analyzed meteorological conditions on the content of these elements in wheat grain (Table 4). However, the amount of rainfall during the spring and summer vegetation season (x_4) had a significant positive impact on the content of magnesium, whereas the average minimum daily temperature (x_3) affected it negatively. Nevertheless, as the standardized regression coefficients (b') for the average minimum daily temperature and the amount of precipitation indicate that the said effects were similar in magnitude but

Table 4

Total impact of the meteorological parameters (x_1, x_2, x_3, x_4, x_5) on yields and the content of macroelements in winter wheat grain

Parameter	Number of vegetation days	Average daily temperature (°C)	Average minimum daily temperature (°C)	Amount of precipitation (mm)	Number of days with precipitation
	x_1	x_2	x_3	x_4	x_5
Grain yield	$Y = 2.716 + 0.009x_1 + 0.392x_2 - 0.564x_3 + 0.0002x_4 + 0.039x_5$; $R^2 = 0.205$				
Standardized regression coefficient (b')	0.101	0.344	-0.475	0.019	0.380
N (g kg^{-1} d.m.)	$Y = 27.493 - 0.048x_1 + 0.482x_2 - 1.344x_3 + 0.017x_4 - 0.049x_5$; $R^2 = 0.428$*				
Standardized regression coefficient (b')	-0.309	0.286	-0.658**	0.892**	-0.343
P (g kg^{-1} d.m.)	$Y = 6.008 - 0.005x_1 - 0.247x_2 + 0.002x_3 - 0.014x_4 + 0.005x_5$; $R^2 = 0.543$**				
Standardized regression coefficient (b')	-0.157	-0.596**	0.592**	-0.040	0.177
K (g kg^{-1} d.m.)	$Y = 11.567 - 0.034x_1 - 0.078x_2 - 0.330x_3 - 0.0001x_4 + 0.014x_5$; $R^2 = 0.267$				
Standardized regression coefficient (b')	-0.678	-0.142	-0.492	-0.0005	0.287
Mg (g kg^{-1} d.m.)	$Y = 2.322 - 0.006x_1 - 0.016x_2 - 0.109x_3 + 0.001x_4 - 0.001x_5$; $R^2 = 0.215$				
Standardized regression coefficient (b')	-0.410	0.114	-0.638*	0.695**	-0.077
Ca (g kg^{-1} d.m.)	$Y = 0.112 + 0.0002x_1 + 0.082x_2 - 0.119x_3 + 0.001x_4 - 0.0001x_5$; $R^2 = 0.310$				
Standardized regression coefficient (b')	0.013	0.553	-0.656	0.516	0.010

* $p < 0.10$, ** $p < 0.05$

Table 5

Linear regression coefficients between the yield (Y) of winter wheat and the selected meorological parameters (x_1, x_2, x_3, x_4, x_5) during the spring and summer vegetative season

Relation of variables	Evaluation of correlation	Number of vegetation days	Average temperature (°C)	Average minimum temperature (°C)	Amount of precipitation (mm)	Number of days with precipitation
		x_1	x_2	x_3	x_4	x_5
Yield (t ha^{-1})	r	0.303	-0.212	-0.280	0.103	0.296
	p	0.194	0.369	0.232	0.666	0.205

opposite (-0.695 and 0.638), the final degree of explanation of the regression model is insignificant.

Numerous authors claim that the course of meteorological conditions in Poland substantially affects the volume and quality of cereal yields (Jaczewska-Kalicka 2008, Orzech et al. 2009, Rachoń, Szumiło 2009, Nogalska et al. 2012, Gaj et al. 2013). In particular, a severe shortage or excess of precipitation as well as highly fluctuating temperatures pose a serious risk to cereal yielding (Mizak et al. 2011). Studies on the response of cereals to the weather conditions during the plant growing season often show that rainfall has had a stronger impact than temperature on the growth and yielding of plants. Koziara (1996) explained it by the higher variability of precipitations than temperature in subsequent years. In addition, according to this author, the low retention capacity of soil explains why the amount and distribution of rainfalls have a decisive impact on the volume of yields. Any precise description of the impact of the weather conditions on yield levels and quality is difficult because of other factors involved, e.g. the type of soil, soil tillage, crop cultivar, fertilization, plant protection treatments and other agronomic factors, whose yield stimulating effects also depend on the course of weather (Orzech et al. 2009). In the research present herein, the average minimum daily temperature in the spring and summer vegetative season had the highest negative impact on the volume of wheat yield. At a higher number of days with precipitation and an average daily temperature during the growing season, the yields of wheat increased. However, these effects were not significant. It may be assumed that, like in the study on winter triticale reported by Rymuza et al. (2012), the grain yield mainly depended on the meteorological conditions during the autumn vegetative and winter resting period. As reported by Jaczewska-Kalicka (2008), the warm and sunny weather is the best at the shooting stage, while moderate temperature and good insolation are favourable at the kernek fillig stage.

Many authors have emphasized the substantial impact of habitat condition, including meteorological conditions, on the chemical composition of grain (Pisulewska et al. 1998, Jaczewska-Kalicka 2008, Kraska, Pałys 2009, Orzech et al. 2009). Pisulewska et al. (1998) believe that the course of meteorological conditions during the growing season ofor cereals interacts with nitrogen fertilization and modifies the content of potassium, calcium and magnesium in winter wheat and winter triticale grain. In the analyzed experiment, the concentration of nitrogen in wheat grain increased in response to higher precipitation in the spring and summer, but decreased together with an increase in the average minimum daily temperature. The content of phosphorus declined together with an increase in the average daily temperature in the spring and summer season, whereas the concentration of potassium decreased under higher average minimum daily temperature. The volume of precipitation in the vegetative season exerted a positive impact on the accumulation of magnesium, while the accumulation of calcium was stimulated by a higher average daily temperature. In the study by KRASKI and PAŁYS (2009) conducted near Lublin, the content of total protein, potassium and magnesium in winter wheat grain was the lowest in 2004 (a cold year) and the concentrations of phosphorus and calcium were the lowest in 2003 (the warmest year).

CONCLUSIONS

1. The examined meteorological conditions during the spring and summer growing season of winter wheat did not exert any significant impact on yielding. However, several results indicated that the average minimum daily temperature had the highest negative impact while the number of days with precipitation had the highest positive impact on winter wheat yields. .

2. The analyzed meteorological parameters had a significant impact on the content of nitrogen and phosphorus, but not on the other analyzed elements (K, Mg, Ca) in winter wheat grain.

3. The accumulation of nitrogen in grain was significantly and positively influenced by the amount of precipitation, while being significantly and negatively affected by the average minimum daily temperature in the spring and summer vegetative season.

4. The concentration of phosphorus in grain was significantly and negatively affected by the average daily temperature in the spring and summer. There was a significant positive impact of the average minimum daily temperature during the plant growing season on this parameter.

REFERENCES

DEPUTAT T., MARCINKOWSKA I. 1999. *Thermal requirements of winter wheat.* Pam. Puł., 118: 87-98. (in Polish)

DUCSAY, L., LOŽEK, O. 2004. *Effect of topdressing with nitrogen on the yield and quality of winter wheat grain.* Plant Soil Environ., 50 (7): 309-314.

GAJ R., GÓRSKI D., PRZYBYŁ J. 2013. *Effect of differentiated phosphorus and potassium fertilization on winter wheat yield and quality.* J. Elem., 18(1): 55-67. DOI: 10.5601/jelem.2013.18.1.04

Jaczewska-Kalicka. 2008. Influence of climate changes on yielding and cereal protection in Poland. Prog. Plant Prot., 48(2): 415-425. (in Polish)

Koziara W. 1996. *Growth, development and yielding of spring and winter triticale depending on meteorological and agricultural factors.* Rocz. AR Poznań, Rozpr. Nauk., 269, 98 pp. (in Polish)

Kraska P., Pałys E. 2009. *Yielding and chemical composition of winter wheat grain cultivated in monoculture in conditions of differentiated doses of herbicides application*. Prog. Plant Prot., 49(1): 441-444. (in Polish)

Makarska E., Ciołek A., Kociuba W. 2010. *Influence of parental forms on changes in the content of mineral elements in grain of new winter triticale hybrid strains.* J. Elem., 15(1): 131-140. DOI: 105601/jelem.2010.15.1

Mizak K., Pudełko R., Kozyra J., Nieróbca A., Doroszewski A., Świtaj Ł., Łopatka A. 2011. *Results of monitoring agriculatural drought in winter wheat crops in Poland in the years 2008-2010.* Water-Environment-Rural Areas, 11(2): 95-107. (in Polish)

Nogalska A., Sienkiewicz S., Czapla J., Skwierawska M. 2012. *The effect of multi-component fertilizers on the yield and mineral composition of winter wheat and macronutrient uptake*. J. Elem., 17(4): 629-638. DOI: 10.5601/jelem.2012.17.4.06

Orzech K., Marks M., Dragańska E., Stępień A. 2009. *Yields of winter wheat in relation to weather conditions and different methods of cultivation of average soil* Ann. UMCS, Lublin, 64(4): 122-129.

Pisulewska E., Zając T., Oleksy A. 1998. *Mineral composition of grain of winter triticale cultivars as affected by increasing nitrogen fertilization.* Biul. IHAR, 205/206: 179-188. (in Polish)

Rachoń L., Szumiło G. 2009. *Comparison of chemical composition of selected winter wheat species.* J. Elem., 14(1): 135-146.

Rymuza K., Marciniuk-Kluska, Bombik A. 2012. *Yielding of winter cereals in relation to thermal and precipitation conditions on Productive Fields in the Zawady Experimental Farm.* Water-Environment-Rural Areas, 12(2): 207-220. (in Polish)

Stankiewicz Cz., Gruszecka D., Steć E., Mitrus J., Walo P., Kamecki M. 2003. *Effect of interaction of parental components on the content of protein and amino acids in triticale grain.* EJPAU, 6(2), #06. http://www.ejpau.media.pl/volume6/issue2/agronomy. art-06.html

J. Elem. s. 401–422 DOI: 10.5601/jelem.2014.19.1.447

DETERMINANTS OF THE DIVERSITY OF MACROPHYTES IN NATURAL LAKES AFFECTED BY LAND USE IN THE CATCHMENT, WATER CHEMISTRY AND MORPHOMETRY LAKES

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Abstract

This study investigated 14 lakes situated in the Borecka Forest, and the analyzed data covered a period of 10 years. The examined water bodies are at low risk of eutrophication. Three factors explaining 79.1% of total variability in the analyzed natural lakes were identified: 1 – morphometry – use of catchment area, 2 – water chemistry, 3 – lake/catchment area. Those factors determined the patterns of macrophyte distribution in the lakes. The variables applied in the CCA ordination plot explained around 59% of the total variability in plant distribution patterns in the examined lakes. Chlorophyll *a* (chl-*a*) was a statistically significant parameter, which explained 12.4% of the total variability in plant distribution patterns in the analyzed lakes. Morphological, physicochemical and catchment area variables have a significant effect on the development of vegetation in the Natura 2000 sites. The results of this study provide the basis for formulating general management guidelines for the investigated lakes and their catchments, which belong to the Natura 2000 ecological network of protected areas. A comprehensive protection plan should be proposed for interdependent habitats. Forest cover in the lakes' catchment areas should be maintained or expanded, and the share of intensively farmed land should be reduced. Clear cutting in areas adjacent to the lakes should be prevented, and the inflow of biogenic elements, nitrogen and phosphorus (which affects Chl-*a* concentrations) should be reduced. The above goals can be achieved, among others, by preserving the existing water relations and species composition of tree stands in the lakes' catchments areas.

Keywords: catchment, CCA, macrophytes, Natura 2000 sites, spatial structure.

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UWARUNKOWANIA RÓŻNORODNOŚCI MAKROFITÓW W JEZIORACH NATURALNYCH POD WPŁYWEM ZAGOSPODAROWANIA TERENU ZLEWNI, CHEMII WODY I MORFOMETRII JEZIOR

Abstrakt

Obiektem badań było 14 jezior usytuowanych na terenie Puszczy Boreckiej. Dane do analiz obejmowały okres 10-letni. Jeziora te należą do zbiorników słabo zagrożonych eutrofizacją. Ustalono 3 czynniki wyjaśniające 79,1% całkowitej zmienności badanych jezior naturalnych. Są to: czynnik 1 – morfometria jeziora/użytkowanie zlewni, czynnik 2 – chemia wody, czynnik 3 – powierzchnia zlewni/jeziora. Czynniki te wpływają na wzorzec rozmieszczenia roślinności w badanych jeziorach. Zastosowane w ordynacji CCA zmienne tłumaczą ok. 59% ogólnej zmienności wzorca rozmieszczenia roślinności badanych jezior. Ustalono, że parametrem istotnym statystycznie jest koncentracja chlorofilu *a*, co tłumaczy 12,4% ogólnej zmienności wzorca rozmieszczenia roślinności w badanych jeziorach. Zróżnicowanie morfologiczne zbiorników, cech chemicznych oraz parametrów zlewniowych jezior ma decydujący wpływ na wykształcanie się poszczególnych typów roślinności, charakteryzujących siedliska Natura 2000. Na podstawie badań możliwe jest sformułowanie generalnych zaleceń, które mimo różnych charakterystyk badanych jezior są podobne. Zarządzanie siedliskami Natura 2000, jakimi są badane jeziora, wymaga właściwego zarządzania na poziomie obszaru zlewni. Właściwym działaniem jest objęcie ochroną całościową wzajemnie współzależnych siedlisk. Korzystne dla utrzymania i zachowania siedlisk Natura 2000 obejmujących badane jeziora jest zwiększanie lub utrzymanie na istniejącym poziomie powierzchni leśnych w zlewni jezior i ograniczanie intensywnego rolniczego użytkowania zlewni jezior. Wskazane jest zapobieżenie całkowitym wyrębom drzewostanu ze stref przyległych do zbiorników, ograniczenie dopływu pierwiastków biogennych: azotu i fosforu, co wpłynie na wielkość koncentracji chlorofilu *a*. Można to osiągnąć, m.in. utrzymując w zlewni istniejące stosunki wodne oraz zachowując zgodny z siedliskiem skład gatunkowy drzewostanów na obszarze zlewni tych jezior.

Słowa kluczowe: zlewnia, CCA, makrofity, Sieć Natura 2000, struktura przestrzenna.

INTRODUCTION

Similarly to most sessile organisms, macrophytes show a long-term response to changes in environmental conditions (NURMINEN 2003, OBOLEWSKI et al. 2009, NAPIÓRKOWSKA-KRZEBIETKE et al. 2012). The discriminating power of macrophytes along lake size and depth gradients is complex (MURPHY 2002, NURMINEN 2003), and the occurrence of species is more likely determined by biological and stochastic factors than by simple environmental determinism (SØNDERGAARD et al. 2010). The main environmental factors affecting macrophyte abundance in lakes are general water chemistry (SPENCE 1982, JEPPESEN et al. 1994, 2000, GLIŃSKA-LEWCZUK 2009, GLIŃSKA-LEWCZUK, BURANDT 2011, GRZYBOWSKI et al. 2010*a*,*b*), the trophic status of a lake (SPENCE 1982, KOLADA 2010) and light availability (CANFIELD et al. 1985). Macrophytes affect the physical, chemical and biological parameters of lakes, and they reflect the impact of various environmental factors such as lake morphometry, water chemistry and biotic interactions (CHERUVELIL, SORANNO 2008, KOLADA 2010). The structure of aquatic vegetation can be used to determine the diversity of flora and lake habitats (CIECIERSKA 2006) and, above all, the factors that change with the depth gradient (MURPHY 2002). The combined, long-term effects of those factors diversify aquatic vegetation along the environmental gradient (SPENCE 1982, BANAŚ et al. 2012).

The objectives of this study were to: (a) determine the factors that affect macrophyte distribution in natural lakes, (b) identify the factors that affect the patterns of plant distribution in the analyzed lakes. The research hypothesis was as follows: macrophyte distribution patterns in natural lakes are determined by lake-type-specific variables. Another aim of the study was to propose management recommendations for lakes which are protected sites included in the Natura 2000 network.

MATERIALS AND METHODS

The analyzed site is situated in the Borecka Forest (geographic coordinates 54°07′31″N; 22°08′54″E) in the north-eastern part of the Masurian Lakeland. This large forest complex occupies a total area of 25 340.1 ha, and it is characterized by low anthropogenic pressure. Agriculture and forestry represent the main types of human activity in the investigated area. Tourism pressure is low, and there are no industrial plants and cities in the vicinity. All of the 14 analyzed lakes (Figure 1, Table 1) are protected sites

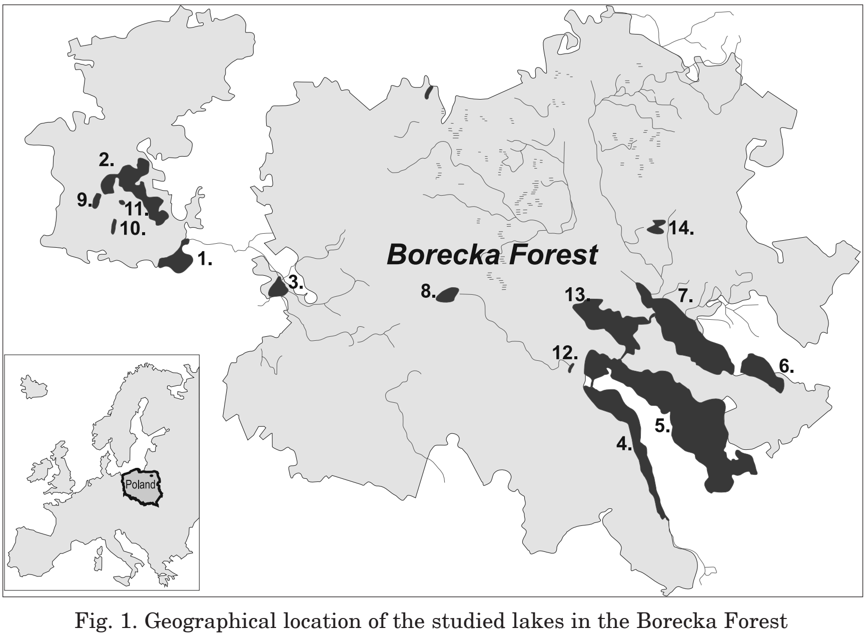

Fig. 1. Geographical location of the studied lakes in the Borecka Forest

Lake types are marked with numbers; stratified lakes: 1 – Zabinki, 2 – Krzywa Kuta, 3 – Lekuk, 4 – Litygajno, 5 – Lazno, 6 – Szwalk Maly, 7 – Szwalk Wielki, 8 – Wolisko; non-stratified lakes: 9 – Biala Kuta, 10 – Smolak, 11 – Kacze, 12 – Dubinek, 13 – Pilwag, 14 – Ciche

Table 1

Characteristic features of the analyzed lakes and their catchments

Lake	Morphometry					Water quality indicators								Catchment				
	no of tran-sect	sur-face area (km^2)	mean depth (m)	max depth (m)	SLD	SD (m)	pH	TP ($mg\ dm^{-3}$)	TN ($mg\ dm^{-3}$)	Chl-*a* ($\mu g\ dm^{-3}$)	Ca ($mg\ dm^{-3}$)	TOC ($mg\ dm^{-3}$)	cond. ($\mu S\ cm^{-1}$)	catch (km^2)	forests (%)	mea-dows (%)	water (%)	agri-cultu-ral (%)
Biała Kuta	4	0.266	1.4	3.2	1.48	1.4	9	0.024	1.32	0.06	27.85	15.83	188	1.53	79.4	3.2	17.40	
Smolak	3	0.063	1.7	5.1	1.34	0.5	7.66	0.026	1.39	7.7	2.87	15.76	27	0.93	93.2		6.80	
Kacze	2	0.018	0.3	0.8	1.07	0.4	6.8	0.032	1.72	3.74	5.71	57.31	40	0.22	91.9		8.10	
Żabinki	5	0.385	9.1	42.5	1.26	3.2	7.93	0.022	0.87	9.41	60.69	13.22	380	24.29	23.2	12.6	1.60	62.6
Dubinek	2	0.182	2.5	6.2	1.10	0.4	7.55	0.022	2.044	4.79	32.13	11.82	204	1.11	83	0.6	16.40	
Pilwąg	14	1.423	1.5	3.6	1.88	0.6	8.05	0.07	2.25	33.5	49.98	18.92	299	9.48	80.79	4.2	15.01	
Litygajno	19	1.668	6	16.4	2.33	1.3	7.78	0.05	1.6	17.2	52.84	12.19	339	16.04	52.3	11.2	10.40	26.1
Łaźno	24	5.712	5.7	18	2.10	4.1	7.75	0.058	1.4	26.7	54.98	12.46	350	142.63	56.2	6.2	4,00	33.6
Szwałk Mały	9	0.711	4.3	6.7	1.64	0.8	7.66	0.043	2.15	23.7	45.7	12.29	302	9.67	56	2.2	7.30	34.5
Szwałk Wielki	15	2.356	4.9	11	1.72	1.1	8.02	0.082	1.9	19.1	53.55	15.51	312	25.35	72.4	12.1	9.30	6.2
Łękuk	3	0.220	4.6	12.5	1.14	1.5	8.05	0.056	1.65	18.6	57.12	18.41	326	14.09	84.9	6.2	1.60	7.3
Ciche	4	0.160	1.0	2.6	1.81	0.5	6.83	0.132	2.488	63.85	59.98	17.33	343	0.98	82.5	1.2	16.30	
Wolisko	2	0.182	4.5	8.6	1.18	1.1	7.74	0.03	2.24	33.4	51.41	11.88	305	2.56	38.6	28.2	7.10	26.1
Krzywa Kuta	15	1.352	6.0	26.5	2.07	2.1	8	0.072	1.82	35.62	41.41	12.67	248	4.13	32.1	7.1	32.70	28.1

No of transect – number of transect, SLD – shoreline development, SD – Secchi depth, TP – total phosphorus, TN – total nitrogen, chl *a* – chlorophyll *a* concentration, TOC – total organic carbon, Cond – conductivity, catch – surface area of catchment of the Natura 2000 network. The studied lakes have a regular shape (ratio of max. length / max. width < 6.0, SLD – shoreline development < 2.3). Their catchment areas are used as farmland and forests (8 lakes) or forests (6 lakes). The lakes were characterized by various water mixing patterns – eight of them were classified as stratified (Zabinki, Krzywa Kuta, Lekuk, Litygajno, Lazno, Szwalk Maly, Szwalk Wielki, Wolisko) and six as shallow, non-stratified water bodies (Biala Kuta, Smolak, Kacze, Dubinek, Pilwag, Ciche).

Water samples were collected twice a year during the summer stagnation period (Table 1), beginning at the depth of 1 m. Three samples were collected each time, and the results are the means of three replicates. Water transparency was measured with the Secchi disc. Physical and chemical analyses of water samples were performed by standard methods (APHA 1999). Total phosphorus, nitrogen and iron content was determined colometrically using the Shimadzu UV 1601 spectrophotometer. Chlorophyll was analyzed by the acetone extraction method (GOLTERMAN 1969) and corrected for pheophytin. Total organic carbon (TOC) concentrations were determined by high-temperature combustion (HTC) using the Schimadzu TOC 5000 analyzer (DUNALSKA 2009). pH and conductivity were determined in situ using the YSI 6600-meter (Yellow Spring Instruments USA).

Aquatic vegetation was investigated in 2002-2012. Field studies were carried out at the peak of every growing season (from the second half of June to the end of August) over the ten-year period, in all lakes. Vegetation was examined from boats along shorelines using a scaled rope with a grapnel end. The surveyed objects (plant communities, species) were marked on bathymetric maps with the use of a GPS navigation device (Garmin 60CSx). The experiment involved inventories of plant communities (phytosociological surveys) in the entire phytolittoral zone and mapping of plant colonies in the littoral zone. Plant communities in the investigated lakes (including Characeans, submerged vascular plants, floating-leaved and emergent rush and sedge rush phytocenoses) were fully identified. The following parameters were also determined: maximum colonization depth, total colonized area, percentage share of every identified community in the total phytolittoral area. Plant communities were identified and classified following a phytosociological approach which is widely applied in Polish studies of aquatic ecosystems (BRAUN-BLANQUET 1964). Species abundance was determined based on the BRAUN-BLANQUET (1964) scale which was modified by dividing it into five units, where: 1 = very rare, 2 = rare, 3 = common, 4 = frequent and 5 = abundant. Aquatic vegetation was classified based on the system proposed for Poland by MATUSZKIEWICZ (2002). The phytosociological system of plant community classification was used to identify protected sites of the Natura 2000 network (CID 2011). In line with the International Code of Phytosociological Nomenclature (WEBER et al. 2000), the inventory of plant communities covered: alliance (All.) *Magnocaricion* Koch 1926 – reed beds composed of large sedges, natural or anthropogenic communities of tall marshland plants, mostly species of the genus *Carex*; All. *Phragmition* Koch 1926 – reed beds characterized by relatively poor floristic composition, usually found between amphiphytes of the littoral zone and floating-leaved or submerged plants; class (Cl.) *Charetea* (FUKAREK 1961 n.n.) KRAUSCH 1964 – macrophytes rooted in the bottom of oligotrophic and mesotrophic water bodies, mostly large algae belonging to *Charophyta*; All. *Potamion* KOCH 1926 em. OBERD. 1957 – mostly submerged plants rooted in the bottom of natural water bodies, artificial water reservoirs, stagnant and slow-flowing waters; All. *Nymphaeion* OBERD. 1953 – macrophytes rooted in the bottom of water bodies, mostly floating-leaved plants found in shallow stagnant waters; Cl. *Lemnetea* R.Tx. 1955 – pleustophytes floating on the surface of stagnant and slow-flowing waters, often found in communities of aquatic and rush plants; Cl. *Utricularietea intermedio-minoris* DEN HARTOG et SEGAL 1964 em. PIETSCH 1965 – plant communities in shallow dystrophic and oligotrophic water bodies with a naturally accumulated layer of peat, dominated by species of the genera *Utricularia* and *Sparganium* as well as rusty peat mosses and peat mosses.

Macrophyte diversity was described using the phytocenotic diversity index (H) calculated based on the Shannon-Wiener index modified by CIECIERSKA (2006).

The species composition of macrophytes in the analyzed lakes was studied in transects. Transects were mapped in accordance with the method proposed by Jensen (1977) to ensure that survey results were representative. Transects were uniformly distributed along the shoreline. They had the width of 100-200 m, and their length was determined based on the maximum reach of macrophyte communities.

The catchment area of the studied lakes varied from 0.22 to 142.63 km^2. Catchment areas were classified into the following categories based on the predominant type of land use: forests – forests and seminatural areas (including raised bogs, transition mires and quaking bogs, low peat bogs, etc.); meadows – semi-natural hay meadows, dry grasslands, pastures, etc. (land principally occupied by agriculture, with significant areas of natural vegetation); agricultural – arable land, permanent crops, homogenous agricultural areas, etc.; and aquatic. Catchment groups were mapped and their percentage cover was determined (Figure 2). The areas of catchment groups are given in square kilometers. The percentage share of forests, meadows and aquatic catchments is shown in Table 1 (square kilometers were used in analyses).

Statistical analysis

Data were analyzed using factor analysis and nonparametric Spearman's correlation analysis in the Statistica 10 package (StatSoft Inc. 2011). A factor analysis of 15 environmental indices (morphological, physicochemi-

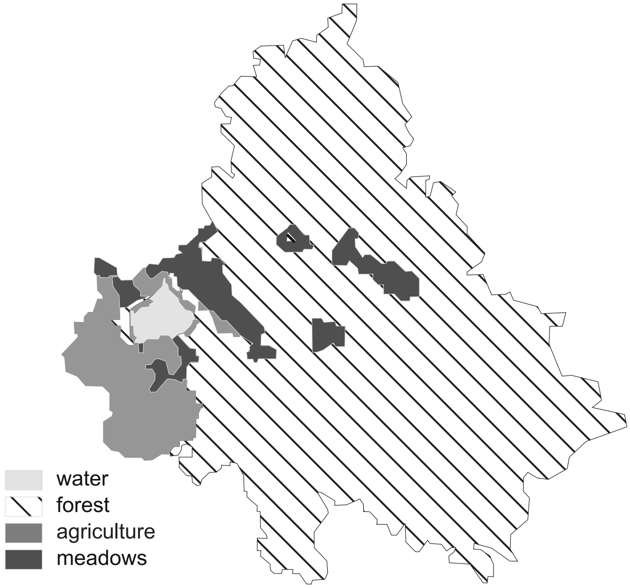

Fig. 2. Land use categories in the catchment area of Lake Leluk

Catchment areas were classified into the following categories: 84.9% – forests (forests and seminatural areas, including raised bogs, transition mires and quaking bogs, low peat bogs, etc.); 6.2% – meadows (semi-natural hay meadows, dry grasslands, pastures, i.e. land principally occupied by agriculture, with significant areas of natural vegetation); 7.3% – agricultural (arable land, permanent crops, homogenous agricultural areas, etc.) and 1.6% – aquatic. Catchment groups were mapped and their percentage cover was determined

cal and catchment area variables) was performed using the 'Varimax-normalized' rotation method. Only statistically significant values were used in further analyses. A preliminary detrended correspondence analysis (DCA) revealed the first gradient length of 2.88 SD, indicating that models based on linear species response models were appropriate for the data structure (Lepš, Šmilauer 2007). A canonical correspondence analysis (CCA) was performed to relate macrophyte species composition to environmental variables in CANOCO (ter Braak, Šmilauer 2002). The composition of aquatic macrophytes in lakes was determined based on the percentage share of species which were reported from more than 5% of transects (i.e. from more than 6 out of the 121 analyzed transects). Statistical significance tests were carried out using Monte Carlo permutation tests. A Monte Carlo test was used to examine the significance of axis eigenvalues generated in the analysis and the species-environmental correlation (using 5000 unrestricted iterations).

RESULTS

Based on the average concentrations of total phosphorus and nitrogen, the studied lakes were classified as mesotrophic and eutrophic water bodies [based on different nutrient (N, P) and chlorophyll criteria in trophic status classification methods proposed by WETZEL (1983), OECD (1982) and NÜRNBERG (2001), but their actual trophic class ranged from oligotrophic to hypertrophic. The majority of the investigated lakes (12) are hard-water bodies with a high calcium content and high alkalinity. In summer, the average total phosphorus concentrations were lower in stratified lakes (0.051±0.035 mg dm^{-3}) than in shallow lakes (0.059±0.064 mg dm^{-3}) – Table 1. The average total nitrogen concentrations were also lower in stratified water bodies (1.71±0.77 mg dm^{-3}) than in non-stratified lakes (1.87±0.73 mg dm^{-3}) in the summer season (Table 1). The average TN/TP ratio in the summer epilimnion was higher in shallow lakes (54) than in deep stratified lakes (38). In summer, chlorophyll concentrations were relatively high (>25 μg dm^{-3} in 6 lakes), ranging from 0.006 ±0.003 μg dm^{-3} (Lake Biala Kuta) to 63.9±0.17 μg dm^{-3} (Lake Ciche) on average (Table 1). Secchi disc visibility was determined in the range of 1.4 ± 3.7 m, and it was higher in deep lakes. Total organic carbon concentrations (TOC) did not exceed 20 mg dm^{-3} in most lakes (13).

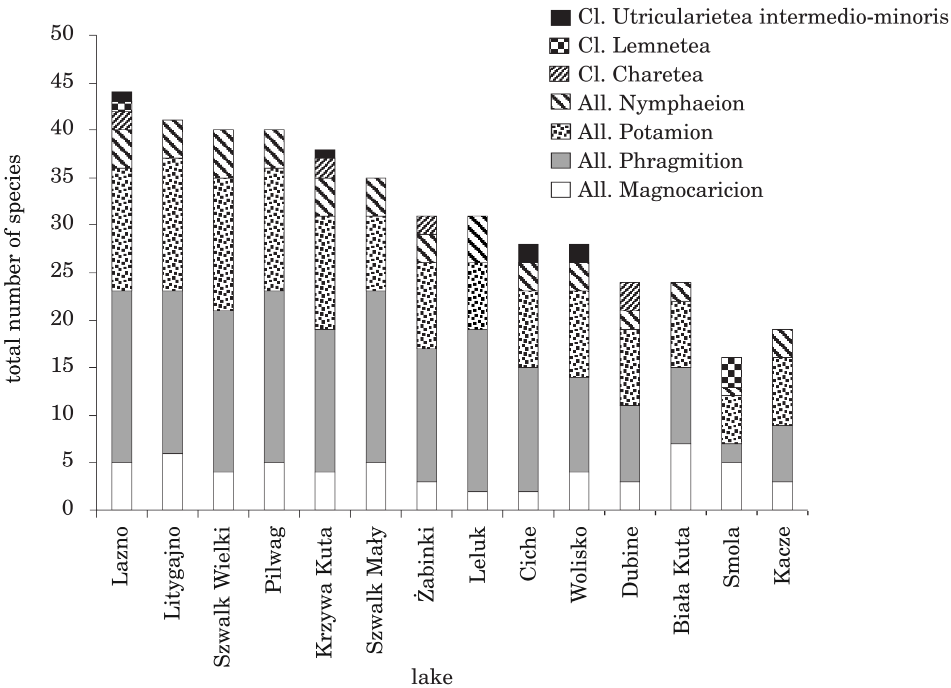

Fig. 3. Total number of macrophyte species from different phytosociological groups in the investigated lakes of the Borecka Forest

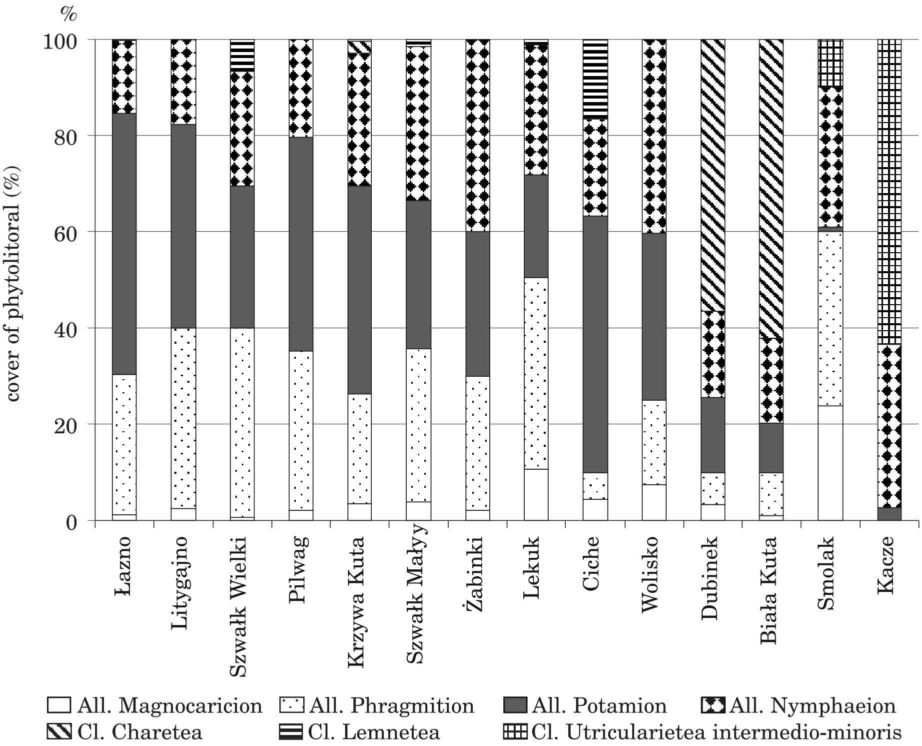

Fig. 4. Variations in aquatic plant communities colonizing the analyzed lakes

In the survey of aquatic vegetation performed in 2002–2012, a total of 123 macrophyte taxa were observed in 14 lakes, including 68 emergent, six floating-leaved, seven free-floating and 42 submerged plant species (including 5 *Characeae* species), although only 19 to 44 macrophyte taxa were determined per lake (Figure 3). The average value of the phytocenotic diversity index (H) was determined at 1.3±0.62 in stratified lakes and at 1.7±0.34 in non-stratified lakes.

Plant communities of the alliances *Phragmition* and *Potamion* had the largest combined share of the phytolittoral zone in most lakes (10). A significant contribution of *Charetea* communities (approximately 60%) was observed in lakes Biala Kuta and Dubinek, and a high share of *Utricularietea intermedio-minoris* communities was noted in lakes Smolak and Kacze (Figure 4).

During factor analysis, abiotic variables (Table 1) were divided into three groups of factors which explained 79.1% of total variability (Table 2, Figure 5). Factor 1 was the morphometry – use of catchment area (mean depth, max. depth, forests and farmland), factor 2 was water chemistry (TP, TN, Chl-a), and factor 3 was the lake/catchment area – (lake area and catchment area).

Table 2

'Varimax-normalized' rotated factor loadings of abiotic variables (abs > 0.7) and the direction of their influence (+ or -) in the investigated lakes

Variable	Factor 1: morphometry – use of catchment area	Factor 2: water chemistry	Factor 3: lake/catchment area
Surface area			0.942
Mean depth	0.880		
Max depth	0.804		
TP		0.764	
TN		0.815	
chl *a*		0.821	
Catch			0.801
Forest	-0.908		
Agricultural	0.827		
Percent of variability	32.3	17.3	29.5

A canonical correspondence analysis (CCA) was performed to relate macrophyte species composition to statistically significant environmental variables. The variables applied in the CCA ordination plot explained around 59% of total variability in plant distribution patterns in the studied lakes. The variables are correlated with the first and second canonical axes, and the longest vectors were Chl-*a* and TP (Figure 6). The first axis expla-

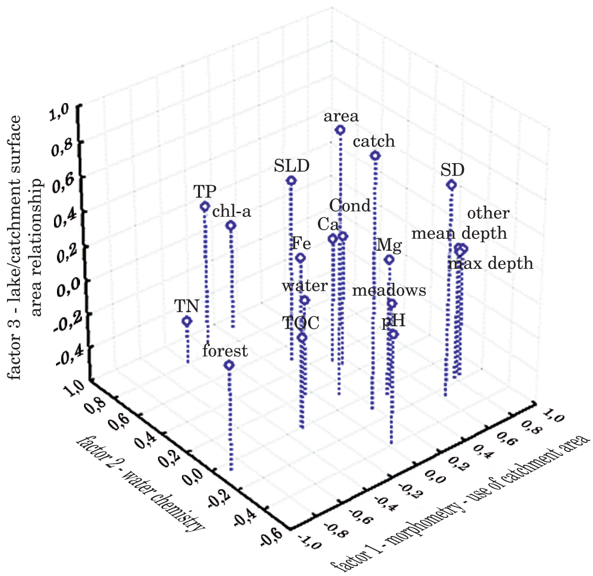

Fig. 5. Abiotic variables described by factors 1-3 in the studied lakes

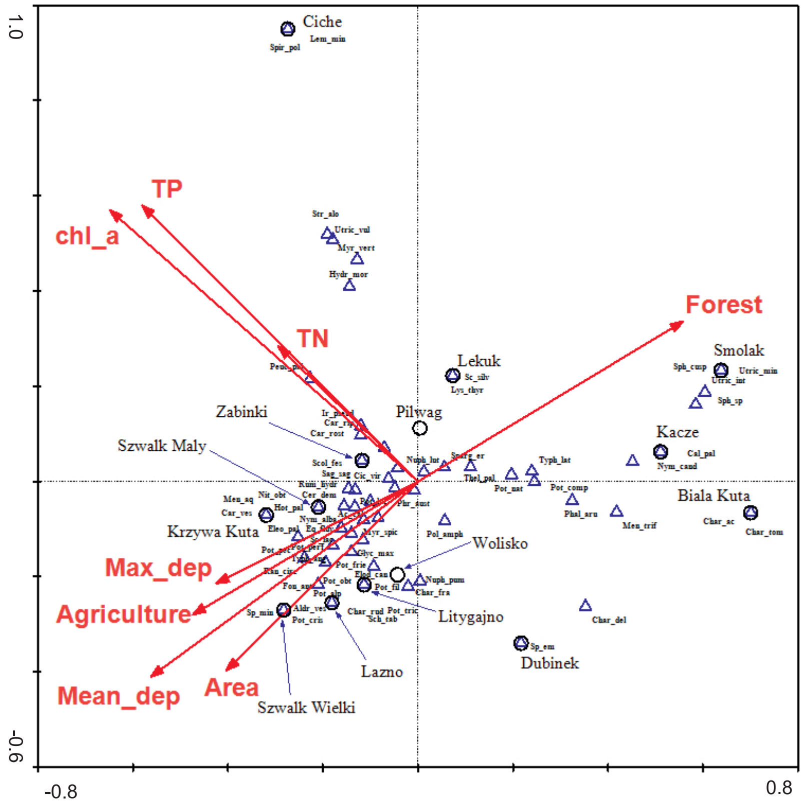

Fig. 6. CCA ordination plots – two first axes

The analysis of the composition of aquatic macrophytes in the lakes was based on the presence and absence of the species and only the common species (present in more than 5% sample sites) were considered in the analysis: *Phr_aust - Phragmites australis, Sc_lac - Scirpus lacustris, Ir_pseud - Iris pseudoacorus, Eleo_pal - Eleocharis palustris, Men_aq - Mentha aquatica, Car_rost - Carex rostrata, Typh_ang - Typha angustifolia, Peuc_pal - Peucedanum palustre, Ac_cal - Acorus calamus, Typh_lat - Typha latifolia, Phal_aru - Phalaris arundinacea, Car_rip - Carex riparia, Eq_fluv - Equisetum fluviatile, Sparg_er - Sparganium erectum, Cic_vir - Cicuta virosa, Glyc_max - Glyceria maxima, Rum_hydr - Rumex hydrolapathum, Sch_tab - Schoenoplectus tabernaemontani, Men_trif - Menyanthes trifoliata, Thel_pal - Thelypteris palustris, Cal_pal - Calla palustris, Scol_fest - Scolochloa festucacea, Lys_thyr -Lysimachia thyrsiflora, Sc_silv - Scirpus silvaticus, Car_ves - Carex vesicaria, Hot_pal - Hottonia palustris, Nuph_lut - Nuphar lutea, Nym_alba - Nymphaea alba, Nuph_pum - Nuphar pumilum, Nym_cand - Nymphaea candida, Pol_amph - Polygonum amphibium f. natans, Pot_nat - Potamogeton natans, Pot_luc - Potamogeton lucens, Pot_pec - Potamogeton pectinatus, Pot_perf - Potamogeton perfoliatus, Pot_frie - Potamogeton friesii, Pot_alp - Potamogeton alpinus, Pot_comp - Potamogeton compressus, Pot_fil - Potamogeton filiformis, Pot_obt - Potamogeton obtusifolius, Pot_trich - Potamogeton trichoides, Pot_crisp - Potamogeton crispus, Cer_dem - Ceratophyllum demersum, Myr_spic - Myriophyllum spicatum, Myr_vert - Myriophyllum verticillatum, Ran_circ - Ranunculus circinatus, Elod_can - Elodea canadensis, Char_tom - Chara tomentosa, Char_ac - Chara aculeolata, Char_del - Chara delicatula, Char_rud - Chara rudis, Char_fra - Chara fragilis, Nit_obt - Nitellopsis obtusa, Hydr_mor - Hydrocharis morsus - ranae, Str_alo - Stratiotes aloides, Sp_em - Sparganium emersum, Sp_min - Sparganium minimum, Sag_sag - Sagittaria sagittifolia, Lem_min - Lemna minor, Spir_pol - Spirodela polyrhiza, Utric_vul - Utricularia vulgaris, Utric_int - Utricularia intermedia, Utric_min - Utricularia minor, Aldr_ves - Aldrovanda vesiculosa, Fon_ant - Fontinalis antipyretica, Sph_sp - Sphagnum sp., Sph_cusp - Sphagnum cuspidatum.*

Circles denote lakes, and triangles denote macrophyte species.

Table 3

Spearman's rank correlation between abiotic variables and aquatic vegetation metrics in two types of lakes in the Borecka Forest ($p < 0.05$)

Macrophyte metrics	Factor 1				Factor 2			Factor 3	
	morphometry – use of catchment area				water chemistry			lake-catchment area	
	mean depth	max depth	forests	agricultural	TP	TN	Chl *a*	lake area	catchment area
Stratified lakes (*n* = 8)									
No of species					-0.51	-0.61			
H	-0.52			-0.38	-0.59				
Proportion of phytocenoses in different syntaxonomic groups									
%Chara			0.39	-0.39	-0.52				
%Pota					-0.61				
%Nymph	-0.59	-0.67		0.40				-0.38	
%Phra	0.52		-0.38		0.38		0.66	0.38	
%Mcar									
%Lemn									
%Utric									
Non-stratified lakes (*n* = 6)									
No of species				-0.91					
H			0.67						
Proportion of phytocenoses in different syntaxonomic groups									
%Chara			0.39	-0.62	-0.63	-0.39	-0.71		
%Pota				0.80	0.39				
%Nymph	-0.61	-0.49			0.39	0.45	0.55		
%Phra	0.39		-0.61		0.38	0.58	0.66		
%Mcar									0.44
%Lemn				0.88			0.78		
%Utric	-0.61	-0.51	0.80		0.68	0.67	0.55		

The table includes only abiotic variables which appeared in the factor analysis.
No of species – number of species; H – Shanon index; Cl. – class, All. – alliance; proportion of phytocenoses: %Chara – class *Charetea* %, Pota – alliance *Potamion*, %Nymph – alliance *Nymphaeion*, %Phra – alliance *Phragmition*, %Mcar – alliance *Magnocaricion*, %Lemn – class *Lemnetea*, %Utric – class *Utricularietea intermedio-minoris*; TP – total phosphorus, TN – total nitrogen, Chl*a* – chlorophyll *a*; Cl. – class, All. – alliance; empty cell – no relationship, correlation values

ins 30.5% of total variability, and the second axis – 19.4%, which accounts for 84.6% of explained variability (with 59% total variability). The test of significance of the first canonical axis revealed a significant gradient that determines differences in plant distribution patterns in the analyzed lakes ($p = 0.05$). The first axis is strongly correlated with Chl-*a* (-0.6), TP (-0.55), Forest (0.53), and Mean depth (-0.53), followed by Agricultural (-0.45) and

Max depth (0.40). The second axis is strongly correlated with TP (0.56) and Chl-*a* (0.55). Chl-*a* (lambda = 0.28, p = 0.002, F = 3.67) was a statistically significant parameter (Monte Carlo permutation test, $p < 0.05$) which explained 12.4% of total variability in plant distribution patterns in the examined lakes.

The correlations between environmental factors and the share of phytosociological groups in stratified and non-stratified lakes were also examined (Table 3). Spearman's correlation analysis examining macrophyte metrics and environmental indices showed that the number of species present in all ecological zones was negatively correlated with nutrient concentrations (TP, TN) in stratified lakes and with agricultural catchments in non-stratified lakes (Table 3). Biological diversity described by SHANNON'S index in stratified lakes increased with a decrease in depth, a decrease in the share of agricultural areas in the catchment and a drop in TP levels (Table 3). The percentage cover of forests in the catchment was positively correlated with biological diversity in non-stratified lakes (Table 3).

The share of phytocenoses of the class *Charetea* increased with the percentage cover of forests in the catchment area, it decreased with a drop in TP concentrations in stratified lakes and with a decrease in TP, TN and Chl-*a* levels in non-stratified lakes (Table 3).

The distribution of nympheids in stratified and non-stratified lakes was negatively correlated with depth (Mean depth, Max. depth), and it was positively correlated with lake area (Area) and the percentage cover of forests in the catchment only in stratified lakes (Table 3). A positive correlation with TP, TN and Chl-*a* was observed in non-stratified lakes (Table 3).

In stratified lakes, the share of *Phragmitetea* class communities was positively correlated with mean depth, TP, Chl-*a* and lake area, and it decreased with an increase in forest cover in the catchment (Table 3). In non -stratified lakes, the share of *Phragmitetea* class communities was positively correlated with mean depth, TP, TN and Chl-*a*. Unlike in stratified lakes, lake area was not a correlated parameter, but correlations were determined with catchment area (Table 3). Similarly to stratified lakes, the share of helophytes of the class *Phragmitetea* decreased with an increase in forest cover in the catchment (Table 3).

In stratified lakes, no significant correlations were noted between the analyzed environmental factors and plant communities of the alliance *Magnocaricion*, class *Lemnetea* or class *Utricularietea intermedio-minoris* (Table 3). In non-stratified lakes, an increase in the share of agricultural catchment area was accompanied by a higher share of pleustophytes in aquatic vegetation, and it was correlated with an increase in chlorophyll a concentrations (Table 3).

No correlations between plant communities of the alliance *Magnocaricion* and environmental factors were determined in stratified lakes. In non -stratified lakes, a positive correlation was observed between the share of plant communities of the alliance *Magnocaricion* and catchment area (Table 3). In stratified lakes, the analyzed environmental factors were not correlated with plant communities of the alliance *Magnocaricion*, class *Lemnetea* or class *Utricularietea intermedio-minoris* (Table 3).

The highest number of significant correlations was observed between plants of the class *Utricularietea intermedio–minoris* in shallow, non-stratified lakes (Table 3). The share of those plant communities increased with the percentage of forest cover in the catchment as well as nutrient and chlorophyll concentrations, and it decreased with depth (Table 3).

DISCUSSION

All of the studied lakes (14) were classified as sites of the Natura 2000 network based on Habitat Directive criteria (EC 1992, CID 2011). The main criterion for classifying water bodies as Natura 2000 sites is the type of aquatic vegetation (plant communities) in lakes. The results of CCA (Figure 7)

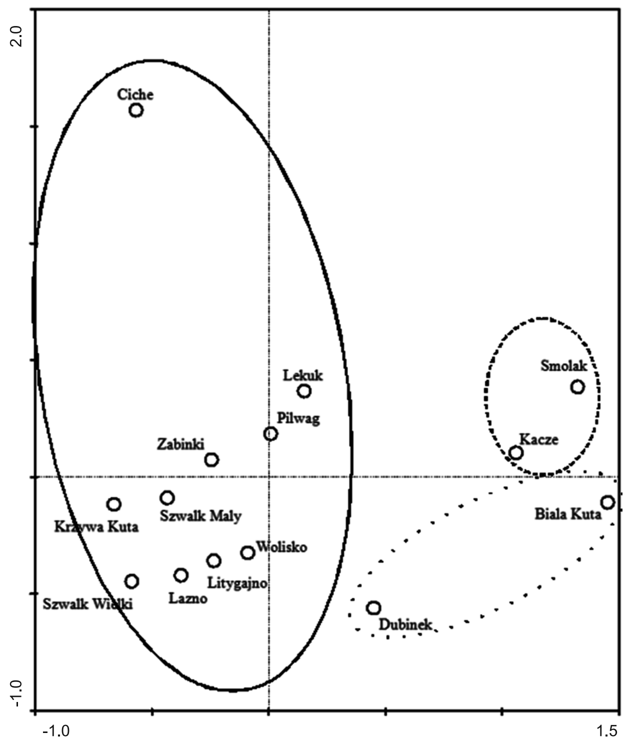

Fig. 7. Generalized CCA ordination plot– two first axes
solid line – eutrophic lakes (3150), dashed line – dystrophic lakes (3160), dotted line – hard oligo-mesotrophic waters with benthic vegetation of Chara spp. (3140); circles denote lakes

supported the identification of eutrophic lakes (Natura 2000 code 3150-1: Litygajno, Żabinki, Szwalk Maly, Lekuk, Szwalk Wielki, Wolisko, Krzywa Kuta, Lazno; Natura 2000 code 3150-2: Pilwag, Ciche). Based on trophic state evaluation criteria (OECD 1982, NÜRNBERG 2001), Lake Ciche was classified as a hypertrophic water body. The above assessment was further confirmed by the results of CCA (Figures 6, 7) which revealed that Lake Ciche had a predominance of pleustophytes (*Lemna minor, Spirodela polyrhiza*) and submerged vegetation (*Myriophyllum verticilatum, Stratiotes aloides, Hydrocharis morsus-ranae, Utricularia vulgaris*) which are characteristic of highly fertile waters (KŁOSOWSKI 2006).

Lakes Biala Kuta and Dubinek are hard oligo-mesotrophic waters with benthic vegetation of *Chara spp*. (Natura 2000 code 3140), whereas lakes Smolak and Kacze were classified as dystrophic water bodies (Natura 2000 code 3160). The variations in plant distribution patters in the above lakes were positively correlated with the first cardinal axis. Lakes Biala Kuta and Dubinek were characterized by low phosphorus concentrations (Table 1). Dense stonewort meadows act as nutrient traps by blocking internal sources of biogenic substances, limiting sediment resuspension and impairing phytoplankton growth (VAN DEN BERG 1999, KUFEL, KUFEL 2002). They secrete growth inhibitors and exert allopathic effects on vascular plants and phytoplankton (VAN DONK, VAN DE BUND 2002). Stoneworts are important regulators of water quality (VAN DER BERG 1999, KUFEL, KUFEL 2002). In those lakes, CCA revealed the presence of species characteristic of the class *Charetea,* and correlations with *Sparganium emersum* were also noted in the phytolittoral zone. In non-stratified lakes, plants of the class *Charetea* were significantly correlated with factor 1 (morphometry – use of catchment area), but only in reference to catchment use, and factor 2 (water chemistry) indicators, which confirms the presence of the above relationships. Similar correlations were noted by other authors (KRAUSE 1981, VAN DEN BERG 1999). They are characteristic of oligotrophic or mesotrophic waters with low phosphorus concentrations and high calcium levels (KRAUSE 1981), and lakes Biala Kuta and Dubinek fit the above description. It is believed that stoneworts have a stabilizing effect when stonewort meadows cover more than 30% of the lake's littoral zone (JEPPESEN et al. 1994, PORTIELJE, RIJSDIJK 2003). This condition was met by lakes Biala Kuta and Dubinek.

The catchments of the analyzed dystrophic lakes (Smolak and Kacze) are largely occupied by raised bogs (<70%) which are a source of humic substances. Most humic substances dissolved in surface waters originate from catchment areas (WILKINSON et al. 1997), and only a small fraction has autochthonic origin (WETZEL 1983, DE HAAN 1992). In general, humic substances stain water, they affect the quality and quantity of light in aquatic ecosystems, increase sedimentation, bind calcium, nitrogen and phosphorus, and neutralize oligotrophic and acidic water bodies (RORSLETT 1987). Dissolved humic substances support the formation of complex phosphorus compounds (De Haan 1992), they accelerate and stabilize calcite precipitation. In acidic environments, humic substances limit the solubility of silica (Conzonno, Cirelli 1995), and similar activity is observed in lakes colonized by stonewort meadows (Kufel, Kufel 2002). Humic substances react with water and sediment components to regulate the quantity and availability of nutrients for plants (Banaś et al. 2012). The above mechanisms explain the CCA results reported in this study (Figures 6, 7). The species identified in lakes Smolak and Kacze are typical of dystrophic waters (mainly species of the class *Utricularietea intermedio–minoris)*, and the positive correlation with the percentage cover of forests in the catchment confirms that the type of land use in catchments affects plant distribution patterns in those lakes. In non-stratified lakes, significant correlations between plants of the class *Utricularietea intermedio-minoris* and factor 1 (morphometry – use of catchment area) and factor 2 (water chemistry) indicators further attests to the presence of the above relationships. The results of the study demonstrate that plant communities of the class *Utricularietea intermedio-minoris* are effective biomarkers of catchment parameters and water quality attributes in both lake types. The ecology of plant communities representing the class *Utricularietea intermedio-minoris* remains poorly investigated. The existing studies suggest that this group of plants is a good indicator of habitat quality, in particular humic substance concentrations in lakes (Adamec, Lev 2002, Ditě et al. 2006). Plant communities of the class *Utricularietea intermedio -minoris* were also found to be correlated with water pH and conductivity (Ditě et al. 2006). The results of this study expand our knowledge about the discussed group of aquatic vegetation.

The proportion of agricultural land in the catchment area was correlated with biological diversity indicators in the examined lakes (Table 3; stratified lakes – agricultural use vs. H, non-stratified lakes – agricultural use vs. number of species). The surface area of stonewort meadows was also found to decrease with an increase in the share of agriculturally used land in the catchment (Table 3). In stratified lakes, the proportion of floating -leaved vegetation increased with the share of agriculturally used land in the catchment. In shallow, non-stratified lakes, an increase in the share of agriculturally used land in the catchment was accompanied by a higher contribution of submerged plants and pleustophytes. These results corroborate the findings of Cheruvelil, Soranno (2008) as well as Kolada (2010) who demonstrated that the type of land use in the catchment is an important indicator of macrophyte distribution patterns in lakes.

The percentage cover of forests in the catchment area was also an indicator of plant structure and diversity in the analyzed lakes. These results are consistent with the findings of Kolada (2010). The values of coefficients of correlation between plant parameters and forest cover in the catchment area were higher in shallow lakes (Table 3). A similar trend was reported by Kolada (2010) in 83 lakes situated in Polish lowlands. The described role of plants of the class *Utricularietea intermedio-minoris* makes a new and original contribution to the existing knowledge.

Most lakes had a considerable share of rush plants of the alliance *Phragmition* (>20% of the phytolittoral zone in 9 lakes). The three identified factors (Tables 2, 3) were correlated with plants of the alliance *Phragmition*. A similar correlation was observed by KOLADA (2010).

Submerged and floating-leaved plants are an indicator of local environmental conditions (SØNDERGAARD et al. 2010). By supplying nutrients and directly influencing habitats, aquatic vegetation also determines abiotic conditions (BARKO et al. 1991, MURPHY 2002) and the flora and fauna of wetlands at various trophic levels (e.g. NORLIN et al. 2005). Yet an analysis of the correlations between submerged vascular plants (all. *Potametea*) and the parameters described by factor 1 (morphometry – use of catchment area) and factor 2 (water chemistry) revealed that submerged and floating-leaved plants play a minor indicative role. They influenced plant distribution patterns in eutrophic lakes classified under code 3150 in the Natura 2000 network (Figure 7). The alliance *Potametea* is composed mainly of ceratophyllids, myriophyllids and potamida. Other authors have demonstrated that this highly varied alliance is represented by plant communities with a broad ecological amplitude (e.g. MURPHY 2002, KŁOSOWSKI 2006). Vascular plants have very different trophic requirements (JEPPESEN et al. 2000). Therefore, the indicative role of communities representing the alliance *Potamion* seems to be very limited, as demonstrated by the results of this study. A positive correlation with TP was noted in stratified lakes, whereas an inverse relationship and a positive correlation with agricultural use of catchment area was observed in non-stratified lakes (Table 3). Floating-leaved plants of the alliance *Nymphaeion* have less diverse environmental requirements than submerged vegetation representing the alliance *Potametea* (KŁOSOWSKI, SZAŃKOWSKI 1999). In both lake types, floating-leaved plants were more robust environmental indicators than submerged vegetation of the alliance *Potamion*. Significant positive correlations were determined between floating-leaved plants and morphometric parameters of the studied lakes (Mean depth, Max. depth). In stratified lakes, floating-leaved plants were additionally correlated with agricultural use of the catchment and were negatively correlated with lake area. Positive correlations with all parameters described by factor 2 (water chemistry) were reported in non-stratified lakes.

Other authors (PORTIELJE, ROIJACKERS 2003, JEPPESEN et al. 2000) have observed that nympheids are highly tolerant of increased turbidity. In this study, this group of plants was a good indicator of water quality in shallow lakes. Similar results were reported by SCHEFFER, VAN NES (2007) who emphasized the growing role of the analyzed plants in shallow lakes and ponds.

Numerous authors have argued that aquatic vegetation in lakes may exhibit a delayed response to nutrient supply because the phosphorus delivered to and present in lakes may be partially unavailable for producers

(Ciecierska 2006). This explains a strong correlation between plant distribution patterns and chlorophyll concentrations and a weaker relationship with the remaining lake parameters in CCA. Despite the above, a significant correlation between nutrient concentrations (TP and TN) and plant communities suggests that those parameters are important drivers of plant distribution patterns in the analyzed lakes. In stratified lakes, weaker correlations were determined between TP levels and plant groups characteristic of eutrophic lakes (*Potamion, Phragmition*), and an inverse relationship was noted between TP concentrations and stonewort meadows. In non-stratified lakes, nutrient concentrations were significantly correlated with vegetation. Significant correlations were noted for two plant groups which determine habitat types in the studied lakes (class *Charetea* and class *Utricularietea intermedio–minoris)*. Nutrient concentrations were also correlated with plant groups encountered in all lake types (%Pota vs. TP; %Phra vs. TP and TN; %Nymph vs. TP and TN). Plant distribution patterns in non-stratified lakes were significantly correlated with chlorophyll concentrations (Table 3). Those relationships are manifested by different plant zones in lakes with varied trophic status. Similar observations were made by Spence (1982) and Rørslett (1987).

The morphological, physicochemical and catchment area parameters of lakes have a significant effect on vegetation development in protected sites of the Natura 2000 network (Figures 6, 7). Lakes of different trophic status differ with respect to the mechanism of eutrophication (Jeppesen et al. 1994, 2000, Murphy 2002, Nurminen 2003, Portielje, Rijsdijk 2003, Norlin et al. 2005, Cheruvelil, Soranno 2008, Kolada 2010). However, the results of the present study provide a basis for formulating general management guidelines for lakes of different types and their catchments, which belong to the Natura 2000 ecological network of protected areas. A comprehensive protection plan should be proposed for interdependent habitats (e.g. lake-forest-peatland). Forest cover in the lakes' catchment areas should be maintained or expanded, and the proportion of intensively farmed land should be reduced. Clearcutting in the areas adjacent to the lakes should be prevented, and the inflow of biogenic elements, nitrogen and phosphorus (which affects Chl-*a* concentrations), should be reduced. The above goals can be achieved, among others, by preserving the existing water relations and species composition of tree stands in the lakes' catchments areas.

Increased human pressure (including agricultural intensification and a decrease in the proportion of forest communities *compatible* with natural *habitat conditions*) in lake catchments leads to increased inflow of biogenic elements and allochthonous matter, which may be followed by eutrophication or even hypertrophication and, ultimately, by the disappearance of submerged vegetation (Ciecierska 2006). In the group of analyzed water bodies, such a situation was observed in Lake Ciche (Table 1, Figure 6).

Effective catchment management is particularly important in shallow, non-stratified lakes (Table 3, Figure 6) whose buffering capacity is low. Dystrophic lakes Smolak and Kacze (code 3160 in the Natura 2000 network) are non-stratified water bodies with a small surface area, surrounded by coniferous forests and peatlands. They are at risk of degradation due to habitat reduction or destruction in their catchments (BANAŚ et al. 2012). Protecting interdependent habitats (lake-forest-peatland) is essential to ecosystem functioning. The priorities for silvicultural management in the catchments of lakes Smolak and Kacze should include the adoption of forest conservation practices and imposing a ban on timber harvesting. Drainage and land reclamation works should also be prohibited, as they could alter the local hydrographic conditions and increase the input of biogenic substances. In dystrophic lakes, eutrophication is usually caused by permanent lowering of the groundwater table in the catchment (due to e.g. long-term drought), lowering of the lake water level, and sphagnum peatland overdrying (CIECIERSKA 2006, BANAŚ et al. 2012), which affect the amount and composition of humic and mineral substances entering the open water zone (DE HAAN 1992).

Lakes Dubinek and Biała Kuta, classified as hard oligo-mesotrophic waters with benthic vegetation of *Chara spp*., seem to be less prone to eutrophication (Figure 6). The key to preserving Natura 2000 3140 sites is maintaining the existing stonewort meadows which serve as buffer zones in lake ecosystems. The implications of charophyte colonization for lake management have been investigated and described (e.g. KRAUSE 1981, VAN DEN BERG 1999). Increased inflow of biogenic elements and a high proportion of farmland and forests in lake catchments contribute to the disappearance of charophyte algae (Table 3). In order to preserve these unique habitats, land use structure in the catchments of Lakes Dubinek and Biała Kuta should remain unchanged. Preferably, the percentage of catchment area under intensive agriculture should be decreased, and the input of biogenic substances from other sources should be limited.

CONCLUSIONS

Morphological, physicochemical and catchment area variables have a significant effect on vegetation development in Natura 2000 sites. The results of this study provide a basis for formulating general management guidelines for the investigated lakes and their catchments, which belong to the Natura 2000 ecological network of protected areas. A comprehensive protection plan should be proposed for interdependent habitats. Forest cover in the lakes' catchment areas should be maintained or expanded, and the proportion of intensively farmed land should be reduced. Clear cutting in the areas adjacent to the lakes should be prevented, and the inflow of biogenic elements, nitrogen and phosphorus (which affects Chl-*a* concentrations), should be reduced. The above goals can be achieved, among others, by preserving the existing water relations and species composition of tree stands in the lakes' catchments areas.

REFERENCES

ADAMEC L., LEV J. 2002. *Ecological differences between Utricularia ochroleuca and U. intermedia habitats.* Carnivorous Plant Newsletter, 31: 14-18.

APHA. 1998. *Standard methods for the examination of water and wastewater*. 20th edition. American Public Health Association, American Water Works Association and Water Environmental Federation, Washington, DC.

BANAŚ K., GOS K., SZMEJA J. 2012. *Factors controlling vegetation structure in peatland lakes - Two conceptual models of plant zonation*. Aquat. Bot., 96: 42-47.

BARKO J.W., GUNNISON D., CARPENTER S.R. 1991. *Sediment interactions with submersed macrophyte growth and community dynamics.* Aquat. Bot., 41: 41-65.

BRAUN-BLANQUET J. 1964. *Pflanzensoziologie*. Springer, Wien, New York.

CANFIELD D.E.JR., LANGELAND K.A., LINDA S.B., HALLER W. 1985. *Relations between water transparency and maximum depth of macrophyte colonization*. *J*. Aquat. Plant Manage., 23: 25-28.

CHERUVELIL K.S., SORANNO P.A. 2008. *Relationships between lake macrophyte cover and lake and landscape features.* Aquat. Bot., 88: 219-227.

CID, 2011. *Commission Implementing Decision of 11 July 2011 concerning a site information format for Natura 2000 sites, (notified under document C(2011) 4892) (2011/484/EU)*. Official Journal of the European Union, L 198/39.

CIECIERSKA H. 2006. *Evolution of the status of lakes located in the city of Olsztyn (Masurian Lake District, N-E Poland) by the macrophytoindication method (MPhI)*. Hydrobiologia, 270: 141-146.

CONZONNO V., CIRELLI A.F. 1995. *Dissolved organic matter in Chascomus Pond (Argentina). Factors influencing distribution and dynamics*. Hydrobiologia, 297: 55-59.

DE HAAN H. 1992. *Impact of environmental changes on the biogeochemistry of aquatic humic substances.* Hydrobiologia, 229: 59-71.

DITĚ D., NAVRATILOVA J., HAJEK M., VALACHOVIČ M., PUKAJOV D. 2006. *Habitat variability and classification of Utricularia communities: comparison of peat depressions in Slovakia and the Třeboň basin.* Preslia, 78: 331-343.

DUNALSKA J. 2009. *Variability of organic carbon forms in lake ecosystems of varying trophic state*. Wyd. UWM, Olsztyn, 110-115. (in Polish)

EC 1992. *Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.* Official Journal of the European Union, L 206, 22.7.

GLIŃSKA-LEWCZUK K. 2009. *Water quality dynamics of oxbow lakes in young glacial landscape of NE Poland in relation to their hydrological connectivity.* Ecol. Engin., 35: 25-37.

GLIŃSKA-LEWCZUK K. BURANDT P. 2011. *Effect of river straightening on the hydrochemical properties of floodplain lakes: Observations from the Łyna and Drwęca Rivers, N Poland.* Ecol. Engin., 37: 786-795.

GOLTERMAN H.L. 1969. *Methods for chemical analysis of fresh waters*. Blackwell Scientific Publications, Oxford and Edinburgh.

GRZYBOWSKI M., SZAREK J., SKIBNIEWSKA K., GUZIUR J. 2010a. *Ecological status and phytocoenotic diversity of macrophytes of lake Szeląg Wielki (north-east Poland).* Pol. J. Nat. Sc., 25(4): 401-414.

GRZYBOWSKI M., SZAREK J., SKIBNIEWSKA K., GUZIUR J. 2010*b*. *Evaluation of diversity of submerged and emergent flora of lake Szeląg Wielki as threatened by a pesticide tomb*. Pol. J. Nat. Sc., 25(4): 154-172.

JENSEN S. 1977. *An objective method for sampling the macrophyte vegetation in lakes.* Vegetatio, 33: 107-118.

JEPPESEN E., SŘNDERGAARD M., KANSTRUP E., PETERSEN B., ERIKSEN R.B., HAMMERSHŘJ M., MORTENSEN M., JENSEN J., HAVE A. 1994. *Does the impact of nutrients on the biological structure and function of brackish and freshwater lakes differ?* Hydrobiologia, 275/276: 15-30.

JEPPESEN E., JENSEN J.P., SŘNDERGAARD M., LAURIDSEN T., LANDKILDEHUS F. 2000. *Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient.* Fresh. Biol., 45: 201-218.

KŁOSOWSKI S. 2006. *The relationships between environmental factors and the submerged Potametea associations in lakes of north-eastern Poland.* Hydrobiologia, 560(1): 15-29.

KŁOSOWSKI S., SZAŃKOWSKI M. 1999. *Habitat conditions of nymphaeid associations in Poland*. Hydrobiologia, 415: 177-185.

KOLADA A. 2010. *The use of aquatic vegetation in lake assessment: testing the sensitivity of macrophyte metrics to anthropogenic pressures and water quality.* Hydrobiologia, 656: 133-147.

KRAUSE W. 1981. *Characeen als Bioindikatoren für den Gewässerzustand.* Limnologica, 13: 399-418.

KUFEL L., KUFEL I. 2002. *Chara beds acting as nutrient sinks in shallow lakes - a review.* Aquat. Bot., 72: 249-260.

LEPŠ J., ŠMILAUER P. 2007. *Multivariate Analysis of Ecological Data using CANOCO.* Cambridge University Press, Cambridge.

MATUSZKIEWICZ W. 2002. *A guide book to identification plant communities in Poland.* PWN, Warszawa. (in Polish)

MURPHY K.J. 2002. *Plant communities and plant diversity in softwater lakes of northern Europe.* Aquat. Bot., 73: 287-324.

NAPIÓRKOWSKA-KRZEBIETKE A., PASZTALENIEC A., HUTOROWICZ A. 2012. *Phytoplankton metrics response to the increasing phosphorus and nitrogen gradient in shallow lakes.* J. Elem., 17(2): 289-303. DOI: 10.5601/jelem.2012.17.2.11

NORLIN J.I., BAYLEY S.E., ROSS L.C.M. 2005. *Submerged macrophytes, zooplankton and the predominance of low- over high-chlorophyll states in western boreal, shallow-water wetlands.* Fresh. Biol., 50: 868-881.

NURMINEN L. 2003. *Macrophyte species composition reflecting water quality changes in adjacent water bodies of lake Hiidenvesi, SW Finland. Ann. Bot. Fennici.*, 40: 199-208.

NÜRNBERG G. 2001. *Eutrophication and trophic state.* Lakeline, 29-33.

OBOLEWSKI K., GLIŃSKA-LEWCZUK K., KOBUS SZ., 2009. *An attempt at evaluating the influence of water quality on the qualitative and quantitative structure of epiphytic fauna dwelling on Stratiotes aloides* L.*, a case study on an oxbow lake of the Łyna river.* J. Elem., 14(1): 119-135.

OECD 1982. Organization for Economic Cooperation and Development. *Eutrophication of waters. Monitoring, assessment and control. Final report, OECD cooperative programme on monitoring of inland waters (eutrophication control)*. Environment Directorate, OECD, Paris.

PORTIELJE R., RIJSDIJK R.E. 2003. *Stochastic modelling of nutrient loading and lake ecosystem response in relation to submerged macrophytes and benthivorous fish*. Fresh. Biol., 48: 741-755.

RORSLETT B. 1987. *A gradient spatial niche model for aquatic macrophytes.* Aquat. Bot., 29: 63-81.

Scheffer M., van Nes E.H. 2007. *Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size*. Hydrobiologia, 584: 455-466.

Søndergaard M., Johansson L., Lauridsen T., Jorgensen T., Liboriussen L. Jeppesen E. 2010. *Submerged macrophytes as indicators of the ecological quality of lakes.* Fresh. Biol., 55: 893-908.

Spence D.H.N. 1982. *The zonation of plants in freshwater lakes.* Adv. *Ecol. Res.,* 12: 37-125.

StatSoft, Inc 2011. Statistica (data analysis software system), version 10. www.statsoft.com.

Ter Braak C.J.F., Šmilauer P. 2002. *CANOCO reference manual and CanoDraw for Windows user's guide: Software for Canonical Community Ordination (version 4.5).* Microcomputer Power, New York.

Van den Berg M.S. 1999. *Charophyte colonization in shallow lakes: processes, ecological effects and implications for lake management.* PhD Thesis. Free University, Amsterdam.

Van Donk E., van de Bund W.J. 2002. *Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: allelopathy versus other mechanisms.* Aquat. Bot., 72: 261-274.

Weber H.E., Moravec J., Theurillat J-P. 2000. *International code of phytosociological nomenclature.* 3rd ed. J. Veget. Sci., 1: 739-768.

Wetzel R.G. 1983. *Limnology*. W.B. Saunders Co., Philadelphia.

Wilkinson K.J., Negre J.C. 1997. *Coagulation of colloidal material in surface waters: the role of natural organic mater*. J. Contamin. Hydrol., 26: 229-243.

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EFFECT OF LIMING AND MINERAL FERTILIZATION ON COPPER CONTENT IN POTATO TUBERS (*SOLANUM TUBEROSUM* L.) AND GREEN MATTER OF FODDER SUNFLOWER (*HELIANTHUS ANNUUS* L.) CULTIVATED ON LOESSIAL SOIL

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Abstract

The paper presents research on the copper content in potato tubers and green matter of fodder sunflower grown in 1986-2001 on podzolic soil developed from loess (static fertilization field). The plant growing experiment was established in a randomized sub-block design on a static fertilization field in a four-year rotation system with mineral NPKMg and NPKMgCa nutrition. The rotation included potato, spring barley, fodder cabbage and winter wheat in 1986-1989, and potato, spring barley, fodder sunflower and winter wheat in 1990-2001. Mineral nutrition consisted of NPK fertilization with constant Mg and varied NPK fertilization with constant Mg and Ca nutrition (liming). Liming was carried out in 1985, 1989, 1993 and in 1997 (4 t ha^{-1} CaO). The copper content in plants was determined with the FAAS technique after digesting plant samples in a mixture of HNO_3, $HClO_4$, H_2SO_4 at a 20:5:1 ratio.

A reduction in the copper level in green biomass of fodder sunflower was observed. Mineral fertilization resulted in an increase of the copper content in sunflower green matter. No interaction between liming and mineral nutrition in shaping the copper content in green biomass of fodder sunflower was recorded. The copper content in potato tubers did not depend on liming, mineral nutrition or on the interaction of these treatments. Some tendency towards decreasing the copper content in potato tubers due to liming and mineral fertilization was noticeable.

Keywords: copper, liming, mineral fertilization NPKMg, potato, fodder sunflower.

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WPŁYW WAPNOWANIA I NAWOŻENIA MINERALNEGO NA ZAWARTOŚĆ MIEDZI W BULWACH ZIEMNIAKA (*SOLANUM TUBEROSUM* L.) I ZIELONEJ MASIE SŁONECZNIKA PASTEWNEGO (*HELIANTHUS ANNUUS* L.) UPRAWIANYCH NA GLEBIE LESSOWEJ

Abstrakt

Badano zawartość miedzi w bulwach ziemniaka i zielonej masie słonecznika pastewnego uprawianych w latach 1986-2001 na glebie płowej wytworzonej z lessu (stałe pole nawozowe). Doświadczenie z uprawą roślin założono metodą podbloków losowanych na stałym polu nawozowym w czteroletnim zmianowaniu z zastosowaniem nawożenia mineralnego NPK Mg i NPK Mg Ca. Zmianowanie obejmowało: ziemniak, jęczmień jary, kapustę pastewną, pszenicę ozimą w latach 1986-1989 oraz ziemniak, jęczmień jary, słonecznik pastewny, pszenicę ozimą w latach 1990-2001. Nawożenie mineralne obejmowało NPK na tle stałego nawożenia Mg i zróżnicowane nawożenie NPK na tle stałego nawożenia Mg i Ca (wapnowanie). Wapnowanie zastosowano w latach: 1985, 1989, 1993, 1997 (4 t ha^{-1} CaO). Zawartość miedzi w roślinach oznaczano metodą FAAS, po mineralizacji próbek roślin w mieszaninie HNO_3, $HClO_4$, H_2SO_4 w proporcji 20:5:1. Zaobserwowano zmniejszenie zawartości miedzi w zielonej biomasie słonecznika pastewnego.

Nawożenie mineralne wpłynęło na wzrost zawartości miedzi w zielonej masie słonecznika. Nie stwierdzono interakcji wapnowania i nawożenia mineralnego w kształtowaniu zawartości miedzi w zielonej biomasie słonecznika pastewnego. Zawartość miedzi w bulwach ziemniaka nie zależała od wapnowania, nawożenia mineralnego i interakcji tych zabiegów. Pod wpływem wapnowania i nawożenia mineralnego wystąpiła tendencja do zmniejszania się zawartości miedzi w bulwach ziemniaka.

Słowa kluczowe: miedź, wapnowanie, nawożenie mineralne NPKMg, ziemniak, słonecznik pastewny.

INTRODUCTION

Soil, whose fertility depends mainly on the type of bedrock, its mineral composition, texture, humus content as well as the course and intensity of soil-typological processes, is the fundamental source of microelements for plants. Anthropogenic factors, including agriculture, as well as atmospheric deposition of gaseous and particulate substances of various origin (e.g. industry, transport) can exert some remarkable impact on the chemical composition of soil (Ciećko, Wyszkowski 2000, Gorlach, Gambuś 2000, Lavado 2006, Strączyński, Strączyńska 2009).

Intensive agriculture, whose aim is to ensure high yields of crops characterized by high nutritional requirements and stimulated by mineral NPK fertilization, contributes to a larger discharge of micronutrients from soils (Czuba 2000, Gembarzewski 2000), but sometimes stimulates their accumulation owing to fertilizing agents and plant protection chemicals added to soil (Gorlach, Gambuś 1997, Kaniuczak 1998). In time, this may result in a secondary reduction of yields and alterations in the chemical composition of crops.

Copper is an essential element for ensuring good plant development, and its role is most often associated with the activation of a spectrum of enzymes enabling specific metabolic processes. Copper deficiency reduces the plant growth and yielding; on the other hand, its excessive amounts in the environment can also lead to the abnormal functioning of organisms (KARA et al. 2004, MUSILOVÁ 2009, ROGÓŻ , TRĄBCZYŃSKA 2009).

Phytoavailability of microelements depends largely on the amount of their bioavailable forms in soil, soil pH, soil chemistry as well as mineral and organic fertilization (KANIUCZAK 1992, 1998, CIEĆKO, WYSZKOWSKI 2000, GORLACH, GAMBUŚ 2000, KANIUCZAK et al. 2003, BEDNAREK et al. 2006). Under natural conditions, there is a large variation of the plant content of micronutrients, including copper, which depends on the species and varieties of plants, their parts, soil properties and growing conditions. Among many crops, two species were chosen for the current study: edible potato, widespread in Polish agriculture, and fodder sunflower, much rarer on Polish farms. The two species differ in economic importance in Poland. Besides, they are grown for different organs.

The aim of this study has been to determine the influence of liming and mineral NPKMg fertilization against the background of constant magnesium nutrition on the copper content in potato tubers (*Solanum tuberosum* L.) and green matter of fodder sunflower (*Helianthus annuus* L.) cultivated in rotation on loessial soil.

MATERIAL AND METHODS

In 1986-2001, the research on the effects of liming (A) and mineral nutrition (B) on the copper content in potato tubers and green matter of fodder sunflower grown in a four-year rotation was carried out on a static fertilization field in Krasne near Rzeszów, situated in the Rzeszów Foothills (*Podgórze Rzeszowskie*).

Prior to the experiment, the soil was highly acid, low in available phosphorus, potassium, boron, zinc and molybdenum, but moderately rich in available magnesium, copper and manganese. The soil was developed from podzolic-type loess (*Haplic luvisol*) and contained 0.087% total N and 0.65% organic C (KANIUCZAK 1998, KANIUCZAK et al. 2011).

The experiment was set up in a random sub-block design with four replicates. The first variable factor was liming (A_2) or its lack (A_1), while the second one consisted of different doses of mineral fertilization (B) with constant magnesium nutrition. The following crops were cultivated in the rotation system: potato, spring barley, fodder sunflower and winter wheat, but in the 1986-1989 rotation cycle fodder cabbage was cultivated instead of sunflower. Potato was grown in 1988, 1992, 1996 and in 2000, while fodder sunflower was grown in three rotations, in 1990, 1994 and 1998.

Basic doses of mineral fertilizers ($N_1P_1K_1$) with constant magnesium nutrition were as follows: potato N_1 = 120 kg N, P_1 = 43.6 kg P, K_1 = 132.8 kg K ha^{-1}; spring barley N_1 = 80 kg N, P_1 = 43.6 kg P, K_1 = 99.6 kg K; fodder sunflower N_1 = 100 kg N, P_1 = 34.9 kg P, K_1 = 99.6 kg K; winter wheat N_1 = 90 kg N, P_1 = 34.9 kg P, K_1 = 83.0 kg K ha^{-1}; fodder cabbage; N_1 = 120 kg N, P_1 = 26.2 kg P, K_1 = 83.0 kg K ha^{-1}. Constant magnesium fertilization was applied before sowing in each experimental sub-block in 1986-1993 at a 24.1 kg Mg ha^{-1} dose for potato, spring barley and winter wheat, and a 72.4 kg Mg ha^{-1} dose for fodder crops. From 1994 on, the magnesium dose was reduced to 24.1 kg Mg ha^{-1}, applied for all experimental crops. Liming with a dose of 4 t CaO ha^{-1} was used in 1985, 1989, 1993 and in 1997, prior to the experiment and after the harvest of the crop last in a rotation. Mineral fertilizers were applied in forms of ammonium nitrate, triple superphosphate, potassium salt KCl (58%), magnesium sulfate and CaO or $CaCO_3$.

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

Plant samples were collected after potato and fodder sunflower (at the flowering stage) harvest. In dry plant material, copper was determined with the atomic spectrophotometric absorbance technique after digesting the samples in a hot mixture of concentrated acids $HClO_4$, HNO_3, and H_2SO_4 (at a 20:5:1 volume proportion) in a Tecator digestion system.

The results were statistically processed by two-factor analysis of variance (liming, mineral NPK fertilization) and calculating the lowest significant difference (LSD) with the Tukey's tests at the significance level of p = 0.05.

RESULTS AND DISCUSSION

The average copper content in potato tubers was approximately 2-fold lower (Table 1) than in sunflower green matter (Table 2). For both crops, however, it ranged between 5 and 15 mg kg^{-1} d.m., which is typical for normal Cu content at plants (Kabata-Pendias et al. 1993, Gorlach, Gambuś 2000). The main EU instrument setting the maximum levels for certain contaminants in foodstuffs is Commission Regulation (EC) No 1881/2006 of 19.12.2006. However, it does not contain standard values for copper. The content of Cu in the tested plants did not exceed 20 mg kg^{-1} d.m., which – according to Kabata-Pendias et al. (1993) – means they are suitable for consumption.

The copper content found in our experiment is similar to the one reported by Rogóż and Trąbczyńska (2009) in potato tuber samples collected from the Wieliczka Foothills. Also Dobrzański et al. (2003) and Strączyński and

Table 1

Mean values and range of copper content in potato tubers depending on liming (A) and mineral fertilization (B) (mg kg^{-1} d.m.)

Treatments of fertilizers	A_1		A_2		Mean
	mean	range	mean	range	
$N_0P_0K_0$	5.87	4.55-7.70	6.30	2.50-8.50	6.09
$N_0P_1K_1$	5.75	3.45-8.18	5.30	2.90-6.90	5.53
$N_{0,5}P_1K_1$	5.63	4.10-6.90	5.35	2.80-6.90	5.49
$N_1P_1K_1$	6.32	3.65-8.80	5.05	2.50-7.50	5.69
$N_{1,5}P_1K_1$	4.90	3.40-4.30	3.97	2.60-3.30	4.44
$N_1P_0K_1$	5.38	3.70-8.30	5.35	3.00-7.80	5.37
$N_1P_{0,5}K_1$	4.71	2.80-7.30	5.24	2.75-7.50	4.98
$N_1P_{1,5}K_1$	5.29	3.80-8.10	4.80	2.20-6.20	5.05
$N_1P_1K_0$	4.72	4.15-6.70	5.42	3.10-7.00	5.07
$N_1P_1K_{0,5}$	5.51	2.70-8.10	5.14	3.00-6.70	5.33
$N_1P_1K_{1,5}$	5.47	2.88-7.30	5.06	3.85-6.30	5.27
$N_{0,5}P_{0,5}K_{0,5}$	5.02	3.62-8.30	4.52	3.00-6.60	4.77
$N_{1,5}P_{1,5}K_{1,5}$	5.24	2.76-7.80	4.59	2.45-6.70	4.92
$N_2P_2K_2$	4.82	2.80-7.60	4.84	3.00-6.80	4.83
Mean of A	5.33		5.07		-
LSD p = 0.05	LSD_A = ns; LSD_B = ns LSD_{AB} = ns				

A_1 – NPK fertilization + Mg constant
A_2 – NPK fertilization + Mg constant, Ca constant
LSD – lowest significant difference for: A – liming, B – mineral fertilization (irrespective of liming), AB – interaction between liming and mineral fertilization
ns – differences not significant

Strączyńska (2009) determined similar quantities of Cu in potato tubers from soils with different contamination degrees caused by copper smelting, although Medyńska et al. (2009) reported a lower content. Ciećko and Wyszkowski (2000), when examining the effects of NPK fertilization on the content of trace elements in potato tubers cultivated on sandy soil, achieved approximately half that amount of Cu in dry matter of tubers. The concentration coefficients calculated by these authors as a ratio of the Cu content in plants from contaminated soils to the metal content in plants from uncontaminated areas only slightly exceeded the value 1. The maximum concentration ratios (1.1) were calculated for plants from soils with a strong contamination degree, according to the IUNG criteria (Kabata-Pendias et al. 1993). This indicates a weak tendency towards accumulating the metal by potato tubers in contrast to potato aerial parts, where the concentration ratio reached 3.43, while the absolute value of Cu content appeared to be more than 4-fold higher. This trend to accumulate heavy metals in aerial plant parts is confirmed by the copper content in the fodder sunflower green matter found in the present study (9.4-17.1 mg kg^{-1} d.m.). In addition, similar trends and concentrations were observed by other authors (Lavado 2006, Fässler et al. 2010).

Table 2

Mean values and range of copper content in green matter of fodder sunflower depending on liming (A) and mineral fertilization (B) (mg kg^{-1} d.m.)

Treatments of fertilizers	A_1		A_2		Mean
	mean	range	mean	range	
$N_0P_0K_0$	12.3	11.5-13.0	11.0	10.7-11.4	11.7
$N_0P_1K_1$	15.3	14.3-16.2	13.3	10.6-16.2	14.3
$N_{0,5}P_1K_1$	15.3	14.2-16.3	13.7	10.6-17.0	14.5
$N_1P_1K_1$	12.1	11.2-12.9	12.6	10.6-14.7	12.4
$N_{1,5}P_1K_1$	11.7	10.7-12.5	9.4	6.1-11.6	10.5
$N_1P_0K_1$	13.4	12.3-14.6	10.8	9.2-12.4	12.1
$N_1P_{0,5}K_1$	17.3	16.6-17.1	11.6	8.5-14.1	14.4
$N_1P_{1,5}K_1$	13.4	12.5-14.1	10.7	9.2-12.5	12.0
$N_1P_1K_0$	11.2	10.1-12.3	10.1	8.6-11.4	10.6
$N_1P_1K_{0,5}$	14.5	13.7-15.1	10.7	9.2-12.3	12.7
$N_1P_1K_{1,5}$	12.7	11.8-13.6	10.5	10.2-10.9	11.6
$N_{0,5}P_{0,5}K_{0,5}$	14.7	13.8-15.4	11.6	11.4-11.8	13.1
$N_{1,5}P_{1,5}K_{1,5}$	14.3	13.3-15.2	11.3	8.8-14.8	12.8
$N_2P_2K_2$	15.2	14.2-16.1	11.0	8.4-14.4	13.1
Mean of A	13.8		11.3		-
LSD $p = 0.05$	$LSD_A = 0.72$; $LSD_B = 3.32$ LSD_{AB} = ns				

A_1 – NPK fertilization + Mg constant
A_2 – NPK fertilization + Mg constant, Ca constant
LSD – lowest significant difference for: A – liming, B – mineral fertilization (irrespective of liming), AB – interaction between liming and mineral fertilization
ns – differences not significant

The copper content in tubers of potato grown in non-limed and limed soils varied between the years in the range of 2.70-8.80 mg kg^{-1} d.m. (Table 1). The course of weather conditions during particular years had a substantial impact on the uptake of elements by plants. The influence of this factor on the chemical composition of crops was also observed by others (Pisulewska et al. 2009, Kraska 2011, Gugała et al. 2012, Knapowski et al. 2012).

The mean copper content in potato tubers grown on non-limed soil ranged from 4.71 to 6.32 mg kg^{-1} d.m., with an overall average content of 5.33 mg kg^{-1} d.m. In general, liming decreased the metal content in potato tubers; an overall average Cu content was 5.07 mg kg^{-1} ranging from 3.97 to 6.30 mg kg^{-1} (Table 1); however, the differences between mean values were statistically insignificant. The difference between the average Cu content in green matter of fodder sunflower harvested from fields treated (11.3 mg kg^{-1}) and not treated with lime (13.8 mg kg^{-1}) was bigger and statistically significant (Table 2). Nevertheless, the copper content in green matter of fodder sunflower grown on non-limed and limed soils was somewhat less varied between years, ranging between 10.1-17.1 and 6.1-17.0 mg kg^{-1}, respectively (Table 2). The effect of soil pH and therefore liming on the content of metal elements at plants was emphasized by many authors (Gorlach, Gambuś

2000, BEDNAREK et al. 2006, BRAVIN et al. 2009, MERCIK et al. 2004, ROGÓŻ, TRĄBCZYŃSKA 2009). Most studies show a decline in the copper content in plants with an increasing soil pH, e.g. ROGÓŻ and TRĄBCZYŃSKA (2009) found a reduction in the average copper content in potato tubers from 6.8 mg kg^{-1} d.m. to 4.7 mg kg^{-1} d.m. along the soil pH increasing from 5.5 to 6.5. This is the consequence of higher solubility and bioavailability of copper as the soil pH beomes lower (BRAVIN et al. 2009). CHAIGNON et al. (2002) observed better copper bioavailability at higher soil pH values around the rhizosphere in acid soils.

Both BRAVIN et al. (2009) and CHAIGNON et al. (2002) point to the influence of fertilization with nitrogen fertilizers on soil pH and copper bioavailability to plants; although usually fertilization with nitrates increases the pH of the rhizosphere, the introduction of ammonium nitrate into soil enhances its acidification and consequently copper absorption, hence the accumulation of copper in plants increases (with the exception of strongly acid soils). In the present study, limited nitrogen fertilization together with a constant dose of phosphorus and potassium (regardless of liming) resulted in a slight increase in the copper content in potato tubers (N_1 dose), while the highest dose ($N_{1,5}$) caused a significant decrease in the content of this element, although the differences between the mean values were statistically insignificant (Table 1). Similarly, fertilization of fodder sunflower with $N_{1,5}P_1K_1$ resulted in a remarkable (and statistically significant compared to $N_0P_1K_1$ and $N_{0,5}P_1K_1$ fertilization variants) reduction of yields of aerial plant parts (Table 2). It is worth noting that the highest content of Cu was characterized by potato tubers grown in the control variant (without NPK fertilization, regardless of liming) - an average of 6.09 mg kg^{-1} d.m. Phosphorus nutrition (at constant fertilization with N_1 and K_1) as well as potassium fertilization (at regular N_1 and P_1 fertilization) did not result in any statistically significant differences in the copper content of potato tubers or green matter of fodder sunflower. Also, the use of increasing NPK doses at a constant N:P:K ratio did not univocally affect the Cu content in the yields of the two crops, although a slight decrease in the Cu content in potato tubers (Table 1) as well as an increase Cu content in the aerial parts of sunflower (Table 2) could be noticed, as compared to the variant without fertilization. CIEĆKO and WYSZKOWSKI (2000) and TRAWCZYŃSKI (2009) found no effect of NPK fertilization on the copper content in potato tubers. However, phosphorus fertilization is a way to immobilize heavy metals in soil and to reduce their bioavailability (GORLACH, GAMBUŚ 2000). This was confirmed by the research performed by GUNES et al. (2009), who reported that the concentrations of Cu and Zn in the analysed plants (including sunflower) were reduced due to phosphorus fertilization.

CONCLUSIONS

1. The copper content in potato tubers did not depend on liming, mineral nutrition or the interaction of these treatments. Under the influence of liming and mineral fertilization, a trend to reduce the copper content in potato tubers was observed.

2. There was no interaction of liming and mineral fertilization in the shaping of the content of copper in green biomass of fodder sunflower.

3. Liming significantly reduced the copper content in green biomass of fodder sunflower, whereas mineral fertilization caused an increase in the copper content in green matter of fodder sunflower.

REFERENCES

Bednarek W., Tkaczyk P., Dresler S. 2006. *Heavy metal content as a criterion for assessment of potato tubers*. Ann., UMCS, Sect. E., 61: 121-131. (in Polish)

Bravin M. N., Marti A. L., Clairotte M., Hinsinger P. 2009. *Rhizosphere alkalisation – a major driver of copper bioavailability over a broad pH range in an acidic, copper-contaminated soil*. Plant Soil, 318: 257-268. DOI: 10.1007/s11104-008-9835-6

Commission Regulation (EC) No 1881/2006.

Chaignon V., Bedin F., Hinsinger P. 2002. *Copper bioavailability and rhizosphere pH changes as affected by nitrogen supply for tomato and oilseed rape cropped on an acidic and a calcareous soil.* Plant Soil, 243: 219-228.

Ciećko Z., Wyszkowski M. 2000. *Effect of NPK and magnesium fertilization on the content of micronutrients (Fe, Cu, Zn, Mn) in potato tubers.* Advances Agricultural Sciences Problem Issues, Polish Academy of Sciences, 471: 197-204. (in Polish)

Czuba R. 2000. *The micronutrients in recent fertilization systems*. Advances of Agricultural Sciences Problem Issues, Polish Academy of Sciences, 471: 161-169. (in Polish)

Dobrzański Z., Kołacz R., Górecka H., Malarz W., Rudnicka A. 2003. *Effect of copper industry on the content of copper, zinc and lead in vegetable fodder.* Acta Agroph., 1(2): 233-238. (in Polish)

Fässler E., Robinson B. H., Gupta S. K., Schulin R. 2010. *Uptake and allocation of plant nutrients and Cd in maize, sunflower and tobacco growing on contaminated soil and effect of soil conditioners under field conditions.* Nutr. Cycl. Agroecosyst., 87: 339-352. DOI: 10.1007/s10705-009-9342-z

Gembarzewski H. 2000. *Microelement content and tendencies of its changes in soils and plants from arable fields in Poland*. Advances of Agricultural Sciences Problem Issues, Polish Academy of Sciences, 471: 171-179. (in Polish)

Gorlach E., Gambuś F. 1997. *Phosphorus and multicomponent fertilizers as a source of soil pollution by heavy metals.* Advances of Agricultural Sciences Problem Issues, Polish Academy of Sciences, 448a: 139-146. (in Polish)

Gorlach E., Gambuś F. 2000. *Potentially toxic trace elements in soils (excess, harmfulness and counteracting).* Advances of Agricultural Sciences Problem Issues, Polish Academy of Sciences, 472: 275-296. (in Polish)

Gugała M., Zarzecka K., Mystkowska I. 2012. *Potato tuber content of magnesium and calcium depending on weed control methods.* J. Elem., 17(2): 247-254. DOI: 10.5601/jelem.2012.17.2.07

GUNES A., INAL A., KADIOGLU Y. 2009. *Determination of mineral element in wheat, sunflower, chickpea and lentil cultivars in response to P fertilization by polarized energy dispersive X-ray fluorescence.* X-Ray Spektrometry, 38(5): 451-462. DOI: 10.1002/xrs.1186

KABATA-PENDIAS A., MOTOWICKA-TERELAK T., PIOTROWSWKA M., TERELAK H., WITEK T. 1993. *Estimation of the degree of soil and crop contamination with heavy metals and sulphur* Framework guideline for agriculture. IUNG Puławy, S. P(53): 20. (in Polish)

KANIUCZAK J. 1992. *Yield and content of macro- and micronutrients in potato tubers against the background of mineral fertilization of brown soil developed from loess*. Scientific Conference "*Determinants of raw materials and food products quality*", Agricultural University of Cracow Publishers, Affiliate in Rzeszów, 1: 303-311. (in Polish)

KANIUCZAK J. 1998. *Research on the evolution of trace elements content in loessial soils.* Scientific Papers of H. Kołłątaj Agricultural University in Cracow, Dissertations, 244: 1-98. (in Polish)

KANIUCZAK J., HAJDUK E., WŁAŚNIEWSKI S. 2011. *Effect of liming and mineral fertilization on cadmium content in grain of spring barley* (*Hordeum vulgare* L.) *and winter wheat (Triticum aestivum* L.*) cultivated on loessial soil.* J. Elem., 16(4): 535-542. DOI: 10.5601/jelem.2011.16.4.03

KANIUCZAK J., NOWAK M., HAJDUK E., WŁAŚNIEWSKI S. 2003. *The influence of liming and mineral fertilization on soluble forms micronutrient content in grey-brown podzolic soils formed from loess*. Advances of Agricultural Sciences Problem Issues, Polish Academy of Sciences, 493: 607-614.

KARA E. E., PIRLAK U., OZDILEK G. H. 2004. *Evaluation of heavy metals (Cd, Cu, Ni, Pb, and Zn) distribution in sowing regions of potato fields in the province of Niğde, Turkey*. Water, Air Soil Pollution, 153: 173-186.

KNAPOWSKI T., RALCEWICZ M., SPYCHAJ-FABISIAK E., MURAWSKA B. 2012. *Effect of the rate of nitrogen and zinc on the zinc and copper accumulation in grain of spring triticale cultivar Kargo*. J. Elem., 17(3): 421 - 429. DOI: 10.5601/jelem.2012.17.3.05

KRASKA P. 2011. *Content of some elements in grain of spring wheat cv. Zebra depending on soil tillage systems and catch crop.* J. Elem., 16(3): 407-419. DOI: 10.5601/jelem.2011.16.3.06.

LAVADO R. S. 2006. *Effects of sewage sludge application on soils and sunflower yield: quality and toxic element accumulation.* J. Plant Nutrit., 29: 975-984.

MEDYŃSKA A., KABAŁA C., CHODAK T., JEZIERSKI P. 2009. *Concentration of copper, zinc, lead and cadmium in plants cultivated in the surroundings of Żelazny Most copper ore tailings impoundment*. J. Elem., 14(4): 729-736. DOI: jelem.2009.14.4.729-736

MERCIK S., STĘPIEŃ W., MATYSIAK B. 2004. *Mobility and uptake of copper and zinc by plants depending on the soil properties.* Advances of Agricultural Sciences Problem Issues, Polish Academy of Sciences, 502: 235-245. (in Polish)

MUSILOVÁ J., BYSTRICKÁ J., TOMAS J., ARVAY J. 2009. *Contamination of potato tuber (Solanum tuberosum* L.*) by nickel and copper*. Ecol. Chem. Engine., A. 16(12): 1605-1616.

PISULEWSKA E., PORADOWSKI R., ANTONKIEWICZ J., WITKOWICZ R. 2009. *The effect of variable mineral fertilization on yield and grain mineral composition of covered and naked oat cultivars*. J. Elem., 14(4): 763-772. DOI: jelem.2009.14.4.763-772.

ROGÓŻ A., TRĄBCZYŃSKA K. 2009. *Trace element content in soils and in selected root crops.* Part I. *The content of copper and zinc*. Advances of Agricultural Sciences Problem Issues, Polish Academy of Sciences, 541: 353-363. (in Polish)

STRĄCZYŃSKI S. J., STRĄCZYŃSKA S. 2009. *Copper bioacumulation in selected plant species cultivated in the area polluted by copper metallurgy.* Advances of Agricultural Sciences Problem Issues, Polish Academy of Sciences, 541: 409-416. (in Polish)

TRAWCZYŃSKI C. 2009. *The influence of manure, brown coal waste fraction and mineral fertilization on some microelement content in soil and potato tubers.* Advances of Agricultural Sciences Problem Issues, Polish Academy of Sciences, 541: 441-448.

J. Elem. s. 433–446 DOI: 10.5601/jelem.2013.18.4.552

EFFECT OF SULFUR FERTILIZATION ON THE CONCENTRATIONS OF COPPER, ZINC AND MANGANESE IN THE ROOTS, STRAW AND OIL CAKE OF RAPESEED (*BRASSICA NAPUS* L. SSP. *OLEIFERA* METZG)*

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Abstract

Sulfur application has a significant effect on the yield of oil bearing plants of the family *Brassicaceae*, especially when the sulfur content of soil is low. Sulfur fertilization also affects the value of plant raw materials, reflected by the concentrations of mineral and biologically active compounds in biomass. The aim of this study was to determine the effect of sulfur application to soil on the concentrations of copper, zinc and manganese in the root residues, straw and oil cake of winter and spring rapeseed. A three-year (2005-2008) field experiment was conducted at the Agricultural Experimentation Station in Bałcyny (NE Poland).

In both spring and winter rapeseed, oil cake contained the highest levels of copper and zinc, followed by root residues and straw. The highest concentrations of manganese per kg dry matter (DM) were found in the root residues of winter rapeseed and in the cake of spring rapeseed. The concentrations of micronutrients (Cu, Zn, Mn) were slightly higher in the roots of winter rapeseed, compared with spring rapeseed. Sulfur fertilization decreased copper levels and increased manganese levels in the root residues of spring and winter rapeseed. Sulfur application to soil increased zinc concentrations in winter rapeseed roots, and it had no significant influence on the zinc content of spring rapeseed roots. Spring rapeseed straw contained considerably higher levels of zinc and manganese than winter rapeseed straw. The copper content of

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straw was comparable in spring and winter rapeseed. Sulfur application to soil increased the concentrations of zinc and manganese in winter rapeseed straw, and it had no significant effect on the levels of those minerals in spring rapeseed straw. Spring rapeseed cake had a significantly higher content of copper and zinc, compared with winter rapeseed cake. Manganese concentrations in the cake of spring and winter rapeseed were similar. Sulfur fertilization contributed to a significant increase in the concentrations of zinc and manganese in winter rapeseed cake. The manganese content of spring rapeseed cake decreased significantly in response to sulfur fertilization, which had no effect on the concentrations of the other micronutrients.

Keywords: winter rapeseed, spring rapeseed, sulfur fertilization, micronutrient content, root residues, straw, cake.

ZAWARTOŚĆ MIEDZI, CYNKU I MANGANU W KORZENIACH, SŁOMIE I WYTŁOKACH RZEPAKU (*BRASSICA NAPUS* L. SSP. *OLEIFERA* METZG) W ZALEŻNOŚCI OD NAWOŻENIA SIARKĄ

Abstrakt

Siarka odgrywa bardzo istotną rolę w plonowaniu roślin oleistych z rodziny *Brassicaceae*, szczególnie w przypadku niskiej jej zawartości w glebie. Wpływa również bardzo silnie na wartość użytkową surowca mierzoną koncentracją w biomasie związków mineralnych oraz biologicznie czynnych. Celem pracy było określenie wpływu doglebowej aplikacji siarki na zawartość miedzi, cynku oraz manganu w resztkach korzeniowych, słomie oraz wytłokach rzepaku ozimego i jarego. Materiał do badań uzyskano w ścisłym doświadczeniu polowym realizowanym w pełnym 3-letnim cyklu badań (2005-2008) na polach doświadczalnych w Bałcynach.

Najwięcej miedzi oraz cynku, niezależnie od formy rzepaku, zawierały wytłoki. Średnią koncentrację tego mikropierwiastka stwierdzono w resztkach korzeniowych, a najmniejszą – w słomie. Najwięcej manganu w 1 kg suchej masy zawierały resztki korzeniowe rzepaku ozimego. Rzepak jary zawierał najwięcej manganu w wytłokach. Koncentracja mikropierwiastków (Cu, Zn, Mn) w korzeniach rzepaku ozimego była nieznacznie większa niż u rzepaku jarego. Nawożenie siarką powodowało zmniejszenie zawartości miedzi oraz wzrost zawartości manganu w resztkach korzeniowych obu form rzepaku. Doglebowa aplikacja siarki powodowała wzrost koncentracji cynku w korzeniach rzepaku ozimego, nie różnicując istotnie jego zawartości w resztkach korzeniowych formy jarej rzepaku. Słoma rzepaku jarego zawierała znacznie więcej cynku i manganu w porównaniu z formą ozimą. Zawartość miedzi w słomie badanych gatunków była na porównywalnym poziomie. Doglebowa aplikacja siarki powodowała wzrost zawartości cynku i manganu w słomie rzepaku ozimego, nie różnicując jego koncentracji w słomie formy jarej. Wytłoki rzepaku jarego zawierały istotnie więcej miedzi i cynku w porównaniu z rzepakiem ozimym. Koncentracja manganu w wytłokach obu form rzepaku była podobna. Nawożenie siarką rzepaku ozimego powodowało istotny wzrost koncentracji cynku i manganu w wytłokach. Nawożenie siarką rzepaku jarego powodowało istotne zmniejszenie zawartości manganu w wytłokach, nie różnicując w nich koncentracji pozostałych mikropierwiastków

Słowa kluczowe: rzepak ozimy, rzepak jary, nawożenie siarką, zawartość mikroelementów, resztki korzeniowe, słoma, wytłoki.

INTRODUCTION

Oil bearing plants of the family *Brassicaceae* are characterized by high sulfur requirements, namely they need 15-20 kg of sulfur to produce 1 Mg of biomass (Zhao et al. 1993). The yield-forming effect of sulfur is particularly notable when winter rapeseed is grown in sulfur-deficient soil. Under such conditions, even low rates of sulfur fertilizer can be highly effective (McGrath, Zhao 1996, Krauze, Bowszys 2001, Zukalová et al. 2001*ab*, Podleśna 2004, Jankowski et al. 2008*ab*, Wielebski 2008, 2011). Sulfur applied to plants of the family *Brassicaceae* can considerably affect the quality of raw products such as oil, fat-free seed residues and straw. Sulfur fertilizers exert the strongest effect on the nutritional value of seeds, including concentrations of numerous biologically active compounds, mostly erucic acid in oil and glucosinolates, fiber, tannins, polyphenols and phytic acid in fat-free seed residues (Rotkiewicz et al. 1996). Sulfur fertilization affects the uptake of other nutrients, too. Ahmad et al. (1999), and McGrath and Zhao (1996) demonstrated that sulfur deficiency inhibits the nitrogen uptake by plants, thus suppressing their growth and development, indirectly modifying the chemical composition of the main and side-line crops.

Sulfur fertilization may significantly influence the chemical composition of postharvest residues of *Brassicaceae* species. These plants are grown on a steadily larger acreage partly because of their economic importance as food crops and energy crops and partly owing to their role in agricultural ecosystems. It should be stressed that although the root residues and straw of oil plants are a rich and often the major (in non-livestock farms) source of soil micronutrients, the micronutrient content of postharvest residues of oil crops still awaits more thorough investigations. Roots of winter rapeseed accumulate large amounts of zinc (*ca* 21.6-48.3 mg kg^{-1} d.m.) (Wiśniowska-Kielian 2003). Root residues of spring rapeseed are also a valuable source of zinc (148 mg kg^{-1} d.m.) (Szczebiot, Ojczyk 2002). High levels of manganese and zinc (10.65-14.28 and 6.15-8.68 mg kg^{-1} d.m., respectively) are found in straw of winter rapeseed. The copper content in aerial parts of winter rapeseed does not exceed 2.5 mg kg^{-1} d.m. (Spiak et al. 2007). It should be noted that in winter rapeseed, the copper content is 1.6-fold higher in vegetative organs than in seeds, and the concentrations of zinc and manganese in seeds are four- and three-fold higher, respectively, than in straw. Spring rapeseed straw contains high levels (34.6-37.2 mg kg^{-1} d.m.) of manganese (Krzywy, Iżewska 2007).

The aim of this study was to determine the effect of sulfur application to soil on the concentrations of micronutrients (copper, zinc and manganese) in root residues, straw and cake of winter and spring rapeseed.

MATERIAL AND METHODS

The experiment was conducted at the Agricultural Experimentation Station in Bałcyny (N = 53°35′49″, E = 19°51′20.3″), in 2005-2008. The experimental variables were:

primary variable – crop plant: winter rapeseed, spring rapeseed;

secondary variable – rate of sulfur fertilizer applied to soil: (+S) winter rapeseed – 60 kg ha^{-1}, spring rapeseed – 40 kg ha^{-1}, (-S) control – no sulfur fertilization.

The experiment had a split-plot design with three replications. The plot size was 18 m^2. Each year, the experiment was established on grey-brown podsolic soil developed from light loam, of good wheat complex. The soil had a slightly acid pH of 5.75-6.39 in 1 M KCl, medium copper and manganese content, and medium to high zinc content (Table 1). The preceding crop was spring barley grown after spring wheat (first and second growing season) or after winter wheat (third growing season).

Table 1

Soil conditions (2005-2008)

Specification	Growing season		
	2005/2006	2006/2007	2007/2008
Soil type	grey-brown podsolic soil		
Soil texture group	light loam		
Soil quality class	IIIa	IIIa	IIIb
Soil suitability for agriculture	good wheat complex		
Organic carbon content of soil (%)	1.47	1.75	1.57
Soil pH (1 M KCl)	6.39	6.08	5.75
Concentrations of available nutrients (mg kg^{-1} soil)			
– P	107	85	143
– K	104	133	104
– Mg	103	85	51
– S	163	140	144
– S-S0$_4$	25	10	10
– Cu	4.4	2.7	2.8
– Zn	23.1	11.1	10.9
– Mn	230	180	235

Winter rapeseed was fertilized pre-sowing with 30 kg N ha^{-1}, 22 kg ha^{-1} P and 166 kg ha^{-1} K. In spring, nitrogen was applied in three split doses: 120 (52.50* + 67.50) kg ha^{-1} before lateral branch development (BBCH 20), 80 kg ha^{-1} at inflorescence emergence (BBCH 50). Spring rapeseed was fertilized pre-sowing with 70 (35*+35) kg N ha^{-1}, 17 kg ha^{-1} P and 100 kg ha^{-1} K. At inflorescence emergence (BBCH 50), supplemental nitrogen was applied at 30 kg ha^{-1}. Boron was sprayed over leaves of winter rapeseed plants (BBCH 53) at 43.75 g ha^{-1}, as aqueous solution of Solubor DF. Phosphorus was applied to soil as triple superphosphate, potassium – as 60% potash salt, sulfur – as ammonium sulfate (*), nitrogen – as ammonium nitrate (treatments without sulfur fertilization) or ammonium sulfate (*) and ammonium nitrate (treatments with sulfur fertilization).

Winter rapeseed cv. Californium was sown in the first week or in the middle of August, at the density of 90 germinating seeds per 1 m^2 of plot area. Spring rapeseed cv. Hunter was sown in the first week or in the middle of April, at the density of 140 germinating seeds per 1 m^2 of plot area. Both winter and spring rapeseeds were dressed with thiamethoxam, metalaxyl-M and fludioxonil. The inter-row spacing was approximately 19 cm.

Dicotyledonous weeds were controlled with metazachlor and quinmerac, at 1 166 g ha^{-1} and 290.5 g ha^{-1} in winter rapeseed, and at 999 g ha^{-1} and 249 g ha^{-1} in spring rapeseed. In the winter rapeseed plantation, haloxyfop-R was applied at 52 g ha^{-1} at the stage of 4-6 leaves unfolded (BBCH 14-16).

In the first growing season, pest control required four and six insecticide treatments in winter and spring rapeseed fields, respectively. In the second and third growing season, high pest infestation coincided with a period of high pest susceptibility of spring rapeseed. Therefore, only one insecticide treatment was carried out in winter rapeseed, and four to six in spring rapeseed plots. Chemical control of plant pathogens was necessary only in winter rapeseed, and it involved the use of 100 g ha^{-1} dimoxystrobin and 100 g ha^{-1} boscalid at the stage of flower fading: most petals fallen (BBCH 66-67).

Harvest was carried out in two stages, at processing maturity. Winter rapeseed was swathed (cutting height of 8 cm) at the end of June or in mid-July. Spring rapeseed reached maturity in the first half of August.

The organic carbon content of soil was determined according to the research protocol of the Chemical and Agricultural Research Laboratory, SCHR PB 24, second edition of 21 June 2004. The available nutrient content and soil pH were determined in the plough layer, in accordance with the Polish Standards: PN ISO 10390:1997 (pH), PN-R-04023:1996 (phosphorus), PN-R-04022/Az1:2002 (potassium), PN-R-04020:1994 (magnesium), PN-92/R-04017 (copper), PN-92/R-04016 (zinc), and PN-93/R-04019 (manganese). The content of total sulfur and sulfate sulfur was calculated according to the research protocol of the Chemical and Agricultural Research Laboratory, SCHR PB 27 second edition of 21 June 2004 and SCHR PB 26, second edition of 21 June 2004.

The concentrations of copper, zinc and manganese were estimated in the dry matter of root residues (roots + stubble), straw and cake of winter and spring rapeseed. Samples for chemical analysis (roots + stubble, straw, and seeds) were collected at harvest. Root and stubble samples were collected with soil, by drilling to the depth of 30 cm with a steel cylinder (diameter – 22.57 cm, area – *ca* 400 cm^2). The samples were washed under running water in a 1 mm mesh sieve. Seeds were cold-pressed in a laboratory press with a pressing capacity of *ca* 50 kg h^{-1}. The oil content of cake ranged from 127 g kg^{-1} d.m. (winter rapeseed) to 129 g kg^{-1} d.m. (spring rapeseed). Dried samples of roots + stubble, straw and cake were ground in a laboratory mill. The concentrations of copper, zinc and manganese in biomass were determined by atomic absorption spectometry (AAS). The chemical properties of soil and the micronutrient content of plant material were analyzed in the Chemical and Agricultural Research Laboratory in Olsztyn.

The results of chemical analyses were processed by Anova in accordance with the experimental method. Mean values from every treatment were compared by the Tukey's test. LSD values were calculated at a 5% error rate.

RESULTS AND DISCUSSION

The application of sulfur fertilizers (ammonium sulfate) to winter and spring rapeseed had no significant effect on changes in the pH of the plough layer, which in turn were largely dependent on the form of rapeseed (Table 2). On average (means from three years), the pH of soil tended to increase under winter rapeseed and to decrease under spring rapeseed (Tables 1 and 2).

Table 2

Soil pH (1 M KCl) after rapeseed harvest

Crop plant	Growing season						Mean	
	2005/2006		2006/2007		2007/2008			
	+S	-S	+S	-S	+S	-S	+S	-S
Winter rapeseed	6.44	6.54	6.16	6.05	5.76	5.76	6.12±0.21	6.11±0.21
Spring rapeseed	6.06	6.23	5.87	5.89	5.44	5.38	5.79±0.22	5.83±0.22

The root dry weight of spring rapeseed in the plough layer (0-30 cm) was higher (by *ca* 12% on average) than that of winter rapeseed. The yields of straw and oil cake per ha were considerably higher (by 67% and over four-fold, respectively) by winter rapeseed than spring rapeseed. Sulfur fertilization had no significant effect on the yields of root residues and straw from winter and spring rapeseed. Sulfur application to soil resulted in a significant decrease in the yield of winter rapeseed cake per ha, and it had no significant effect on the yield of spring rapeseed cake (Figure 1).

The postharvest biomass of winter and spring rapeseed (root residues, straw and cake) differed considerably with respect to the concentrations of micronutrients (Cu, Zn, Mn) – Figure 2. In the biomass of winter and spring rapeseed, the cake was the richest source of copper and zinc, followed by root residues and straw. Manganese concentrations in the postharvest residues of winter and spring rapeseed were different. Winter rapeseed contained the largest amounts of manganese in root residues (46.4 mg kg^{-1} d.m.).

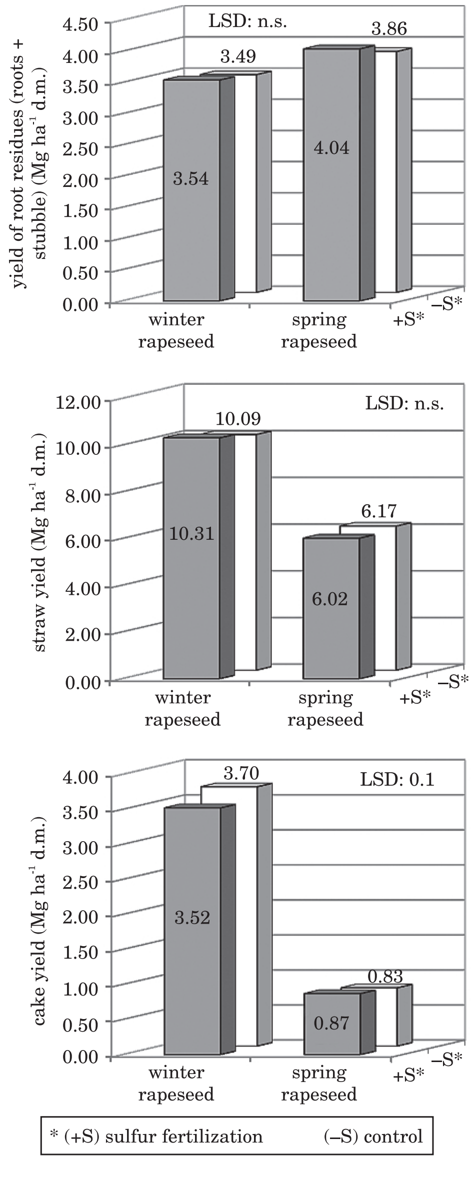

Fig. 1. Effect of sulfur fertilization on the biomass yield of winter and spring rapeseed in 2005-2008

The manganese content of cake and straw was lower by 8.6 and 36.2 mg kg^{-1} d.m., respectively. In spring rapeseed, manganese concentrations were the highest in cake (40.8 mg kg^{-1} d.m.), and lower in root residues (36.1 mg kg^{-1} d.m.) and straw (20.9 mg kg^{-1} d.m.) – Figure 2.

In the study by Gondek and Filipek-Mazur (2003), roots of winter rapeseed accumulated considerable amounts of zinc (*ca* 21.6-48.3 mg kg^{-1} d.m.).

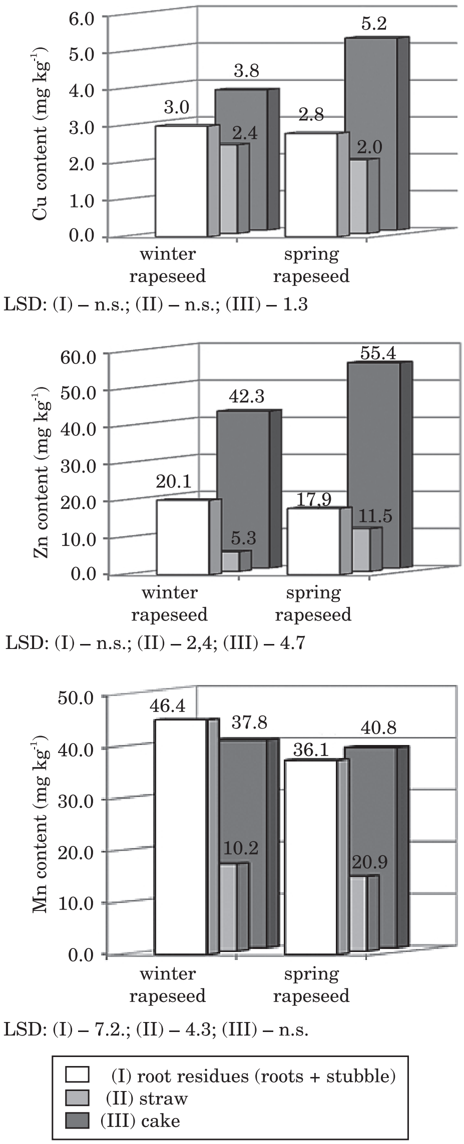

Fig. 2. Concentrations of copper, zinc and manganese in the root residues (roots + stubble), straw and cake of winter and spring rapeseed (means for crops), 2005-2008

Szczebiot and Ojczyk (2002) also demonstrated that roots of spring oil plants contained mostly zinc; the highest zinc concentrations (148 mg kg^{-1} d.m.) were determined in spring rapeseed roots, followed by roots of Indian mustard (123 mg kg^{-1} d.m.) and white mustard (110 mg kg^{-1} d.m.). The post-harvest residues (roots + stubble) of spring oil crops are also a valuable source of manganese. In the study by Szczebiot and Ojczyk (2002), root residues of white mustard were characterized by the highest manganese concentrations (124 mg kg^{-1} d.m.). The manganese content in roots of Indian mustard and spring rapeseed were by around 27% and 44% lower, respectively. The copper content of the root residues of winter rapeseed determined by Gondek and Filipek-Mazur (2003) was 2.6-3.1 mg kg^{-1} d.m., whereas Szczebiot and Ojczyk (2002) reported that the copper content of spring rapeseed roots reached 3.0-3.5 mg kg^{-1} d.m.

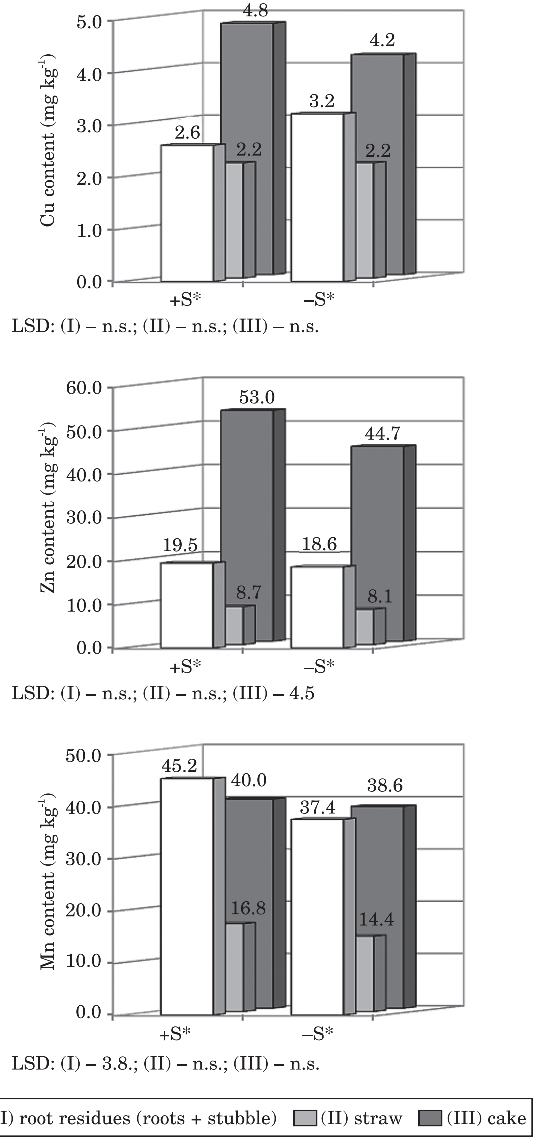

Fig. 3. Concentrations of copper, zinc and manganese in the root residues (roots + stubble), straw and cake of winter and spring rapeseed (means for sulfur fertilization), 2005-2008

The analysis of the data presented in Figure 3 shows that sulfur fertilization had no significant effect on copper concentrations in roots of winter and spring rapeseed. Sulfur application to soil caused a significant (14%) increase in zinc concentrations in roots of winter rapeseed, having no significant influence on the zinc content of spring rapeseed roots (Figure 4). Manganese content per kg root d.m. increased (by ca. 21% on average) in response to sulfur fertilization in both winter and spring rapeseed (Figures 3).

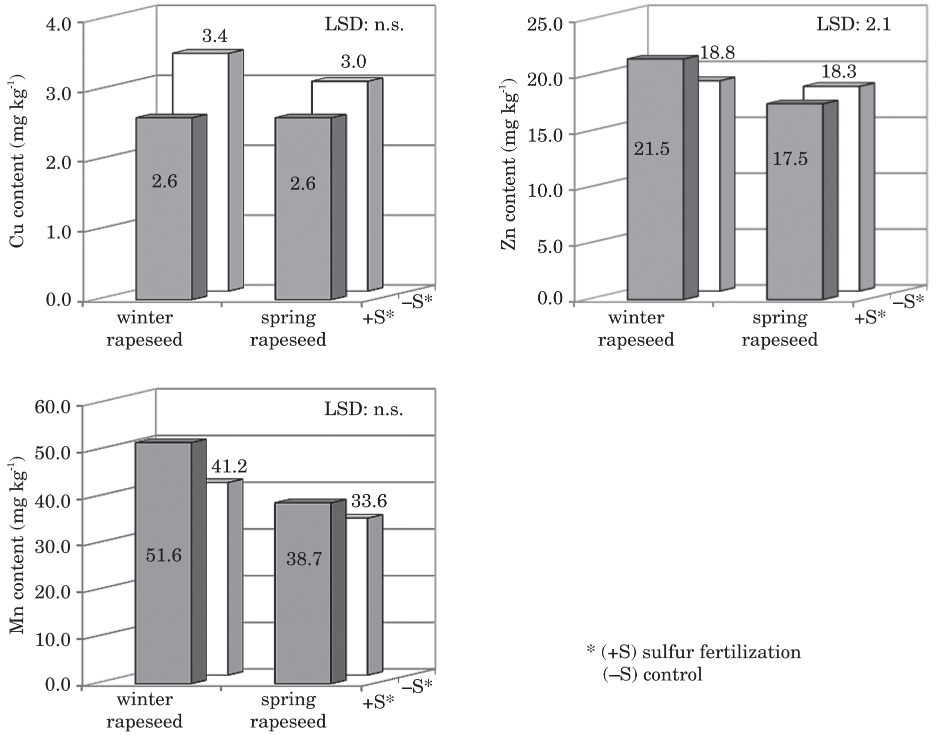

Fig. 4. Effect of sulfur fertilization on the concentrations of copper, zinc and manganese in the root residues (roots + stubble) of winter and spring rapeseed (interaction: crop · sulfur fertilization), 2005-2008

Spiak et al. (2007) reported that 1 kg d.m. of winter rapeseed straw contained 2.4-2.7 mg Cu, 6.2-8.7 mg Zn and 10.7-14.3 mg Mn. The straw of spring rapeseed is a rich source of manganese, whose content ranges from 34.6 to 37.2 mg kg^{-1} d.m. Krzywy and Iżewska (2007) reported that the zinc and copper content of spring rapeseed straw reached 4.0-4.4 mg kg^{-1} d.m. and 27.9-41.9 mg kg^{-1} d.m., respectively. In our study, the straw of winter and spring rapeseed contained primarily manganese (8.3-21.4 mg kg^{-1} d.m.) and zinc (4.7-11.6 mg kg^{-1} d.m.) – Figure 5. Spring rapeseed straw was a richer source of zinc and manganese than winter rapeseed straw. The concentrations of these minerals were over twofold higher in the straw of spring rapeseed than winter rapeseed. The copper content of straw was comparable in winter and spring rapeseed (2.0-2.4 mg kg^{-1} d.m.), and the noted differences were within the margin of error (Figure 2). Sulfur fertilization had no significant effect on copper concentrations in the straw of winter and spring rapeseed (Figure 3). Sulfur application to soil contributed to a substantial increase in the zinc and manganese levels in straw of winter rapeseed (by 23% and 46%, respectively), but it had no significant effect on the concentrations of those micronutrients in straw of spring rapeseed (Figure 5).

The fat-free seed residues of oil plants are a rich source of iron. The cake of spring oil-bearing crops (spring rapeseed, Indian mustard) contains higher levels of iron (138-266 and 133-162 mg kg^{-1} d.m.), compared with winter rapeseed (111-175 mg mg kg^{-1} d.m.). The concentrations of zinc and manganese in cake of winter rapeseed are similar (37-64 mg Zn and 41-63 Mn mg kg^{-1} d.m.) (Banaszkiewicz 1998, Kalembasa, Adamiak 2010). Fat -free seed residues of spring rapeseed are a richer source of manganese (52-61 mg kg^{-1} d.m.) than of zinc (37-43 mg kg^{-1} d.m.) (Bell et al. 1999). The copper content of fat-free seed residues was comparable in winter and spring rapeseed (*ca* 4.3-6.1 mg kg^{-1} d.m.). The cake of mustards contains higher concentrations of copper (by *ca* 35-57% on average) than the cake of rapeseed (Banaszkiewicz 1998, Bell et al. 1999, Kalembasa, Adamiak 2010).

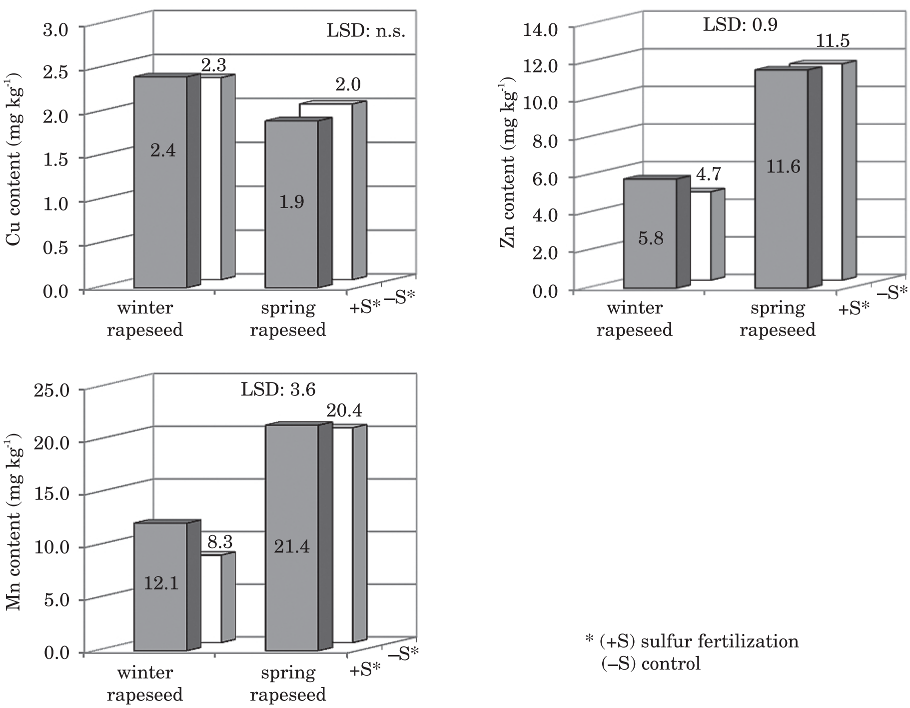

Fig. 5. Effect of sulfur fertilization on the concentrations of copper, zinc and manganese in the straw of winter and spring rapeseed (interaction: crop · sulfur fertilization), 2005-2008

In the present study, the cake of winter rapeseed contained the following amounts of copper, zinc and manganese: 3.8, 42.3 and 37.8 mg kg^{-1} d.m., respectively. The cake of spring rapeseed contained significantly higher concentrations of copper (by 1.4 mg kg^{-1} d.m.) and zinc (by 13.1 mg kg^{-1} d.m.) than the cake of winter rapeseed. Manganese levels in the cake of spring and winter rapeseed were similar (37.8-40.8 mg kg^{-1} d.m.) – Figure 2. Sulfur fertilization contributed to a significant increase in the concentrations of zinc and manganese in winter rapeseed cake. Sulfur application to soil considerably decreased the manganese content of spring rapeseed cake (by 24%), having no effect on the concentrations of copper and zinc (Figure 6).

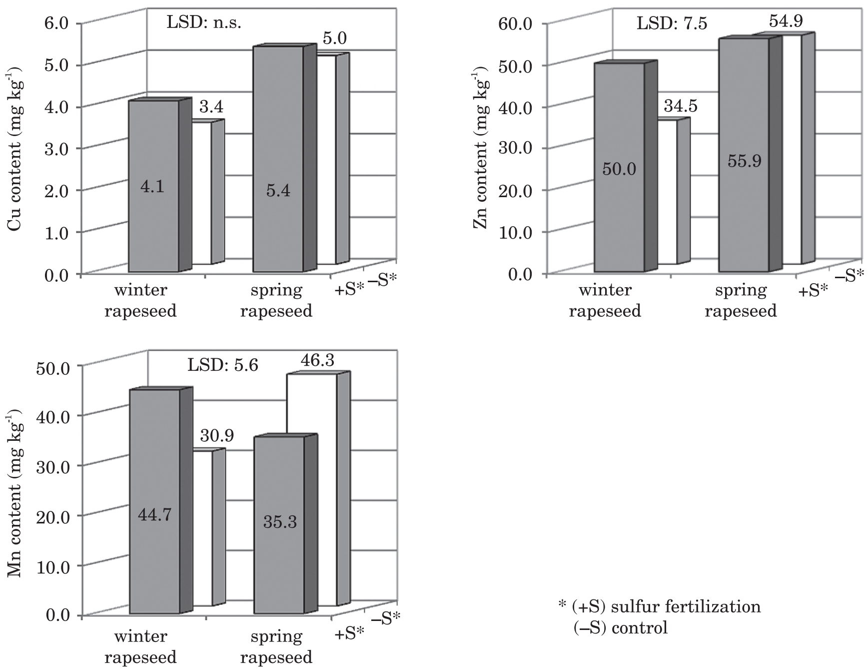

Fig. 6. Effect of sulfur fertilization on the concentrations of copper, zinc and manganese in the cake of winter and spring rapeseed (interaction: crop · sulfur fertilization), 2005-2008

CONCLUSIONS

1. In both spring and winter rapeseed, oil cake contained the highest levels of copper and zinc, followed by root residues and straw. The highest concentrations of manganese were found in the root residues of winter rapeseed and in the cake of spring rapeseed.

2. The concentrations of micronutrients were higher in roots of winter rapeseed than in roots of spring rapeseed. Sulfur fertilization decreased copper levels and increased manganese levels in the root residues of spring

and winter rapeseed, whereas zinc concentrations increased only in winter rapeseed roots.

3. Spring rapeseed straw contained considerably higher levels of zinc and manganese than winter rapeseed straw. The copper content of straw was comparable in spring and winter rapeseed. Sulfur application to soil increased the concentrations of zinc and manganese in winter rapeseed straw, but it had no significant effect on the levels of these minerals in spring rapeseed straw.

4. Spring rapeseed cake had a significantly higher content of copper and zinc than winter rapeseed cake. Manganese concentrations in the cake of spring and winter rapeseed were similar. Sulfur fertilization contributed to a significant increase in the concentrations of zinc and manganese in winter rapeseed cake. The manganese content of spring rapeseed cake decreased significantly in response to sulfur fertilization.

REFERENCES

AHMAD A., ABROL Y.P., ABDIN M.Z. 1999. *Effect of split application of sulphur and nitrogen on growth and yield attributes of Brassica genotypes differing in time of flowering*. Can. J. Plant Sci., 79(2): 175-180.

BANASZKIEWICZ T. 1998. *Mineral content in seeds and cakes of three domestic winter rape varieties.* Rośliny Oleiste - Oilseed Crops, 19(2): 556-561. (in Polish)

BELL J.M., RAKOW G., DOWNEY R.K. 1999. *Mineral composition of oil-free seeds of Brasica napus, B. rapa and B. juncea as affected by location and year*. Can. J. Anim. Sci, 79: 405-408.

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

JANKOWSKI K., BUDZYŃSKI W., SZYMANOWSKI A. 2008*a*. *Influence of the rate and timing of sulphur fertilization on winter oilseed rape yield*. Rośliny Oleiste - Oilseed Crops, 29(1): 75-89.

JANKOWSKI K., BUDZYŃSKI W., SZYMANOWSKI A. 2008*b*. *Effect of sulfur on the quality of winter rape seeds.* J. Elem., 13(4): 521-534.

KALEMBASA S., ADAMIAK E. A. 2010. *Determination of chemical composition of rapeseed* cake. Acta Agroph., 15(2): 323-332.

KRAUZE A., BOWSZYS T. 2001. *Effect of time of sulphur fertilization of spring oilseed rape cv. Star on seed yield, sulphur content and crude oil.* Rośliny Oleiste - Oilseed Crops, 22(1): 285-290. (in Polish)

KRZYWY E., IŻEWSKA A. 2007. *Impact of mature and organic fertilizers on the quantity and content yield of heavy metals in spring rape*. Pol. J. Chem. Tech., 9: 60-63.

MCGRATH S.P., ZHAO F.J. 1996. *Sulphur uptake, yield responses and interaction between nitrogen and sulphur in winter rape (Brassica napus).* J. Agric. Sci. Camb., 126(1): 53-62.

PODLEŚNA A. 2004. *The effect of sulfur fertilization on concentration and uptake of nutrients by winter oilseed rape.* Rośliny Oleiste - Oilseed Crops, 25: 627-636. (in Polish)

ROTKIEWICZ D., OJCZYK T., KONOPKA I. 1996. *Influence of sulphur fertilization on the technological and useful value of winter repeseed.* Rośliny Oleiste - Oilseed Crops, 17(2): 257-264. (in Polish)

SPIAK Z., PISZCZ U., ZBROSZCZYK T. 2007. *Disposition of nutrients in seeds and straw of selected Winter rape cultivars.* Part II. *Micronutrients*. Zesz. Nauk. AR Wroc., Rol., XC, 553: 107-115. (in Polish)

SZCZEBIOT M., OJCZYK T. 2002. *Fertilization value of post-harvest residues of oil crops for winter wheat*. Fragm. Agron., 2: 198-206. (in Polish)

WIELEBSKI F. 2008. *Efficiency of sulphur fertilization of different types of oilseed rape varieties on the basis of the results from field experiments of many years*. Rośliny Oleiste - Oilseed Crops, 29(1): 91-103. (in Polish)

WIELEBSKI F. 2011. *The effect of sulphur fertilization on chemical composition of seeds of different breeding forms of winter oilseed rape in the conditions of diverse nitrogen rates*. Rośliny Oleiste - Oilseed Crops, 32(1): 79-95. (in Polish)

WIŚNIOWSKA-KIELIAN B. 2003. *Trace element contents in soil and winter wheat*. Part I. *Microelements content*. Chem. Inż. Ekol., 10(9): 1011-1019.

ZHAO F.J., EVANS E. J., BILSBORROW P. E., SYERS J. K. 1993. *Influence of sulphur and nitrogen on seed yield and quality of low glucosinolate oilseed rape (Brassica napus* L.*)*. J. Sci. Food Agric., 63: 29-37.

ZUKALOVÁ H., MATULA J., KUCHTOVÁ P., MIKŠÍK V. 2001a. *Influence of sulphur on the yield and quality of winter oilseed rape*. Rośliny Oleiste - Oilseed Crops, 22(2): 587-596.

ZUKALOVÁ H., MATULA J., VAŠÁK J. 2001b. *Effect of sulphur fertilization upon biomass dry matter production dynamics and glucosinolate biosynthesis in three types of winter oilseed rape (Brassica napus* L.*)*. Rośliny Oleiste - Oilseed Crops, 22(1): 273-284.

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INDICATORS OF MINERAL AND ENERGY METABOLISM IN THE THREE DAYS FOLLOWING MILK FEVER SYMPTOMS IN DAIRY COWS

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Abstract

A total of 60 dairy cows met the requirements of the study (50 disease cases and 10 as the control group), located on 10 farms in the central Lublin region. The cows were aged 3 to 8 years, with good or very good milk production and about a week after parturition. They were first divided into two groups based on their clinical symptoms of milk fever. During the experiment, two subgroups were distinguished from the groups, based on the condition of the animals during the observation period. At 24, 48 and 72 hour,s blood was taken from the animals after no clinical symptoms were detected and at least 36 hours after the last administration of medicines. In the first days of the analysis, low levels of minerals (calcium – Ca and inorganic phosphorus – Pi) and low concentrations of glucose and cholesterol with simultaneously higher amounts of free fatty acids (FFA) were observed in the serum. On the second day of the analysis, these levels had dropped and on the third day some of the cows were observed to have small problems with appetite or difficulty getting up; therefore, after taking material for evaluation and analysis, treatment was begun or prophylaxis increased. Based on the results, the authors can conclude that the major health threat to cows recovering from milk fever appears 2 to 3 day after the subsidence of any signs of acute hypocalcaemia, and 3 days after the last application of medicines. Cows which had been clinically healthy in this period showed significant differences in biochemistry results versus the control groups and needed to receive prophylactic preparations to resolve the mineral and energy insufficiencies. Our observations suggest the necessity for check-up blood tests in cows for 3 days after an episode of milk fever, as well as mineral and energy prophylactic supplementation during that time.

Keywords: milk fever, dairy cows health, hypocalcaemia, phosphorus, glucose.

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WSKAŹNIKI PRZEMIANY MINERALNEJ I ENERGETYCZNEJ W PIERWSZYCH TRZECH DOBACH PO USTĄPIENIU OBJAWÓW PORAŻENIA POPORODOWEGO U KRÓW MLECZNYCH

Abstrakt

Do badań zakwalifikowano 60 krów mlecznych (50 szt. przypadki chorobowe i 10 szt. grupa kontrolna) pochodzących z 10 gospodarstw centralnej Lubelszczyzny. Zwierzęta miały od 3 do 8 lat, cechowały się dobrą lub bardzo dobrą produkcją mleczną i znajdowały w okresie ok. tygodnia po porodzie. Początkowo podzielono je na dwie grupy na podstawie przebiegu objawów klinicznych porażenia poporodowego. W trakcie badań wydzielono z poszczególnych grup po dwie podgrupy, ze względu na stan zwierząt podczas okresu obserwacji. Od zwierząt pobierano krew po 24, 48 i 72 godzinach od ustąpienia wszystkich objawów klinicznych i jednocześnie przynajmniej 36 godzin po ostatniej podaży leków. W pierwszym dniu analiz stwierdzono niski poziom wskaźników mineralnych (Ca i Pn) oraz glukozy i cholesterolu, ale jednocześnie wyższą wartość wolnych kwasów tłuszczowych (WKT) w surowicy krów. Natomiast w drugim dniu analiz poziomy te obniżyły się, a trzeciego dnia u części krów wystąpiły nieznaczne problemy z apetytem lub problemy ze wstawaniem, dlatego po pobraniu materiału do badań i wykonaniu analiz u tych krów ponownie rozpoczęto terapię lub wzmożono profilaktykę. Stwierdzono, że największe zagrożenie dla zdrowia krów w okresie rekonwalescencji po porażeniu poporodowym występuje po 2 – 3 dniach od ustąpienia objawów ostrej hipokalcemii i po 3 dniach od ostatniej podaży leków. Krowy, które w tym okresie były klinicznie zdrowe, w badaniach biochemicznych wykazywały duże różnice w porównaniu z grupą kontrolną i powinny otrzymywać preparaty zapobiegające niedoborom mineralnym i energetycznym. Z obserwacji własnych wynika, że istnieje konieczność wykonywania kontrolnych badań krwi u krów w okresie 3 dni po przebytym porażeniu poporodowym i profilaktycznego suplementowania preparatami mineralno-energetycznymi.

Słowa kluczowe: porażenie poporodowe, zdrowotność krów mlecznych, hipokalcemia, fosfor, glukoza.

INTRODUCTION

During the postpartum period, different metabolic disorders of varying intensity can be observed including hypocalcemia, which is the consequence of hormonal, production and feeding changes. In this period the organism has to deal with an increased use of its own reserves and nutrients absorbed from the digestive tract (Goff, Horst 1997, Kondracki, Bednarek 1997, Stec et al. 2000). Appropriate calcium-phosphorus metabolism is maintained mainly by hormonal regulation, based mostly on parathormone (PTH), calcitonin (Calc) and vitamin D metabolite activity as well as some glucocorticoid and sex hormone activity (Thiede 1994, Goff, Horst 1997, Kurek, Stec 2005). Modern prophylaxis of hypocalcaemia is based on this regulation, decreasing the calcium content in the diet before parturition to cause increased concentration of parathormone in the blood and simultaneously better usage of calcium from the feed and reserves mobilization from the bone system (Goff 2008).

In the postpartum period, animals need more energy, which coinciding with a higher risk of hypocalcaemia, makes milk fever more likely to occur (GOFF, HORST 1997, VERNON 2002, SOBIECH et al. 2010). Acute clinical hypocalcaemia of the peripartum period, known as milk fever, is a condition described as resulting from a low calcium concentration in the extracellular space in cows. However, the phosphorus and magnesium concentration, and the dynamics of energy conversions in the organism, affect the clinical symptoms and total calcium concentration in cows (SANSOM et al. 1983, FENWICK 1988, PEHRSON et al. 1998, LARSEN et al. 2001). In dairy herds, the comatose form of milk fever is most often diagnosed, characterized by progressing neural signs (consciousness disorders) from drowsiness to coma and characteristic lateral recumbence with the cow's head on the back. Other developments are the loss of milk yield, appetite and thirst. Another frequent form is a milk fever syndrome characterized by psychomotor excitation in the standing position.

According to our own experiment, these two forms of milk fever differ in their magnesium levels with very low concentrations of calcium (Ca) and inorganic phosphorus (Pi). The concentration of magnesium (Mg) is much lower (although not below physiological values) in hyper excitability and high in the comatose form (KUREK, STEC 2004*a*, 2005). While describing the clinical forms of hypocalcaemia, SANSOM et al. (1983) and FENWICK (1988) observed changes in magnesium levels, some researchers recorded only hypermagnesemia (BJÖRKMAN et al. 1994), while others did not notice any changes in Mg concentration (PEHRSON et al. 1998). Acute hypocalcaemia affects mostly cows after 3 lactations (≥ 5 years), but may be observed in young animals as well. It is mostly observed within 72 hours of parturition. Based on our own observations, the incidence clinical cases in different dairy cow herds, despite the constant improvement in housing and nutrition, is about 2-8 % of livestock, which is confirmed in other publications (ESSLEMONT, KOSSAIBATI 1996, HOUE et al. 2001, HORST et al. 2003). The course of milk fever and the condition of cows which recover from it cause large economic losses and often lead to the culling of many animals. The most frequent complications responsible for losses in dairy herds are: metritis, fetal membranes retention, abomasal displacement or ulceration, limb and muscle injuries, or udder injuries or inflammations (HORST et al. 1997, HOUE et al. 2001, DEGARIS, LEAN 2009). The current understanding of milk fever and losses caused in dairy herds by its consequences is unsatisfactory. In particular, there are very few publications concerning systemic changes and decreased farming efficiency in the days following the treatment and the subsidence of the clinical symptoms, which is especially important for herd management. Such data are available only regarding animals with complications during the illness or immediately afterwards. For this reason, a decision was made to examine animals which were considered healthy by framers after acute hypocalcaemia, and to analyze their basic biochemistry profile during the recovery. The aim was to determine if, and to what extent, high-yielding cows after an incident of milk fever require some prophylactic or medical treatment once the clinical symptoms are subsiding in order to normalize the mineral and energy metabolism.

MATERIAL AND METHODS

In 2005-2011, a study was conducted on a group of 60 HF cows aged between 4 and 9 years. 50 cows were diseased animals and 10 healthy individuals were the control group. The animals came from 10 farms housing between 30 to 300 dairy cows, located in the central Lublin region. The farms had similar housing conditions and a feeding system based on the TMR technology. The feeds were corn silage, haylage, silage from grass or beet tops, beet pulp, hay, straw, concentrates with the addition of cereals, as well protein and mineral supplements. The feed ration was based on the production level, physiological period and the animals' age. Feeding animals on the farms was based on the fodders/24-hours/animal: corn silage (15-18 kg), hay silage (16-17 kg), hay (1-2 kg), wheat or barley straw (1-2 kg), grass silage, beet tops or beet pulp (4-5 kg), home-made concentrate from cereal grains – triticale, wheat, oats (4-5 kg) as well as supplements in doses dependent on the productivity and the commercial preparation (approximately 0.05-0.2 kg), all-mash feed with the protein content of 18-36% depending on milk yield (2-5 kg), mineral and vitamin supplements in doses dependent on the productivity and applied supplement (approximately 0.1-0.3 kg). The experiment encompassed cows with milk fever during the period from a few hours to 3 days after delivery. Sick cows presented clinical signs from the gastrointestinal tract, nervous and musculoskeletal system, typical for hypocalcaemia. Animals with clinical changes had medium or good body condition, and good or very good milk yield (35-50 l daily at peak lactation before sickness). Based on the rapidity and character of the clinical symptoms, as well biochemistry results confirming different kinds of hypocalcaemia, two groups of animals with different clinical image of the disease were distinguished. Group I included 20 cows with excitation and hypersensitivity, occasional tremors and contractions of single muscle groups; cold peripheral body parts, lack of appetite or thirst, difficulty in defecating, and different milk yield loss. Besides, the cows were able to stand up, although the standing position seemed hesitant or animals would lie down and then attempted to stand up again during the treatment. The remission of clinical symptoms was observed 24-48 hours from the start of treatment. The moment the cows fell ill, they showed a significantly decreased Ca concentration (average 1.58 mmol l^{-1}) in the serum, quite a large decrease of Pi (1.01 mmol l^{-1}) and a low concentration of Mg (0.7 mmol l^{-1}). Glucose and cholesterol in the serum were within the lower physiological norm limits. Group II included 30 cows which had milk fever with rapid consciousness disorders, from drowsiness to coma. During the course of the disease, these animals showed no appetite or thirst, ceased milk production, had a characteristic downer cow's syndrome with lateral recumbence, holding the head on the back, kept the limbs spread forward, unable stand up, their peripheral body parts were cold, urination or defecation stopped and some cows suffered from tympanites. At the onset of the condition, the Ca concentration was very low (average 1.4 mmol l^{-1}), Pi was 0.29 mmol· l^{-1} and Mg concentration was 1.4 mmol l^{-1}. Glucose and cholesterol were on the lower level of the physiological norm (WINNICKA 2011). In both groups during milk fever there were no significant changes in potassium (K) levels in the serum and free fatty acids (FFA) in the plasma. During the study, group II was additionally divided into two subgroups according to the animals' health status. Group IIa (25 animals) included cows with higher milk yield after the illness and in which the farmers saw no health changes. Group IIb (5 animals) included cows which, 3-4 days after milk fever had subsided, preferred the lying position and had difficulty standing up. Group III, the control, included healthy animals (10 cows), which were in the same physiological condition and came from the same farms as the cows with clinical hypocalcaemia.

Clinical examination and blood sampling were done in both groups 24, 48 and 72 hours after all clinical symptoms had subsided and at least 24 hours from the last administration of medications. In the control group, blood was taken 96, 120 and 144 hours after delivery, therefore material collection from clinically healthy cows was optimized to 24 hours after possible hypocalcaemia. Blood was drawn from the jugular vein into heparinized tubes. After centrifugation, total calcium (Ca), total magnesium (Mg), inorganic phosphorus (Pi), glucose and cholesterol in the serum were determined by colormetry with commercial tests on a BS-130 MINDRAY. The potassium level was assayed by the ion selective electrode method on an AVL 9180. The concentration of free fatty acids (FFA) was assessed by the titrimetric method.

All results were statistically analyzed using Statistica 5.0 PL software. Significance of differences between averaged values was estimated by the *t*-Student test, at significance levels of $p \leq 0.05$ and $p \leq 0.01$.

RESULTS

Animals which had milk fever with excitatory symptoms (group I) at the beginning of the experiment did not show any clinical signs and yielded approximately 30 liters of milk per day. By the third day, 3 cows (15 %) had worse appetite and lower milk production, although the test results were close to other cows in the same group (therefore they did not form another subgroup). The results from the third blood sample justified a decision to start mineral, vitamin and energy supplementation for the whole group. In group II cows, which had the comatose form of milk fever two cows could not stand up between the second and third day of the study. During the next few hours, 4 other cows were observed to have similar symptoms. The animals were reluctant to remain standing or chose to lie down. The laboratory tests results led to an immediate decision to commence treatment and to isolate this group from other animals. Based on the farmers' experience, such cases had happened before in cows which had recovered from milk fever and clinically did not need prophylactic treatment with vitamin and mineral supplements.

The lowest calcium levels (but not statistically significant) in the first blood sample were observed in animals which had previously exhibited the excitatory symptoms. The lowest Pi levels were observed in those groups which had previously had comatose milk fever (Table 1), although the results for group I were much lower than the concentrations observed in the control group. The concentrations of Mg and K were within physiological norm (WINNICKA 2011). Group IIb featured the highest values of FFA with a low glucose level (Table 2). On the second day, group I showed a decrease of the Ca level relative to the first blood sample, with values statistically significantly lower than in the control group. This group had the lowest Mg concentration albeit within normal limit. The lowest average Pi values were observed in group IIb, statistically lower than the results in group III. The largest FFA concentration and the lowest glucose concentration were observed in group IIb, although these values were not statistically significant

Table 1

The mean concentration of ions in the groups

Groups	Sampling hours	Analyzed parameters (mmol l^{-1})			
		Ca	Mg	Pi	K
I	24	2.01±0.17^{Aa}	0.93±0.2	1.23±0.19	4.6±0.7
	48	1.87±0.19^{ABb}*	0.91±0.2*	1.25±0.14	4.5±0.8
	72	1.74±0.17^{Bc}**	0.82±0.2*	1.36±0.18	4.3±0.5
II a	24	2.09±0.34	1.14±0.2	1.20±0.34*	4.5±0.5
	48	1.99±0.13	1.12±0.1	1.24±0.22*	4.4±0.3
	72	1.98±0.17*	1.14±0.2	1.17±0.27**	4.6±0.6
II b	24	2.08±0.22	1.12±0.1	1.12±0.21*	4.2±0.4
	48	2.01±0.11	1.15±0.1	1.03±0.28**	4.1±0.6
	72	1.99±0.19*	1.13±0.1	1.01±0.32**	4.3±0.2
III	96	2.11±0.31	1.01±0.3	1.64±0.33	4.7±0.3
	120	2.13±0.35	1.10±0.2	1.67±0.24	4.6±0.5
	144	2.14±0.41	1.09±0.2	1.82±0.22	4.7±0.4

a, *b*, *c* – statistically significant difference between the means at $p \le 0,05$
A, *B*, *C* – statistically significant difference between the means at $p \le 0,01$
* – statistically significance of differences between control and experimental group at the same sampling time at $p \le 0,05$
** – statistically significance of differences between control and experimental group at the same sampling time at $p \le 0,01$

in comparison to the other groups and previous samples. At 72 hours after the subsidence of milk fever clinical symptoms group I cows, very low concentrations of Ca (statistically significantly lower than in the control group) and low (but within normal limit) Mg concentrations were found (statistically lower than samples from other groups). On the same sampling time, low levels of calcium and very low levels of phosphorus were found in group II, markedly low in group IIb, where the average inorganic phosphorus level was statistically significantly lower than in the analogous samples in the control group. This group had the highest levels of FFA, exceeding the physiological norm and significantly higher than values from other animals, and the lowest glucose and cholesterol levels (Table 2). All the groups showed

Table 2

The mean content of FFA, glucose and cholesterol in serum

Groups	Sampling hours	Analyzed parameters (mmol l^{-1})		
		glucose	cholesterol	FFA
I	24	3.3±0.6	3.4±0.8	608.4±123.1[a]
	48	3.0±0.5	3.3±0.5	624.4±78.4[ab]
	72	2.6±0.9	2.7±0.6	688.3±108.3[b]
II a	24	3.1±0.3	2.9±0.3	668.4±182.2
	48	2.9±0.4	2.9±0.2	602.3±192.3
	72	2.9±0.3	3.1±0.5	617.9±104.3
II b	24	2.7±0.2	2.2±0.2*	723.4±133.2
	48	2.7±0.2	2.0±0.1*	766.4±100.2*
	72	2.6±0.1	2.0±0.2*	728.5±83.4*
III	96	3.5±0.3	3.9±0.7	618.8±125.4
	120	3.6±0.4	4.2±0.6	612.3±143.3
	144	3.6±0.3	4.3±0.7	588.5±173.3

a, *b* – statistically significant difference between the means at $p \le 0,05$
A, *B* – statistically significant difference between the means at $p \le 0,01$
* – statistically significance of differences between control and experimental group at the same sampling time at $p \le 0,05$
** – statistically significance of differences between control and experimental group at the same sampling time at $p \le 0,01$

decreasing average Ca levels during the whole study, contrary to the control group. The average potassium value also fluctuated, and was lower in the experimental groups than in the control groups, although the difference during recovery were not significant.

DISCUSSION

Postpartum paresis causes the largest economic losses in dairy herds, due to complications either during the course of the illness or later because of the postpartum immunosuppression (Kimura et al. 2006). In cows with hypocalcaemia, the percentage of cases with fetal membranes retention and uterine disorders is several-fold more significant (ERB et al. 1985, CORREA et al. 1993, HOUE et al. 2001). In cows with hypocalcaemia, the incidence of uterine inflammation increases (WHITEFORD, SHELDON 2005, KIMURA et al. 2006), the process of uterus involution slows down and reproduction failures occur (BORSBERRY, DOBSON 1989, KAMGARPOUR et al. 1999, WHITEFORD, SHELDON 2005). There are reports on increased cases of ketosis and abomasum dysfunction (HOUE et al. 2001, GOFF 2003). Additionally, during hypophosfatemia there may be complications involving lameness and different peripartum recumbence (KUREK et al. 2010). However, most of these complications are not combined directly with the downer syndrome but with subclinical hypocalcaemia during the postpartum period (HOUE et al. 2001, GOFF 2003, KIMURA et al. 2006). These facts justify the assumption that the subclinical disorders observed during our study between the first and third day after the clinical symptoms had subsided can be as threatening and dangerous as milk fever itself. For herd management, it is necessary to prevent such a development. In some animals, this condition can lead to clinical disorders like the postpartum downer syndrome, worse appetite and lower milk yield, as was observed during this study.

Apparently healthy cows without any clinical symptoms showed significantly different laboratory test results than other cows from the same farms not suffering from milk fever. These animals were poor in mineral deficiency and feed conversion rate. Nevertheless, a large percentage of cows not suffering from milk fever often had different levels of subclinical hypocalcaemia and subclinical phosphorus deficiency, as observed during studies concerning high-yield dairy herds (ESSLEMONT, KOSSAIBATI 1996, GOFF, HORST 1997, HORST et al. 2003, GOFF 2008, HOUE et al. 2006). The biochemistry tests show the necessity for increased mineral supplementation (Ca and Pi) in all cows after milk fever, and simultaneous Mg supplementation in animals which had excitatory parturient paresis. Our results confirm the need for milk fever prevention and simultaneous prevention of subclinical hypocalcaemia, not only before delivery but also during the whole early postpartum period, which can be more significant (GOFF, HORST 1997, KONDRACKI, BEDNAREK 1997, PEHRSON et al. 1998, BEDNAREK, KONDRACKI 2000, STEC et al. 2000, HORST et al. 2003, GOFF 2008). Following the comatose form of milk fever, cows show significantly lower phosphorus concentrations than in the excitatory form, which poses a risk for postpartum downer cow syndrome with typical symptoms (KUREK, STEC 2004*a*,*b*, KUREK et al. 2010). Ca, Mg and Pi concentrations during the study show that correct parenteral treatment of milk fever maintains the stability of mineral metabolism for as long as 48 hours after mineral preparation administration. Afterwards, apart from slightly increased milk production and adequate nutrition of cows after an episode of the disease, the significantly lower concentrations of these minerals justify further supplementation. In our study, the cows which had had the comatose form of milk fever revealed energy balance disturbances with a significant

FFA increase and glucose decrease, especially in group IIb (free fatty acids > 700 μmol^{-1}/l). Some animals in this group could be considered as having subclinical ketosis. The results prove that it is necessary to develop a more precise energy balance management plan for cows which had the comatose form of postpartum paresis, both during the illness and afterwards, as also shown in other studies (Stec et al. 2000, Kurek, Stec 2004*a*,*b*, Vernon 2002). Many farmers use energy-rich substances added directly to water just after delivery. The study shows that this practice is advisable not only after delivery but also in cows which have previously been afflicted by milk fever.

CONCLUSIONS

Most studies on milk fever focus on the course of the illness, and observations concerning animal health afterwards are scanty (Sansom et al. 1983, Fenwick 1988, Björkman et al. 1994, Larsen et al. 2001, Kurek, Stec 2004*a*,*b*, 2005, Sobiech et al. 2010). Also, supplementation is aimed at preventing the disease only during the first 3 days after delivery (Horst et al. 1997, Bednarek et al. 2000, Stec et al. 2000, Goff 2008). Our study shows the necessity for longer monitoring periods of the animal's condition following delivery (with or without milk fever signs). For several days postpartum cows should receive higher doses of calcium and phosphorus preparations as well as energy supplements, due to significantly low calcium levels found in the third blood samples. Considering the low calcium levels in the control group during the whole study, full mineral prophylactics in high yielding cows should be an ordinary practice, and for a week after delivery doses of minerals cannot be adjusted to milk yields alone. The check-up of animals' condition cannot be based solely on clinical examination, as all the animals in group IIb showed no changes until the third to fourth day after the treatment of the comatose form of milk fever. Some animals with subclinical changes will not show significant symptoms (group IIb), but can produce significantly less milk than animals with an adquate level of macronutrients. Subclinical signs may be overlooked, and this can to large economic losses.

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REFERENCES

Bednarek D., Kondracki M., Grzęda M. 2000. *Magnesium and calcium in prophylaxis of parturient paresis in dairy cows.* Med Wet., 56: 367-371.

Björkman C., Jönsson G., Wroblewski R. 1994. *Concentrations of sodium, potassium, calcium, magnesium and chlorine in the muscle cells of downer cows and cows with parturient paresis.* Res. Vet. Sci., 57: 53-57.

Borsberry S., Dobson H. 1989. *Periparturient diseases and their effect on reproductive performance in five dairy herds.* Vet. Rec., 124: 217-219.

DeGaris P.J., Lean I.J. 2009. *Milk fever in dairy cows: A review of pathophysiology and control principles.* Vet. J., 176: 58-69

Erb, H.N., Smith, R.D., Oltenacu, P.A., Guard, C.L., Hilman, R.B., Powers, P.A., Smith, M.C.,White, M.E. 1985. *Path model of reproductive disorders and performance, milk fever, mastitis, milk yield and culling in Holstein cows.* J. Dairy Sci., 68: 3337-3349.

Esslemont R.J., Kossaibati M.A. 1996. *Incidence of production diseases and other health problems in a group of dairy herds in England.* Vet. Rec., 139: 486-490.

Fenwick D.C. 1988. *The relationship between certain blood cations in cows with milk fever and both the state of consciousness and the position of cows when attended.* Aust. Vet. J., 65: 374-375.

Goff J.P., Horst R.L. 1997. *Physiological changes at parturition and their relationship to metabolic disorders.* J. Dairy. Sci., 80: 1260.

Goff, J.P. 2003. *Managing the transition cow – considerations for optimising energy and protein balance and immune function*. Cattle Practice, 11: 51-63.

Goff J.P. 2008. *The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows.* Vet. J., 176: 50-57.

Horst R.L., Goff J.P., Reinhardt T.A., Buxton D.R. 1997. *Strategies for preventing milk fever in dairy cattle.* J. Dairy Sci., 80: 1269.

Horst R.L., Goff J.P., McCluskey B.J. 2003. *Prevalence of subclinical hypocalcemia in US dairy operations.* J. Dairy Sci., 86 (Suppl. 1): 247.

Houe H., Ostergaard S., Thilsing-Hansen T., Jorgensen R.J., Larsen T, Sorensen J.T., Agger J.F., Blom J.Y. 2001. *Milk fever and subclinical hypocalcaemia – an evaluation of parameters on incidence risk, diagnosis, risk factors and biological effects as input for a decision support system for disease control.* Acta Vet. Scand., 42: 1-29.

Kamgarpour, R., Daniel, R.C., Fenwick, D.C., McGuigan, K., Murphy G. 1999. *Postpartum subclinical hypocalcaemia and effects on ovarian function and uterine involution in a dairy herd.* Vet. J., 158: 59-67.

Kimura, K., Reinhardt, T.A., Goff, J.P. 2006. *Parturition and hypocalcaemia blunts calcium signals and immune cells of dairy cattle.* J. Dairy Sci., 89: 2588-2595.

Kondracki M., Bednarek D. 1997. *Effect of orally administered calcium chloride on concentration of caclium in the blood serum of cows after parturition*. Med. Wet., 53: 150. (in Polish)

Kurek Ł., Lutnicki K., Banach A. 2010. *Various types of hypophosphataemia of dairy cows and clinical implications depending on intensity of the deficiency*. Bull. Vet. Inst. Pulawy, 54: 35-41.

Kurek Ł., Stec A. 2004*a*. *Levels chosen macroelements and their fractions in various forms of clinical hypocalcemia in dairy cows*. Med. Wet., 60: 985-989. (in Polish)

Kurek Ł., Stec A. 2004*b*. *Level of indicators of parenchymatous organ functions and energy in clinical hypocalcemia in dairy cows.* Med. Wet., 60: 1084-1088. (in Polish)

Kurek Ł., Stec A. 2005. *Parathyroid hormone level in blood of cows with different forms of clinical hypocalcaemia*. Bull. Vet. Inst. Pulawy, 49: 129-132.

LARSEN T., MOLLER G., BELGIO R. 2001. *Evaluation of clinical and clinical chemical parameters in parturient paresis cows.* J. Dairy Sci., 84: 1749-1758.

PEHRSON B., SVENSSON C., JONSSON M. 1998. *A comparative study of the effectiveness of calcium propionate and calcium chloride for the prevention of parturient paresis in dairy cows.* J. Dairy Sci., 81: 2011-2016.

SANSOM B.F, MANSTON R., VAGG M.J. 1983. *Magnesium and milk fever*. Vet. Rec., 112: 447-449.

SOBIECH P., RYPUŁA K., WOJEWODA-KOTWICA B., MICHALSKI S. 2010. *Usefulness of calcium-magnesium products in parturientparesis in HF cows.* J. Elem., 15(4): 693-704.

STEC A., KUREK Ł., STUDZIŃSKA M. 2000. *Effect of Propical the formation of calcium and energy metabolism in dairy cows.* Życie Wet., 75: 95. (in Polish)

THIEDE M.A. 1994. *Parathyroid hormone-related protein: a regulated calcium- mobilizing product of the mammary gland.* J. Dairy Sci., 77: 1952-1963.

VERNON R.G. 2002. *Nutrient partitioning, lipid metabolism and relevant imbalances*. Proc. XXII World Buiatrics Congress Hannover, 210-223.

WHITEFORD L.C., SHELDON I.M. 2005. *Association between clinical hypocalcaemia and postpartum endometritis*. Vet. Rec., 157: 202-204.

WINNICKA A. 2011. *Reference values of basic laboratory examinations in veterinary medicine*. SGGW, Warszawa. (in Polish)

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IMPACT OF A SPENT MUSHROOM SUBSTRATE, *AGARICUS BISPORUS* ON CHROMIUM AND COPPER SPECIATION IN THE HUMUS HORIZON

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Abstract

Spent mushroom substrate used as fertilizing material provides nutrients for plants in forms with a different degree of availability. A two-year experiment was conducted in central eastern Poland (Siedlecka High Plain) to determine the fertilizing effect of substrate previously used to grow mushrooms *(Agaricus bisporus)* on the total content chromium and copper content and their quantitative content in the fraction in humus horizon pseudogley loessive soil. The experiment included the control (without fertilization), and several fertilized variants: NPK, swine manure, swine manure + NPK, spent mushroom substrate and spent mushroom substrate +NPK. The sequential fractionation of chromium and copper, carried out according to the BCR protocol, in the soil humus horizon under the above treatments demonstrated various concentration of these metals in the extracted fractions and their shares in the total content. Fertilization with spent mushroom substrate alone and with NPK contributed to a decrease in the Cr content in the F2 and F3 fractions, but resulted in an increase in the Cu content in the F1, F2 and F3 fractions after the second year of plant cultivation in comparison with the first year. The highest share of the tested metals in the total content was detected in the residual fraction F4: after the second year for chromium and after the first year of the experiment for copper.

Keywords: fractions of chromium and copper, BCR method, spent mushroom substrate, Luvisol.

WPŁYW PODŁOŻA PO UPRAWIE PIECZARKI *AGARICUS BISPORUS* NA SPECJACJĘ CHROMU I MIEDZI W POZIOMIE PRÓCHNICZNYM GLEBY

Abstrakt

Zużyte podłoże po uprawie pieczarki zastosowane jako nawóz dostarcza do gleby składniki pokarmowe dla roślin w formie różnie dostępnej. W dwuletnim doświadczeniu polowym, zlokalizowanym w środkowo-wschodniej Polsce (Wysoczyzna Siedlecka), określono wpływ nawożenia

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podłożem po uprawie pieczarki białej *(Agaricus bisporus)* na zawartość ogólną chromu i miedzi oraz ich ilościowy udział w wydzielonych frakcjach ornego poziomu próchniczego gleby płowej opadowo-glejowej użytkowanej rolniczo. Obiektami doświadczalnymi były: obiekt kontrolny (bez nawożenia); nawożony mineralnie NPK; nawożony obornikiem trzody chlewnej; nawożony obornikiem trzody chlewnej + NPK; nawożony podłożem po uprawie pieczarki; nawożony podłożem po uprawie pieczarki + NPK. Frakcjonowanie sekwencyjne chromu i miedzi, wg procedury BCR, w poziomie próchnicznym gleby poszczególnych obiektów doświadczenia wykazało zróżnicowaną zawartość tych metali w wydzielonych frakcjach oraz ich udział w zawartości ogólnej. Wprowadzone podłoża: popieczarkowe oraz popieczarkowe z dodatkiem NPK wpłynęły na zmniejszenie udziału chromu we frakcji F2 i F3, zwiększenie zaś udziału miedzi we frakcji F1, F2 i F3 po 2. roku uprawy w stosunku do 1. roku. Największy udział badanych metali w zawartości ogólnej stwierdzono we frakcji rezydualnej F4 – chromu po 2. roku badań, a miedzi po 1. roku.

Słowa kluczowe: frakcje chromu i miedzi, metoda BCR, podłoże popieczarkowe, gleba płowa.

INTRODUCTION

In soil, metal elements such as chromium and copper are found in different chemical compounds and forms, mainly mineral or organic ones, as ions in the soil solution or bound to the solid phase. They have different bioavailability to plants and soil organisms, being most easily absorbed as simple ions from highly soluble compounds, but less readily as complex ions. The soil's supply of easily absorbable compounds determines the degree of plant nutrition and is used to assess fertilization requirements of cultivated soil. Fertilizers introduced to cropped soil increase the content of compounds, including metals, in their active forms mainly in the soil solution, while their excess is absorbed by the solid phase. The deficit of natural fertilization in Poland has encouraged the search for new technologies to increase the content of organic matter in cultivated soil. New developments include the use of waste organic materials such as spent mushroom substrate (Kalembasa, Wiśniewska 2004). Such substrate used as fertilizing material provides nutrients for plants in forms characterized by different degrees of availability (Herrero-Hernandez et al. 2011). The content of heavy metals in substrate mainly depends on the composition of components used during the manufacturing processes, but generally does not exceed the permissible limits specified in the Polish regulations (Kalembasa, Majchrowska-Safaryan 2009).

The goal is to simplify the methods for fractionating heavy metals. The BCR method, approved by the European Union, is the basic procedure used to fractionate metals with different bioavailability in soil, sewage sludge and marine deposits (Mosso, Davidson 2003).

The aim of this study was to determine the impact of fertilization with substrate previously used to grow mushrooms *(Agaricus bisporus)* on the total content of chromium and copper and their quantitative content in fractions. The concentrations of the metals were determined with the sequential

BCR method in tilled humus horizon of cultivated pseudogley loessive soil during a two-year field experiment located in central eastern Poland (Siedlecka High Plain).

MATERIAL AND METHODS

The plant growing experiment was carried out in 2008 and 2009, on a production field (52°20′00″N and 22°03′00″E). The duration of the experiment was set on the assumption that spent mushroom substrate used as a fertiliser had a beneficial impact on plant yield mainly during the first two years after its introduction into soil. The experiment was designed in a randomized block design with four replications on 7×7 m plots on pseudogley soil lessive with granule composition typical of sandy loam (74% sand, 19% silt, and 7% clay). The experiment included the following treatments: the control (without fertilization), fertilized with NPK, with swine manure – 25 t ha^{-1} (used routinely because of the hog fattening production on the farm), with swine manure (25 t ha^{-1}) + NPK, with spent mushroom substrate (20 t ha^{-1}), and with spent mushroom substrate (20 t ha^{-1}) +NPK.

The starch cultivar of potato (*Solanum tuberosum* L.) Pasat was the test plant in the first year, replaced by winter wheat (cv. Finezja) in the second year. Fertilization with organic materials was performed in spring. The doses of manure and spent mushroom substrate were established based on their nitrogen content. Mineral fertilization with NPK was carried out in spring before potato and in autumn before winter wheat. After harvest, samples of soil were collected from the ploughed humus horizon (0-25 cm) and sieved through 2 mm mesh. The following parameters were determined: pH in 1 mol KCl dm^{-3} with the potentiometric method; cation exchange capacity (CEC) calculated from hydrolytic acidity (Hh) and the sum of exchangeable base cations (S) determined with the Kappen's method; carbon in organic compounds (C_{org}) with the oxidation-titration method (Kalembasa, Kalembasa 1992); total content of chromium (Cr_t) and copper (Cu_t) after initial mineralization of the tested material in a mixture of concentrated acids HCl + HNO_3 at the 3:1 ratio; metals extracted in a fraction with the BCR procedure – Table 1 (Ure et al. 1993) determined with ICP – AES. The analyses were performed in three replications. The reference material WEPAL Soil Reference Material BCR 142R (light sandy soil) was used to verify the accuracy of measurements.

Spent substrate obtained after 6-week cultivation of common mushroom, *Agaricus bisporus,* originated from a mushroom farm where mushrooms were cultivated on phase III substrate (after fermentation and inoculation with mycelium). The cover consisted of raised peat, chalk, urea and protein supplement as well as swine manure obtained from deep litter pig pens. The

Table 1

Scheme of sequential extraction of heavy metals by the BCR method

Fraction	Name	Extraction reagent	Extraction time (h)	pH
F1	exchangeable/ acid extractable	0.11 mol dm^{-3} CH_3COOH	16	3.00
F2	reducible – bound to Fe and Mn oxyhydroxides	0.5 mol dm^{-3} NH_2OH HCl	16	2.00
F3	oxidizable - bond to organic matter	30% H_2O_2 (1 h, 85°C) + 1 mol dm^{-3} CH_3COONH_4	16	2.00
F4	residual	calculated as the difference between the total content of the metal and the sum of the above determined fractions	-	-

soil: solution ratio 1 g : 10 cm^3

content of dry matter was determined in the substrate and manure with the drying-weighing method (at 105°C) and the other parameters were tested as explained above.

The results were statistically analysed with a two-way analysis of variance (Anova). The F Fisher-Snedecor test was used to determine the significance of impact of the experimental factors on the tested parameters. $LSD_{0.05}$ was calculated with the Tukey's test. The calculations were performed with Analwar-5FR software. The coefficients of simple correlation were determined with the Pearson's method using a Statistica 9.1 software package.

RESULTS AND DISCUSSION

The chemical analysis of swine manure and spent mushroom substrate used as fertilizers revealed that they differed in the content of dry matter, carbon in organic compounds and pH (Table 2). In comparison with swine manure (assumed as 100%), spent mushroom substrate had a higher content of dry matter (by 23%), a slightly higher pH (by 2.6%), a lower content of carbon in organic compounds (by 27.4%), a higher total content of chromium (by 66%) and a four times lower amount of copper.

The extracted fractions with the BCR procedure revealed various percentages (%) of Cr and Cu in the total content (Figure 1). The highest content of the metals was detected in manure in the residual fraction F4, i.e. 52.5% Cr and 76.8% Cu, respectively, and in substrate in the oxidative fraction F3 (42.6% Cr) and for Cu (50.7%) in F4 fraction. The content of these metals in the fraction F2 bound to iron and manganese oxides and

Table 2

The properties of swine manure and spent mushroom substrate used to fertilize the soil

Organic material	DM in 105°C	OrgC	Cr_t	Cu_t	pH_{KCl}
	(g kg^{-1})		(mg kg^{-1})		
Swine manure	250	383	2.82	41.3	6.97
SMS	309	278	4.20	11.6	7.15

SMS – spent mushroom substrate

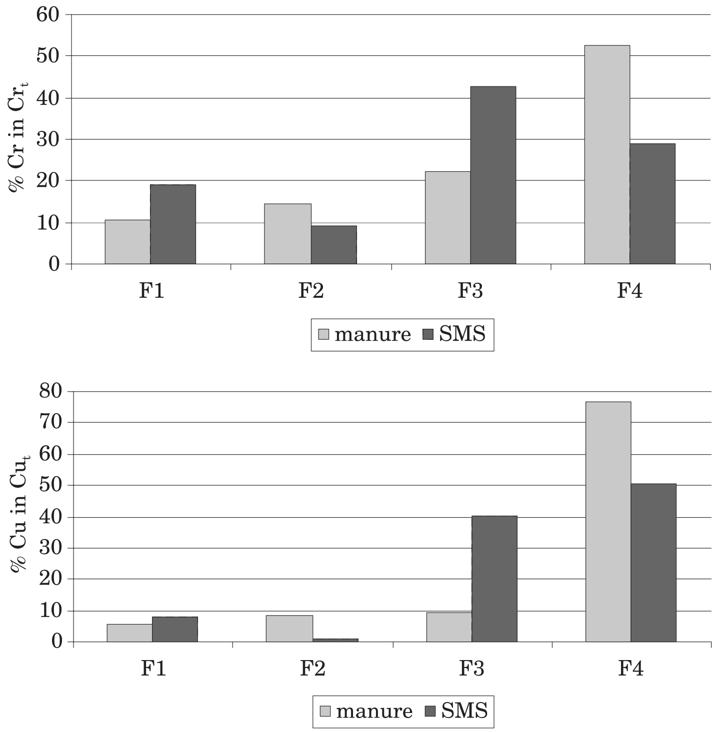

Fig 1. The percentages of chromium and copper in the total content in the separated fractions of organic materials used for fertilizing

manure – swine manure; SMS – spent mushroom substrate; Fractions: F1 – exchangeable; F2 – reducible; F3 – oxidizable; F4 – residual

hydroxides, exposed to transformations under reductive conditions, was considerably diverse: 9.2% of chromium and 1.04% of copper were bound to this fraction. The metals constituted from 8.04% for Cu to 19.1% for Cr of the total content of the fraction easily available to plants (F1). Similar results of fractionating heavy metals in spent mushroom substrate which originated from three different farms were reported by KALEMBASA, MAJCHROWSKA-SAFARYAN (2009). HERRERO-HERNANDEZ et al. (2011) fractionated spent mushroom substrate in accordance with the BCR procedure and detected approx. 5% of copper in the F1 fraction, 2% in the F2 fraction, 60% in the F3 fraction and 38% in the F4 fraction.

The content of chromium and copper in the humus horizon under the individual treatments was low and within the natural range, never exceeding the permissible limits set for cultivated soil in Poland (*The Regulation*... 2002). After two seasons of plant growing, the soil contained between 4.72 and 4.85 mg kg^{-1} of Cr and between 2.03 and 1.85 mg kg^{-1} of Cu (Table 3). The analysis of variance revealed that the total content of chromium did not depend on the experimental factors, unlike total chromium, which was significantly differentiated by the applied fertilization ($LSD_{0.05}$ = 0.627). Kalembasa and Wiśniewska (2004) and Herrero-Hernandez et al. (2011) claim that the application of spent mushroom substrate increases the content of

Table 3

The properties of soil humus horizon of individual fertilizer objects after first and second year of cultivation in a field experiment

Experimental objects	pH	OrgC	CEC	Cr_t	Cu_t
	KCl	(g kg^{-1})	(mmol(+) kg^{-1})	(mg kg^{-1})	
First year of cultivation (after the potato cultivation)					
Control objects	4.79	6.02	67.6	4.770	1.745
NPK	5.18	7.00	76.9	4.897	1.838
Swine manure	5.07	7.40	71.4	4.832	2.687
Swine manure + NPK	4.44	7.72	68.4	4.618	1.872
SMS	5.11	7.65	90.3	4.702	2.189
SMS + NPK	4.92	7.67	87.8	4.498	1.825
Mean	-	7.24	77.1	4.719	2.026
Second year of cultivation (after the wheat cultivation)					
Control objects	4.61	6.09	64.5	4.715	1.511
NPK	4.58	6.40	67.6	5.130	2.061
Swine manure	4.69	8.30	77.1	4.979	2.256
Swine manure + NPK	4.90	6.75	75.7	5.099	2.127
SMS	4.59	7.65	79.2	4.323	1.682
SMS + NPK	4.27	8.00	81.3	4.856	1.484
Mean	-	7.20	74.2	4.850	1.853
$LSD_{0.05}$ for: A(years) B(fertilization) B/A interaction A/B interaction	 - - - -	 n.s. 0.353 0.499 0.333	 - - - -	 n.s. n.s. n.s. n.s.	 n.s. 0.612 n.s. n.s.

OrgC – carbon in organic compounds; CEC – cation exchanage capacity; Cr_t – total content of chromium; Cu_t – total content of copper; SMS – spent mushroom substrate; n.s. – not significant difference

Table 4

The chromium content (mg kg^{-1}) in separated fractions and the percentage (%) of the total content in the humus horizon of individual objects soil of field experiment

Experimental objects	F1		F2		F3		F4	
	(mg kg^{-1})	(%)	(mg kg^{-1})	(%)	(mg kg^{-1})	(%)	(mg kg^{-1})	(%)
First year of cultivation (after the potato cultivation)								
Control objects	0.134	2.80	0.652	13.7	1.476	30.9	2.508	52.6
NPK	0.137	2.79	0.668	13.6	1.515	30.9	2.577	52.6
Swine manure	0.154	3.18	0.660	13.7	1.671	34.6	2.347	48.6
Swine manure + NPK	0.172	3.72	0.715	15.5	1.551	33.5	2.180	47.2
SMS	0.143	3.04	0.735	15.6	1.644	35.0	2.180	46.4
SMS + NPK	0.170	3.78	0.677	15.1	1.584	35.3	2.058	45.8
Mean	0.152	3.22	0.684	14.5	1.574	33.4	2.308	48.9
Second year of cultivation (after the wheat cultivation)								
Control objects	0.150	3.18	0.521	11.0	1.048	22.2	2.996	63.5
NPK	0.148	2.88	0.593	11.5	1.038	20.2	3.351	65.3
Swine manure	0.200	4.01	0.558	11.2	0.930	18.7	3.291	66.1
Swine manure + NPK	0.167	3.27	0.510	10.0	1.031	20.2	3.391	66.5
SMS	0.166	3.83	0.549	12.6	0.906	20.9	2.702	62.5
SMS + NPK	0.159	3.27	0.543	11.2	1.148	23.6	3.006	61.9
Mean	0.165	3.41	0.546	11.3	1.017	21.0	3.123	64.3
$LSD_{0.05}$ for:								
A(years)	0.008		0.015		0.008		0.011	
B(fertilization)	0.020		0.038		0.019		0.025	
B/A interaction	0.029		0.054		0.028		0.036	
A/B interaction	0.019		0.036		0.018		0.030	

SMS – spent mushroom substrate; Fractions: F1 – exchangeable; F2 – reducible; F3 – oxidizable; F4 – residual

copper, which is strongly bound by organic matter and clay minerals in humus horizons. The use of substrate did not increase the content of chromium in soil, the finding which was also reported by Kalembasa and Wiśniewska (2004). The import of elements (particularly from atmospheric deposition and waste organic matter) and their loss (mainly by leaching into the depth of the soil profile and being absorbed by plants) are the essential factors that explain fluctuations of heavy metals in soil and their final balance (Karczewska 2002).

The sequential analysis of chromium and copper in the soil from the treatments after the first and second year of cultivation revealed their different content in the extracted fractions (Tables 4, 5). In the bioavailable fraction F1, the amount of Cr was lower than in the other fractions and its concentration in the total content was slightly higher (on average) after the second

Table 5

The copper content (mg kg^{-1}) in separated fractions and the percentage (%) of the total content in the humus horizon of individual objects soil of field experiment

Experimental objects	F1		F2		F3		F4	
	(mg kg^{-1})	(%)	(mg kg^{-1})	(%)	(mg kg^{-1})	(%)	(mg kg^{-1})	(%)
First year of cultivation (after the potato cultivation)								
Control objects	0.066	3.78	0.056	3.21	0.536	30.7	1.087	62.3
NPK	0.098	5.33	0.065	3.54	0.566	30.8	1.109	60.3
Swine manure	0.149	5.54	0.166	6.18	0.884	32.9	1.488	55.4
Swine manure + NPK	0.066	3.52	0.084	4.48	0.585	31.2	1.137	60.7
SMS	0.074	3.38	0.097	4.43	0.635	29.0	1.383	63.2
SMS + NPK	0.085	4.65	0.106	5.80	0.564	30.9	1.070	58.6
Mean	0.089	4.36	0.096	4.60	0.629	31.0	1.212	60.1
Second year of cultivation (after the wheat cultivation)								
Control objects	0.066	4.36	0.047	3.11	0.484	32.0	0.914	60.5
NPK	0.083	4.03	0.059	2.86	0.670	32.5	1.249	60.6
Swine manure	0.085	3.77	0.065	2.88	0.833	36.9	1.273	56.4
Swine manure + NPK	0.069	3.24	0.093	4.37	0.808	38.0	1.157	54.4
SMS	0.081	4.82	0.093	5.52	0.600	36.7	0.908	53.9
SMS + NPK	0.090	6.06	0.105	7.08	0.565	38.1	0.724	48.8
Mean	0.079	4.38	0.077	4.30	0.660	35.5	1.037	55.8
$LSD_{0.05}$ for:								
A(years)	n.s.		0.011		0.059		0.054	
B(fertilization)	0.043		0.028		0.152		0.137	
B/A interaction	n.s.		0.040		n.s.		0.193	
A/B interaction	n.s.		0.026		n.s.		0.133	

Explanations see Table 4; n.s. - not significant difference

year than after the first year of cultivation; it was also higher in the soil of fertilized treatments than in the non-fertilized control.

After the first year, the highest content of Cr was detected in the object with spent mushroom substrate + NPK (3.72%), whereas after the second year it was the most elevated in the variants with manure (4.01%) and substrate (3.83%). Kalembasa and Pakuła (2009*a*) determined from 1.59 to 3.58% of chromium in the exchangeable fraction F1 in the surface layers of cultivated brown forest soil in central eastern Poland. The Cu content in the F1 and F2 fractions was similar (on average) after the first and the second year of cultivation. Fertilization significantly differentiated this content. The concentration of this metal in soil after the first year was the highest in the object fertilized with manure (5.54%) and after the second year – with spent mushroom substrate + NPK (6.06%). These results indicate that the soil processes during the two years of the experiment, which involved the cultivation of two different plants, enhanced the copper solubility in some objects, especially with spent mushroom substrate + NPK. Herrero-Hernandez et al. (2011) reports that one year after the application of spent mushroom substrate at 40 t ha^{-1}, the percentage of copper in the F1 fraction was 4.22%. Kalembasa and Majchrowska-Safaryan (2011) detected, on average, 4.06% of copper in the bioavailable fractions (F1 and F2), extracted with the Zeien -Brummen method, of the humus horizons of different types of soil. This proves that only a small amount of copper appeared in easily soluble fractions, indicating copper mobility in soil environment, which has also been shown by Domańska and Filipek (2011).

The content of chromium in the reductive fraction F2 (in total content) was higher than in the F1 fraction; it was higher after the first year of cultivation (14.5%) than after the second year (11.3%). The application of spent mushroom substrate alone and with NPK in the first year of cultivation contributed to the increase in Cr content in this fraction to a higher degree than after the second year. The introduction of organic matter into soil has an impact on proportions of chromium in individual fractions, indicating that the amount of this metal increases significantly in complexes with iron, manganese and organic compounds (Kalembasa, Pakuła 2009*a*). The sequential analysis revealed three-fold less copper than chromium in the F2 fraction. The proportion of F2 Cu in the total content was comparable in the objects (4.60% after the first year of cultivation and 4.30% after the second year of cultivation). The highest content of Cu after the first year of cultivation was recorded in the object fertilized with manure (5.80%) and after the second year in the object fertilized with spent mushroom substrate + NPK (7.08%). The concentration of Cu in the F2 fraction after the first year of cultivation was higher in the treatments fertilized with organic materials, whereas after the second year it went up in the treatments fertilized with spent mushroom substrate, both compared with the controls. The analysis of variance revealed that the content of chromium and copper in the F2 fraction significantly depended on the year of study, fertilization and interaction between these factors.

In the organic fraction F3, the content of chromium was much higher than in the reductive fraction F2, i.e. 33.4% on average after the first year and 21.0% after the second year. The use of spent mushroom substrate alone (35.0%) and with NPK (35.3%), particularly in the first year of cultivation, contributed to the increase in Cr content in relation to the control object. Kalembasa, Pakuła (2009*a*,*b*) detected a comparable proportion of Cr in the organic fraction in the humus horizons of brown forest soil in the Central-East Poland (18.5-41.4%). The content of copper in the F3 fraction was on average higher after the second year of cultivation (35.5%) than after the first year (31.0%). After the first year, the highest content of Cu was detected in the objects fertilized with manure alone and with NPK, whereas after the second year it was with manure and substrate + NPK. After the second year of cultivation, an increase in Cu content was recorded in all fertilized objects in comparison with the first year with the highest gain in the object fertilized with spent mushroom substrate alone (by 7.7%) and with NPK (by 7.2%). An increase in copper in the organic fraction by 30% in soil supplemented with a spent mushroom substrate in relation to a control object has been also reported by Herrero-Hernandez et al. (2011). Copper is found in considerable amounts in organic complexes (the oxidative fraction F3), which confirms its high affinity to the formation of complexes with functional groups of humic and fulvic acids (Karczewska 2002). The content of chromium and copper in the organic fraction F3 significantly depended on the year of study and type of fertilization.

The highest content of chromium and copper in the tested objects was detected in the residual fraction F4 after the first and the second year of cultivation. A higher proportion of Cr in this fraction was determined after the second year of cultivation (64.3%) than in the first year (48.9%). The lowest content of Cr in this fraction was recorded in the object fertilized with spent mushroom substrate alone and with NPK. The easy reduction of soluble Cr^{6+} to Cr^{3+} of low solubility in soil environment results in strong binding of this metal to soil solid phase (Kalembasa, Pakuła 2009*a*). The content of copper in the F4 fraction was on average higher after the first year of cultivation than after the second year (60.1% and 55.8% respectively). After the first year of cultivation, the highest content of Cu of this fraction was detected in the object fertilized with spent mushroom substrate alone (63.2%), whereas after the second year in the object fertilized with NPK (60.6%). After the second year of cultivation in the objects supplemented with spent mushroom substrate alone and with NPK, the content of Cu in this fraction decreased in comparison with the first year. Kalembasa and Pakuła (2009*b*), Wójcikowska-Kapusta and Niemczuk (2009), Guan et al. (2011) and Herrero-Hernandez et al. (2011) fractionated copper with the BCR method and found that this metal accumulated mainly in two frac-

Table 6

The correlation coefficients between the fractions of chromium and copper (mg kg^{-1}) and some properties after two years of field experiment

Parametr	Element	F1	F2	F3	F4
Total content	Cr Cu	-0.05 0.61*	-0.28 0.54	-0.22 0.88*	0.67* 0.93*
pH_{KCl}	Cr Cu	-0.34 0.37	0.46 0.25	0.55 0.21	-0.36 0.63*
OrgC	Cr Cu	0.66* 0.26	0.16 0.46	0.05 0.68*	-0.22 -0.14
CEC	Cr Cu	0.17 0.06	0.26 0.36	0.22 -0.03	-0.33 0.05

* significant α =0.05.

tions: residual F4 and oxidative F3. An analysis of variance showed that the content of both tested metals in F4 fraction depended on the year of study, type of fertilization and interaction between these factors. The statistical calculations (Table 6) revealed that the total content of copper was significantly correlated with the concentration of this metal in the easily soluble and exchangeable fraction F1 and the organic fraction F3, whereas for both these metals with the residual fraction F4. The pH value of the tested soil had a significant impact on the content of chromium in the F4 fraction and the content of carbon in organic compounds significantly correlated with the amount of chromium in the F1 fraction and copper in the F3 fraction.

CONCLUSIONS

1. Spent mushroom substrate, used for growing *Agaricus bisporus* and afterwards applied to soil significantly increased the total content of copper in the humus horizon. The content of chromium and copper in the soil under a two-year field experiment did not exceed the permissible limits specified in the Regulation of the Minister of the Environment and stayed with the natural value range.

2. The sequential fractionation of chromium and copper with the BCR procedure in the humus horizon of soil in the individual experimental objects revealed diversified concentration of these metals in the extracted fractions and their share in the total content. The spent mushroom substrate alone and with NPK contributed to a decrease in Cr content in the F2 and F3 fractions and to an increase in Cu content in the F1, F2 and F3 fractions after the second year of the cultivation in comparison with the first year. The highest concentration of tested metals in the total content was detected in the residual fraction F4: for chromium after the second year and for copper after the first year of the experiment.

3. The concentration of tested metals in the total content, in the extracted fractions and in the soil of experimental objects after two years of cultivation was arranged in the following order of decreasing values: for chromium F4>F3>F2>F1; and for copper F4>F3>F2⇔F1.

4. The analysis of variance showed that the content of chromium and copper in the extracted fractions depended significantly on the year of study and type of fertilization.

REFERENCES

Domańska J., Filipek T. 2011. *Content of Cu bound to soil fractions as affected by soil pH and organic matter content.* Environ. Protect. Natur. Res., 48: 74-79.

Guan T. X., He H. B., Zhang X. D., Bai Z. 2011. *Cu fractions, mobility and bioavailability in soil-wheat system after Cu-enriched livestock manure applications.* Chemosphere, 82: 215-22.

HERRERO-HERNANDEZ E., ANDRADES M.S., RODRIGUEZ-CRUZ M.S., SCHANCHEZ-MARTIN M.J. 2011. *Effect of spent mushroom substrate applied to vineyard soil on the behavior of copper-based fungicide residues.* J. Environ. Manage., 92: 1849-1857.

KALEMBASA S., KALEMBASA D. 1992. *The quick method for the determination of C:N ratio in mineral soils.* Pol. J. Soil Sci., 25(1): 41-46.

KALEMBASA D., MAJCHROWSKA-SAFARYAN A. 2009. *Fraction of heavy metals in the beds after the cultivation mushroom from mushroom factory.* Environ. Protect. Natur. Res., 41: 572-577.

KALEMBASA D., MAJCHROWSKA-SAFARYAN A., CHROMIŃSKA M. 2011. *Dynamics of change in the total content of copper in soils and in sequential- separated fractions*. Acta Agrophys., 18(1): 67-75.

KALEMBASA D., PAKUŁA K. 2009*a*. *Chromium in sequential extracted fractions in Cambisols and Luvisols of the Siedlce Upland.* Adv. Agri. Sci. Probl., 542: 721-728.

KALEMBASA D., PAKUŁA K. 2009*b*. *Heavy metal fractions in soils fertilized with sewage sludge.* Environ. Protect. Eng., 35(2): 157-164.

KALEMBASA D., WIŚNIEWSKA B. 2004. *The utilization of mushroom bed for the recultivation of sois.* Soil Sci. Annual, 55(2): 209-217.

KARCZEWSKA A. 2002. *Heavy metals in soils polluted emissions from copper smelters – forms and solubility.* Zesz. Nauk. AR, Wrocław, 432.

MOSSOP K.F., DAVIDSON CH.M. 2003. *Comparison of original and modified BCR sequential extraction procedures for the fractionation of copper, iron, lead, manganese and zinc in soils and sediments.* Anal. Chem. Acta, 478: 11-118.

Regulation of the Minister of Environment of 9 September 2002 on standards for soil quality and soil quality standards. Dz. U. Nr 165, Poz. 1359.

UREA A.M., QUEVAUVILLER PH., MUNTAU H., GRIEPINK B. 1993. *Speciation of heavy metals in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commision of the European Communities.* Intern. J. Environ. Anal. Chem., 51: 135-151.

WÓJCIKOWSKA-KAPUSTA A., NIEMCZUK B. 2009. *Copper speciation in different-type soil profiles.* J. Elementol., 14(4): 815-824. 478: 11-118.

J. Elem. s. 471–482
DOI: 10.5601/jelem.2014.19.2.460

ACCUMULATION OF HEAVY METALS IN MOSS SPECIES *PLEUROZIUM SCHREBERI* (BRID.) MITT. AND *HYLOCOMIUM SPLENDENS* (HEDW.) B.S.G. IN SŁOWIŃSKI NATIONAL PARK

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Abstract

This paper presents the results of research into the heavy metal content in the moss species *Pleurozium schreberi* and *Hylocomium splendens* and in the organic and humus horizons of soil under coniferous pine forests in Słowiński National Park. The test samples were taken in September 2011 from 15 research stations situated in the park. The research stations were set up in places most frequently visited by tourists and in the vicinity of car parks. The examined soil was characterized by strong acid reaction, which had impact on an increased accessibility of the examined heavy metals to plants. Variation coefficients for active acidity and exchangeable acidity were from 5 to 8%. The highest level of organic matter was in the subhorizons Ol, slightly lower in the subhorizons Ofh, and the lowest in the A horizons. It was demonstrated that the active acidity of the soil's surface genetic levels was signnificantly correlated with the manganese and zinc content (in both moss species) and wth zinc (in *H. splendens*). Relationships among the determined heavy metals in both species of moss made the following decreasing series: Fe>Mn>Zn>Cu. The data revealed varied accumulation characteristics of the moss species. The U Mann-Whitney's test on the significance of variation in the content of selected heavy metals in *Pleurozium schreberi* and *Hylocomium splendens* demonstrated statistical differences in the concentration of Cu ($p<0.01$). According to the values of the *Pleurozium*/*Hylocomium* coefficient, *P. schreberi* had higher accumulation potential in relation to Zn, and *H. splendens* in relation to Fe and Cu. Both tested moss species presented similar accumulation characteristics in reference to Mn. The levels of enrichment factors (*EF*) confirmed weak contribution of heavy metals accumulated in soil to the formation of the Fe, Zn, Mn and Cu content in *P. schreberi* and *H. splendens*.

Keywords: *Pleurozium schreberi*, *Hylocomium splendens*, accumulation of Zn, Fe, Cu, Mn.

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AKUMULACJA METALI CIĘŻKICH W MCHACH *PLEUROZIUM SCHREBERI* (BRID.) MITT. I *HYLOCOMIUM SPLENDENS* (HEDW.) B.S.G. W SŁOWIŃSKIM PARKU NARODOWYM

Abstrakt

Badano zawartość metali ciężkich w mchach: *Pleurozium schreberi* i *Hylocomium splendens* oraz w poziomach organicznych i próchnicznych borów sosnowych Słowińskiego Parku Narodowego. Próbki do badań pobierano we wrześniu 2011 r. z 15 stanowisk zlokalizowanych na terenie parku. Stanowiska badawcze zlokalizowano w miejscach najczęściej uczęszczanych przez turystów oraz w sąsiedztwie parkingów samochodowych. Badane gleby charakteryzują się odczynem silnie kwaśnym, co wpływa na zwiększoną dostępność badanych metali ciężkich dla roślin. Współczynniki zmienności dla kwasowości czynnej oraz kwasowości wymiennej wynosiły od 5 do 8%. Największą zawartość materii organicznej stwierdzono w podpoziomach Ol, nieco mniejszą w podpoziomach Ofh, a najmniejszą w poziomach A. Wykazano, że kwasowość czynna wierzchnich poziomów genetycznych gleby pozostaje w istotnym statystycznie związku z zawartością manganu i cynku (w obu gatunkach mchów) oraz cynku (w *H. splendens*). Relacje między oznaczanymi metalami ciężkimi w obu gatunkach mchów układają się w następujący szereg malejący: Fe>Mn>Zn>Cu. Wykazano zróżnicowane właściwości akumulacyjne badanych gatunków mchów. Wyniki testu U Manna-Whitneya istotności zróżnicowania wybranych metali ciężkich w *Pleurozium schreberi* i *Hylocomium splendens* wskazują na istotne statystycznie różnice w koncentracji Cu ($p < 0.01$). Zgodnie z wartościami współczynnika *Pleurozium*/*Hylocomium* większe właściwości akumulacyjne ma *P. schreberi* w stosunku do Zn, a *H. splendens* w stosunku do Fe i Cu. Oba badane gatunki mchów wykazują zbliżone właściwości akumulacyjne w stosunku do Mn. Wartości współczynników wzbogacenia (*EF*) potwierdzają niewielki udział metali ciężkich zawartych w glebie w kształtowaniu zawartości Fe, Zn, Mn i Cu w *P. schreberi* i *H. splendens*.

Słowa kluczowe: *Pleurozium schreberi*, *Hylocomium splendens*, akumulacja Zn, Fe, Cu, Mn.

INTRODUCTION

Heavy metals as natural components of ecosystems are necessary in small quantities for sustaining proper functions of plants, although their excess in the environment is harmful. In extremely high concentrations, they disturb whole ecosystems and pose a threat to plants, animals and people (Gruca-Królikowska, Wacławek 2006, Malzahn 2009, Medyńska-Juraszek, Kabała 2012). Heavy metals are bio-accumulated in plant and animal tissues, thus the risk of intoxication grows in subsequent links of the trophic chain. Plants react differently to increased heavy metal concentrations in the environment. The most sensitive ones are used in bio-monitoring to acquire information about the quality of the environment (Migaszewski et al. 2009). Among plant bio-indicators, moss species are useful in monitoring studies. Lacking natural protective barriers such as the epidermis and cuticle, they easily absorb substances, especially metals, which are deposited on their surface (Gjengedal, Steinnes 1990, Berg, Steinnes 1997, Mankovska 1998, Grodzińska, Szarek-Łukaszewska 2001, Reiman et al. 2001, Gerdol et al. 2002, Szczepaniak, Biziuk, 2003, Gałuszka 2006, 2007, Samecka-Cymerman et al., 2006, Dećkowska et al. 2008, Harmens et al. 2010). Moss absorbs nutritional elements mainly from precipitation and dry deposition. Absorption from the substratum is limited due to the lack of roots (Grodzińska 1980). The influence of soil and soil solutions on the heavy metal content in moss cannot be neglected (Steinnes 1995, Wells, Brown 1996, Økland et al. 1999, Gerdol et al., 2002). *Pleurozium schreberi* and *Hylocomium splendens* found both in pure and polluted habitats are the most popular species, with a widespread geographical distribution, used for evaluation of the air quality. Inter-species comparisons of their chemical content indicate differences in the accumulation of elements. In most cases, higher concentrations were found in *H. splendens*, but this tendency has not been explained in detail. One possible explanation could lie in morphological differences (Halleraker et al. 1998, Grodzińska et al. 1999, Migaszewski et al. 2009).

The objectives of the study were (i) determination of the content of selected heavy metals in *Pleurozium schreberi* and *Hylocomium splendens* growing in a protected area, (ii) comparison of their accumulative characteristics, (iii) evaluation of the influence of heavy metals found in soil on the concentration of heavy metals in moss, and (iv) evaluation of air pollution levels in Słowiński National Park.

SAMPLING SITES AND METHODS

The research was carried out in a forest in Słowiński National Park (SNP), which lies on the Łeba Sandbar. Samples of moss (*Pleurozium schreberi*, *Hylocomium splendens*) and of the organic and humus soil layers were collected in September 2011. They were taken from 15 research stations located in Park's ecosystems of *Empetro nigri-Pinetum*, growing on the Dystric Arenosols and Podzols developed from deep sand dunes (SGP 2011). The research stations were situated in places most frequently visited by tourists and in the vicinity of car parks. The soil samples were submitted to the following determinations: pH in H_2O and 1 M dm^{-3} measured with a potentiometer in a solution of KCl; the organic matter content in a muffle furnace at the temperature of 550°C. The plant material was transported to a laboratory, where it was refined of mineral parts of soil, dried to constant mass at 65°C and homogenized in a grinder (Namieśnik et al., 2000). Moss and soil samples were wet mineralized in a closed system in a mixture of HNO_3 and 30% H_2O_2. In the solutions, the concentrations of Zn, Fe, Mn and Cu were determined by atomic absorption spectrometry (Aanalyst 300, Perkin Elmer) according to Ostrowska et al. (2001). The results were compared to standards (Merck KGaA, 1 g/1000 ml).

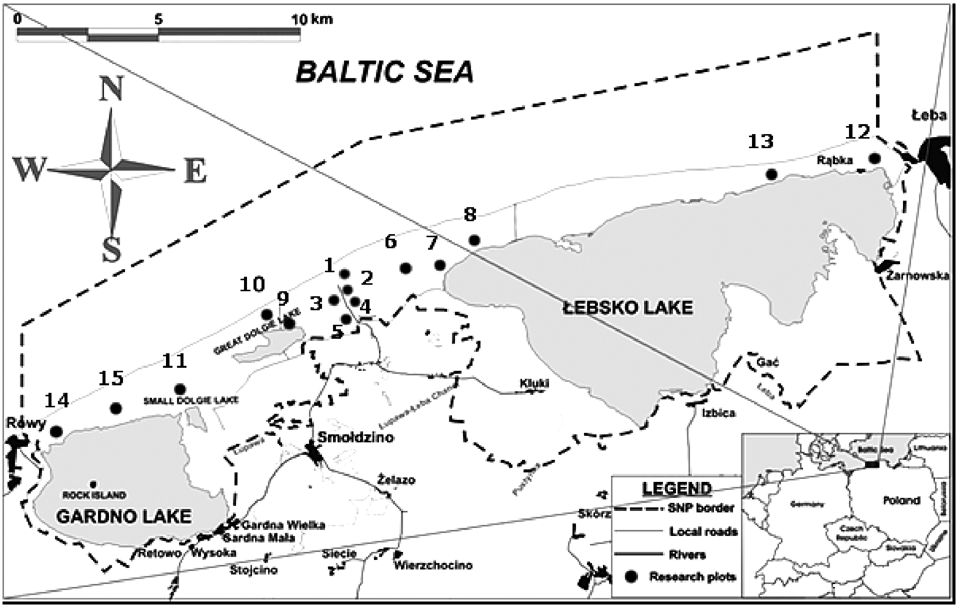

Fig. 1. Situation plan of the Słowiński National Park – locations of the study sites

STATISTICAL ANALYSIS

In order to characterize and compare concentrations of the selected heavy metals in the examined moss species, average, minimum, maximum, medians, standard deviations, variation coefficients (CV), Spearman's correlation coefficients, *Pleurozium*/*Hylocomium* coefficients and enrichment factors (*EF*) were calculated. The validity of statistical variation between the moss species and the heavy metal content in the soil was checked by the non-parametric U Mann-Whitney's test. Statistica software was used for calculations (7.1).

RESULTS AND DISCUSSION

The samples of organic and humus horizons taken from 15 research stations within the area of the Łeba Sandbar had strong acid reaction (Table 1). The variation coefficients for active acidity (pH, H_2O) and exchangeable acidity (pH, KCl) were from 5 to 8%. The highest clevel of organic matter was in the subhorizons Ol, slightly lower in the subhorizons Ofh, and the lowest in the A horizons. The organic matter content decreased with the depth, conversely to the diversity of this parameter, which increased (CV 2 59%).

Table 1

pH and organic matter in organic and humic horizons in SNP

Specification	pH (H_2O)			pH (KCl)			Organic matter (%)		
	Ol	Ofh	A/AC	Ol	Ofh	A/AC	Ol	Ofh	A/AC
Average	4.4	3.8	4.4	3.5	2.8	3.3	96.6	76.9	3.1
Minimum	4.1	3.1	3.9	3.0	2.5	2.9	91.6	39.0	0.6
Maximum	4.7	4.1	5.0	3.8	3.1	3.6	98.5	97.2	5.9
Median	4.4	3.9	4.4	3.6	2.8	3.4	97.5	73.8	2.6
Standard deviation	0.2	0.2	0.3	0.2	0.2	0.2	0.02	0.2	0.01
CV (%)	5	7	7	8	6	8	2	23	59

The content of the analyzed metals in the soil was varied. The largest quantities of Zn, Fe and Mn were found in the Ol subhorizons, slightly smaller ain Ofh, and the smallest in the A horizons. The highest concentrations of Cu and Fe were found in the Ofh subhorizons, and Zn and Mn were most abundant in the Ol subhorizons. The variation coefficients for the zinc content were from 28 to 38 %, for iron – from 35 to 134 %, manganese – from 52 to 55 %, and copper – from 18 to 35 %, depending on the genetic level of soil (Table 2).

Table 2

Heavy metals content (mg kg^{-1}) in organic and humus horizons in SNP

Specification	Zn			Fe			Mn			Cu		
	Ol	Ofh	A/AC	Ol	Ofh	A/AC	Ol	Ofh	A/AC	Ol	Ofh	A/AC
Average	68.9	47.0	2.89	469	1609	346	206.2	40.0	4.17	0.80	0.90	0.09
Minimum	37.1	24.2	1.28	118	478	124	48.7	12.2	1.15	0.50	0.60	0.06
Maximum	101.0	82.0	5.68	2071	8517	568	415.0	86.2	8.3	1.10	1.70	0.19
Median	65.5	42.7	2.7	279	907	381.1	198.9	37.4	3.7	0.8	0.9	0.08
Standard deviation	19.0	15.4	1.08	505	2161	120	112.6	23.1	2.15	0.14	0.29	0.03
CV (%)	28	33	38	108	134	35	55	58	52	18	31	35

The moss samples collected from the Łeba Sandbar in Słowiński National Park were determined to contain different levels of Zn, Fe, Mn and Cu. The zinc content varied from 47.9 to 95.2 (mg kg^{-1}) in *Pleurozium schreberi* and from 38.7 to 84.9 mg kg^{-1} in *Hylocomium splendens* (Figure 2, Table 3). The Zn concentration in the tested moss revealed was highly varied. The mean variability coefficients (CV) of the Zn content were 21.0% for *P. schreberi* and 24.0% for *H. splendens*. At most research stations, higher accumulation of zinc in *P. schreberi* than in w *H. splendens* was discovered. The Zn content in *P. schreberi* and *H. splendens* moss in the examined coniferous forests is comparable to the results obtained by other, e.g. GAŁUSZKA (2006) 33-54 mg kg^{-1} in Wigierski National Park, KOZANECKA et al. (2002)

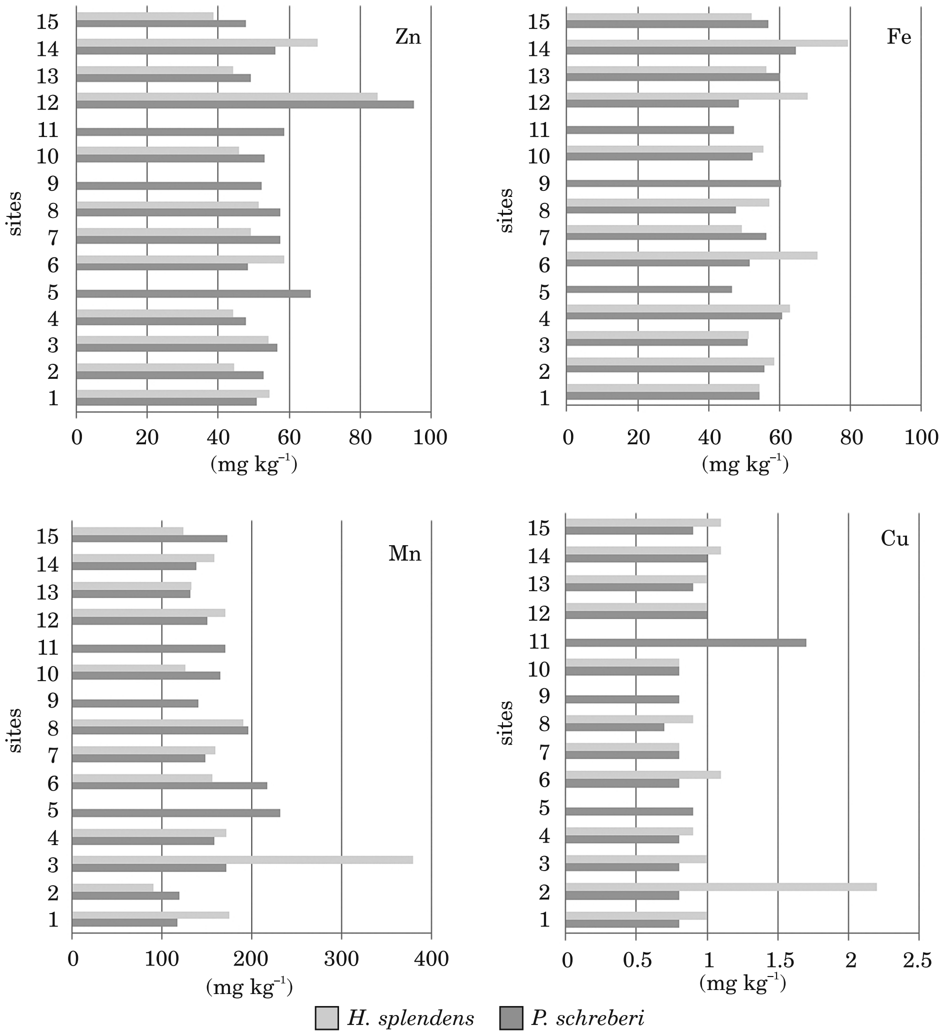

Fig. 2. Content of selected elements (mg kg-1) in *Pleurozium schreberi* and *Hylocomium splendens*

33-43 mg kg-1 in Biała Primeval Forest, or MALZAHN (2009) 46 mg kg-1 in Białowieska Primeval Forest.

The iron content in moss was also diverse. In *P. schreberi* the Fe level ranged from 233.0 to 323.0 (mg kg-1), and in *H. splendens* it varied from 247.0 to 396.0 (mg kg-1) (Figure 2, Table 3). Variability coefficients for the Fe concentration were from 10.4% (*P. schreberi*) to 15.0% (*H. splendens*). These values of the Fe content in moss in SNP are substantially lower than determined earlier by GRODZIŃSKA (1980). The current results indicate that the pollution in the park had decreased. Similar iron concentrations in *P.*

schreberi moss were obtained by: GAŁUSZKA (2006), 194-795 mg kg^{-1}, and KOZANECKA et al. (2002), from 326 to 650 mg kg^{-1}.

The manganese content in the tested moss was from 116.3 to 231.4 mg kg^{-1} (*P. schreberi*) and from 90.5 to 379.0 mg kg^{-1} (*H. splendens*) – Figure 2, Table 3. The Mn concentration in *H. splendens* was twice as varied as in *P. schreberi* (CV = 42.3% and CV = 20.7%, respectively). According to KABATA-PENDIAS, PENDIAS (1999), the plant's demand for manganese is diverse but in most cases 10-25 mg kg^{-1} is sufficient. A concentration of about 500 mg kg^{-1} can be toxic for most plants. The average manganese content in *P. schreberi* from Biała Primeval Forest is about 383 mg kg^{-1} (KOZANECKA et al. (2002). The Mn content in both moss species originating from Lithuania was from 50.0 to 744.0 mg kg^{-1} (ČEBURNIS, STEINNES 2000).

The copper content in moss was from 0.7 to 1.7 mg kg^{-1} in *P. schreberi* and from 0.8 to 2.2 mg kg^{-1} in *H. splendens*, showing much higher variation among the stations. The mean variation coefficients of the Cu concentration in moss were 26.2% (*P. schreberi*) and 34.4% (*H. splendens*) – Figure 2, Table 3. Some earlier tests of the chemical composition of *P. schreberi* by GRODZIŃSKA et al. (1999) in SNP demonstrated the concentration of Cu equal 7 mg kg^{-1}. The average Cu content in *P. schreberi* in Poland, according to DEĆKOWSKA et al. (2008), is 10.7 mg kg^{-1}. The Cu content in moss from SNP determined in our study is very low and sufficient just to cover the plant's physiological needs.

Relationships among the determined heavy metals in both species of moss made the following decreasing series: Fe>Mn>Zn>Cu. Identical relationships among the tested elements were discovered by KOZANECKA et al. (2002) and MALZAHN (2009).

Table 3

Mean, standard deviation (SD), coefficient of variation (CV), minimum (min), maximum (max), median concentrations of different elements (mg kg^{-1}) in *Pleurozium schreberi* and *Hylocomium splendens* and correlation coefficient

Plant		Zn	Fe	Mn	Cu
Pleurozium schreberi	mean (mg kg^{-1})	56.7	271.1	161.5	0.9
	SD	11.8	28.2	33.4	0.24
	CV (%)	21.0	10.4	20.7	26.2
	min	47.9	233.0	116.3	0.7
	max	95.2	323.0	231.4	1.7
	median	53.0	271.0	158.2	0.8
Hylocomium splendens	mean (mg kg^{-1})	53.2	298.1	169.1	1.1
	SD	12.7	44.8	71.2	0.37
	CV (%)	24.0	15.0	42.3	34.4
	min	38.7	247.0	90.5	0.8
	max	84.9	396.0	379.0	2.2
	median	50.3	283.5	158.2	1.0
Correlation *P. schreberi-H. splendens*		**0.81**[a]	0.26[b]	0.21[b]	0.27[b]

[a] statistically significant for the $p < 0.01$, [b] – not statistically significant

Comparing the accumulation of the tested metals in *P. schreberi* and *H. splendens*, a statistically significant, positive correlation was demonstrated only for the zinc content (r = 0.81, n = 36, $p < 0.05$) – Table 3. Correlations between Fe, Mn and Cu in both moss species were statistically unimportant.

The results of the U Mann-Whitney's test on the significance of variation in the content of the heavy metals in the moss species *Pleurozium schreberi* and *Hylocomium splendens* sampled in SNP demonstrate statistically significant differences only for the Cu concentration ($p < 0.01$).

The *Pleurozium/Hylocomium* coefficients calculated for moss from SNP demonstrate higher accumulation potential of *P. schreberi* in relation to Zn, and *H. splendens* in relation to Fe and Cu (Table 4). Similar results were obtained by REIMAN et al. (2001). In the case of iron and copper, a higher accumulation potential of *H. splendens* than *P. schreberi* was proven (0.96 and 0.80 respectively). Similar relationships between the content of these metals in moss were discovered by GAŁUSZKA (2007) or BERG, STEINNES (1997). As for manganese, similar accumulative values were discovered for *P. schreberi* and in *H. splendens.*

Table 4

Pleurozium/Hylocomium concentration (median) ratios of 4 elements in *H. splendens* and *P. schreberi* derived from SNP

Zn	Fe	Mn	Cu	References
1.05	0.96	1.00	0.80	Results of their own, SNP - 2011
0.97	0.95	0.81	0.89	GAŁUSZKA (2007)
1.03	0.89	0.95	0.80	REIMAN et al. (2001)
-	0.63	1.30	0.76	BERG and STEINNES (1997)

In order to examine the influence of the soil's chemical composition on the content of heavy metals in moss, the Spearman's correlation coefficients were calculated (Table 5). It was demonstrated that the active acidity (pH, H_2O) had a significant statistical correlation with Mn (r = -0.33 *P. schreberi* and r = -0,32 *H. splendens*, where n = 36, $p < 0.05$) (r = 0.32, n = 45, $p < 0.05$, *P. schreberi*). Exchangeable acidity (pH, KCl) was statistically significantly and positively correlated with iron contained in both moss species (r = 0.41, n = 45 and r = 0.41, n = 36, $p < 0.05$) and with manganese (r = -0.33, n = 36, $p < 0.05$) in the case of *H. splendens*. The organic matter accumulated in the surface layers of soil had strong impact on the Zn content in *P. schreberi* (r = -0.37, n = 45, $p < 0.05$) and on the Mn content in *H. splendens* (r = 0.48, n = 36, $p < 0.05$). The content of heavy metals in the soil demonstrated a very weak impact on the content of these metals in moss. A positive correlation was demonstrated in the case of zinc in the soil and in *P. schreberi* (r = 0.38, n = 45, $p < 0.05$). As the iron content in the soil increased, the Cu concetration in moss (r = -0.31, *P. schreberi* and

Table 5

The correlation coefficients Spearmann selected soil properties and heavy metals in mosses

Plant	Elements	pH, H_2O	pH, KCl	Organic mater	Zn	Fe	Mn	Cu
Pleurozium schreberi (*n* = 45, *p* < 0.05, $r_{crit.}$ = 0.30)	Zn	**0.32**	-0.01	**-0.37**	**0.38**	-0.16	0.17	0.01
	Fe	-0.05	**0.41**	0.17	0.02	-0.09	0.29	0.08
	Mn	**-0.33**	**-0.33**	0.22	0.19	0.22	-0.28	0.08
	Cu	0.08	-0.06	-0.05	0.09	**-0.31**	0.09	0.05
Hylocomium splendens (*n* = 36, *p* < 0.05, $r_{crit.}$ = 0.30)	Zn	-0.08	0.15	0.16	-0.16	**0.41**	**-0.42**	0.56
	Fe	-0.23	**0.40**	0.01	0.16	0.04	-0.03	0.45
	Mn	**-0.32**	-0.25	0.48	0.13	0.13	**-0.39**	-0.15
	Cu	-0.15	0.16	0.14	0.17	-0.43	0.08	-0.22

r = -0.43, *H. splendens*) decreased while the Zn content rose (r = 0.41, n =36, $p < 0.05$). Moreover, manganese included in the soil had some impact on decreasing the content of Zn (r = -0.42) and Mn (r = -0.39), while soil copper resulted in an increase in Zn (r = 0.56) and Fe (r = 0.45) in *H. splendens* (Table 5).

The heavy metal content in the soil had a weak influence on the Zn, Fe, Mn and Cu content in moss. Similar relationships were obtained by GERDOL et al. (2002). A small content of the metals in soils and moss implies relatively clean air (BROŻEK, ZAREMBSKI 2011) and translates into small levels of the enrichment factor (*EF*) – Figure 3. The lowest enrichment factor was discovered in the case of iron (average 0.3 for *P. schreberi* and *H. splendens*), copper (averge 1.5 for *P. schreberi* and 1.7 for *H. splendens*) and zinc (average 1.5 for *P. schreberi* and 1.4 for *H. splendens)*. The highest *EF* was found only in the case of manganese (average 2.8 for *P. schreberi* and *H. splendens*).

Little quantities of heavy metals in the soil suggested an alluvial form of pollution, e.g. brought by dry and wet precipitation. However, in the examined case, quantities of heavy metals originating from such sources were minimal. This was supported by the results of tests on suspended particulate matter PM10 (a potential source of heavy metals) held in 2010 in Słowiński National Park – 17 μg m^{-3} (BROŻEK, ZAREMBSKI 2011). In 1995, the amount of emitted dust containing heavy metals (529 t) decreased substantially in comparison to 1990 (1165 t) (GUS 1991, 1995). The area of Słowiński National Park has been recognized by many scholars as one of the cleanest nature reserves in Poland (GRODZIŃSKA 1980, GRODZIŃSKA et al. 1990). The enrichment factor (*EF*) values confirm small contribution of heavy metals in the soil to the formation of the Fe, Zn, Mn and Cu content in *P. schreberi* and *H. splendens* (Figure 3). Similar levels of the EF coefficients for the *EF* were achieved in *H. splendens* by BARGAGLI et al. (1995).

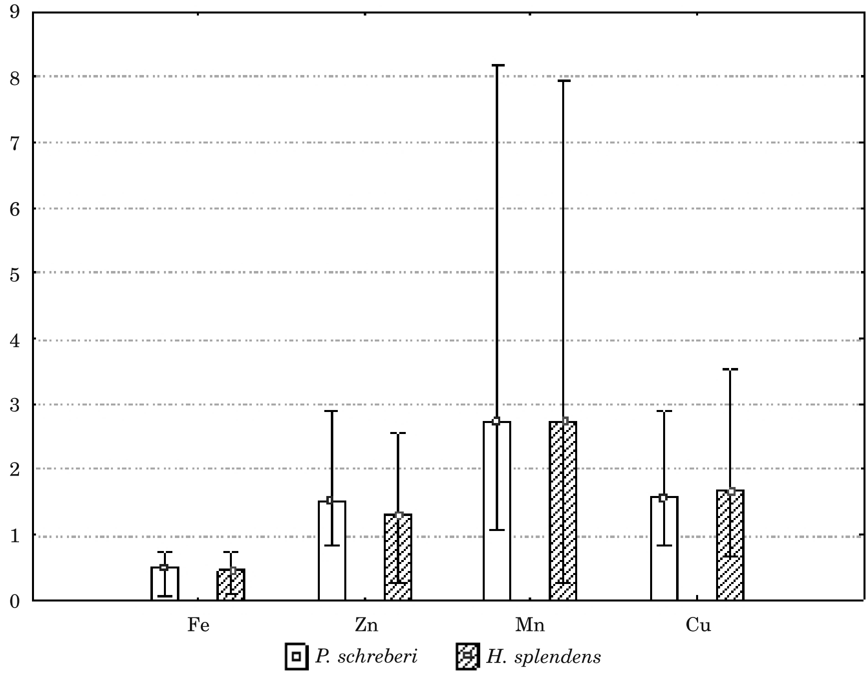

Fig. 3. Enrichment factor of *P. schreberi* and *H. splendens* in heavy metals, point (mean), rectangle (standard deviation), whiskers (minimum-maximum)

CONCLUSIONS

The moss samples from Słowiński National Park had varied concentrations of Zn, Fe, Mn and Cu. The examined soil was characterized by strong acid reaction, which had impact on an increased accessibility of the examined heavy metals to plants. It was demonstrated that the active acidity (pH, H_2O) of the soil's surface genetic levels had a significant statistical correlation with the manganese and zinc content (in both moss species) and wih zinc (in *H. splendens*). The Mn content in the tested moss was from 116.3 to 231.4 mg kg^{-1} in *P. schreberi* and from 90.5 to 379.0 mg kg^{-1} in (*H. splendens*) (CV = 42.3% and CV = 20.7% respectively). The relationships between the determined heavy metals in both moss species made an identical decreasing series: Fe>Mn>Zn>Cu.

Comparing the accumulation of the examined heavy metals in *P. schreberi* and *H. splendens*, a statistically significant and positive correlation was demonstrated for zinc ($r = 0.81$, $n = 36$, $p < 0.05$). Correlations between Fe, Mn and Cu in both species of moss proved to be statistically unimportant. The results of the U Mann-Whitney's test concerning the significance of variations of selected heavy metals in the moss *Pleurozium schreberi* and *Hylocomium splendens* demonstrates substantial differences in the Cu content ($p < 0.01$).

The *Pleurozium/Hylocomium* coefficients demonstrate higher accumulation potential of *P. schreberi* in relation to zinc and *H. splendens* in relation to iron and copper. Both examined moss species demonstrated similar accumulation capabilities regarding manganese. The values of enrichment factors (*EF*) confirm small contribution of heavy metals in soil to the formation of the Fe, Zn, Mn and Cu content in *P. schreberi* and *H. splendens.*

REFERENCES

Bargagli R., Brown D.H., Nelli L. 1995. *Metal biomonitoring with mosses: procedures for correcting for soil contamination*, Environ. Pollut., 89(2): 169-175. http://vls2.icm.edu.pl/pdflinks/14010413155414302.pdf

Berg T., Steinnes E. 1997. *Use of mosses (Hylocomium splendens and Pleurozium schreberi) as biomonitors of heavy metal deposition: from relative to absolute deposition values*. Environ. Pollut., 98: 61-71. http://vls2.icm.edu.pl/pdflinks/14010413180914456.pdf

Brożek A., Zarembski A., 2011. *The annual assessment of air quality in the province of Pomerania. The report of 2010*. Woj. Insp. Ochr. Środ., Gdańsk. (in Polish)

Čeburnis D., Steinnes E. 2000. *Conifer needles as biomonitors of atmospheric heavy metal deposition: comparison with mosses and precipitation, role of the canopy*. Atmos. Environ., 34: 4265-4271. http://vls2.icm.edu.pl/pdflinks/14010413201414621.pdf

Dećkowska A., Pierścieniak M., Gworek B., Maciaszek D. 2008. *Chosen the plant's species as coefficients of changes in environment*. Ochr. Środ. Zasob. Nat., 37: 128-138. (in Polish) http://www.ios.edu.pl/pol/nr37

Gałuszka A. 2006. *Biogeochemical background of selected trace elements in mosses Pleurozium schreberi* (Brid.) Mitt. *and Hylocomium splendens* (Hedw.) *B.S.G. from Wigierski National Park*. Pol. J. Environ. Stud., 15(2a): 72-77.

Gałuszka A., 2007. *Distribution patterns of PAHs and trace elements in mosses Hylocomium splendens (*Hedw.*) B.S.G. and Pleurozium schreberi* (Brid.) Mitt. *from different forest communities: a casy study, south central Poland.* Chemosphere, 7: 1415- 1422. DOI: 10.1016/j.chemosphere.2006.10.010

Gerdol R., Bragazza L., Marchesini R. 2002. *Element concentrations in the forest mosses Hylocomium splendens: variation associated with altitude, net primary production and soil chemistry*. Environ. Pollut., 116:129-135. http://vls2.icm.edu.pl/pdflinks/14010413474516796.pdf

Gjengedal E., Steinnes E. 1990. *Uptake of metal ions in moss from artificial precipitation*. Environ. Monit. Assess., 14:77-87.

Grodzińska K. 1980. *Heavy metals pollution of Polish National Parks*. Ochr. Przyr., 43: 9. (in Polish)

Grodzińska K., Szarek-Łukaszewska G. 2001. *Response of mosses to the heavy metal deposition in Poland – an overview*. Environ. Pollut., 114: 443-451. http://vls2.icm.edu.pl/pdflinks/14010413491916918.pdf

Grodzińska K., Szarek G., Godzik B. 1990. *Heavy metal deposition in Polish National Parks – changes during ten years*. Water Air Soil Pollut., 49: 409-419.

Grodzińska K., Szarek-Łukaszewska G., Godzik B. 1999. *Survey of heavy metal deposition in Poland using mosses as indicators*, Sci. Total Environ., 229: 41-51. http://vls1.icm.edu.pl/pdflinks/13081312411220148.

Gruca-Królikowska S.,Wacławek W. 2006. *Metals in the environment.* Part II. *Impact of he-*

avy metals on plants. Chemia Dydaktyka Ekologia Metrologia, 11(1-2): 41-56. (in Polish) http://tchie.uni.opole.pl/CDEMfree/Gruca_metale.pdf

GUS, 1991. *Environmental protection. Materials and statistical studies*. Warszawa. (in Polish)

GUS, 1995. *Environmental protection. Materials and statistical studies*. Warszawa. (in Polish)

Halleraker J. H., Reimann C., Caritat De P., Finne T.E., Kashulina G., Niskavaara H., Bogatyrev I. 1998. *Reliability of moss (Hylocomium splendens and Pleurozium schreberi) as a bioindicators of atmospheric chemistry in the Barents region: Interspecies and field duplicate variability*. Sci. Total Environ., 218:123-139. http://vls1.icm.edu.pl/pdflinks/13081312395319919.

Harmens H., Norris D.A., Steinnes E. 2010. *Mosses as biomonitors of atmospheric heavy metal deposition: Spatial patterns and temporal trends in Europe.* Environ. Pollut., 158: 3144-3156. DOI: 1016/j.envpol.2010.06.039.

Kabata-Pendias A., Pendias H. 1999. *Biogeochemistry of trace elements*. PWN, Warszawa. (in Polish)

Kozanecka T., Chojnicki J., Kwasowski W. 2002. *Content of heavy metals in plant from pollution-free regions*. Pol. J. Environ. Stud., 11(4): 395-399.

Malzahn E., 2009. *Biomonitoring of the forest environment of the Białowieża Primeval Forest*. Ochr. Środ. Zasob. Nat., 40: 439-447. (in Polish) http://www.ios.edu.pl/pol/nr40

Mankovska B. 1998. *The chemical composition of spruce and beech foliage as an environmental indicator in Slovakia*. Chemosphere, 36: 949-953. http://vls2.icm.edu.pl/pdflinks/14010413445716607.pdf

Medyńska-Juraszek A., Kabała C. 2012. *Heavy metal pollution of forest soils affected by the copper industry.* J. Elem., 17(3): 441-451. DOI: 10.5601/jelem.2012.17.3.07.

Migaszewski Z., Gałuszka A., Crock J.G., Lamothe P.J., Dołęgowska S. 2009. *Interspecies and interregional comparisons of the chemistry of PAHs and trace elements in mosses Hylocomium splendens* (Hedw.) *B.S.G. and Pleurozium schreberi* (Brid.) Mitt. *from Poland and Alaska*. Atmos. Environ., 43: 1464-1473. http://vls1.icm.edu.pl/pdflinks/13081312240718138.

Namieśnik J., Jamrógiewicz Z., Pilarczyk M., Torres L. 2000. *Preparation of samples for the analysis of environmental*. Wyd. Nauk. Tech., Warszawa. (in Polish)

Økland T., Økland R.H., Steinnes E. 1999. *Element concentrations in the boreal forest moss Hylocomium splendens: variation due to segment size. branching patterns and pigmentation*. J. Bryol., 19: 671-684.

Ostrowska A., Gawliński S., Szczubiałka Z. 2001. *Methods of analysis and evaluation properties of soils and plants.* Inst. Ochr. Środ., Warszawa. (in Polish)

Polish Soil Classification. 2011. Soil Sci. Ann., 62(3): 5-142. (in Polish)

Reimann C., Niskavaara H., Kashulina G., Filzmoser P., Boyd R., Volden T., Tomilina O., Bogatyrev I. 2001. *Critical remarks on the use of terrestrial moss (Hylocomium splendens and Pleurozium schreberi) for monitoring of airborne pollution*. Environ. Pollut., 113: 41-57. http://vls1.icm.edu.pl/pdflinks/13081312190217178.

Samecka-Cymerman A., Kosior G., Kempers A.J., 2006. *Comparison of the moss Pleurozium schreberi with needles and bark of Pinus sylvesrtis as biomonitors of pollution by industry in Stalowa Wola (southeast Poland)*. Ecotox. Environ. Safe, 65: 251-258. DOI: 10.1016/j.ecoenv.2005.05.009.

Steinnes E. 1995. *A critical evaluation of the use of naturally growing moss to monitor the deposition of atmospheric metals*. Sci. Environ., 160(161): 243-249. http://vls1.icm.edu.pl/pdflinks/13081312372619674.

Szczepaniak K., Biziuk M., 2003. *Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution*. Environ. Res., 93: 221-230. DOI: 10.1016/S0013-9351(03)00141-5.

Wells J.M., Brown D.H. 1996. *Mineral nutrient recycling within shoots of the moss Rhytidiadelphus squarrosus in relation to growth*. J. Bryol., 19: 1-17.

J. Elem. s. 483–494

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IMPACT OF PHYSICAL AND CHEMICAL PARAMETERS OF THE SUBSOIL ON THE BOTANICAL COMPOSITION OF SPORTS FIELD TURF

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Abstract

This paper presents results of a 2-year study on the impact of physical and chemical characteristics of the subsoil on the herbaceous composition of a sports field turf at the Centre for Sport and Recreation in Olsztyn. Samples of soil for physical and chemical analyses as well as samples of plant material for botanical and weight analyses were collected from the top soil (0-15 cm) and drainage layer (15-30 cm) at five sampling points across the sports field. The physical parameters were determined with the methods used in soil science studies. The mass loss during furnace drying was assumed to represent the content of organic matter. The following were analyzed: the hydrolytic acidity of soil, content of organic carbon in soil and concentrations of N, P, K, Ca, Mg, Na, as well as phytoavailable macro- and micronutrients (P, K, Mg, Cu, Mn, Fe and Zn) determined in 0.5 mol HCl dm^{-3}. Detailed botanical and weight analysis of plant material was performed. The results were statistically processed with Statistica 10.0 software. It was shown that the top soil of the sports field had a grain-size composition of poor loamy sand, whereas the drainage layer resembled loose sand and poor loamy sand. The content of floatable parts in excess of the standard value as well as the physical properties such as bulk density, specific density and porosity indicate high compactness and density of the subsoil. It was demonstrated that the top soil had an average-to-high content of phosphorus and magnesium, low-to-average content of potassium, average concentrations of copper, manganese and iron, and a high content of zinc. A high turf share of monocotyledonous and dicotyledonous weeds in the sward, which are resistant to treading, indicates excessive load and high compactness of the sports field as well as worse physical and chemical properties of the subsoil.

Keywords: physical and chemical properties of subsoil, floral composition, sports field.

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WPŁYW WŁAŚCIWOŚCI FIZYKOCHEMICZNYCH PODŁOŻA NA SKŁAD BOTANICZNY MURAWY BOISKA SPORTOWEGO

Abstrakt

Przedstawiono wyniki 2-letnich badań dotyczących wpływu właściwości fizykochemicznych podłoża na skład florystyczny murawy boiska sportowego Ośrodka Sportu i Rekreacji OSIR w Olsztynie. W pięciu reprezentatywnych powierzchniach boiska, z warstwy nośnej (0-15 cm) i warstwy filtrującej (15-30 cm), pobrano próbki glebowe do analiz fizykochemicznych i materiał roślinny do analiz botaniczno-wagowych. W próbkach glebowych oznaczono właściwości fizyczne metodami stosowanymi w gleboznawstwie. Straty masy podczas spalania przyjęto za zawartość materii organicznej. Oznaczono kwasowość hydrolityczną gleby, zawartość węgla organicznego w glebie, N, P, K, Ca, Mg, Na. Makro- i mikroskładniki przyswajalne (P, K, Mg, Cu, Mn, Fe i Zn) oznaczono w 0,5 mol HCl dm^{-3}. Dokonano szczegółowej analizy botaniczno-wagowej materiału roślinnego. Wyniki badań opracowano statystycznie z użyciem programu Statistica 10.0. Wykazano, że warstwę nośną płyty boiska stanowi piasek słabogliniasty, natomiast filtracyjną – piasek luźny i słabogliniasty. Zawartość części spławianych przekraczających przyjętą normę oraz właściwości fizyczne, m. in. gęstość objętościowa, gęstość właściwa i porowatość, świadczą o zagęszczeniu podłoża. Wykazano, że w warstwie nośnej zawartość fosforu i magnezu wynosiła od średniej do wysokiej, potasu – od niskiej do średniej, zawartość miedzi, manganu i żelaza była średnia, cynku – wysoka.

Duży udział chwastów jedno- i dwuliściennych w runi odpornych na udeptywanie świadczy o nadmiernym obciążeniu boiska, zagęszczeniu i pogorszeniu właściwości fizykochemicznych podłoża.

Słowa kluczowe: właściwości fizykochemiczne podłoża, skład florystyczny, boisko sportowe.

INTRODUCTION

Sports field turf should be low, compact, elastic and resistant to treading and damage; it should ensure the safe movement of contestants and create optimal conditions for kicking, rolling and passing a ball (KINDS 1985, TURGEON 2005). In order to meet these requirements, the process of constructing a sports field should include preparation of special subsoil (top soil and drainage layer), selection of proper lawn grass varieties and regular maintenance (KINDS 1985, CANAWAY 1990, THORGOOD 2003, ŁACHACZ et al. 2007, GRABOWSKI et al. 2008). The grass species chosen for sports field turf should withstand intensive use of a field.

Excessive compactness of the upper layer of subsoil, a common cause of turf degradation, leads to a change in the soil physical properties, reduced porosity and worse subsoil permeability (DIN 18035, GIBBS et al. 1993, HUFF 2003). Compaction of the subsoil results in the elimination of grasses and expansion of other, unwanted plants.

The objective of the study was to determine the impact of physical and chemical properties of soil on the botanical composition of sports field turf at the Centre for Sport and Recreation (CSR) in Olsztyn.

MATERIAL AND METHODS

In 2009-2010, a study was carried out at the main sports field located at the Centre for Sports and Recreation in Olsztyn, which was constructed in the late 1970s.

The field turf was rolled with a drum roller in early spring each year. It was fertilized twice with phosphorus and potassium: in early spring (45 kg P_2O_5 and 70 kg K_2O ha^{-1}) and in autumn (35 kg P_2O_5 and 80 kg K_2O ha^{-1}), and several times with nitrogen: at the onset of the plant growing season and then every 2-3 cuts (20-30 kg N ha^{-1}). The grass sward was mowed at least once a week in May and June, and then every two weeks in late summer and autumn. Irrigation was done during spells of dry weather.

Each year in the spring, the herbicide Starane 250 EC at 1.0-1.2 l ha^{-1} was applied for selective weed control. During a break in the sporting season (in June 2010), the grass layer was rejuvenated by direct grass seeding into the sward. The turf was mowed low (at app. 2 cm), dragbrushed and groomed. These treatments were followed by sand application, pulverization by harrowing and direct seeding into the sward (four times on crossing lines and on diagonals) of a lawn grass mixture (made by the SHR Sport Nieznanice) at 250 kg ha^{-1} (4 times at 62.5 kg ha^{-1}). A Vredo disc seeder and a light drum roller were employed. Under-sowing was performed with a mixture of lawn grasses composed of *Lolium perenne* cultivars Niga (44.5%), Nira (3.6%) and Inka (11.9%); *Poa pratensis* culivars Amason (19.6%) and Ani (0.4%); *Festuca rubra* cultivars Adio (9.97%), Nimba (10.0%) and Leo (0.03%). On five representative sub-fields set out in the middle of a field (points 1, 2, 4 and 5) and in its centre (point 3), soil samples for physical and chemical analyses and plant samples for botanical and weight analyses were taken from the top soil (0-15 cm) and drainage (15-30 cm) layers. The ash content was determined in soil samples after combustion in a muffle furnace at 550°C. The mass loss in the furnace was assumed to represent the content of organic matter (SAPEK, SAPEK 1997). The following determinations were made: total porosity from the formula: fc = 1 – (gc : gw) · 100 (% vol.); real-time moisture of the subsoil with the drying method; bulk density (gc) measured in small, 100 cm^3 cylinders by drying the samples at 105°C; specific density (gw) calculated from a regression equation (OKRUSZKO 1971); hydrolytic acidity with the Kappen's method; total nitrogen with the Kiejdahl's method; potassium and sodium with flame photometry; total phosphorus with the colorimetric method after mineralization of soil samples in sulphuric acid; total calcium with complex titration, and total magnesium by measuring the absorption of radiation of magnesium ions on an ASA apparatus. The content of organic carbon was measured by colorimetry with oxidant mixture of 0.2 M potassium dichromate solution in sulphuric acid and the detection of absorption on a spectrophotometer. The available forms of macro- and microelements (P, K, Mg, Cu, Mn, Fe and Zn) were measured in

0.5 mol HCl dm^{-3}. In the autumn of 2009 and 2010, 100 g plant samples were randomly collected from each sub-area of the field. A detailed botanical and weight analysis was performed on green material. Lawn grasses, other grasses, legumes, herbs and weeds were identified and their per cent shares in the sward were determined after drying.

The results were statistically processed with Statistica 10.0 software (Data Analysis Software System, Statsoft Inc. 2011). Correlation analysis between the examined factors was performed with the Pearson's linear correlation. The values of correlation coefficients were calculated between the basic physical and chemical parameters of the top soil layer and the share of lawn grasses and other types of plants in the sward.

RESULTS AND DISCUSSION

The surface layer of soil under a sports field, called the top soil (0-15 cm), in our study was sampled to the right of the penalty area, where it was composed of grains representing the texture of light loamy sand and loamy sand (*Classification of soil granulation*… 2009). The content of skeletal particles in the top soil ranged from 18 to 25%, sand fractions (with the predominance of coarse and medium sand) varied from 71 to 88%, dust fraction constituted from 8 to 22% and silt fraction made up from 4 to 7% (Table 1). The percentage of floatable fraction (<0.02 mm) ranged between 7 and 18%, which was above the required minimum set for a sports field, except for points 2 and 3 (DIN 18035).

The drainage layer (15-30 cm) represented the grain-size composition of loose sand and poor loamy sand (point 5). The content of skeletal parts ranged from 13 to 21%, sand fraction varied from 87 to 93% (with the predominance of coarse and medium sand), dust fraction constituted from 4 to 9%, and silt fraction made up from 2 to 4%. The percentage of floatable fraction (<0.02 mm) ranged from 5 to 9% and did not exceed the norm, except point 5 (DIN 18035).

Based on the grain-size composition of the subsoil, this layer was categorized as light and medium skeletal formation (*Classification of soil granulation* … 2009). The physical properties of the subsoil (the top soil and drainage layers) are typical of poor loamy sands and loose sands. In the examined soil formations, the ash content was significant, ranging from 66.0 to 89.1% in the top soil and from 67.8 to 91.7% in the drainage layer (Table 2).

The bulk density of the tested subsoil layers was high and ranged, on average, from 1.55 to 1.67 Mg m^{-3}, with the higher values determined for the top soil (Table 2). The recorded bulk density values indicate high compactness of the subsoil, especially in the top soil layer.

On average, the specific density was 2.45 Mg m^{-3} in the top soil and

Table 1

Grain-size composition of the subsoil under a sport field turf

Research point number	Layer type	Layer depth (cm)	Fraction content (%)							Definition of soil formation acc. PTG*
			skeletal parts	granular parts						
			gravel fraction	sand fraction			dust fraction		silt fraction	
				coarse sand	medium sand	fine and very fine sand	coarse dust	fine dust		
			particle diameter (mm)							
			>2.0	2.0-0.5	0.5-0.25	0.25-0.05	0.05-0.02	0.02-0.002	<0.002	
1	top soil	0-15	21	11	39	37	4	5	4	ps
2			24	18	32	36	5	3	4	ps
3			18	26	33	29	4	4	4	ps
4			18	38	30	20	3	5	4	ps
5			25	31	29	11	11	11	7	pg
Mean			21	25	33	26	5	6	5	
1	drainage	15-30	20	39	42	12	2	2	3	pl
2			16	55	25	12	3	3	2	pl
3			16	44	31	17	3	3	2	pl
4			13	32	31	30	2	2	3	pl
5			21	34	23	30	4	5	4	ps
Mean			17	41	30	20	3	3	3	

* Classification of soil granulation and mineral formations (Rocz. Glebozn. 2009)

Table 2

Physical properties of the subsoil

Sampling point	Layer type	Layer depth (cm)	Ash content (%)	Bulk density (Mg m^{-3})	Specific density (Mg m^{-3})	Total porosity (%)	Sandle - time moisture content layers (%)
1	top soil	0-15	81.0	1.67	2.43	31.3	29.4
2			76.0	1.64	2.36	30.1	27.2
3			66.0	1.67	2.43	31.3	25.8
4			86.1	1.69	2.48	31.9	27.9
5			89.1	1.66	2.54	34.6	27.3
Mean			79,6	1,67	2.45	31.8	27.5
1	drainage	15-30	83.1	1.57	2.38	34.0	26.9
2			78.3	1.55	2.28	32.0	23.7
3			67.8	1.58	2.39	34.0	20.3
4			89.3	1.56	2.37	35.0	17.5
5			91.7	1.57	2.48	36.7	26.4
Mean			82.0	1.57	2.38	34.3	23.0

2.38 Mg m^{-3} in the drainage layer. The recorded values approximate the density of mineral soil, which ranges from 2.50 to 2.80 Mg m^{-3} (OKRUSZKO 1971).

The total porosity of the top soil ranged between 30.1 and 34.5%, being close to the upper values of poor loamy sands. In the drainage layer, it was higher (32.0-36.7%) and similar to the total porosity of loose sands. Soil porosity depends on a variety of factors, such as the grain-size composition, humus content, mesofauna and plant root activity, soil tillage, fertilization and the weather (KONSTANKIEWICZ 1985). An optimal total porosity of the surface layer of a sports field ranges from 35 to 40% (DIN18035, ŁACHACZ et al. 2007).

The porosity of the examined soil, except at sampling point 5, was less than the standard DIN 18035, thus being responsible for worse aerial and hydrous soil properties. Another consequence was a more difficult access of the turf rhizosphere to oxygen, causing poor root growth, especially at high irrigation doses. The results showed a significant correlation coefficient between the specific density and porosity of the top soil of the examined sports field versus the proportion of common meadow grass, red fescue and legumes (Table 3). The physical properties of the top soil under the sports field decided about the shares of particular plants in the sward: common meadow grass (from 47.6 to 66.6%), red fescue (54.8 to 86.5%) and legumes (79.2 to 90.2%).

The fertility of the top soil of the sports field is specified in Table 4. The reaction of this layer was only weakly varied, ranging from neutral to alkaline, and being above the required pH (pH from 5.5 to 6.5). An increase in pH to 7.5 reduces the amount of absorbable iron, manganese and zinc. In comparison with the content of macronutrients (P, K, Mg, Ca, Mg, Na and N, in total) in cultivated soils, the top soil was rich in nitrogen (1.18-2.40 N g kg^{-1}) and calcium (11.70-20.70 Ca g kg^{-1}), moderate in phosphorus (0.90-1.00 P g kg^{-1}), and poor in potassium (1.00-1.30 K g kg^{-1}), magnesium (1.90-3.90 Mg g kg^{-1}) and sodium (0.04-0.12 Na g kg^{-1}). The statistical analyses demonstrated that potassium in the top soil was the only analysed macronutrient which affected significantly the share of grasses, herbs and weeds in the sward in the individual years of the research. In 2009 and 2010, respectively, the values of correlation coefficients reported for perennial ryegrass (R = 0.62, 0.84), common meadow grass (R = 0.68, 0.65), herbs and weeds (R = -0.57, -0.96) and other grasses (R = -0.84, -0.83) indicate the beneficial effect of potassium on the share of perennial ryegrass and common meadow grass in the sward, limiting the contribution of other grasses and herbs and weeds (Table 3).

In the top soil, total nitrogen was strongly connected with organic carbon (Table 4). The C : N ratio in the surface layer was on average 11.76 : 1.0, which implies good microbiological activity in this soil layer. According to the content of organic matter (up to 3.0%), the subsoil is classified as mineral formation at sampling points 1, 3 and 4 (up to 3.0% organic substance) and humus mineral formation at points 2 and 5 (4.62% to 4.72% of organic matter) – Table 4.

Table 3

The Pearson's linear correlation coefficients (R) for species and groups of plants in the sports field turf, content of potassium and physical properties of the top soil

Year	Species and groups of plants	K	Specific density	Total porosity
2009	*Lolium perenne* L.	0.62*	0.25	0.26
	Poa pratensis L.	0.68*	-0.81*	-0.74*
	Festuca rubra L.	-0.19	0.82*	0.93**
	Other grasses	-0.84*	-0.21	-0.30
	Legumes	0.11	0.95**	0.90**
	Herbs and weeds	-0.57	-0.51	-0.64
2010	*Lolium perenne* L.	0.84*	-0.04	-0.02
	Poa pratensis L.	0.65*	-0.70*	-0.69*
	Festuca rubra L.	0.41	0.74*	0.83*
	Other grasses	-0.83*	-0.21	-0.30
	Legumes	-0.39	0.89**	0.92**
	Herbs and weeds	-0.96*	0.35	0.28

* significant for α = 0.05, ** significant for α = 0.01

The content of organic matter in the top soil under a sports field should be within 2 to 4.0% (DIN 18035, ŁACHACZ et al. 2007). The study demonstrated that the content of organic matter, except for points 2 and 5, did not exceed these parameters.

Apart from the humus content, the fertility of the subsoil also depends on the abundance of macronutrients available to plants (KALISZ, ŁACHACZ 2009, GRZEGORCZYK et al. 2011, 2013) – Table 4. According to the norms proposed by the IUNG Puływay (1990) for evaluation of concentrations of macro- and micronutrients in soil, the content of phosphorus was moderate at point 1 and high at points 2, 3, 4 and 5 (ranging from 62.3 to 87.2 mg P kg^{-1}). The content of potassium ranged from 74.7 to 107.9 mg K kg^{-1} of soil, whereas the content of magnesium (45.0-62 Mg mg kg^{-1}) was within the moderate-to-high range. The content of most microelements (Cu, Mn and Fe) was average but high for zinc (Table 4).

Table 4

Chemical properties of the subsoil

Specification	Sampling point number					Mean
	1	2	3	4	5	
pH KCl	7.09	7.17	7.45	7.22	7.22	7.23
pH H_2O	7.68	7.82	7.98	7.76	7.78	7.80
Total content (g kg^{-1})						
N	1.18	2.40	1.53	1.35	2.16	1.72
P	1.00	0.90	1.00	0.90	0.90	0.90
K	1.30	1.30	1.00	1.00	1.00	1.00
Ca	11.70	20.70	20.10	15.14	17.50	17.10
Mg	2.40	2.40	1.90	2.00	3.90	2.52
Na	0.09	0.12	0.08	0.06	0.04	0.08
C-org.	13.7	27.4	17.4	16.2	26.80	20.30
Organic matter (%)	2.36	4.72	3.00	2.79	4.62	3.50
C:N	11.61	11.42	11.37	12.00	12.41	11.76
Content of available (g kg^{-1})						
P	6.23	8.72	7.19	8.11	7.63	7.58
K	8.30	9.13	10.79	7.47	8.30	8.80
Mg	4.50	6.20	5.00	5.10	6.00	5.40
Content of available (mg kg^{-1})						
Cu	1.7	2.2	3.6	2.2	3.8	3.5
Mn	206	143	181	177	178	177
Fe	1900	2000	2100	1700	2700	2080
Zn	10.2	22.4	15.6	15.9	17.3	16.3

Table 5

Share of grasses and other plants in the sward (% d.m.)

Species and groups of plants	Sampling point number									
	1		2		3		4		5	
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Lolium perenne L.	22.5	28.6	26.3	31.0	20.7	25.7	27.0	30.5	26.2	29.4
Poa pratensis L.	25.0	33.0	27.0	35.9	21.4	31.0	22.8	33.6	21.1	31.0
Festuca rubra L.	13.1	18.1	11.0	15.0	13.5	14.4	12.0	16.0	17.4	20.5
Other grasses	27.9	17.8	25.8	15.7	31.8	21.5	26.8	16.6	25.5	15.4
Legumes	1.0	0.3	0.6	0.1	0.8	0.4	1.8	0.3	2.2	0.6
Herbs and weeds	10.5	2.2	9.3	2.3	11.8	4.0	9.6	3.0	7.6	3.1
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

The botanical composition of the sports field sward is presented in Table 5. The results of our detailed botanical and weight analyses show that apart from grass species sown spontaneously, the sward included some other species of monocotyledons and dicotyledons. In total, 16 plant species were identified in the sward, including 5 grass, 2 legume, 1 sedge and 8 herb and weed species. The under-sown species (*Lolium perenne* L., *Poa pratensis* L., *Festuca rubra* L.), regardless of the sub-area, increased their proportion in the sward (from 55.6 to 81.9% d.m.). *Poa pratensis* L. was the predominant species, with only slightly less abundant *Lolium perenne* L., in contrast to *Festuca rubra* L., which was the least numerous (Thorogood 2003, Łachacz et al. 2007, Grabowski et al. 2008). Of the other grasses, *Poa annua* L. was identified most often, especially in the centre of the sports field (Table 5). Legumes such as *Trifolium repens* L. and *Medicago lupulina* L. were rare in the sward. Herbs and weeds were represented by the following species: *Plantago media* L., *Plantago lanceolata* L., *Bellis perennis* L., *Taraxacum officinale* F.H. Wigg, *Achillea millefolium* L., *Ranunculus repens* L. and *Carex hirta* L. The presence of undesired species in the sward indicates excessive load (utilization) of the sports field, compactness of the top soil as well as worse physical properties of the subsoil, the findings consistent with the reports in literature (Beard et al. 1978, Gibbs et al. 1993, Huff 2003, Turgeon 2005, Grzegorczyk et al. 2011, 2013).

CONCLUSIONS

1. The subsoil under the examined sports field presented the grain-size composition of poor loamy sand and loose sand. Medium sand (0.25-0.50 mm) and coarse sand (0.50-1.00 mm) sub-fractions were predominant in the sand fraction.

2. Substantial amounts of floatable parts (<0.02 mm), slightly exceeding the standard value, high bulk and specific density as well as low porosity indicate high compactness and density of the subsoil.

3. The reaction of the subsoil ranges from neutral to alkaline, and exceeds the optimal value for grasses (pH_{HCl} 5.6-6.5).

4. The top soil layer had an average-to-high content of phosphorus and magnesium, low-to-average content of potassium, average content of copper, manganese and iron and a high concentration of zinc.

5. A substantial proportion of monocotyledonous and dicotyledonous weeds in the sward, which are more resistant to treading, suggest excessive load, high compactness and worse physical and chemical properties of the subsoil.

6. The low-to-average content of potassium in the top soil under the sports field significantly contributed to an increase in the proportion of under-sown grass species, such as *Lolium perenne* L., *Poa pratensis* L., *Festuca rubra* L., and to a smaller share of undesired plants in the sward.

REFERENCES

Beard I.B., Rieke P.E. Turgeon A.I., Vargas J.M. 1978. *Annual bluegrass (Poa annua* L.*) description, adaptation, culture and control*. Michigan Agr. Expt. Res. Rept. 352.

Canaway P.M. 1990. *A comparison of different methods of establishment using sec and sod on the cover and playing quality of football turf*. J. Sports, Turf Res. Inst., 66: 9-28.

DIN 18035. 1991, Deutsche Normen, 4: 1-12.

Gibbs R.I., Adams W., Baker S. 1993. *Changes in soil physical properties of different construction methods for soccer pitches under intensive use*. Int. Turfgrass Soc. Turgrass Soc. Res., 1(7): 413-421.

Grabowski K., Grzegorczyk S., Głowacka-Gil A. 2008. *The effect of sludge on the initial growth and the development of lawn grasses in the background of different mix type and sowing time.* Pol. J. Environ. Stud., 17(6): 975-980.

Grzegorczyk S., Alberski J., Olszewska M. 2013. *Accumulation of potassium, calcium and magnesium by selected species of grassland legumes and herbs.* J. Elem., 18(1): 69-78.

Grzegorczyk S., Grabowski K., Alberski J. 2011. *Nitrogen accumulation by selected species of leguminous plants and meadow herbs.* Ecol. Chem. Engin., A, 18(4): 531-536.

Huff D.R. 2003. *Annual bluegrass.* In: *Turfgrass biology, genetics and breeding*. Casler M., Duncan R. (eds.) John Wiley and Sons Hoboken, N.I., 27-38.

Kalisz B., Łachacz A. 2009. *Content of nutrients, heavy metals and exchangeable cations in riverine organic soils.* Pol. J. Soil Sci., 42(1): 43-52.

Kinds H.P.P. 1985. *Development in sports turf grasses over the past 25 years*. Zeitschrift für Wegetationstechnik, 8: 64-70.

Classification of soil granulation and mineral formations 2009. Rocz. Glebozn., 60(2): 5-16. (in Polish)

Konstankiewicz K. 1985. *Soil porosity, definitions, methods of determination*. Prob. Agrofiz., 47: 1-70. (in Polish)

Okruszko H. 1971. *The specific gravity of hydrogenic soils on the basis of mineral content.* Wiad. IMUZ, 10(1): 47-54. (in Polish)

ŁACHACZ A., GRABOWSKI K., KALISZ B., BIEDRZYCKA A. 2007. *Effects of DANO compost and sewage sludge on some physicochemical properties and organic matter quality of soil under lawn grasses.* Pol. J. Environ. Stud., 16(3B): 299-304.

SAPEK A., SAPEK B. 1997. *Methods of chemical analysis of organic soils.* Mat. Instr. IMUZ Falenty, 115: pp. 81. (in Polish)

THOROGOOD D. 2003. Perennial ryegrass. In: *Turfgrass biology, genetics and breeding*. M.D. CASLER and R.R. DUNCAN. HOBOKEN, N.J.: JOHN WILEY S sons (eds.), INC., 75-106.

TURGEON A.I. 2005. *Turfgrass management. Pearson Prentice Hall*. Phoenix Book Tech., 1-13.

Fertilising recommendations. IUNG Puławy, 1990, 1-26. (in Polish)

J. Elem. s. 495–506

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INFLUENCE OF COBALT CONCENTRATION ON THE GROWTH AND DEVELOPMENT OF *DENDROBIUM KINGIANUM* BIDWILL ORCHID IN AN *IN VITRO* CULTURE

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Abstract

The study investigated the influence of increased cobalt content in Murashige and Skoog (1962) solid medium on the growth and development of *Dendrobium kingianum* Bidwill orchid plants. Explants of shoots were used for micropropagation of the orchid plants on MS regeneration medium supplemented with 0.5 mg dm^{-3} NAA and 1.0 mg dm^{-3} kinetin. Cobalt (as $CoCl_2 \cdot 6H_2O$) was added to all treatments in concentrations of 0.025 (control), 0.625, 1.25 and 2.5 mg dm^{-3}. The results obtained after eight months showed that treatments with the cobalt chloride in concentrations 0.25-1.25 mg dm^{-3} did not influence the number of shoots and roots, and the length of shoots of the orchids. The treatment with the cobalt chloride in concentration 0.625 mg dm^{-3} positively influenced on the length of roots and increment of the fresh weight of plantlets. However, in media with the highest cobalt concentration (2.5 mg dm^{-3} $CoCl_2 \cdot 6H_2O$), a negative influence of the metal on the number of shoots of the orchids was noted.

Spectrophotometric analysis (ASA) showed that cobalt accumulation increased in both the shoots and the roots with the increase in the external Co level, whereas iron accumulation in these organs decreased. Cobalt and iron accumulation in the roots was 3-4 times higher than in the shoots.

Keywords: biometrical features, cobalt chloride, orchids, tissue culture.

WPŁYW STĘŻENIA KOBALTU NA WZROST I ROZWÓJ STORCZYKA *DENDROBIUM KINGIANUM* BIDWILL W KULTURZE *IN VITRO*

Abstrakt

Celem badań była ocena wpływu zwiększonego stężenia kobaltu w pożywce Murashige i Skooga (1962) na wzrost i rozwój roślin storczyka *Dendrobium kingianum* Bidwill. Do mikrorozmnażania roślin storczyka wykorzystano jego pędy, które umieszczono na stałej pożywce

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regeneracyjnej MS z dodatkiem 0,5 mg dm^{-3} NAA i 1,0 mg dm^{-3} kinetyny. Kobalt (w formie $CoCl_2 \cdot 6H_2O$) dodano do pożywek w stężeniach: 0,025 (kontrola), 0,625, 1,25 i 2,5 mg dm^{-3}. Po 8 miesiącach nie wykazano wpływu chloru kobaltu w stężeniu 0,25-1,25 mg dm^{-3} na liczbę uzyskanych pędów i korzeni oraz na długość pędów. Odnotowano natomiast dodatni wpływ chlorku kobaltu w stężeniu 0,625 mg dm^{-3} na długość korzeni oraz przyrost świeżej masy. Na pożywce o największym stężeniu kobaltu (2,5 mg $CoCl_2 \cdot 6H_2O$ dm^{-3}) stwierdzono istotnie mniej pędów niż w pożywce kontrolnej.

Analiza (ASA) zawartości kobaltu i żelaza w suchej masie pędów i korzeni storczyka wykazała, że wraz ze wzrostem stężenia kobaltu w pożywce MS następował znaczny wzrost akumulacji kobaltu oraz spadek akumulacji żelaza w tych organach. Zawartość kobaltu i żelaza była 3-4-krotnie większa w korzeniach niż w pędach.

Słowa kluczowe: chlorek kobaltu, cechy biometryczne, storczyki, kultury tkankowe.

INTRODUCTION

Many morphological factors control the processes of plant growth and development in *in vitro* culture (Prażak 2001*a*, Soontornchainaksaeng et al. 2001, Puchooa 2004, De Faria et al. 2004, Alvarez-Pardo et al. 2006, Aziz et al. 2010). One of the most important factors of physical environment in plant tissue culture is ethylene (C_2H_4), a gaseous plant hormone that plays an important role in plant growth and development (Yang, Hoffman 1984, Trujillo-Moya, Gisbert 2012.). The same research was done in the past using ethylene inhibitors, that is, for example, cobalt chloride ($CoCl_2$), for promoting shoot organogenesis in several plant species which has been reviewed by Samimy (1978), Mohiuddin et al. (1995) and Kumar et al. (1998). Cobalt chloride may extend the life of cut roses (Venkatarayappa et al. 1980). Cobalt, a component of vitamin B_{12}, has been regarded as an essential element for animals and microorganisms but has not been recognized as such for plants. Its only physiological role is in the fixation of molecular nitrogen in root nodules of leguminous plants which possess vitamin B_{12} and cobamide coenzymes and in the non-legumes *Alnus glutinosa* and *Casuarina cunninghamiana* (Bond, Hewitt 1962). Cobalt, in the form of fertilizer and pre-seeding and pre-sowing chemicals, has increased yield in many plants (Palit, Sharma 1994). Gad and Kandil (2010) reported that cobalt addition enhanced all parameters of tomato growth and yield with all sources of phosphorus fertilizers especially mono super phosphate.

The main purpose of this study was to investigate the influence of cobalt in concentrations 25, 50 and 100 times higher, than the standard content in MS medium on the growth and development of the orchid *Dendrobium kingianum* Bidwill.

MATERIAL AND METHODS

Dendrobium kingianum Bidwill are extremely variable, lithophytic plants which often grow in large masses in Australia (New South Wales and Queensland). *Dendrobium kingianum* Bidwill plants were collected from a greenhouse. To develop an aseptic culture, orchid shoots (pseudobulbs 15-30 mm long with two terminal leaves) were surface sterilized with 0.1% $HgCl_2$ solution for 3 minutes, washed several times with distilled water and transferred to an initial MURASHIGE and SKOOG (1962) medium (MS) supplemented with IAA (indolyl-3-acetic acid) at 0.5 mg dm^{-3} and BA (6-benzylaminopurine) at 1.0 mg dm^{-3}.

The pH of the medium was adjusted to 5.2 before gelled with agar. All cultures were incubated at 22-24°C with 16 h light (at an irradiance of 54 μmol m^{-2} s^{-1})/ 8 h dark cycle. After two months, all newly formed about 20 mm long shoots were separated, weighed (about 0.150 g/1 shoot) and individually transferred (5 shoots per vessel, and 25 per treatment) to an MS multiplication medium (Figure 1) supplemented with NAA (1-naphthalene acetic acid) at 0.5 mg dm^{-3} and kinetin (6-furfurylaminopurine) at 1.0 mg dm^{-3} (PRAŻAK 2001b). Cobalt (as $CoCl_2 \cdot 6H_2O$) was added to all treatments in concentrations 25, 50 and 100 times higher (0.625, 1.25, 2.5 mg dm^{-3}) than the standard content in MS medium (0.025 mg dm^{-3}). All media contained 3% sucrose and 0,8% Difco bacto-agar. Test glass culture vessels (100 ml) with Magenta B-caps as closures were dispensed with 20 ml medium respectively. The number and length of shoots and roots and

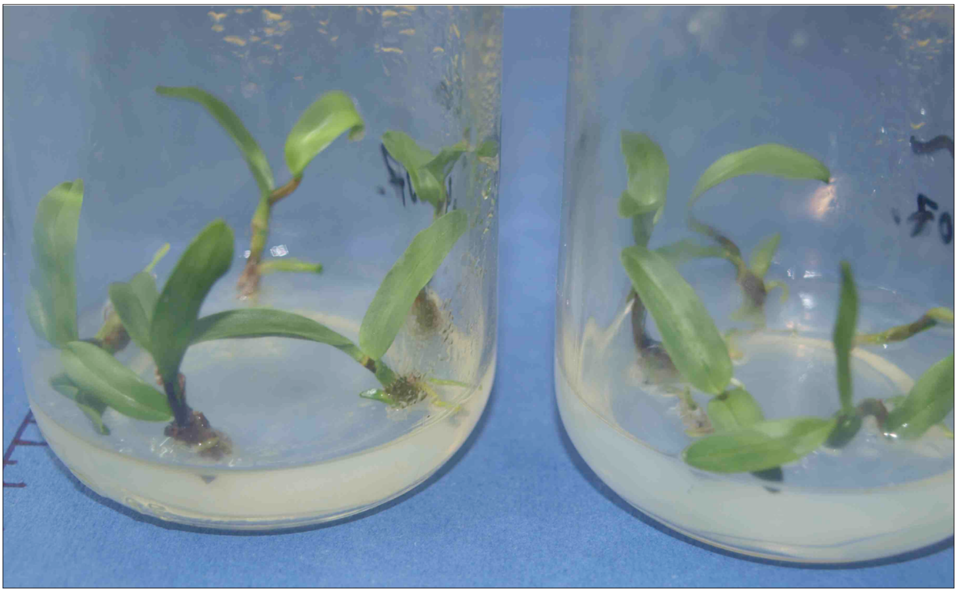

Fig. 1. *Dendrobium kingianum* Bidwill shoot explants in glass culture vessels

the fresh weight were analysed after four and eight months of growth (after 2 and 4 passages) in 22-25 plantlets from each treatment. The orchids had strong roots hence there was no risk to tear them off while measuring. The experiment was repeated twice (2 x 8 months).

After eight months all plants from the different treatments were separated into roots and shoots. Then both shoot and root samples were washed in distilled water and placed in a forced air oven to dry at 70°C for 72 hrs. The dried plants material was digested using a diacid (HNO_3-$HClO_4$) mixture. After dilution of the digests, they were processed for cobalt (Co) and iron (Fe) analysis using the ASA method.

Statistical analysis of the results was performed using analysis of variance on mean values, applying Tukey's test for difference assessment, at the significance level $\alpha = 0.05$.

RESULTS

In this study, the formation of multiple shoots was successfully induced from single shoot explants of *Dendrobium kingianum* Bidwill. The results obtained after four months showed that the 25-, 50- and 100-fold increases in cobalt content in MS solid medium did not influence the number and length of shoots and roots, and fresh weight of the plantlets (Table 1). In the

Table 1

The influence of increased cobalt (as $CoCl_2 \cdot 6H_2O$) content in MS medium on biometrical features of *Dendrobium kingianum* Bidwill after 4 and 8 months *in vitro* culture (mean value of feature/1 explant)

Biometrical feature	Months	Concentration of $CoCl_2 \cdot 6H_2O$ (mg dm^{-3})				$LSD_{p=0.05}$
		0.025 (control)**	0.625 (25 · 0.025)	1.25 (50 · 0.025)	2.5 (100 · 0.025)	
Number of shoots	4	4.02	4.09	3.92	3.50	n.s.
	8	12.22	11.56	8.89	7.91*	3.92
Shoot length (mm)	4	38.50	37.87	37.50	38.35	n.s.
	8	44.00	46.11	38.56	41.46	n.s.
Number of roots	4	11.01	10.91	8.50	8.22	n.s.
	8	19.38	21.44	14.67	14.36	n.s.
Root length (mm)	4	15.30	16.46	16.83	18.46	n.s.
	8	34.60	49.78*	35.33	34.91	11.33
Fresh weight of plant Increment (g)	4	0.632 (0.482)	0.791 (0.641)	0.553 (0.423)	0.540 (0.390)	n.s. n.s.
	8	2.018 (1.868)	3.233* (3.083*)	1.692 (1.542)	1.654 (1.504)	1.024 (1.012)

* - result significantly different in relation to the control at p=0.05
** - standard content of Co in MS medium
n.s. - not significant in relation to the control at p=0.05

Fig. 2. *Dendrobium kingianum* Bidwill plantles after 8 months growing in an *in vitro* culture

series with cobalt concentration 25 times higher than the standard content in MS medium means of 4.09 shoots and 0.791 g fresh weight per 1 explant were obtained. They were higher than the means in the control treatment, but the differences were not statistically significant (Table 1). The increment of orchid plant fresh weight after four months of *in vitro* culture was 0.641 g in the case of the cobalt concentration increased 25-fold and 0.482 g for the control. However, the 50-, 100-fold increases in cobalt content in MS medium led to a lower mean number of shoots and roots and lower fresh weight of orchid plants than in the control (Table 1).

The results after eight months of *Dendrobium kingianum* Bidwill *in vitro* culture (Figure 2) show that the 25-fold increase in cobalt content in MS solid medium positively influenced root length and plantlets fresh weight (Table 1). In the series with the 25-fold increase in cobalt concentration, significantly longer roots per 1 explant (49.78 mm) were obtained than in the control treatment (34.60 mm). In the same series, significantly higher fresh weight of plant per 1 explant (3.233 g) was noted in comparison with in the control (2.018 g) – Table 1.

In treatment with the highest cobalt concentration (2.5 mg dm^{-3} $CoCl_2 \cdot 6H_2O$) a significantly negative influence of the metal on the number of shoots of *Dendrobium kingianum* Bidwill was noted. The effect of Co toxicity was more severe on shoots than on roots. The percentage decrease in number of shoots relative to the control was greatest (35%) at the highest Co level (2.5 mg dm^{-3} $CoCl_2 \cdot 6H_2O$), followed by a 27% at 1.25 mg $CoCl_2 \cdot 6H_2O$ dm^{-3} of MS medium, and 5% at 0.625 mg dm^{-3} $CoCl_2 \cdot 6H_2O$

of MS medium. The reduction in the number of roots was 27% at 2.5 mg $CoCl_2 \cdot 6H_2O$ dm^{-3} of MS medium, and 26% at 1.25 mg $CoCl_2 \cdot 6H_2O$ dm^{-3} of MS medium.

The significant increment of orchid plant fresh weight after eight months of *in vitro* culture was 3.083 g in the case of the cobalt concentration increased 25-fold and 1.868 g for the control (Table 1). In media with cobalt content increased 50 and 100 times a negative influence of Co on the number and length of shoots and roots, and on the weight of plant fresh mass were noted. The treatment with a 100-fold increase cobalt led to a significantly smaller number of shoots and roots than in the control (Table 1).

Cobalt accumulation in both shoots and roots increased with the increase in external Co level. Cobalt accumulation in the roots was higher than in the shoots in all treatments (Figure 3). The maximum Co contents was observed in both the roots (3978.71 μg kg^{-1} DW) and the shoots (1126.6 μg kg^{-1} DW) at the highest level of Co (2.5 mg dm^{-3} $CoCl_2 \cdot 6H_2O$), followed by 1675.38 and 430.07 μg Co kg^{-1} DW of roots and shoots, respectively, at 1.25 mg $CoCl_2 \cdot 6H_2O$ dm^{-3} of MS medium, and 999.89 and 239.91 μg Co kg^{-1} DW of roots and shoots, respectively, at 0.625 mg $CoCl_2 \cdot 6H_2O$ dm^{-3} of MS medium.

Iron content in the roots and shoots showed a decreasing trend with increasing Co concentration in the growth medium (Figure 2). Iron concentration decreased by 35% (266 to 173 mg Fe kg^{-1} DW from 0.025 to 2.5 mg $CoCl_2 \cdot 6H_2O$ dm^{-3} of MS medium) in the roots and by 18% (67.4 to 55.3 mg Fe kg^{-1} DW from 0.025 to 2.5 mg $CoCl_2 \cdot 6H_2O$ dm^{-3} of MS medium) in the shoots (Figure 4).

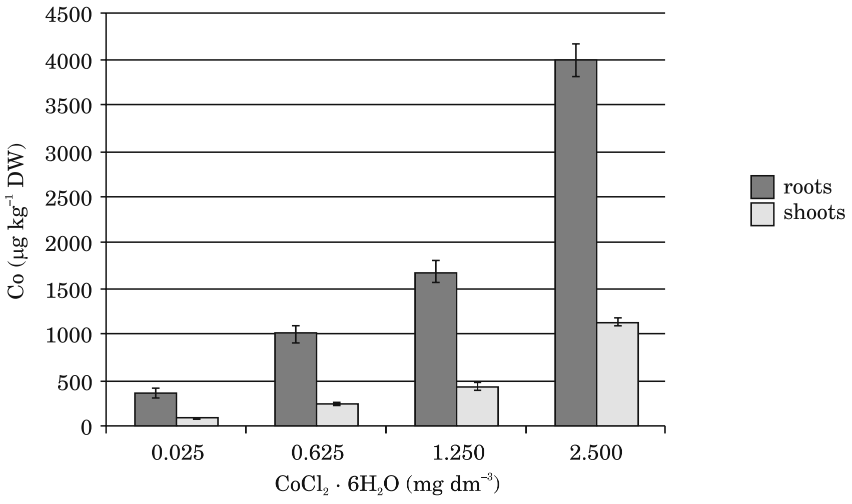

Fig. 3. Cobalt content in the roots and shoots of *Dendrobium kingianum* Bidwill after 8 months growing in MS medium supplemented with different concentrations of cobalt (as $CoCl_2 \cdot 6\,H_2O$)

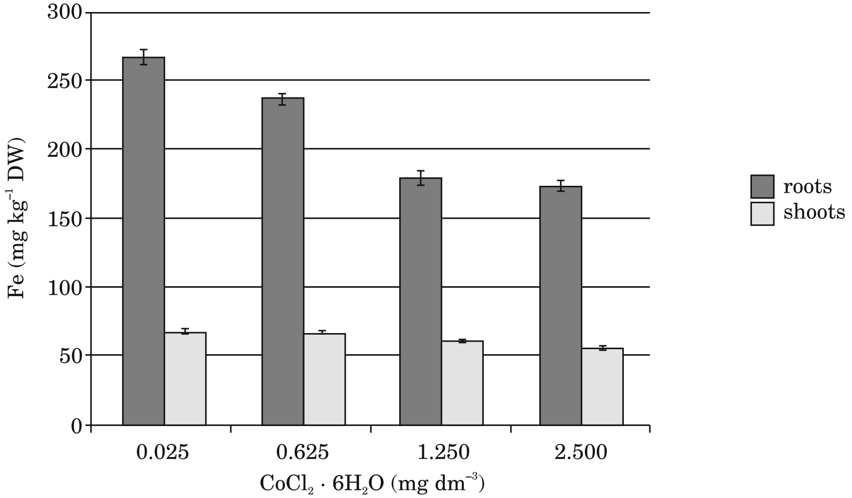

Fig. 4. Iron content in the roots and shoots of *Dendrobium kingianum* Bidwill after 8 months growing in MS-medium supplemented with different concentrations of cobalt (as $CoCl_2 \cdot 6\ H_2O$)

DISCUSSION

The experiment showed that 25- and 50-fold increase cobalt concentration in MS medium (0.625, 1.25 mg dm^{-3}) did not affect the number of shoots in *Dendrobium kingianum* Bidwill orchid plants growing in *in vitro* conditions. The 25-fold increase in cobalt concentration in MS medium strongly stimulated root length and plant fresh weight increment.

Chae et al. (2012) reported that shoot organogenesis and plant regeneration in gloxinia (*Sinningia speciosa Bail)* were improved by using ethylene inhibitors such as, inter alia, cobalt chloride ($CoCl_2$). The leaf explants were cultured by them on initial shoot regeneration media (MS media with BAP at 2 mg dm^{-3} + NAA at 0.1 mg dm^{-3}) supplemented with different concentrations of cobalt chloride ($CoCl_2$). The addition of $CoCl_2$ significantly improved the regeneration frequency giving higher shoots per explant and longer shoots. In treatment of 1 mg dm^{-3} $CoCl_2$ 12% more shoots were produced by per explant compared to control. Inhibition of ethylene formation by cobalt chloride did not confirm Santana-Buzzy et al. (2006) in case of habanero peppers (*Capsicum sinense* Murray) and Chae and Park (2012) in case of purple coneflower (*Echinacea angustifolia* D.C.). Their results showed that $CoCl_2$ added to the culture medium did not help to inhibit the production of ethylene.

Amarasinghe (2009) reported that the rate of callus proliferation in the screened traditional indica rice (*Oryza sativa* L.) varieties in Sri Lanka was significantly higher in the medium supplemented with both 5 mg dm^{-3} copper sulphate and 5-10 mg dm^{-3} cobalt chloride together. In regeneration, the highest number of normal plants with the least number of albino plants could be obtained in the media containing 5 mg dm^{-3} copper sulphate in combination with 5 mg dm^{-3} cobalt chloride. The results indicate that the *in vitro* performance in Sri Lanka traditional indica rice varieties can be improved by using the media containing both copper sulphate and cobalt chloride.

Atta-Aly et al. (1991) found that supplementing nutrient solution with a low level of cobalt (0.25 mg dm^{-3}) improved growth of tomato plants and enhanced both flowering and fruiting. Gad (2005*a*) demonstrated that cobalt at 7.5 mg dm^{-3} significantly increased growth parameters, fruit yield and nutrient concentration in tomatoes, as well as total soluble solids, total soluble sugars and L-Ascorbic acid, while titratable acidity decreased. On the other hand, supplementing nutrient solution with a higher level of cobalt resulted in a negative response.

Concentrations of 2.5 mg $CoCl_2 \cdot 6H_2O$ dm^{-3} in MS medium the most negatively influenced the number of shoots of *Dendrobium kingianum* Bidwill. Liu et al. (1995) showed that with a higher level of cobalt in medium the growth of onion roots increased more than that of shoots. Samimy (1978) reported that cobalt promotes the elongation of the hypocotyl of soybean seedlings and decreases their thickness, and concluded that cobalt exerts its effects on soybean hypocotyl growth, at least in part, by inhibiting ethylene formation. Jayakumar and Jaleel (2009) found that higher concentrations of Co (level of 100-200 mg kg^{-1} in the soil) resulted in maximum accumulation in all parts of soybean plants, while low concentrations of cobalt (50 mg kg^{-1} Co level) proved to be favourable to the overall growth of soybean plants.

As in many other plants, differential Co transport may antagonistically affect the uptake and accumulation of other essential inorganic nutrients, such as iron. High levels of many trace elements are known to commonly induce iron deficiency in plants (Hunter, Verghano 1953).

In the present study, an increase in cobalt accumulation both in shoots and roots was associated with an increase in external Co level. Increased cobalt content in MS medium led to a decrease in Fe accumulation in these organs. Cobalt and iron accumulation in the roots was 3-4 times higher than in the shoots.

Gad and Kandil (2008) reported that increasing cobalt levels in plant media from 5 up to 15 mg dm^{-3} increased cobalt content in the roots of sweet potato plants as compared to the control treatment. These results clearly indicated that cobalt content increases with the concentration of added *cobalt*. Increasing cobalt concentration in the plant media was also shown to result in a progressive depression effect on iron content in the tubers of sweet potato plants. This may be explained by results obtained by Blaylock et al. (1993) and Gad (2005*a* and 2006), who showed certain antagonistic relationships between cobalt and iron. They also stated that cobalt contributes to a wilted appearance and reduces net photosynthesis. Data obtained by

GAD and KANDIL (2010) showed that cobalt increased the content of micronutrients, with the exception of iron. The reduction rate of Fe indicates the competition between Fe and Co (GAD 2005*b*). ROMERA and ALCANARA (1994) reported that the presence of the ethylene inhibitor, such as, cobalt chloride in the nutrient solution inhibited the Fe- deficiency stress responses ferric-reducing capacity and subapical cucumber root swelling. LUCENA et al. (2006) and WATERS et al. (2007) showed that treatment of *Arabidopsis*, tomato, cucumber plants with 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene, induced the expression of ferric reductase (*AtFRO2*, *LeFRO1*, *CsFRO1*), iron transporter (*AtIRT1*, *LeIRT1*, *CsIRT1*), H^+-ATPases (*CsHA1*), and transcription factors (*LeFER*, *AtFIT*), that regulate the iron deficiency response. Conversely, the addition of ethylene inhibitors, such as Co^{2+}, markedly repressed the expression of these genes.

Under lower cobalt application, the improved root system has helped plants to better absorb water and other nutrients (JAYAKUMAR, JALEEL 2009). Improvement of water uptake associated with Co may be attributed to its action on inhibiting vascular blockage and closing stomata (REDDY 1988). It probably influenced a higher increment of fresh weight and root growth in orchid plantlets.

CONCLUSIONS

1. Ethylene inhibitor cobalt chloride significantly did not promote the shoot regeneration of *Dendrobium kingianum* Bidwill. The experiment showed that 25-, 50- and 100-fold increases in cobalt concentration in MS medium (0.625, 1.25, 2.5 mg dm^{-3}) did not affect positively the number of shoots in *Dendrobium kingianum* Bidwill orchid plants growing in *in vitro* conditions.

2. The increase 25-fold in cobalt content (0.625 mg dm^{-3}) positively influenced the number and length of the roots and fresh weight of *Dendrobium kingianum* Bidwill.

3. Spectrophotometric analysis (ASA) of cobalt and iron content in the roots and shoots of orchid plants confirmed an antagonistic relationships between these metals – an increase in cobalt accumulation both in the shoots and the roots as the external cobalt level increased, and a decrease in iron in these organs.

REFERENCES

AMARASINGHE A.A.Y. 2009. *Effects of copper sulphate and cobalt chloride on in vitro performances of traditional indica rice (Oryza sativa* L.*) varieties in Sri Lanka.* J. Agric. Sci., 4(3):132-141.

ATTA-ALY M. A., SHEHATA N. G., EL-KOBBIA T., M. 1991. *Effect of cobalt on tomato plant growth and mineral content.* Ann. Agric. Sci., Ain Shams Univ., Cairo., 36: 617-624.

Alvarez-Pardo V.M., Ferreira G., Nunes V.F. 2006. *Seed disinfestations methods for in vitro cultivation of epiphyte orchids from Southern Brazil.* Horticultura Brasileira, 24: 217-220.

Aziz E. E., Azza A., El-Din E., E.A., Omer E.A. 2010. *Influence of zinc and iron on plant growth and chemical constituents of Cymbopogon citratus* L. *grown in newly reclaimed land.* Int. J. Acad. Res., 2(4): 278-283.

Blaylock A.D., Davis T. D., Jolly V. D., Walser R. H. 1993. *Influence of cobalt and iron on photosynthesis, chlorophyll and nutrient in regreening chlorotic tomatoes and soybeans.* J. Plant Nutr., 8: 823-838.

Bond G., Hewitt E.J. 1962. *Cobalt and the fixation of nitrogen by root nodules of Alnus and Casuarina.* Nature, 195: 94-95.

Chae S.C., Park S. U. 2012. *Improved shoot organogenesis of Echinacea angustifolia DC treated with ethylene inhibitors.* Life Sci. J. 9(4): 1725-1728.

Chae S.C., Kim H.H., Park S. U. 2012. *Ethylene inhibitors enhance shoot organogenesis of gloxinia (Sinningia speciosa).* Sci. Word J., DOI: 10.1100/2012/859381. Epub 2012 Oct 17.

De Faria R.T., Rodrigues F.N., Oliveira L.V.R., Müller C. 2004. *In vitro Dendrobium nobile plant growth and rooting in different sucrose concentrations.* Horticultura Brasileira, 22(4): 780-783.

Gad N. 2005*a*. *Effect of cobalt on tomato growth, yield and fruit quality*. Egypt. J. Appl. Sci., 20(4): 260-270.

Gad N. 2005*b*. *Interactive effect of cobalt and salinity on tomato plants. I – Growth and mineral composition as affected by cobalt and salinity.* Res. J. Agric. Biol. Sci., 1(3): 261-269.

Gad N. 2006. *Increasing the efficiency of water consumption through cobalt application in the newly reclaimed soils.* J. Appl. Sci. Res. 2(11): 1081-1091.

Gad N., Kandil H. 2008. *Response of sweet potato (Ipomoea batatas* L.*) plants to different levels of cobalt.* Aust. J. Basic Appl. Sci., 2(4): 949-955.

Gad N., Kandil H. 2010. *Influence of cobalt on phosphorus uptake, growth and yield of tomato.* Agric. Biol. J. North Am., 1(5): 1069-1075.

Hunter J.G., Verghano O. 1953. *Trace-element toxicities in oat plants.* Ann. Appl. Biol., 40: 761-777.

Jayakumar K., Jaleel C. A. 2009. *Uptake and accumulation of cobalt in plants: a study based on exogenous cobalt in soybean.* Bot. Res. Intl., 2 (4): 310-314.

Kumar P.P., Lakshmanan P., Thorpe T.A. 1998. *Regulation of morphogenesis in plant tissue culture by ethylene.* In Vitro Cell. Develop. Biol., 34(2): 94-103.

Liu D., Wang W., Zhai L., Jiang W. 1995. *Effect of* Mg^{2+}, Co^{2+} *and* Hg^{2+} *on the nucleus in root tip cells of Allium cepa.* Bull. of Environ. Contamination Toxicol., 55: 779-787.

Lucena C., Waters B.M., Romera F.J., García M.J., Morales M., Alcántara E., Pérez-Vicente R. 2006. *Ethylene could influence ferric reductase, iron transporter, and H+-ATPase gene expression by affecting FER (or FER-like) gene activity.* J. Exp. Bot., 57(15): 4145-4154. PMID: 17085755

Mohiuddin A.K.M., Chowdhury M.K.U., Abdullah Z.C., Napis S.1995. *The influence of cobalt chloride on in vitro shoot proliferation in cucumber (Cucumis sativus* L.*).* Asian Pacific J. Mol. Biol. Biotechnol., 3(4): 332-338.

Murashige T., Skoog F. 1962. *A revised medium for rapid growth and bioassay with tobacco tissue cultures.* Plant Physiol., 15: 473-497.

Palit S., Sharma A. 1994. *Effects of cobalt on plants.* Bot. Rev., 60(2): 149-181.

Prażak R. 2001*a*. *Influence of aluminium from* $AlCl_3$ *on differentiation and growth of Dendrobium kingianum Bidwill in in vitro conditions.* Develop. Plant Soil Sci. Plant Nutrit, 92: 178-179.

Prażak R. 2001*b*. *Micropropagation of Dendrobium kingianum Bidwill orchid.* Biotechnologia, 2(53): 144-147. (in Polish)

PUCHOOA D. 2004. *Comparison of different culture media for the in vitro culture of Dendrobium (Orchidaceae).* Int. J. Agri. Biol., 6(5): 884-888.

REDDY T. V. 1988. *Mode of action of cobalt extending the vase life of cut roses.* Sci. Hort., 36: 303-314.

ROMERA F.J., ALCANTARA E. 1994. *Iron-deficiency stress responses in cucumber (Cucumis sativus* L.*) roots (A possible role for ethylene?).* Plant Physiol., 105(4): 1133-1138. PMCID: PMC159441.

SAMIMY C. 1978. *Influence of cobalt on soybean hypocotyl growth and its ethylene evolution.* Plant Physiol., 62: 1005-1006.

SANTANA-BUZZY N., IGLESIAS-ANDREU L.G., MONTALVO-PENICHE M. DEL C., LÓPEZ-PUC G., BARAHONA-PÉREZ F. 2006. *Improvement of in vitro culturing of habanero pepper by inhibition of ethylene effects.* Hort. Sci., 41(2): 405-409.

SOONTORNCHAINAKSAENG P., CHAICHAROEN S., SIRIJUNTARUT M., KRUATRACHUE M. 2001. *In vitro studies on the effect of light intensity on plant growth of Phaius tanervilliae (Banks ex L'Herit.) Bl. and Vanda coerulea Griff.* Scienceasia, 27: 233-237.

TRUJILLO-MOYA C., GISBERT C. 2012. *The influence of ethylene and ethylene modulators on shoot organogenesis in tomato.* Plant Cell Tiss. Organ Cult., 111(1): 41-48.

VENKATARAYAPPA T., TSUJITA M. J., MURR D. P. 1980. *Influence of cobaltous ion (Co^{2+}) on the postharvest behaviour of Samantha roses.* J. Amer. Soc. Hort. Sci., 105: 148-151.

WATERS B.M., LUCENA C., ROMERA F.J., JESTER G.G., WYNN A.N., ROJAS C.L., ALCÁNTARA E., PÉREZ-VICENTE R. 2007. *Ethylene involvement in the regulation of the H(+)-ATPase CsHA1 gene and of the new isolated ferric reductase CsFRO1 and iron transporter CsIRT1 genes in cucumber plants.* Plant Physiol Biochem., 45(5):293-301. PMID: 17468001.

YANG S.F., HOFFMAN N.E. 1984. *Ethylene biosynthesis and its regulation in higher plants.* Ann. Rev. Plant Physiol., 35(1): 155-189.

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CONTENT OF CALCIUM AND PHOSPHORUS IN THE MEAT, GILLS AND LIVER OF PERCH (*PERCA FLUVIATILIS* L.) FROM THE WIELKOPOLSKA LAKES DISTRICT (POLAND)

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Abstract

The aim of this work was to compare the concentration of calcium and phosphorus and Ca/P ratio in the meat, gills and liver of perch (*Perca fluviatilis* L.) caught from the Lake Góreckie, Strzeszyńskie and Wędromierz (the Wielkopolska Lake District). The study involved 30 individuals of fish caught in spring. The muscles samples for analyses were taken from the large side muscle of the fish body above the lateral line. Calcium concentration was determined by atomic absorption spectrophotometer Solar 969, Unicam. Phosphorus content was analyzed with colorimetric method, by spectrophotometer Lambda 25, Perkin-Elmer (at wavelength 430 nm). The mean content of Ca in the meat of fish caught from Lake Góreckie, Strzeszyńskie and Wędromierz was 3.175, 0,516 and 2.498 g kg^{-1}, respectively. The significantly higher amounts of Ca were determined in the gills than in the meat and liver and ranged from 63.09 g kg^{-1} (the Lake Góreckie) to 70.24 g kg^{-1} (the Lake Strzeszyńskie). The mean content of P was at the same level in the meat and in the liver and ranged from 2.703 g·kg^{-1} to 2.812 g kg^{-1}. Ratio of calcium to phosphorus in the meat of fish caught from the lake Góreckie, Wędromierz and Strzeszyńskie was 1.174:1, 0.254:1 and 0,888:1, respectively.

Keywords: calcium, phosphorus, organs, perch.

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ZAWARTOŚĆ WAPNIA I FOSFORU W MIĘSIE, SKRZELACH I WĄTROBIE OKONIA (*PERCA FLUVIATILIS* L.) Z POJEZIERZA WIELKOPOLSKIEGO

Abstrakt

Celem pracy było oznaczenie stężenia wapnia i fosforu oraz obliczenie stosunku Ca do P w mięśniach skrzelach i wątrobie okonia (*Perca fluviatilis* L.) odłowionego z Jezior: Góreckiego, Strzeszyńskiego i Wędromierz (Pojezierze Wielkopolskie). Badaniami objęto 30 osobników pozyskanych wiosną 2012 roku. Materiał do analiz stanowił mięsień najdłuższy grzbietu z nadosiowej części ciała, skrzela i wątroba. Stężenie wapnia oznaczono z użyciem spektrofotometru absorpcji atomowej Solar 969, Unicam. Stężenie fosforu oznaczono metodą kolorymetryczną stosując spektrofotometr Lambda 25, Perkin-Elmer (długość fali 430 nm). Średnia zawartość Ca w mięsniach ryb odłowionych z Jezior Góreckiego, Strzeszyńskiego i Wędromierz wynosiła odpowiednio: 3,175, 0,516 i 2,498 g kg^{-1}. Znacząco więcej Ca oznaczono w skrzelach, w porównaniu z próbami mięśni i wątroby; wartości te mieściły się w zakresie od 63,09 g kg^{-1} (Jezioro Góreckie) do 70,24 g kg^{-1} (Jezioro Strzeszyńskie). Średnia zawartość P była bardzo zbliżona w próbach mięśni oraz wątroby i wynosiła od 2,703 g kg^{-1} do 2,812 g kg^{-1}. Stosunek zawartości wapnia do fosforu w mięśniach ryb odłowionych z Jezior Góreckiego, Wędromierz i Strzeszyńskiego wynosił odpowiednio: 1,174:1, 0,254:1 i 0,888:1.

Słowa kluczowe: wapń, fosfor, narządy, okoń.

INTRODUCTION

Fish meat is an important source of amino acids, proteins, vitamins dissolved in lipids, long chain fatty acids and lipids as a valuable energy source. Through their high nutritive values fish meat should be a very significant part of the human diet, because it have low risk of coronary heart disease, hypertension and cancer (MENDIL et al. 2010). Metals can be accumulated by fish, both through the food chain and water (MENDIL, ULUÖZLÜ 2007). Fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human organism (AL-YOUSUF et al. 2000). The level and intensity of the accumulation depends on many factors, such as: species of fish, size, sex, seasonal changes and environmental factors (YILMAZ et al. 2010). Trace elements are introduced into aquatic system (lakes, rivers) through atmospheric fallen dumping wastes and geological weathering (AL-YOUSUF et al. 2000).

Tissue metals concentrations are influenced by an environmental contamination. Fish from more contaminated or cooler lakes had lower indicators of physical condition than individuals from cleaner reservoirs (EASTWOOD, COUTURE 2002). There were carried out analyses about variations in heavy metals pollution of the Lake Balaton and accumulation capacity especially for Zn, Cu, Cd and Pb in common bream. There were observed significant positive correlations between the level of heavy metals accumulated in the organs of fish and the pollutant load of the water (FARKAS et al. 2003). As CHEN and CHEN (2001) indicated, metal content in fish tissues are related to the pollution status of the environment. Except for Zn and Mn concentrations in the muscles of *Sardinella lemuru* being higher than those of the slightly polluted Chi-Ku Lagoon, the metals concentrations in the fish of Ann-Ping coastal were similar to those of slightly polluted regions. EASTWOOD and COUTURE (2002) investigated seasonal variations in the liver metal contamination of yellow perch (*Perca flavescens*) caught from seven northeastern Ontario Lakes. There were much higher concentrations of metals in the spring. It may be due to increased metal input or bioavailability caused by snowmelt events or lake turnover that affect water quality parameters. The same results were observed by LAITINEN (1994).

Phosphorus is one of the most essential mineral, necessary for normal growth, bone mineralization, reproduction and energy metabolism in fish (NAKAMURA 1982, ALBREKTSEN et al. 2009). The availability of phosphorus on the organism depends on the chemical form and solubility of the mineral. As ALBREKTSEN et al. (2009) indicated in Atlantic salmon, primary inorganic salts of phosphorus are more available than secondary salts, whole phosphorus bound to calcium in the bone tissue is the last available. Calcium and phosphorus are essential elements for human being which means that those metals should be part of human diet (CELIK et al. 2004). Calcium and phosphorus plays a very important roles in several physiological processes and are directly involved in the development and maintenance of the skeletal system. In vertebrates calcium is complexed with phosphorus in hydroxyapatite to form the principal crystalline of bone (YE et al. 2006). Ca/P ratio is the most important indicator for good bone health.

The daily dose of phosphorus in the diet of an adult should be 800 mg (SAPEK 2009), and approximately 1000 mg of calcium (1200 mg per day for women and men aged 25 years and younger, and 1100 mg per day for women over 60 years old due to progressing in loss in bone mineral pass) (LIDWIN-KAŹMIERKIEWICZ et al. 2009). The ideal would be to consume the same amount of phosphorus as calcium (calcium/phosphorus balance). The ratio between phosphorus and calcium greater than 3:2 may cause metabolic disorders. Fish meat is a rich source of phosphorus and those can be the order of 150-200 mg 100 g^{-1} of product. This element occurs in almost all species of fish. Species which are rich in calcium are sardines, herring, sprat and salmon. Approximate share of phosphorus and calcium in the fish meat should be at 1.8% (dry weight).

The aim of this work was to determine calcium and phosphorus content and Ca/P ratio in the meat, gills and liver of perch caught from Lake Góreckie, Strzeszyńskie and Wędromierz during spring and analyze content of the elements in the different organs. Muscles are not always a good indicator of the whole body fish metal contamination and, therefore, it is important to analyze other tissues, such as the liver and gills (JARIĆ et al. 2011).

MATERIAL AND METHODS

Study area

The Lake Góreckie is a dimictic lake with a surface area of ca. 104 ha, maximum and mean depths of 17.25 m and 8.97 m, respectively and a shore line of 8300 m. The degree of antropopressure from the annual nitrogen and phosphorus for the eutrophic Lake Góreckie is estimated at 9.5 g m^{-2} $year^{-1}$ for total nitrogen and 0.36 for total phosphorus g m^{-2} $year^{-1}$ (PEŁECHATY, OWSIANY 2003). The Lake Strzeszyńskie has got a surface area of 34.9 ha and is located in the administrative boundaries of the city of Poznań. The maximum depth is 18.8 m and the mean depth is 8.2 m. A shore line of 4500 m is mostly covered with deciduous and pine forests used mainly for recreational destination. The lake has purity class No. 2. and it is used by the Polish Fishing Association. The Lake Wędromierz is a flow reservoir, with an surface area of 73.8 ha, maximum and mean depths of 11.8 m and 4.9 m, respectively and a shore line covered with a mixed forests.

Trophic level of the lakes was based on Carlson's index (CARLSON 1977) calculated as an average for three seasons (spring, summer and autumn). The basis for the calculation were the results of analysis: Secchi disk visibility, chlorophyll concentration and the concentration of total phosphorus in the surface layer carried out in 2012.

The obtained values of Carlson index were for particular lakes: the Lake Góreckie – 71, the Lake Strzeszyńskie – 63 and the Lake Wędromierz – 83. On this basis, all the lakes were classified as eutrophic.

Fish samples

The study involved 30 individuals of perch (*Perca fluviatilis* L.) caught in spring (April, 13-25 2010). The experimental fish were obtained in natural condition from the Wielkopolska Lake District. Measurements of the mass of the fish body – BW (± 0.01 g) and body length – Lc (± 0.1 cm) and the total and body length – Lt (± 0.1 cm) were taken on the each individuals. The meat samples for analyses were taken from the large side muscle of fish body above the lateral line. Due to a relatively low amounts of liver and gills obtained from one individuals, the material was combined (about 2 pieces of each). There were chosen for analysis individuals with similar biometric measurements. Body weight ranged from 160 to 295 g, and body length and total length was from 20.0 to 23.5 cm and from 21.5 to 28.0, respectively (Table 1).

The samples of fish organs were immediately frozen after the preparation and kept in the deep freezer before analyzing. All frozen samples were freeze dried in a Finn-Aqua Lyovac GT2 freeze drier (parameters: temperature -40°C, pressure $6 \cdot 10^{-2}$ mbar, duration at least 48 h).

The freeze dried samples were mineralized in microwave mineraliza-

Table 1

The mean values of the biometric measurements of perch (*Perca fluviatilis* L.) from Lake Góreckie, Strzeszyńskie and Wędromierz

Place of catch	Total length (cm) (range and mean)	Body length (cm) (range and mean)	Body mass (g) (range and mean)
Lake Góreckie	21.5-27.5 25.5	18.0-22.5 20.0	170.5-270.5 182.2
Lake Strzeszyńskie	22.0-27.0 23.8	18.5-23.5 20.5	165.0-255.0 170.6
Lake Wędromierz	23.0-28.0 24.7	19.5-24.0 21.2	160.0-295.0 195.6

tor Ethos Plus, Milestone. For the mineralization 0.1 g of the tissue was weighted and then HNO_3 and H_2O_2 were added in ratio 4:1. During the first 10 minutes, the temperature was increased to 190°C. During the next 7 minutes the temperature was kept at a level of 190 ± 5°C. The mineralized samples have been carried quantitatively to the measuring flask with a capacity of 50 cm^3.

Calcium concentration was determined by atomic absorption spectrophotometer Solar 969, Unicam. Phosphorus content was analyzed with colorimetric method, by spectrophotometer Lambda 25, Perkin-Elmer (at wavelength 430 nm). Analyses were carried out according to PN-EN 13805/2003, PN-EN 15505/2009 and PN-ISO 13730. Tissue concentrations of minerals have been reported as g kg^{-1} dry weight (g kg^{-1} d.w.).

The accuracy of the analyses was controlled by adding standard solutions. As a standards were used calcium standard solution $Ca(NO_3)_2$ in HNO_3 (Merck, Germany) and KH_2PO_4 (POCH S.A., Poland) dissolved in water.

Statistical analyses

Data analyses were performed by using the Statistica 8.0 software (StatSoft, USA). Significance of differences in the average content of calcium and phosphorus in the meat, gills and liver of perch were statistically analysed with the Tukey's test. In this case, a significance of differences in the average content of Ca and P (between the lakes within a single tissue) were calculated by one-way analysis of variance (Anova).

RESULTS AND DISCUSSION

It is very difficult to compare a metal concentrations between the same tissue of two different species or the same species lives in a different reservoir, because of different feeding habits, differences in aquatic environment, growing rates of the species, types of analyzed tissues and other factors (YILMAZ et al. 2010). As BRUCKA-JASTRZĘBSKA, PROTASOWICKI (2006) indicated levels of bio-elements and harmful metals in the carp body depend on the culture method, water quality and type of the feed. The rate at which metals affect the organism is related to the metal transporting function of the blood.

Table 2

The mean content of calcium and phosphorus (g kg^{-1} dry weight) in the meat, liver and gills of perch (*Perca fluviatilis* L.) from Lake Góreckie, Strzeszyńskie and Wędromierz

Place of catch	Tissues	*n*	Ca (g kg^{-1})	P (g kg^{-1})	Ca:P ratio
Lake Góreckie	meat	10	3.175±0.508^{a}	2.633±0.520^{a}	1.174:1
	liver	5	2.043±0.067^{a}	2.703±0.013^{a}	0.756:1
	gills	5	63.09±1.969^{a}	7.079±0.407^{a}	8.912:1
Lake Strzeszyńskie	meat	10	0.516±0.058^{b}	2.028±0.270^{b}	0.254:1
	liver	5	1.870±0.399^{a}	2.703±0.216^{a}	0.692:1
	gills	5	70.24±3.061^{b}	7.054±0.511^{a}	9.958:1
Lake Wędromierz	meat	10	2.498±0.305^{c}	2.812±0.337^{a}	0.888:1
	liver	5	1.123±0.235^{b}	2.773±0.155^{a}	0.405:1
	gills	5	70.17±5.000^{b}	6.942±0.334^{a}	10.107:1

The values for the same tissues marked with different letters in the same column are significantly different ($p \leq 0,05$, Tukey's test).

As analyses indicated the mean content of Ca in the meat of perch caught from the Lake Góreckie, Strzeszyńskie and Wędromierz was 3.175, 0.516 and 2.498 g kg^{-1}, respectively. Those values differed statistically significantly (one-way Avova, Tukey test) – Table 2. The highest Ca concentrations in the liver were determined in fish collected from the Lake Wędromierz (1.123 g kg^{-1}) and this value differed statistically significantly from those indicated in the liver of fish from the Lake Góreckie and Strzeszyńskie. The mean content of Ca in the gills was significantly higher than in the meat and liver and ranged from 63.09 g kg^{-1} (the Lake Góreckie) to 70.24 g kg^{-1} (the Lake Strzeszyńskie). Analyses indicated that the mean content of Ca decreased in the following sequence: gills > meat > liver (the Lake Góreckie and Wędromierz) and gills > liver > meat (the Lake Strzeszyńskie) – Figure 1.

ŁUCZYŃSKA et al. (2009) determined an essential mineral components in the muscles of six freshwater fish from Mazurian Great Lakes. They examined that the non-predatory fish (bream, roach and vendance) contained more calcium (74.6 mg) than the predatory fish (perch, pike and burbot) (59.5 mg), but there were no statistically significant differences between these values. The mean content of calcium in the meat of perch ranged from 43.7 to 94.8 mg 100 g^{-1}. A large amounts of calcium are accumulated in the bones, therefore the fish eaten with the bones (for example, sardines) are the best source of calcium. Analyses of the whole body of Prussian carp (*Carassius auratus gibelio*) indicated that the mean content of calcium was 5.63 g kg^{-1} wet weight (STANEK et al. 2013) – Table 3. Calcium concentra-

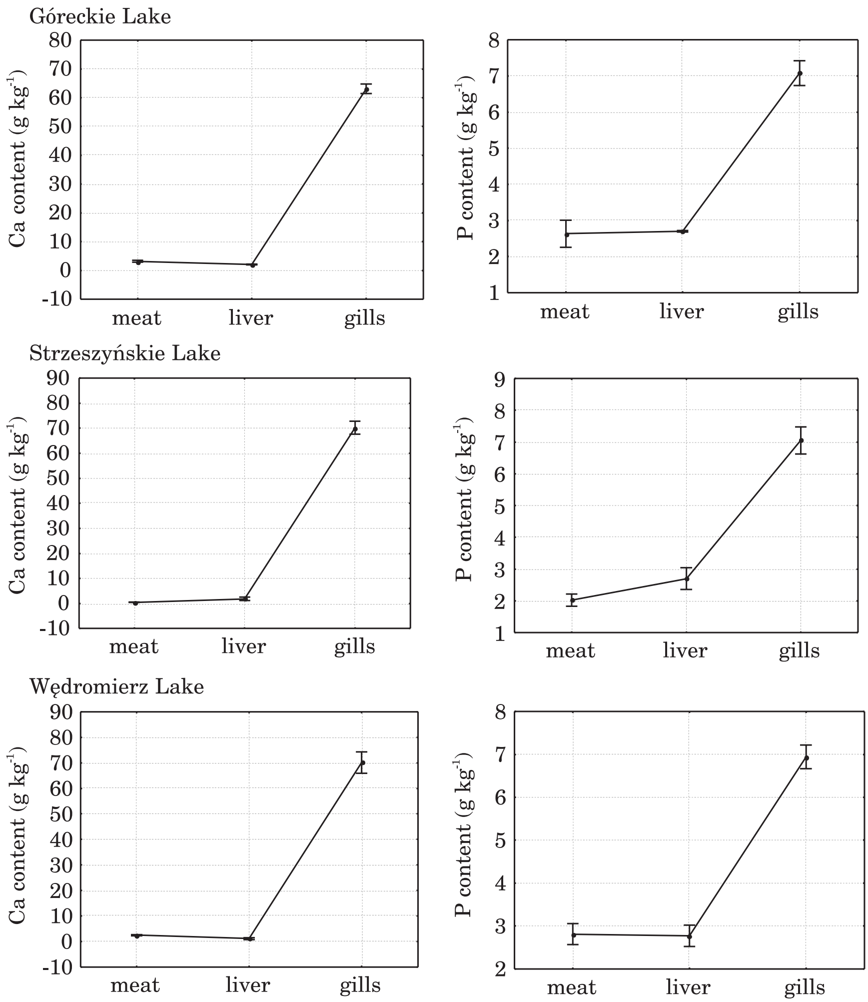

Fig. 1. The mean content of calcium and phosphorus (g kg^{-1} dry weight) in the meat, liver and gills of perch (*Perca fluviatilis* L.) from Lake Góreckie, Strzeszyńskie and Wędromierz

tion in the large side muscle of the fish body above the lateral line of roach from the Brda River was 1.82 g kg^{-1} in females and 1.93 g kg^{-1} in males (in spring). In the individuals from autumn it was 0.83 and 1.10 g kg^{-1}, respectively (STANEK, JANICKI 2011). These analyses indicated that calcium concentration was much higher in the whole body of fish than in a different parts of body. Content of calcium differed statistically significant between individuals caught in a different seasons, but there were not statistically significant differences between samples taken from females and males caught within one season. Meat of the analysed roach were not a rich source of calcium.

Analyses of variance indicated that the mean content of P (g kg^{-1} wet weight) was in the same level in the meat and liver of perch and ranged from 2.028 to 2.812 g kg^{-1} and from 2.703 to 2.773 g kg^{-1}, respectively. The average content of phosphorus in the gills was significantly higher than in the other tissues and ranged from 6.942 to 7.079 g kg^{-1} (Table 2). Analyses indicated that the mean content of P decreased in the following sequence: gills > meat > liver (the Lake Wędromierz) and gills > liver > meat (the Lake Góreckie and Strzeszyńskie) – Figure 1.

Łuczyńska et al. (2009) examined that the mean content of phosphorus in the meat of perch ranged from 1068.3 to 1265.4 mg 100 g^{-1}. Analyses carried out by Stanek et al. (2013) indicated that the mean concentration of phosphorus in the whole body of Prussian carp was 2.38 g kg^{-1}. Two-way analyses of variance indicated, that the mean value of phosphorus in the meat of analyzed roach from Brda River caught in spring was higher than in the fish from autumn, and it was 2.24 g kg^{-1} in females and 2.30 g kg^{-1} in males from spring, respectively. There were no statistical significant differences between those values. In the meat of fish caught in autumn the mean value of phosphorus was 1.89 g kg^{-1} in the tissue of females and 2.01 g kg^{-1} in males. There were no statistical significant differences between these values (Stanek, Janicki 2011).

As indicated analyses about perch and those previous about roach and Prussian carp, the muscle tissue are not considered to the specific physiological sites for calcium and phosphorus (Al-Yousuf et al. 2000). Phosphorus and calcium accumulate in the largest amounts in bones. Borucka-Jastrzębska et al. (2009) determinated micro- and macroelements concentration in the different tissues of freshwater fish (rainbow trout, common carp and Siberian sturgeon (*Acipenser baeri* B.). And they reported that calcium distribution followed the same pattern for all the three analyzed species in decreasing order: gills > muscles > skin > liver > kidney > blood. As Perkowska, Protasowicki (2000) indicated metals accumulate in a different ratio in the liver and kidney. Thus, fish livers represent a good biomonitors of metals present in the surrounding environment. Analyses showed that high levels of the heavy metals were in the liver while the lowest ones were in the muscles. The difference in accumulation potential between these two tissues can be explained by the activity of metallothioeins, proteins that are present in liver but not in the muscle, which have the ability to bind certain metals and thus allow the tissue to accumulate them at a high degree (Visnjic-Jeftic et al. 2010). Liver as a particularly metabolically active tissue, has a tendency to accumulate metals to higher degree than muscle tissue (Yilmaz et al. 2010). Elements have a tendency to react with the oxygen carboxylate, amino group, nitrogen and/or sulphur of the mercapto group in the metallothionein protein which is at highest concentration in the liver (Al-Yousuf et al. 2000). In all fish species analyzed by Roméro et al. (1999) the content of metals were higher in the gills than in the muscles. It proves that the respiratory system is the main way of asquisition of this metals by fish. Gills and liver are chosen as target organs for assessing metal accumulation. The highest concentrations of elements (for Fe, Cu, Mn, Sr, Cr, Cd, Co and Pb) were found in the liver and skin, while the lowest ones were in the muscle. Main concentrations of other studied metals (e.g. Na, As) were found more in the muscle tissue than in liver and skin. Analyses of the heavy metals carried out by CANLI, ATLI (2003) indicated that the highest cadmium concentrations were found in the liver of red gurnard (*Trigla cuculus*), whole the lowest cadmium levels were always found in muscles tissues of the analyzed fish. Lead concentration were much higher than cadmium and the liver and gills accumulated the great amounts of this metal. As JARIĆ et al. (2011) indicated, liver was the main heavy metal storage tissue, while the muscle had the lowest amounts of the analyzed metals.

EASTWOOD, COUTURE (2002) investigated seasonal variations in liver metal contamination of yellow perch (*Perca flavescens*) caught from seven northeastern Ontario Lakes. There were much higher concentration of metals in the spring. It may be due to increased metal input or bioavailability caused by snowmelt events or lake turnover that affect water quality parameters. DRĄG-KOZAK et al. (2011) determined heavy metals concentration in some tissues and organs of rainbow trout (*Oncorhynchus mykiss*) and they reported that higher concentration of the metals were found in the spring compared with those during the autumn season. The same results were observed by LAITINEN (1994). The mean values of Ca concentration were significantly higher in the muscle of Eurasian perch caught in spring, relative to samples taken from fish caught in winter and autumn. As investigated MENDIL et al. (2010) concentration of most determined metals have been reported higher in the fish samples of summer season than other. The seasonal variations in the heavy metals load of bream could be attributed rather to the seasonal change in the factor condition of fish than to variations in the pollutant load of the site (FARKAS et al. 2003, DURAL et al. 2006).

As analyses indicated Ca/P ratio in the meat of fish caught from the Lake Góreckie, Wędromierz and Strzeszyńskie was 1.174:1, 0.254:1 and 0.888:1, respectively (Table 2). As numerous studies show the value of this ratio should be 1:1 in the consumed products, because when there is an excess of calcium over phosphorus, phosphorus is not absorbed, because this form calcium phosphates is not biologically available (CHAVEZ-SANCHEZ et al. 2000). Ca/P ratio in the meat of Prussian carp was 2.37:1 (STANEK et al. 2013) – Table 3. Ratio of calcium to phosphorus in the meat of wild roach from Brda River was ranged from 0.43:1 to 0.82:1 (STANEK, JANICKI 2011) – Table 3. PORN-NGAN et al. (1993) investigated which portion of calcium phosphate and ratio phosphorus to calcium inhibits zinc availability in rainbow trout by giving diets with different amounts of P and Ca. They observed that increase Ca levels slightly reduced the average body weight. NAKAMURA (1982) investigated a negative relationship between the amount of P absorbed by carp and the dietary Ca content. And numerous studies show

Table 3

The mean content of calcium and phosphorus (g kg^{-1} dry weight) in the meat of Prussian carp (*Carassius auratus gibelio*) from Lake Gopło and roach (*Rutilus rutilus*) from Brda River

Species	Place of catch	*n*	Ca (g kg^{-1})	P (g kg^{-1})	Ca:P ratio
Prussian carp* *Carassius auratus gibelio*	Lake Gopło	14	5.63 ± 1,24	2.38 ± 0,31	2.37:1
Roach** *Rutilus rutilus*	Brda River	40	1.41 ± 0,76	2.11 ± 0,27	0.67:1

* Stanek et al. (2013)
** Stanek and Janicki (2011)

that excess of phosphorus in the body causes calcium malabsorption, which can lead to decalcification of the bones.

CONCLUSION

1. Perch caught from lakes of similar trophic level were characterized by differences in the content of calcium in the muscle, gills and liver.

2. There were no statistically significant differences in the mean content of phosphorus determined in the liver and gills of fish collected from the lake Góreckie, Strzeszyńskie and Wędromierz.

REFERENCES

Albrektsen, S., Hope, B., Aksnes, A. 2009. *Phosphorus (P) deficiency due to low P availability in fishmeal produced from blue whiting (Micromesistius poutassou) in feed for under-yearling Atlantic salmon (Salmo salar) smolt*. Aquaculture, 296: 318-328. DOI: 10,1016/j.aquaculture.2009.08.033

Al-Yousuf M.H., El-Shahawi M.S., Al-Ghais S.M. 2000. *Trace metals in liver, skin and muscle of Lethrinus lentjan fish species in relation to body length and sex*. Sci. Total Environ., 256: 87-94.

Borucka-Jastrzębska E., Protasowicki M. 2006. *Levels of selected metals in tissues and organs of 5-month-old carp (Cyprinus carpio* L.*)*. Acta Sci. Pol., Pisc., 5(2): 3-16.

Borucka-Jastrzębska E., Kawczuga D., Rajkowska M., Protasowicki M. 2009. *Levels of microelements (Cu, Zn, Fe) and macroelements (Mg, Ca) in freshwater fish*. J. Elementol., 14(3): 437-447.

Cantli M., Atli G. 2003. *The relationship between heavy metals (Cd, Cr, Cu, Fe, Pb and Zn) levels and the size of six Mediterranean fish species*. Environ. Pollut., 121: 129-136.

Carlson R.E. 1977. *A trophic state index for lakes*. Limnology Oceanography, 22(2): 361-369.

Celik U., Oehlenschläger J. 2004. *Determination of zinc and copper in fish samples collect-*

ed from Northeast Atlantic by DPSAV. Food Chem., 87: 343-347. DOI: 10.1016/j.foodchem.2003.11.018

Chavez-Sanchez C., Martinez-Palacios C.A., Martinez-Perez G., Ross L.G. 2000. *Phosphorus and calcium requirements in the diet of the American cichlid Cichlasoma urophthalmus (Günther)*. Aquacult. Nutr., 6: 1-9.

Chen Y-Ch., Chen M-Ch. 2001. *Heavy metals concentrations in nine species of fishes caught in coastal waters off Ann-Ping, S.W. Taiwan*. J. Food Drug Anal., 9(2): 107-114.

Drąg-Kozak E., Łuszczek-Trojnar E., Popek W. 2011. *Heavy metals In some tissues and organs of rainbow trout (Oncorhynchus mykiss) fish species in relation to age and season.* Ochr. Środ. Zas. Nat., 48: 161-169. (in Polish)

Dural M., Lugal Göksu M.Z., Akif Özak A., Derici B. 2006. *Bioaccumulation of some heavy metals in different tissues of Dicentrarchus labrax* L. *1758, Sparus aurata* L. *1785, and Mugil cephalus* L. *1758 from the Çamlik Lagoon of the eastern cost of Mediterranean (Turkey).* Environ. Monit. Assess., 118: 65-74.

Eastwood S., Couture P. 2002. *Seasonal variation in condition and liver metal concentration of yellow perch (Perca flavescens) from a metal-contaminated environment.* Aquat. Toxicol., 58: 43-56.

Farkas A., Salánki J., Specziár A. 2003. *Age- and size-specific patterns of heavy metals In the organs of freshwater fish Abramis brama L. populating a low-contaminated site*. Water Res., 37: 959-964.

Jarić I., Višnjić-Jeftić Ž., Cvijanović G., Gačić Z., Jovanović L., Skorić S., Lenhardt M. 2011. *Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of starlet (Acipenser ruthenus) from the Danube River in Serbia by ICP-OES*. Microchem. J., 98: 77-81. DOI: 10.1016/j.microc.2010.11.008

Laitinen M. 1994. *Calcium and magnesium concentrations in ova, bone and muscle and quality of reproductive products of the perch (Perca fluviatilis) in an acid and neutral lake*. Pol. Arch. Hydrobiol., 41(4): 495-506.

Lidwin-Kaźmierkiwicz M., Pokorska K., Protasowicki M., Rajkowska M., Wechterowicz Z. 2009. *Content of selected essentials and toxic metals in meat of freshwater fish from West Pomeranian, Poland.* Pol. J. Food Nutr. Sci., 59(3): 219-224.

Litwińczuk A., Skałecki P., Florek M., Grodzicki T. 2006. *Utility value and physicochemical quality of silver Ginel carp meat (Carassius auratus gibelin* L.*) caught in spring and autumn season*. Rocz. Nauk. Pol. Tow. Zootech., 2(4): 97-102. (in Polish)

Łuczyńska J., Borejszo Z., Łuczyński M.J. 2009. *The composition of fatty acids in muscles of six freshwater fish species from the Mazurian Great Lakes (Northwestern Poland).* Arch. Pol. Fish., 16: 167-178. DOI: 10.5513/JCEA01/12.4.962

Mendil D., Uluözlü, Ö.D. 2007. *Determination of trace metal levels in sediment and five fish species from lakes in Tokat, Turkey*. Food Chem., 101: 739-745. DOI: 10.1016/j.foodchem.2006.01.050.

Mendil D., Demirci Z., Tuzen M., Soylak M. 2010. *Seasonal investigation of trace element contents in commercially valuable fish species from the Black sea, Turkey*. Food Chem. Toxicol., 48: 865-870.

Nakamura Y. 1982. *Effects of dietary phosphorus and calcium contents on the absorption of phosphorus in the digestive tract carp*. Bull. Japan. Soc. Sci. Fish., 48(3): 409-413.

Pełechaty M., Owsianny P.M. 2003. *Horizontal distribution of phytoplankton as related to the spatial heterogeneity of a lake- a case study from two lakes of the Wielkopolski National Parka (western Poland).* Hydrobiology, 510: 195-205.

Perkowska A., Protasowicki M. 2000*. Cadmium and lead in fishes in selected elements of Świdwie Lake ecosystem*. Acta Ichthyol. Piscat., 30(2): 71-84.

PN-EN 13805/2003 – *Foodstuffs. Determination of trace elements. Mineralization under pressure*. (in Polish)

PN-EN 15505/2009 – *Foodstuffs. Determination of trace elements. Annex B for the determination of calcium*. (in Polish)

PN-ISO 13730 – *Determination of total phosphorus content.* (in Polish)

Porn-Ngam N., Satoh S., Takeuchi T., Watanabe T. 1993. *Effect of the ratio of phosphorus to calcium on zinc availability to rainbow trout in high phosphorus diet.* Nippon Suisan Gakkaishi, 59(12): 2065-2070.

Roméo M., Siau Y., Sidoumou Z., Gnassia-Barelli M. 1999. *Heavy metal distribution in different fish species from the Mauritania coast*. Sci. Total Environ., 232: 169-175.

Sapek A. 2009. *Phosphorus in the human food chain and the environment in Poland*. Inż. Ekol., 21**:** 62-72. (in Polish)

Stanek M., Peter E., Janicki B. 2012. *Content of the calcium and phosphorus in the meat of Prussian carp (Carassius auratus gibelio BLOCH, 1783) from the Lake Gopło (Poland)*, JCEA (in press)

Stanek M., Janicki B. 2011. *Impact of season and sex on calcium and phosphorus content in the meat of roach (Rutilus rutilus L.) from the Brda River (Poland, Bydgoszcz)*. Fol. Biol., 59: 189-194. DOI: 10.3409/fb59_3-4.189-194.

Ye Ch-X, Liu Y-J, Tian L-X, Mai K-S, Du Z-Y, Yang H-J, Niu J. 2006. *Effect of dietary calcium and phosphorus on growth, feed efficiency, mineral content and body composition of juvenile grouper*, *Epinephelus coioides.* Aquaculture, 255: 263-271. DOI: 10.1016/j.aquacuture.2005.12.028.

Yilmaz A.B., Sangün M.K., Yağlioğlu D., Turan C. 2010. *Metals (major, essential to non-essential) composition of the different tissues of three demersal fish species from Iskenderun Bay, Turkey*. Food Chem., 123: 410-415. DOI: 10.1016/j.foodchem.2010.04.057.

Visnjic-Jeftic Z., Jaric I., Jovanovic L., Skoric S., Smederevac-Lalic M., Nikcevic M., Lenhardt M. 2010. *Heavy metal and trace element accumulation in muscle, liver and gills of the Pontic shad (Alosa immaculata Bennet 1835) from the Danube River (Serbia).* Microchem. J., 95: 341-344. DOI: 10.1016/j.microc.2010.02.004.

J. Elem. s. 519–532 DOI: 10.5601/jelem.2014.19.2.653

INTERACTION OF ORGANIC FERTILISATION WITH MULTI-COMPONENT MINERAL FERTILISERS AND THEIR EFFECT ON THE CONTENT OF MICROELEMENTS IN PERENNIAL RYEGRASS*

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Abstract

In 2007-2008, a plant growing pot experiment was carried out, in which the content of microelements, including heavy metals, in soil material and in compost produced by the GWDA method was found equal or less than the threshold values set by the Regulation of the Ministry of the Environment. Thus, the aim of this study was to determine the interaction between organic fertilisation and selected multi-component mineral fertilisers (SuproFoska 20, Suprofos 25 and Inmarc 4 with added urea) as well as their influence on the development of some qualitative traits of perennial rye-grass. The results showed that double doses of multi-component mineral fertilisers with urea contributed to a higher increase in the content of microelements in biomass of perennial ryegrass *Lolium perenne* than their single doses. The highest concentrations of cadmium, copper and lead were contained by ryegrass biomass after the application of a double dose of Inmarc 4, i.e. 9.37%, 11.0% and 2.81% more, respectively, than after the application of a single dose of SuproFoska 20 or Suprofos 25. The experiment demonstrated the highest content of cadmium, copper, manganese and lead in perennial ryegrass biomass in the treatments with combined organic (compost) and mineral (SuproFoska 20 with urea) fertilisation. However, the nickel and zinc content in biomass were the highest in the variant with combined organic (compost) and mineral (Inmarc 4 with urea) fertilisation. Multi-component mineral fertilisers and urea applied in combination with compost made of municipal sewage sludge increased the content of microelements in the biomass of cv. Stadion perennial rye-grass *Lolium perenne* when compared to the fertilisation objects with organic fertilisation alone.

Keywords: *Lolium perenne Stadion* cultivar, compost, multiple mineral fertilizers, content of heavy metal.

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WSPÓŁDZIAŁANIE NAWOŻENIA ORGANICZNEGO Z WIELOSKŁADNIKOWYMI NAWOZAMI MINERALNYMI NA ZAWARTOŚĆ MIKROSKŁADNIKÓW W ŻYCICY TRWAŁEJ

Abstrakt

W latach 2007-2008 przeprowadzono doświadczenie wegetacyjno-wazonowe. Zawartość mikroskładników, a wśród nich metali ciężkich, w materiale glebowym oraz użytym do badań kompoście wyprodukowanym metodą GWDA nie przekraczała wartości dopuszczalnych określonych w przepisach ministerialnych. Celem badań było określenie współdziałania nawożenia organicznego z wybranymi wieloskładnikowymi nawozami mineralnymi (SuproFoska 20, Suprofos 25 i Inmarc 4 z dodatkiem mocznika) w kształtowaniu niektórych cech jakościowych życicy trwałej. Wykazano, że podwojone dawki wieloskładnikowych nawozów mineralnych z dodatkiem mocznika przyczyniły się do zwiększenia zawartości mikroskładników w biomasie życicy trwałej *Lolium perenne* w porównaniu z dawkami pojedynczymi. Najwięcej kadmu, miedzi i ołowiu zawierała biomasa rośliny testowej po zastosowaniu podwojonej dawki Inmarc 4, odpowiednio o 9,37%, 11,0% i 2,81% więcej w porównaniu z wprowadzoną pojedynczą dawką SuproFoski 20 i Suprofosu 25. Stwierdzono, że najwięcej kadmu, miedzi, manganu i ołowiu w biomasie życicy trwałej było na obiektach z łącznym nawożeniem organicznym (kompost) i mineralnym (SuproFoska 20 z mocznikiem). Natomiast zawartość niklu i cynku w biomasie rośliny testowej była także największa na obiektach z łącznym nawożeniem organicznym i Inmarc 4 z mocznikiem w porównaniu z pozostałymi obiektami nawozowymi. Wieloskładnikowe nawozy mineralne i mocznik stosowane łącznie z kompostem wpływały na zwiększenie zawartości analizowanych mikroskładników w biomasie życicy trwałej *Lolium perenne* odmiany *Stadion* w porównaniu z obiektami, w których stosowano wyłącznie nawożenie organiczne.

Słowa kluczowe: życica trwała odmiana *Stadion*, kompost, wieloskładnikowe nawozy mineralne, zawartość metali ciężkich.

INTRODUCTION

The livestock of sheep, cattle and horses kept on farms has decreased considerably over the last several years. On the other hand, natural fertiliser prices have increased, while farmland liming has been neglected. These are the causes of growing acidification of over 50% of arable land. Changes have also taken place in the crop structure in favour of cereals and industrial crops. This leads to the imbalance of organic matter and nutrients in soils. Therefore, multidirectional research has been initiated with the aim to acquire new, cheap and environmentally safe sources of organic matter and nutrients for plants. Many authors claim that municipal sewage sludge may be used for fertilisation purposes (Aggelides, Londra 2000, Jakubus 2006, Selivanovskaya, Latypova 2006, Jasiewicz et al. 2007, Singh, Agrawal 2008, Hargreaves et al. 2008, Czekała 2008, Torri, Lavado 2008, Chiba et al. 2009) because it is rich in organic substances and some macro- and microelements (Walter et al. 2006, Haroun et al 2007, Ngole 2007, He et al. 2009). However, municipal sewage sludge may contain excessive amounts of heavy metals or be contaminated with pathogenic microorganisms, parasites and their live eggs. For this reason, municipal sewage sludge should meet the standards defined in the *Regulation of the Ministry of the Environment* (Official Journal of Law No. 137, item 934 of 2010) before being introduced into soil.

Saha et al. (2007) and Zhao et al. (2009) conclude that tests should be run on the presence of heavy metals as well as organic pollutants in sewage sludge. Organic pollutants are persistent organic compounds comprising carbon. The research carried out by Venkatesan and Senturpandian (2006) implies a very important role played by organic fertilisation, which determines the carbon content in soil and in general is positively correlated with soil activity. Dinesh et al. (2004) and Cai et al. (2007) claim that organic fertilisers are beneficial for the relationships between the soil tilth and its physical and chemical properties.

According to Kabata-Pendias and Pendias (1999), the main sources of cadmium and lead in arable soils unexposed to industrial emissions are mineral fertilisers. Gorlach and Gambuś (1997) believe that the degree of pollution, including heavy metals, caused by application of mineral fertilisers is most strongly affected by the raw material from which fertilisers are made, and by the technological processes through which they are manufactured. The fertilisers most heavily polluted with heavy metals include phosphate, lime, potassium and nitrogen formulas. Phosphate fertilisers may constitute a considerable source of soil pollution with heavy metals, particularly with cadmium. The average content of heavy metals in these fertilisers is arranged in the following order: Cd < Cu < Pb < Ni < Zn, with the level of their content being largely dependent on the form of fertiliser.

The aim of this study was to determine the interaction of organic fertilisation (compost produced from municipal sewage sludge) and selected multi-component mineral fertilisers (SuproFoska 20, Suprofos 25 and Inmarc 4 with added urea) as well as their impact on the development of some qualitative traits of perennial ryegrass.

MATERIAL AND METHODS

In spring 2007, a pot experiment was set in a greenhouse of the Western Pomeranian University of Technology in Szczecin. The soil material used in the experiment had the texture of heavy loamy sand and was classified as very good rye complex, soil quality class IVa.

The soil was characterised by reaction close to neutral (6.50). The content of plant-assimilable phosphorus, potassium and magnesium forms (64.5, 123.0 and 8.4 mg kg^{-1}, respectively) was average. The soil application of municipal sewage sludge composts necessitated determinations of the total content of heavy metals in the soil material (Table 1). The data in Table 1 show that the content of microelements, including heavy metals, did not exceed the acceptable levels specified in the *Regulation of the Ministry of the Environment* (Official Journal of Laws No. 137, item 934 of 2010) for the use of municipal sewage sludge.

Table 1

The content of microelements, including heavy metals, in the soil material used in the study

Cadmium	Copper	Manganese	Nickiel	Lead	Zinc
0.50	5.50	2.71	7.55	8.00	46.8

The compost used in the study was produced by the GWDA method and came from the Municipal Sewage Treatment Plant in Stargard Szczeciński. It contained more nitrogen and phosphorus (28.0 and 12.5 g kg^{-1} d.m., respectively) than potassium (6.00 g·kg^{-1} d.m.). The cadmium, copper, chromium, manganese, nickel, lead and zinc content (0.90, 67.0, 26.7, 259.0, 8.61, 56.0 and 143.0 mg kg^{-1} d.m.) in this compost was within the standards set by the *Regulation of the Ministry of Agriculture and Rural Development* (Official Journal of Laws No. 119, item 765 of 2008). Microbiological analyses received from the Municipal Sewage Treatment Plant in Stargard Szczeciński show that the compost made of municipal sewage sludge met the sanitary and hygienic requirements specified in ministerial regulations.

Table 2

The content of microelements in multi-component mineral fertilisers

Type of fertiliser	Microelement (mg kg^{-1} d.m)					
	Cd	Cu	Mn	Ni	Pb	Zn
SuproFoska 20	16.8	16.0	50.3	6.80	6.60	26.0
Suprofos 25	16.4	16.2	50.8	6.80	6.70	26.2
Inmarc 4	17.8	16.4	55.2	6.25	6.45	26.5

Based on the data comprised in Table 2, it is possible to conclude that the content of microelements, including heavy metals (such as cadmium, copper, manganese, nickel, lead and zinc), was within the standards set for mineral fertilisers (Official Journal of Laws No. 119, item 765 of 2008).

The study design included three factors (Table 3). The first factor was a series with and without municipal sewage sludge compost; the second factor was the type of multi-component mineral fertiliser (SuproFoska 20, Suprofos 25, Inmarc 4 with added urea), while the third one was the dose of multi-component mineral fertilisers. The test plant was perennial ryegrass (*Lolium perenne*) cultivar *Stadion*. Each of the experimental variants was run in four replications.

The soil material collected for analyses was sieved through a 5 mm mesh to remove larger impurities. Next, batches of 9 kg of the soil were put into pots. The dose of compost was determined at a level of 100 kg N ha^{-1}; this corresponded to 16.92 Mg fresh compost mass per 1 ha. When converted per pot, the compost dose was 50.8 g of fresh mass. In the treatments with planned application of municipal sewage sludge compost, the fertiliser was

Table 3

The design of pot vegetation experiment

Fertilisation objects and doses of mineral fertiliser	Without compost	With municipal sewage sludge compost at a dose corresponding to 100 kg N ha^{-1}
Control	+	+
SuproFoska 20 + urea – dose I	+	+
SuproFoska 20 + urea – dose II	+	+
Suprofos 25 + urea – dose I	+	+
Suprofos 25 + urea – dose II	+	+
Inmarc 4 + urea – dose I	+	+

spread on the soil surface and then mixed with the soil to the depth of 8-10 cm. After 7 days, mineral fertilisers were introduced into the soil material.

The dose of mineral fertilisers (SuproFoska 20, Suprofos 25 and Inmarc 4) was determined at a level of 200 kg ha^{-1} (dose I) and 400 kg ha^{-1} (dose II). When converted per pot, the fertilisers were applied in the amount of 0.6 g (dose I) and 1.2 g (dose II). Due to the low nitrogen content in the above multi-component mineral fertilisers, any possible nitrogen deficiency was prevented by the application of urea.

The multi-component mineral fertilisers and a 1/3 of the dose of urea in the form of aqueous solution were introduced into the soil material in spring 2007, prior to sowing ryegrass. The fertilisers were mixed with the soil material to the depth of 5-7 cm. After 5 days, perennial ryegrass was sown on the soil surface in pots, 50 seeds per each pot. The seeds were covered with 1 cm layer of quartz sand. Afterwards, the pots were placed under a foil roof. In order to maintain the moisture of soil material at 60% of full water capacity, the soil material and the plants in pots were sprinkled with redistilled water. The remaining 2/3 of the nitrogen dose in the form of urea were divided into 2 parts and applied in the form of aqueous solution after the first and the second cut of perennial rye-grass.

Each year, three cuts of the grass were harvested. In 2007, perennial ryegrass was sown on 19 April, the first cut harvested on 2 June, the second one was carried out 15 July and the third one took place on 26 August. After the last cut, the pots with grass were wintered in a greenhouse.

In spring 2008, the pots with grass were placed again under a foil roof. According to the design, doses of multi-component mineral fertilisers and 1/3 of the total dose of aqueous urea solution were introduced into the pots. The remaining doses of urea were introduced into soil after the first and the second cut of perennial ryegrass. The mineral fertiliser doses and plant care measures were the same as in 2007.

In 2008, three cuts of perennial ryegrass biomass were harvested, with the first cut made on 31 May, the second one – on 13 July and the third one – on 30 August. During the experiment, the yield of perennial ryegrass dry matter from each replication of every fertilisation treatment was determined in 2007 and 2008. The grass plants from the four replications of a given fertilisation object were mixed and ground. This way, averaged samples of the ryegrass biomass was obtained for all fertilisation variants. The total cadmium, copper, manganese, nickel, lead and zinc content was determined in the dry matter of collected perennial ryegrass samples after their previous wet mineralisation according to the Polish standards PN–ISO 11466 and PN-ISO 11047. The determinations were carried out with the method of atomic absorption spectrometry on a Perkin Elmer AAS 300 spectrometer.

The experiment was performed in a randomised complete block design with four replications and a control group. Statistical calculations on the significance of differences in the content of microelements, including some heavy metals, were conducted using a three factorial analysis of variance and FR-ANALWAR computer software. Confidence half-intervals were calculated at the significance level $p = 0.05$, using the Tukey's test.

RESULTS AND DISCUSSION

The average content of microelements in perennial ryegrass, including some heavy metals, obtained in the pot experiment is presented in Tables 4 and 5.

The data comprised in Tables 4 and 5 prove that the content of microelements in perennial ryegrass biomass was within the average values given by Kabata-Pendias and Pendias (1999). Whenever the multi-component mineral fertilisers SuproFoska 20, Suprofos 25 and Inmarc 4, used in the current experiment, are mentioned while describing the experimental results, it must be remembered that they were applied with urea due to their low nitrogen content.

The highest average content of cadmium in ryegrass biomass was determined in the variant with the application of the multi-component mineral fertiliser Inmarc 4 (0.34 mg Cd kg^{-1} d.m.). The cadmium concentrations in perennial ryegrass in the fertilisation treatments with SuproFoska 20 and Suprofos 25 were similar and 3.03% lower than in the variant with Inmarc 4. The type of multi-component mineral fertiliser with the urea supplementation did not have any significant effect on the differences in the cadmium content in the analysed ryegrass biomass. In the series without municipal sewage sludge compost, most cadmium was in perennial ryegrass biomass from the fertilisation object with Inmarc 4 (0.31 mg Cd kg^{-1} d.m.). Less cadmium was determined in plants fertilised with Suprofos 25 and SuproFoska 20 (6.89% and 10.7% less, respectively). The cadmium content in perennial ryegrass biomass in the fertilisation treatments with Suprofos 25 and Inmarc 4 was the same (0.36 mg Cd kg^{-1} d.m). Double doses of multi-compo-

Table 4

The effect of multi-component mineral fertilisers and urea applied with and without municipal sewage sludge compost on the average content of cadmium, copper and manganese in perennial ryegrass biomass. Mean values from two years (2007-2008) in mg kg^{-1} d.m. grass

Specification		Fertilisation variants											
		cadmmium				copper				manganese			
		fertilisers types (T)											
		Supro-Foska 20	Suprofos 25	Inmarc 4	mean	Supro-Foska 20	Suprofos 25	Inmarc 4	mean	Supro-Foska 20	Suprofos 25	Inmarc 4	mean
		+ urea				+ urea				+ urea			
Without compost	(C)	0.28	0.29	0.31	0.29	6.56	6.61	6.75	6.64	67.1	68.7	67.8	67.8
With compost		0.37	0.36	0.36	0.36	7.59	7.62	7.53	7.58	74.6	76.1	73.6	74.8
Dose I	(D)	0.32	0.32	0.33	0.32	6.91	7.01	7.00	6.97	69.7	71.0	69.1	69.9
Dose II		0.33	0.33	0.35	0.34	7.24	7.22	7.28	7.24	71.9	73.8	72.3	72.7
Mean		0.33	0.33	0.34		7.07	7.11	7.14		70.8	72.4	70.7	
Control without compost		0.25				5.59				62.7			
Control with compost		0.34				6.73				67.2			
LSD $_{0.05:}$													
Fertilisers types (T)		n.s.				n.s.				n.s.			
Fertilisation doses (D)		n.s.				n.s.				1.481			
Without and with addition of compost (C)		0.022				0.307				1.481			
Interaction TxC		n.s.				n.s.				n.s.			

Table 5

The effect of multi-component mineral fertilisers and urea applied with and without municipal sewage sludge compost on the average content of nickel, lead and zinc in perennial ryegrass biomass. Mean values from two years (2007-2008) in mg kg^{-1} d.m. grass

Specification		Fertilisation variants											
		nickiel				lead				zinc			
		fertilisers types (T)											
		Supro-Foska 20	Suprofos 25	Inmarc 4	mean	Supro-Foska 20	Suprofos 25	Inmarc 4	mean	Supro-Foska 20	Suprofos 25	Inmarc 4	mean
		+ urea				+ urea				+ urea			
Without compost	(C)	0.93	0.93	0.91	0.92	1.39	1.39	1.40	1.39	24.6	25.3	23.8	24.5
With compost		1.01	0.98	0.90	0.96	1.46	1.48	1.49	1.48	27.8	27.4	27.0	27.4
Dose I	(D)	0.97	0.95	0.93	0.95	1.42	1.42	1.43	1.42	25.5	25.6	25.0	25.4
Dose II		0.97	0.97	0.88	0.94	1.44	1.45	1.46	1.45	26.9	27.0	25.9	26.6
Mean		0.97	0.96	0.90		1.43	1.43	1.44		26.2	26.3	25.4	
Control without compost		0.87				1.32				21.4			
Control with compost		0.95				1.43				23.7			
LSD $_{0.05}$:													
Fertiliser types (T)		n.s.				n.s.				n.s.			
Fertilisation doses (D)		n.s.				0.022				0.691			
Without and with addition of compost (C)		n.s.				0.022				0.691			
Interaction TxC		n.s.				n.s.				n.s.			
Interaction TxD													
Interaction TxDxC													

nent mineral fertilisers and urea did not have any significant effect on the increase in the cadmium content in the examined biomass when compared to their single doses (Table 4). The largest increase in the cadmium content, by 9.37%, was found between the fertilisation variants with a single dose of SuproFoska 20 and Suprofos 25 versus the one with a double dose of Inmar 4. As reported by CZUBA (1996), in order to achieve high and good quality plant yields while preserving a high level of soil fertility, attention should be paid to the close interaction between organic and mineral fertilisers. This interaction grows stronger when optimal doses, terms and forms of applied mineral, natural and organic fertilisers are selected and the plant rotation system is likewise optimal.

The average content of copper in perennial ryegrass biomass ranged from 7.07 to 7.14 mg Cu kg^{-1} d.m. The highest copper concentration was found in ryegrass biomass in the fertilisation treatment with Inmarc 4, while the lowest one appeared in the pots fertilised with SuproFoska 20. Differences in the effect between the multi-component mineral fertilisers with urea on the copper content in perennial ryegrass biomass were not significant.

In the fertilisation objects with and without municipal sewage sludge compost introduced to soil, most copper was detected in perennial ryegrass biomass from pots with the multi-component mineral fertiliser Inmarc4 (6.75 and 7.53 mg Cu kg^{-1} d.m.). Less copper was obtained in the fertilisation objects with SuproFoska 20 and Suprofos 25, namely 2.1 and 2.89% less, respectively, than in pots with Inmarc 4 had. The doubling of the doses of multi-component mineral fertilisers and urea did not have any significant effect on the increase in the copper content in perennial ryegrass biomass when compared to their single doses (Table 4). Nevertheless, an increase was observed in the copper content, on average by 29.5%, when compared to the control object.

The average content of manganese in perennial ryegrass biomass in fertilisation treatments with multi-component mineral fertilisers and urea ranged from 70.7 to 72.4 mg Mn kg^{-1} d.m. The type of multi-component mineral fertilisers did not have any significant effect on the manganese content in the biomass but an average increase of 13.7% was found in its content when compared to the control object. Doubled doses of the mineral fertilisers added to soil significantly increased the manganese content in perennial ryegrass biomass when compared to their single doses.

The highest content of manganese in the experimental series without municipal sewage sludge compost was in perennial ryegrass biomass from the fertilisation variant with Suprofos 25 (68.7 mg Mn kg^{-1} d.m.), while the least manganese was in ryegrass biomass harvested from the pots with SuproFoska 20 (67.1 mg Mn kg^{-1} d.m.). After the application of the organic fertiliser, most manganese in biomass was obtained after the application of Suprofos 25, while the least was determined in the fertilisation variant with Inmarc 4.

Regarding single doses of the mineral fertilisers, most manganese was found in perennial ryegrass biomass from the fertilisation variant with Suprofos 25 (71.0 mg Mn kg^{-1} d.m.), while the least was in biomass harvested from the pots with Inmarc 4 (69.1 mg Mn kg^{-1} d.m). Double doses of mineral fertilisers induced the highest accumulation of manganese in perennial ryegrass biomass in the fertilisation treatment with Suprofos 25, while the lowest one was in the variant with SuproFoska 20. An increase in the manganese content in ryegrass biomass between these fertilisation objects reached 13.4%. An increase in the manganese content following the application of mineral fertilisation between dose I and dose II was 3.15, 3.94 and 4.63%, respectively. The highest increase in the manganese content in ryegrass biomass was obtained in the fertilisation treatments where organic fertilisation and a double dose of mineral fertilisers had been applied, reaching 19.3% and 15.9% respectively, when compared to the control (Table 4). Similar results were obtained by ANTONKIEWICZ et al. (2003), ANTONIEWICZ and JASIŃSKA (2009) and KASPERCZYK et al. (2001), who reported that organic fertilisers interact with mineral ones because they are rich in organic matter and some microelements which mineral fertilisers do not contain.

The nickel content in perennial ryegrass biomass did not undergo significant changes in response to the application of multi-component mineral fertilisers in the experimental series without municipal sewage sludge compost. Double doses of mineral fertilisers did not have any significant effect on the increase in the nickel content in biomass compared to their single doses. Nevertheless, an average increase was observed in the nickel content, by 8.33%, between the fertilisation objects and the control. Municipal sewage sludge compost introduced to soil did not have any significant effect on changes in the nickel content in perennial ryegrass biomass compared to exclusive organic fertilisation. The mineral fertilisers and their doses applied in conjunction with municipal sewage sludge compost did not have any significant effect on changes in the nickel content in ryegrass biomass (Table 5).

The average content of lead in perennial ryegrass biomass in the fertilisation variants with SuproFoska 20 and Suprofos 25 reached the same values (1.43 mg Pb kg^{-1} d.m.) and was slightly smaller (by 0.70%) than that in the fertilisation treatment with Inmarc 4. The doubling of doses of mineral fertilisers and urea significantly increased the lead content in biomass compared to single doses (Table 5). The highest average increase in the lead content in ryegrass biomass (by 12.1% versus the control) was obtained in the fertilisation variants with combined organic and mineral fertilisation.

In the experimental series without municipal sewage sludge compost, the highest zinc content in perennial ryegrass biomass was obtained after the introduction of Suprofos 25. This content was higher by 6.30 and 2.84% compared to the fertilisation variants with Inmarc 4 and SuproFoska 20. In the experimental series with municipal sewage sludge compost, the highest increase in the zinc content (by 2.96%) was obtained between the fertilisation treatments with SuproFoska 20 and Inmarc 4. When taking into account single and a double doses of the multi-component mineral fertilisers with urea, the highest increase in the zinc content (by 4.24%) occurred between the fertilisation variants with Suprofos 25 and Inmarc 4 (Table 6). The highest average increase in the zinc content in perennial ryegrass biomass appeared between the fertilisation objects without and with organic fertilisation as well as the one with mineral fertilisation (by 21.5% versus the control; Table 5). The multi-component mineral fertilisers used in the study did not have any significant effect on differences in the average content of zinc in perennial ryegrass biomass, whereas their doubling significantly increased its amount in biomass compared to their single doses. The applied organic fertilisation significantly affected the increase in the average cadmium, copper, manganese, lead and zinc content in perennial ryegrass biomass compared to the fertilisation treatments with municipal sewage sludge compost. In the opinion of GORLACH (1992), the risk of environmental loading with organic fertilisers is often larger than with mineral ones. The main reason is the inability to synchronise the release of nutrients from organic fertilisers with their demand by plants and with their large amounts of various organic compounds and heavy metals.

In brief, it is possible to state that most cadmium, copper and lead was accumulated in ryegrass biomass after the application of a double dose of Inmarc 4, more by 9.37%, 11.0% and 2.81% respectively, compared to single doses of SuproFoska 20 and Suprofos 25 introduced into soil. The results show that the highest cadmium, copper, manganese and lead content in perennial ryegrass biomass occurred in the fertilisation treatments with combined organic (municipal sewage sludge compost) and mineral (SuproFoska 20 with urea) fertilisation. However, the nickel and zinc content in ryegrass biomass was also the highest in the fertilisation variant with combined organic (compost) and mineral (Inmarc 4 with urea) fertilisation compared to the other fertilisation objects.

Similar results were obtained by KRZYWY-GAWROŃSKA and GUTOWSKA (2007) and KRZYWY-GAWROŃSKA (2009), who showed that organic fertilisation contributes to some increase in the cadmium, copper, manganese, nickel and lead content in test plants. IŻEWSKA (2007) reported that application of moderate doses of composts (5-20 Mg d.m. ha^{-1}) free from excessive amounts of heavy metals induced limited accumulation of the above metals in the biomass of energy crops, including grasses.

As reported by KABATA-PENDIAS and PENDIAS (1999), the abundance of cadmium, copper, manganese, nickel, lead and zinc in grasses is 0.05 to 0.8, 2.20 to 21.0, 20 to 665, 0.4 to 1.70, 0.30 to 3.50 and 3.70 to 29.8 mg kg^{-1} d.m., respectively. In our two-year experiment, the content of cadmium, copper, manganese, nickel, lead and zinc in perennial ryegrass biomass did not exceed the values given by KABATA-PENDIAS and PENDIAS (1999) in any of the tested fertilisation variants.

CONCLUSIONS

1. Municipal sewage sludge compost introduced into soil significantly affected the increase in the average content of cadmium, copper, manganese, lead and zinc in the biomass of perennial ryegrass *(Lolium perenne)* of the cultivar *Stadion* compared to the fertilisation variants without organic fertilisation.

2. Multi-component mineral fertilisers and urea applied with municipal sewage sludge compost increased the content of cadmium, copper, nickel, manganese, lead and zinc in perennial ryegrass biomass compared to the fertilisation variants with exclusive organic fertilisation.

3. As affected by single and double doses of multi-component mineral fertilisers and urea, the content of cadmium, copper, nickel, manganese, lead and zinc in perennial ryegrass biomass was higher than in the control.

4. Double doses of multi-component mineral fertilisers with urea contributed to the increase in the content of microelements in perennial ryegrass biomass when compared to their single doses.

5. The average content of cadmium, copper, manganese, nickel, lead and zinc in perennial ryegrass biomass did not exceed the standard values given in literature concerning the application of organic and mineral fertilisation during two-year experiments.

6. The results indicate that it is possible to combine the application of organic and mineral fertilisers when using their optimal doses and dates of fertilisation treatments.

REFERENCE

AGGELIDES S.M., LONDRA P.A. 2000. *Effects of compost produced from town wastes and sewage sludge on the physical properties of a loamy and a clay soil.* Biores. Techn., 71(3): 53-259. DOI:10.1016/S0960-8524(99)00074-7

ANTONKIEWICZ J., RADKOWSKI A., JASIEWICZ C. 2003. *Effect of manure on the content and uptake of macroelements by meadow sward.* Zesz. Probl. Post. Nauk Rol. 494: 17-26. (in Polish)

ANTONKIEWICZ J., JASIEWICZ C. 2009. *Organic pollutants in sewage sludge*. Zesz. Probl. Post. Nauk Rol., 537: 15-23. (in Polish)

CAI Q.Y., MO C.H., WU Q.T., ZENG Q.Y. & KATSOYIANNIS A. 2007. *Concentration and speciation of heavy metals in six different sewage sludge-composts*. J. Hazard. Mat., 147: 1063-1072. DOI. 10.1016/j.jhazmet.2007.01.142.

CHIBA M.K., MATTIAZZO M.E., OLIVEIRA F.C. 2009. *Sugarcane field in Untisol, Rusing sewage sludge as a phosphorus source.* Acta Sci-Agron., 31(3):495-501.

CZEKAŁA J. 2008. *Chemical properties of compost produced from municipal sewage sludge and various bio-waste.* J. Res. Appl. Agric. Engine., 53(3): 35-41. (in Polish)

CZUBA R. 1996. *Mineral fertilization of crops*. Wyd. Zak. Chem. Police, ss 413. (in Polish)

DINESH R., CHAMDHURI S.G., SHEEJA T.E., 2004. *Soil biochemical and microbial indices in wet tropical forests: Effect of deforestation and cultivation.* J. Plant Nutr. Soil Sci., 167:(1), 24-32. DOI:10.1002/jpln.200321254

GORLACH E. 1992. *Some ecological aspects of production and use of organic fertilizers.* Mat. Konf. Nauk. „Nawozy organiczne", Szczecin 8-9 września 1992, 5-13. (in Polish)

GORLACH E., GAMBUŚ F. 1997. *Phosphate and compound fertilizers as a source of soil contamination with heavy metals*. Zesz. Probl. Post. Nauk Rol., 448a:139-146. (in Polish)

HARGREAVES J.C., ADL M.S., WARMAN P.R. 2008. *A review of the use of composted municipal solid waste in agriculture*. Agric. Ecosys. Environ**.,** 123, 1-14. DOI. 10.1016/j.agee.2007.07.004

HAROUN, M., IDRIS, A., OMAR, S.R.S. 2007. *A study of heavy metals and their fate in the composting of tannery sludge.* Waste Manage., 27(11): 1541-1550. DOI:10.1016/j.wasman.2006.09.006

HE M., TLAN G., LIANG X. 2009. *Phytotoxicity and speciation of copper, zinc and lead during the aerobics composting of savage sludge*. J. Hazard. Mater., 163: 671-677. DOI.10.1016/j.jhazmat.2008.07.01

IŻEWSKA A. 2007. *Effect of fertilization with manure, sewage sludge and sludge compost on soil properties.* Zesz. Probl. Post. Nauk Rol., 518, 85-92. (in Polish)

JASIEWICZ CZ., ANTONIEWICZ J., MAZUR Z., KRAJEWSKI W. 2007. *Agrochemical properties of soil fertilized with sewage sludge from sewage treatment plant in Olecko*. Ecol. Chem. Eng., 14(5-6): 457-463. (in Polish)

JAKUBUS M. 2006. *Effect of long-term applications of sewage sludge on changes in selected soil chemical properties*. Zesz. Probl. Post. Nauk. Rol., 512: 209-219. (in Polish)

KABATA-PENDIAS A., PENDIAS H. 1999. *Biogeochemistry of trace elements.* Wyd. Nauk. PWN, Warszawa, ss 364. (in Polish)

KASPERCZYK M., RADKOWSKI A., KACORZYK P. 2001. *Force of fertilizer bovine manure.* Pam. Puł., 125: 37-42. (in Polish)

KRZYWY-GAWROŃSKA E. GUTOWSKA I. 2007. *The content of macro-and micronutrients in perennial ryegrass after application of compost from potato pulp and municipal sewage sludge.* Wyd. IMUZ Raszyn, Woda, Środowisko, Obszary Wiejskie, 7, 2a(20): 231-244. (in Polish)

KRZYWY-GAWROŃSKA E. 2009. *Investigations of the effect of compost from municipal sewage sludge and the active substance PRP Sol on soil fertility and productivity*. Monografia, Wyd. ZUT, Szczecin, 96. (in Polish)

NGOLE V.M., 2007. *Response of copper, lead and zinc mobility and bioavailability to sludge application on different soils*. Pol. J. Soil Sci., 40(2): 125-138.

Regulation of the Ministry of the Environment on municipal sewage sludge. Official Journal of Law No. 137, item 924 of 2010. (in Polish)

Regulation of the Ministry of Agriculture and Rural Development of 18 June 2008 on the execution of some provisions of the Act on fertilisers and fertilisation. Official Journal of Law No. 119, item 765 of 2 July 2008. (in Polish)

SAHA S., PRAKASH V., KUNDU S., KUMAR N., MINA B.L. 2008. *Soil enzymatic activity as affected by long-term application of farm yard manure and mineral fertilizer under a rainfed soybean wheat system in N-W Himalaya.* Europ. J. Soil Biol., 44: 309-315.

SELIVANOVSKAYA S.YU., LATYPOVA V.Z. 2006. *Effect of composted sewage sludge on microbial biomass, activity and pine seedlings in nursery forest.* Waste Manage., 26: 1253-1258. DOI: 10.1016/j.wasman.2005.09.018

SINGH R.P., AGROWAL M. 2007. *Potential benefits and risks of land application of sewage sludge*. Waste Manage., 28:347-358. DOI: 10.1016/j.wasman.2006.12.010

TORRI S.I., LAVADO R. 2008. *Zinc distribution in soils amended with different kinds of sewage sludge*. J. Environ. Manage., 88(4): 1571-1579. DOI: 10.1016/j.jenvman.2007.07.026

WALTER I., MARTINEZ F., CALA V. 2006. *Heavy metal speciation of phototoxic effects of three representative sewage sludges for agricultural uses*. Environ. Pollut, 139: 507-514. DOI.10.1016/j.envpol.2005.05.020

VENKATESAN S., SENTHURPANDIAN V.K. 2006. *Comparison of enzyme activity with depth under tea plantations and forested sites in south India.* Geoderma, 137: 212-216.

ZHAO Y., WANG P., LI J., CHEN Y., YING X., LIU S. 2009. *The effect of two organic manures on soil properties and crop yields on a temperate calcareous soil under a wheat-maize cropping system.* Europ. J. Agron., 31(1): 36-42. DOI:10.10164/eja.2009.03.001

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AN ASSESSMENT OF THE EFFECT OF POTASSIUM FERTILIZING SYSTEMS ON MAIZE NUTRITIONAL STATUS IN CRITICAL STAGES OF GROWTH BY PLANT ANALYSIS

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Abstract

Yield of maize depends on nitrogen supply to the plant at critical stages of the formation of main yield components. In the rain-fed production system, the productivity of applied nitrogen is conditioned by water supply to growing crop. Potassium is considered to be a nutrient significantly affecting water use by crops. Therefore, an in-season nitrogen balance is needed to adjust N levels and predict yields. These assumptions were validated in a long-term static field experiment with four levels of potassium fertilizing. They were differentiated by two soil fertility levels (Medium, High) and applied fertilizer (K-, K+). The nutritional status of maize was evaluated at two stages: 5th leaf and the beginning of flowering, using three approaches: i) nutrient concentration, ii) nitrogen to other nutrient ratios, iii) DRIS (Diagnosis Recommended Integrated System) method. These studies showed that potassium management was the key contributor to the year-to-year grain yield variability. Thus, grain yield was the basis for verification of the tested indices. At the 5th leaf stage only phosphorus (concentration and N/P ratio) can be considered as a potential predictor of the final yield of grain. The ear leaf as a vegetative part proved to be useful for evaluation of nutritional indices in maize and for making predictions about yield. The optimal system of potassium management can be defined according to its impact on the N/K, N/Mg and N/Ca ratios and, consequently, on yields of maize. The N/K ratio reached the saturation status, as presented by the quadratic regression model. The other two ratios affected grain yield in accordance to the linear regression model. The applied DRIS procedure corroborated the importance of potassium management as a factor significantly affecting the nutritional balance in maize. This crop has a potential to produce high yield of grain on medium K fertile soil provided potassium fertilizer is supplied whenever needed.

Keywords: maize, nutrient concentration, nitrogen to nutrient ratios, DRIS, critical stadia of nutrient evaluation.

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OCENA WPŁYWU SYSTEMU NAWOŻENIA POTASEM NA STAN ODŻYWIENIA KUKURYDZY W KRYTYCZNYCH FAZACH WZROSTU METODĄ ANALIZY ROŚLINNEJ

Abstrakt

Plon kukurydzy zależy od stanu zaopatrzenia rośliny w azot w krytycznych fazach formowania elementów struktury plonu ziarna. W naturalnych warunkach produkcji efektywność azotu jest uwarunkowana stanem zaopatrzenia rośliny w wodę. Potas jest uznawany za czynnik wpływający na wykorzystanie wody przez rośliny uprawne. Zatem w okresie wegetacji jest konieczny bilans żywieniowy azotu, zarówno do jego korekty, jak i predykcji plonu. Przyjęte założenia zweryfikowano na podstawie wieloletniego stałego doświadczenia polowego z zastosowaniem 4 systemów gospodarki potasem określonej na podstawie poziomów żyzności gleby (średni, wysoki) i bieżąco stosowanego nawożenia potasem (K-, K+). Stan odżywienia kukurydzy określono w stadium 5. liścia i na początku kwitnienia. Sposób gospodarowania potasem okazał się głównym czynnikiem zmienności plonów ziarna w latach, co przyjęto jako podstawę do weryfikacji testowanych indeksów. W stadium 5. liścia tylko fosfor (koncentracja, stosunek N do P) można rozważać jako potencjalny predykator plonu ziarna. Liść podkolbowy okazał się częścią rośliny przydatną do opracowania zarówno indeksów stanu odżywienia, jak i predykcji plonu ziarna. Optymalny system gospodarki potasem można określić, bazując na wpływie tego czynnika na wielkość stosunków N do K, N do Mg i N do Ca, a w konsekwencji na plon ziarna. Stosunek N do K osiągnął stan wysycenia, na co wskazuje uzyskany model regresji 2° i wyznaczone optimum. Pozostałe stosunki kształtowały plon ziarna zgodnie z modelem regresji liniowej. Zastosowana procedura DRIS potwierdziła znaczenie gospodarki potasem jako czynnika istotnie kształtującego stan bilansu żywieniowego kukurydzy. Ta roślinna ma duży potencjał do produkcji ziarna na glebach o średniej zasobności w potas, pod warunkiem bieżącego nawożenia tym składnikiem.

Słowa kluczowe: kukurydza, koncentracja składników pokarmowych, stosunek azotu do składników pokarmowych, DRIS, krytyczne stadia oceny stanu odżywienia.

INTRODUCTION

Yield of maize depends on nitrogen supply to plant at critical stages of two principal components of grain yield formation. The first one, establishing during the vegetative period of maize growth, is the number of kernels per plan (NKP). The second one, weight of the individual kernel, in practice used as 1000 kernel weight (TKW), reveals during the grain filling period (Otegui, Bonhomme 1998). Both components create as a sink for carbohydrates produced by leaves, considered as the source of assimilates. The critical period for the NKP extends from the stage of 8^{th} leaf (BBCH 18) up to the stage of watery ripe of maize (BBCH 71) (Grzebisz et al. 2008*b*, Subedi, Ma 2005). In rain-fed agricultural systems, the productivity of applied nitrogen is conditioned by water supply to growing crop. Water, because of its scarcity should be used with the highest efficiency, which in turn is governed by plant nutritional status. Potassium due to its impact on many physiological processes plays an important role in water use by crop plants (Grzebisz et al. 2013, Oosterhuis et al. 2013).

An effective system of maize plant nutritional status evaluation requires an implementation of very reliable analytical tools. Tissue analysis seems to be the useful method for making a quick assessment of nitrogen status in maize during the whole period of the NKP formation. The reliability of tissue analysis and resulting fertilization recommendations require an accuracy in six following steps of conducted diagnosis: i) method of plant part sampling, ii) laboratory methods of nutrient concentration determination, iii) standard nutrient ranges for laboratory data comparison, iv) interpretation procedures of the obtained data, v) methods for working out fertilizing recommendations, and vi) a simplicity of the advisory report (PARENT 2011). Most of this method relies on a fixed concentration range of a given nutrient in the indicative plant part, termed as the saturation level/range (JONES et al. 1990). It is assumed that a crop at the saturation range can reach the maximum productivity. Tissue analysis in maize is the most advanced for the ear leaf, considered as the stage the most sensitive with respect to the NKP formation (JONES et al. 1996). Therefore, the most standard ranges and research studies are broadly devoted to this particular stage of maize development (CAMPBELL, PLANK 2000, JONES et al. 1990, POTARZYCKI 2010). The Diagnosis and Recommended Integrated System is one of the most sophisticated analytical tool for maize nutritional status assessment. This method relies on the relationship between studied nutrients and defined standards (ELWALI et al. 1985).

The most advantage of ear leaf analysis is to make a yield prediction (SOLTANPOUR et al. 1995). The main disadvantage is low practical applicability of obtained models. In spite of this stage proximity to flowering, there is no chance to make corrections of maize nutritional status. Simply, this is the end of vegetative growth. For diagnostic purposes much more important are the earliest stages, just before the inflorescences set up. At this particular stage, maize reaches the highest rate of absolute growth, significantly affected by nitrogen supply (GRZEBISZ et al. 2010*a*). There are only a few papers concerning maize nutritional status at the stage of 5th leaf (GRZEBISZ et al. 2008*a*, MALLARINO, HIGASHI 2009).

The key objective of the conducted study was to assess maize nutritional status, oriented on nitrogen balancing, under conditions of four long-term potassium fertilizing systems. The second objective was to compare the final yield predictive usability of two stages of maize growth, i) 5th leaf, and ii) ear leaf at the beginning of flowering.

MATERIALS AND METHODS

Study on nutritional status of maize (variety *Eurostar,* FAO 240) in response to four potassium fertilizing treatments, existing in the long-term experiment (1991), was carried out during three consecutive growing seasons

2004, 2005 and 2006 at RGD Brody (Poznan University of Live Sciences Experimental Station; 16°28'E i 52°44'N). The experimental trial was established on a soil originated from a loamy sand underlined by light loam soil and classified accordingly to Polish as the light soil. The field trial arranged as a three-factorial split-block design, replicated four times, consisted of following factors:

1. Potassium soil fertility level: M (medium); H (high);
2. Potassium rates: - K; + K (100 kg K ha^{-1});
3. N rates: 0, 100, 150 and 200 kg N ha^{-1}.

The tested systems of potassium fertilization are a combination of: i) medium K fertility level without and with fertilizer potassium application (acronym MK-, MK+, respectively), ii) high soil K fertility level without and with fertilizer potassium application (HK-, HK+).

The plant material for the analysis was collected at two stages of maize growth: 5^{th} leaf (BBCH 15) and at the beginning of anthesis (BBCH 61). The individual sample comprised 12 plants per plot, or ear leaves. The samples were dried in 65°C to the constant weight, ground and analyzed for concentration of nitrogen, phosphorus, potassium, magnesium, calcium, copper, zinc and manganese. Nitrogen concentration in plant material was determined by the Kjeldahl method; potassium, calcium, magnesium, zinc, copper and manganese by the FAAS method (Flame Atomic Absorption Spectrophotometry, Varian 250 plus); phosphorus – colorimetrically (Analitykjena Specord 40). Concentrations of all nutrients were expressed on a dry weight basis.

The interpretation of data was conducted by three analytical tools. The first, based on *the saturation level approach*, allows to compare data sets of a given nutrient concentration in plant samples with standard ranges. The second, applied in this study relied on nitrogen ratios with other studied nutrients. Following sets of nutrient's pair was calculated: N/P, N/K, N/Mg, and N/Ca. The Diagnosis and Recommendation Integrated System (DRIS) was used to interpret the complex plant tissue nutrient ratios (WALWORTH, SUMMER 1987).

DRIS indices were then calculated according to following formulae, example for nitrogen:

$$I(N) = (f(N/P) + f(N/K) + f(Mg/N) + f(Ca/N))/ 4$$

when N/P > n/p than $$f(N/P) = \left[\frac{N/P}{n/p} - 1\right] \cdot \frac{1000}{CV}$$

when N/P < n/p than $$f(N/P) = 1 - \left[\frac{n/p}{N/P}\right] \cdot \frac{1000}{CV}$$

where,

N/P – nutrient ratio of N to P contents in the studied crop,
n/p – nutrient ratio of N to P in the DRIS norm (Table 1)
CV – coefficient of variation for n/p ratio for the DRIS norm,
1000 – coefficient of recalculation.

Table 1

A statistical evaluation of nutrient relationships in the ear leaf of maize at the stage of the beginning of anthesis (ELWALI et al. 1985)

Nutrient ratios	Number of observations in the data bank	Mean*	Standard deviation (SD*)
n/p	1909	9.035	2.136
n/k	1908	1.463	0.426
p/k	1909	0.169	0.054
ca/n	1553	0.160	0.057
ca/P	1554	1.447	0.612
ca/k	1553	0.237	0.122
mg/n	1556	0.071	0.029
mg/p	1557	0.639	0.330
mg/k	1556	0.104	0.063
mg/ca	1554	0.465	0.182

* for yielding population in the whole set of examined data

The diagnostic procedure consists of two related stages, calculation of nutrient indices and their interpretation against the DRIS norms. The DRIS norms for maize by ELWALI et al. (1985) were used in this study (Table 1). The sum of indices with recognition of its signs (plus and minus) is always zero. The sum of indices without recognition of its signs, called absolute sum of indices ASI, is as closer to zero as more balanced plant nutritional status is.

RESULTS AND DISCUSSION

Maize at the stage of 5th leaf – BBCH 15

Up to the stage of 5^{th} leaf maize was supplied with 100 kg N ha^{-1} (Table 2). Under Polish conditions, this rate is considered as an optimal for maximizing maize grain yield (KRUCZEK 2005, GRZEBISZ et al. 2010*a*). At this stage of growth maize achieved a sufficiency level of nutrient concentration for nitrogen, potassium, but not for phosphorus and magnesium (SCHULTE, KELLING 1991). The concentration of all nutrients showed a significant year-to-year variability. The highest was observed for potassium. In maize plants grown in 2004, K concentration was twice as low compared to 2005. The latter was the year with the highest yield of grain. *The dilution effect* was noted for magnesium and calcium. Effect of potassium fertilizing systems was significant for all nutrients. Except potassium, a slight declination trend in response to freshly applied potassium fertilizer was also observed for magnesium and calcium. Therefore, this relationship can be considered as the first signal for potential antagonism on plots fertilized with potassium.

Table 2

Nutrient concentration in maize at the stage of 5th leaf (g kg^{-1} DM)

Main factor	Level of the factor	N	P	K	Mg	Ca
Years	2004	38.46[b]	2.822[a]	21.72[a]	1.907[b]	7.322[b]
	2005	40.53[a]	2.929[a]	43.77[c]	1.294[a]	6.457[a]
	2006	41.22[a]	3.794[b]	35.83[b]	1.939[b]	7.793[c]
	F	6.230**	23.70***	123.0***	23.11***	21.89***
Potassium fertilizing systems	MK-	41.53[b]	3.286[b]	27.00[a]	2.079[b]	7.775[b]
	MK+	39.23[a]	3.061[a]	34.51[ab]	1.713[ab]	6.891[a]
	HK-	40.47[a]	3.414[b]	35.35[ab]	1.649[ab]	7.380[ab]
	HK+	39.06[a]	2.966[a]	38.24[b]	1.413[a]	6.716[a]
	F	3.010*	2.618*	16.97***	9.985***	8.258***
Nitrogen rates	0	38.54[a]	3.158	34.35	1.633[a]	6.784[a]
	100	41.60[b]	3.205	33.20	1.794[b]	7.597[b]
	F	21.10***	0.141	0.980	3.396*	23.61***

[a] numbers marked with the same letters are not significantly different; *** -. **. * - probability level at 0.001; 0.01; 0.05 respectively

Among the considered nutrients, only potassium showed a significant year-to-year variability (Figure 1). In the semi-dry, 2004, K concentration was low, on average amounting to 20 g kg^{-1}, in fact, indicating a status of maize plant malnutrition. In addition, any impact of potassium management on its concentration was observed. In 2005, K concentration was the highest, showing the increasing trend in accordance to supply of potassium, both from soil and fertilizer resources (GRZEBISZ, OERTLI 1994). This is in

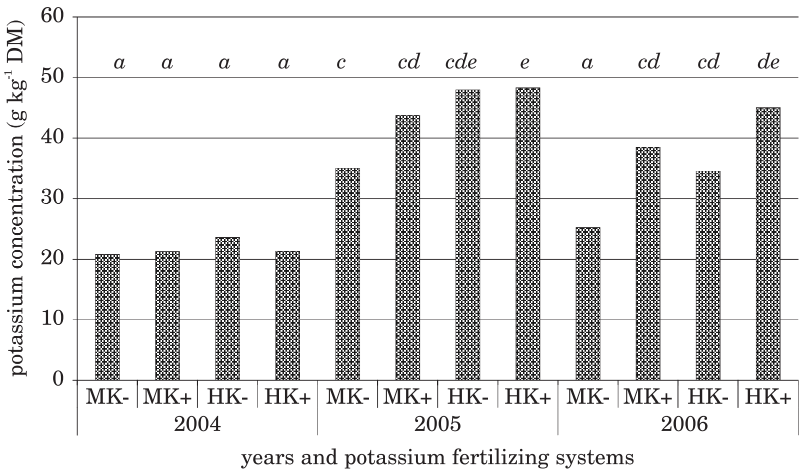

Fig. 1. Effect of potassium fertilizing systems on potassium concentration in maize at the stage of 5th leaf in consecutive years

agreement with Grzebisz et al. (2013), who presented an opinion that the elevated K nutritional status at initial stages of crop growth is a prerequisite of high yield. In 2006, at the stage of 5th leaf, K concentration in maize was significantly affected by freshly applied K fertilizer. It can be therefore concluded, that at the stage of 5th leaf, K nutritional status is not a decisive factor of final grain yield of maize.

The analysis of ratios between nitrogen and other nutrients showed a decisive impact of the course of weather, followed by potassium fertilizing systems (Table 3). The highest year-to-year variability was noted for N/K pair, followed by N/Mg and N/P. In the first case, this ratio was the narrowest in 2005, and the broadest in 2004. The reverse relation was found for N/Ca. Effect of potassium fertilizing systems was significant for two pairs, i.e., N/K and N/Mg. In both cases it was the broadest for maize plants

Table 3

A statistical evaluation of nutrient ratios in maize at the stage of 5th leaf

Main factor	Level of the factor	N/P	N/K	N/Mg	N/Ca
Years	2004	14.16[b]	1.811[c]	20.73[a]	5.282[a]
	2005	14.03[b]	0.959[a]	33.79[b]	6.376[b]
	2006	11.03[a]	1.248[b]	22.90[a]	5.379[a]
	F	32.45***	100.5***	51.31***	23.80***
Potassium fertilizing systems	MK-	13.18	1.655[b]	21.22[a]	5.466
	MK+	13.08	1.277[a]	25.97[b]	5.768
	HK-	12.61	1.267[a]	27.08[b]	5.576
	HK+	13.43	1.158[a]	28.95[b]	5.906
	F	0.916	19.00***	8.507***	1.874
Nitrogen rates	0	12.60[a]	1.296	26.26	5.816
	100	13.55[b]	1.383	25.35	5.543
	F	6.907*	3.020	0.655	3.634

[a] numbers marked with the same letters are not significantly different; ***. **. * – probability level at 0.001; 0.01; 0.05 respectively

grown in the control K plot (MK-). In addition, both pairs showed a considerable year-to-year variability. Therefore, the respective ratios were regressed against yield of grain (GY). The N/K relationship was significant provided years with drought were considered:

$$GY = 1758N/K + 4117 \text{ for } n = 8, R^2 = 0.6 \text{ and } P \leq 0.01$$

The N/Mg relationship was, however, significant when the semi-dry year, i.e., 2004, was excluded from calculation:

$$GY = 93.85N/Mg + 4202 \text{ for } n = 8, R^2 = 0.5 \text{ and } P \leq 0.05$$

Table 4

Coefficients of correlation for grain yield versus nutrient ratios at the stage of 5th leaf (n = 6)

Main factor	Level of the factor	N/P	N/K	N/Mg	N/Ca
Potassium fertilizing systems	MK-	0.891**	-0.43	0.637	0.477
	MK+	0.773*	0.160	0.581	0.522
	HK-	0.708*	-0.01	0.255	0.122
	HK+	0.856**	0.306	0.389	0.382

**. * – probability level at 0.01; 0.05 respectively

It can be concluded, based on the obtained data that both ratios limited yield of grain, but only in years with pronounced drought. This conclusion is in agreement with an opinion expressed by Grzebisz et al. (2013), Grzebisz (2013), Potarzycki (2011) concerning yield forming effect of potassium and magnesium in crop plants.

The detailed study on the effect of potassium fertilizing systems on ratios between nitrogen and other nutrients, implicitly showed on the decisive rule of N/P (Table 4). It was reliable, irrespective of the tested system of potassium fertilizing. Therefore, this pair can be used as a predictor of final yield of maize. This conclusion corroborates studies by Kruczek (2005). The author showed that maize is highly sensitive to phosphorus supply, and its effect is first measurable at the stage of 5th leaves.

The ear leaf – BBCH 61

The ear leaf is the most frequently plant part used in evaluation of maize nutritional status at the beginning of maize flowering (Jones et al. 1990, Campbell et al. 2000). The highest year-to-year variability of nutrient concentration in the tested maize part was observed for phosphorus, followed by nitrogen and magnesium (Table 5). Phosphorus concentration in 2005 was twice as high as in 2006. It is necessary to stress that the highest yield of maize was harvested in 2005 and the lowest in 2006. The same trend, but with slightly lower differences between years, were noted for magnesium. The obtained data sets, based on averages for each particular year, were compared to published standards and/or to be recently published research data (Table 6). Nitrogen and phosphorus concentrations, except 2006, were within standard ranges. Potassium concentration was in the sufficiency range, but only in two contrasting years, 2005 and 2006. The much lower value, as determined, in 2004 can be considered as *the effect of K dilution*. Concentration of magnesium fulfilled the condition of the range by Campbell et al. (2000). It was, however, much below the lower limit in years with water stress. In the case of calcium, its concentration was above standard ranges by Jones et al. (1990) and Potarzycki (2010).

Effect of potassium fertilizing systems on nutrient concentration in the ear leaf were significant for potassium, magnesium and calcium. Potassium concentration was significantly higher in plants grown on the K HK system

Table 5

Nutrient concentration in the cob leaf at the stage of the beginning of anthesis (g kg^{-1} DM)

Main factor	Level of the factor	N	P	K	Mg	Ca
Years	2004	34.77[c]	2.686[b]	14.58[a]	2.169[b]	6.302[b]
	2005	31.15[b]	3.887[c]	18.76[b]	2.678[c]	5.623[a]
	2006	18.33[a]	1.987[a]	18.66[b]	1.831[a]	6.115[b]
	F	442.2***	150.7***	75.06***	76.35***	20.14***
Potassium fertilizing systems	MK-	27.21	2.675	16.47[a]	2.636[c]	6.540[c]
	MK+	28.06	2.925	16.71[a]	2.179[b]	5.911[b]
	HK-	28.11	2.814	18.10[b]	2.194[b]	6.024[b]
	HK+	28.94	3.000	18.05[b]	1.895[a]	5.580[a]
	F	2.220	2.457	7.300***	29.56***	19.48***
Nitrogen rates	0	24.31[a]	2.754	17.76	1.882[a]	5.451[a]
	100	28.43[b]	2.894	17.44	2.331[b]	6.024[b]
	150	29.64[b]	2.880	17.03	2.368[b]	6.339[b]
	200	29.94[b]	2.885	17.09	2.324[b]	6.240[b]
	F	30.00***	0.537	1.130	16.75***	19.39***

[a] numbers marked with the same letters are not significantly different; ***. **. * – probability level at 0.001; 0.01; 0.05 respectively

compared to MK system. No significant differences between objects MK- and MK+, and HK- and HK+ were found. For magnesium and calcium, it was, however, observed an antagonism, as documented by concentration decrease in response to increased supply of potassium. Effect of increasing nitrogen rates revealed for nitrogen, magnesium and calcium. In each case, the N rate of 100 kg N ha^{-1}, was high enough to increase significantly concentration of each of these nutrients. However, only nitrogen concentration responded considerably to the weather course in consecutive seasons.

Concentration of all determined nutrients was significantly modified by interaction of potassium fertilizing systems, which showed a significant year

Table 6

Nutrient sufficiency ranges in maize ear leaf at the stage of the beginning of anthesis, (g kg^{-1} DM)

Nutrients	Jones et al. 1990	Campbell, Plank 2000	Potarzycki 2010
N	26-36	28-40	21.3-33.3
P	2.2-4.0	2.5-5.0	2.3-3.5
K	18-45	18-30	18.8-25.1
Mg	4.3-10.0	2.5-8.0	4.1-6.7
Ca	2.7-3.4	1.5-6.0	2.8-3.6

-to-year variability. The predicted value of each nutrient has been evaluated by simple regression against grain yield. For nitrogen (N) it was significant, but the R^2 increased, when the tested set of data was limited only to dry years:

1. All years: GY = 1039N + 4187 for n = 12 , R^2 = 0.7738 and $P \le 0.001$
2. Years with drought: GY = 936.9N + 4311 for n = 8, R^2 = 0.88 and $P \le 0.001$.

Both equations implicitly indicate the importance of potassium in improvement the N management during the critical stage of yield formation by maize plant. Effect of nitrogen was also supported by higher concentration of magnesium, especially in years with drought:

$$GY = 29909Mg + 886 \text{ for } n = 8, R^2 = 0.59 \text{ and } P \le 0.01$$

The variability of phosphorus concentration in the ear leaf was affected by potassium fertilizing treatments, but showed a quite reverse trend to N and Mg. In this particular case, any increase of P concentration, especially in years with water shortage resulted in the grain yield (GY) decrease:

$$GY = -8642P + 9572 \text{ for } n = 12 , R^2 = 0.69 \text{ and } P \le 0.001.$$

The ameliorative effect of potassium fertilizing systems on K concentration was even more striking. Its significance reveals to provide considering just dry years:

$$GY = -3518K + 12644 \text{ for } n = 8, R^2 = 0.89 \text{ and } P \le 0.001.$$

These trends simple inform, that maize nutritional status at the beginning of maize flowering was significantly affected by water shortage. This trend clearly underlines a disturbance of nutrient management in maize during the period critical for components of yield formation (Jones et al. 1996). The growth disturbance resulted in the lower number of kernels per cob. In consequence, the size of the cob, which is considered as the physiological sink for carbohydrates, produced by maize during the grain filling period, was significantly decreased.

The calculated ratios of nitrogen to other nutrients showed a high response to all studied factors (Table 7). The impact of years was nutrient specific. The narrowest ratio, except N/P, was observed in 2006. Effect of potassium fertilizing systems was significant for each of the studied nutrient ratios, but the most pronounced was noted for N/Mg. In this case, an increase of potassium supply, resulted in the ratio increase. This trend corroborates the above presented hypothesis, concerning a close physiological relation between nitrogen and magnesium. The presented facts are in accordance with Grzebisz et al. (2010b) and Grzebisz et al. (2013), who underlined an importance of the improved management of magnesium for N economy of crop plants. Two of studied ratios, i.e., N/K and N/Ca were significantly affected by potassium fertilizing systems, but underlined a seasonal variability. In the first case, as presented in Figure 2, the N/K increase up to 2.17 resulted in the net yield of the grain increase. At this particular value, a

Table 7

A statistical evaluation of nutrient ratios in maize ear leaf at the stage of the beginning of anthesis

Main factor	Level of the factor	N/P	N/K	N/Mg	N/Ca
Years	2004	13.28[c]	2.440[c]	16.62[c]	5.584[b]
	2005	8.318[a]	1.702[b]	12.45[b]	5.623[b]
	2006	9.287[b]	0.994[a]	10.30[a]	3.020[a]
	F	117.9***	340.3***	92.21***	279.2***
Potassium fertilizing systems	MK-	10.47[ab]	1.718[ab]	10.83[a]	4.180[a]
	MK+	9.756[a]	1.796[b]	13.05[b]	4.862[b]
	HK-	10.84[b]	1.598[a]	13.01[b]	4.678[b]
	HK+	10.11[ab]	1.737[ab]	15.60[c]	5.249[c]
	F	2.791*	3.351*	25.42***	18.53***
Nitrogen rates	0	9.242[a]	1.431[a]	13.47	4.532
	100	10.27[b]	1.708[b]	12.72	4.787
	150	10.88[b]	1.854[b]	13.04	4.756
	200	10.78[b]	1.856[b]	13.27	4.892
	F	7.211***	19.48***	0.695	2.158

[a] numbers marked with the same letters are not significantly different; ***. **. * – probability level at 0.001; 0.01; 0.05 respectively

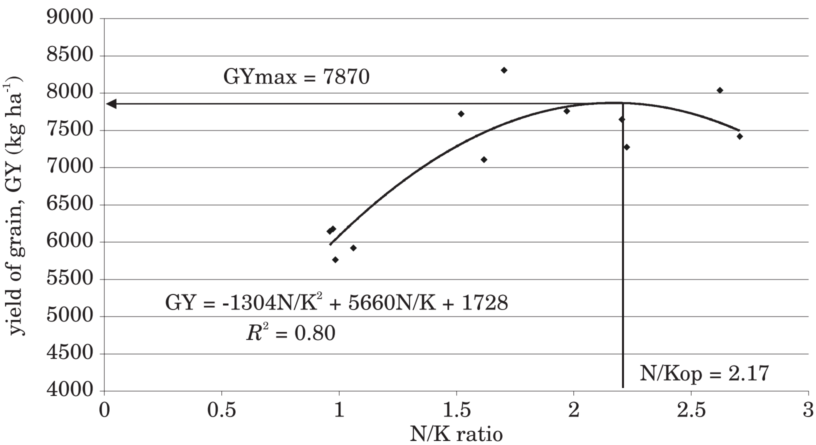

Fig. 2. Grain yield of maize as a function of N/K relationships in the ear leaf at anthesis

maximum yield of 7.87 t ha^{-1} was produced by maize. Further increase in this ratio resulted in grain yield decrease. This negative trend has revealed in the HK+ system of K management. The second case, referred to the N/Ca ratio, implicitly indicates on importance of N supply to plants during the period of growth impedance by stress:

$$GY = 580.2N/Ca + 4355 \text{ for } n = 12, R^2 = 0.80 \text{ and } P \leq 0.001.$$

Based on above presented data, it can be assumed, that any factor improving N economy of maize crop during the period of water stress, in turn increases yield of grain. In the studied case, effect of potassium fertilizing systems revealed via improvement in potassium, magnesium, calcium productivity. In has been observed the best growing conditions for maize revealed in treatments with broadened ratios of N/K, N/Mg, and N/Ca. These explanations have been fully corroborated by the detailed analysis of nutrient ratios on the harvested yield of grain (Table 8). The N/K ratios significantly affected yield of maize in all, except the HK+, systems. In this treatment, the applied K fertilizer resulted in the decrease of impact of this ratio on final yield of maize. The N/Mg ratio was significant only in the MK+ system, stressing on importance of magnesium for N management. The N/Ca ratio significantly influenced the grain yield, irrespectively on K fertilizing system. The highest production effect was related to the medium K fertile soil.

Table 8

Coefficients of correlation for grain yield versus nutrient relationship in the ear leaf, at the stage of the beginning of anthesis (n = 12)

Main factor	Level of the factor	N/P	N/K	N/Mg	N/Ca
Potassium fertilizing systems	MK-	0.227	0.780**	0.083	0.808***
	MK+	0.125	0.667**	0.700**	0.902***
	HK-	0.447	0.798***	0.414	0.689**
	HK+	0.065	0.494	0.390	0.718**

***-. **. * – probability level at 0.001; 0.01; 0.05 respectively

An evaluation of maize nutritional by the DRIS method

The DRIS method was applied to evaluate nutritional status of maize, taking into account all tested elements and ratios between them (Table 9). The Absolute Sum of Indices (ASI) is a good indicator of nutritional trends as imposed by increasing N rates on the background of potassium fertilizing systems. In general, the ASI significantly decreased, when maize was grown both on K fertile soil (HK main plot) and at the same time fertilized currently with potassium (K+ treatments). Consequently, the lowest ASI, amounting to 16.76 was noted for the most intensive K system, i.e., KH+.

Table 9

As assessment of maize nutritional status by the DRIS procedure at the stage of the beginning of anthesis

Potassium fertilizing systems	N rate, (kg ha^{-1})	Nutrient indices (DRIS)					Limiting nutrients	Absolute sum indices, (ASI)
		N	P	K	Mg	Ca		
MK-	0	-7.55	-7.10	-1.45	3.99	12.11	N ≥ P > K	32.20
	100	-1.95	-8.63	-6.04	5.50	11.13	P > K > N	32.25
	150	-2.66	-9.63	-9.25	8.10	13.45	P > K > N	43.09
	200	-2.01	-8.36	-7.87	6.40	11.85	P ≥ K > N	36.49
MK+	0	-3.01	-2.37	-1.83	-0.73	7.95	N > P > K > Mg	15.89
	100	-1.62	-2.75	-5.72	1.42	8.67	K > P > N	20.18
	150	0.59	-4.93	-6.75	2.13	8.96	K > P	23.36
	200	0.54	-4.00	-5.76	0.19	9.02	K > P	19.51
HK-	0	-6.72	0.13	1.48	-2.81	7.92	N > Mg	19.06
	100	-0.70	-7.69	-2.89	2.54	8.74	P > K > N	22.56
	150	0.96	-8.05	-4.31	1.32	10.08	P > K	24.72
	200	0.78	-7.46	-4.15	1.27	9.56	P > K	23.22
HK+	0	-0.04	-2.99	1.12	-5.6	7.51	P > Mg	17.26
	100	-1.30	-1.86	-1.95	-1.15	6.25	K ≥ P > N ≥ Mg	12.51
	150	1.18	-1.72	-3.25	-3.71	7.50	Mg ≥ K > P	17.36
	200	2.95	-3.48	-4.65	-1.83	7.01	K > P > Mg	19.92

The increasing N rates, except the KH+ plot, resulted in the ASI increase. However, it was not the most productive system. The main reason was to broaden N/K ratio.

The DRIS procedure allows also to indicate a nutrient(s) limiting yield of a given crop. In the studied case, the limiting nutrient was dependent on the potassium management system. It is obvious that nitrogen was the grain yield limited nutrient in the N fertilizing control. The exception was the HK+ plot, where N was in balance. In both treatments without fresh potassium application (K-), yield of grain was the most limited by phosphorus, followed by potassium. This place for phosphorus seems to be strange, in the light of its negative relationship with grain yield. This unexpected result is due to its low concentration in ear leaf in years with water shortages. It simply indicates on disturbance of P uptake by water stressed plants. The negative P impact on final grain yield was probably not due to its surplus but due to low capacity of maize cob to accumulate assimilates produced by maize during the grain filling period.

CONCLUSIONS

1. At the stage of 5th of maize growth N/P concentration relationship can be used as the first predictor of final yield of maize.

2. Potassium concentration in maize at the stage of 5th leaf impacts significantly maize nutritional status. The N/K ratio can be used for yield prediction only in years with temporary drought.

3. At the beginning of flowering nitrogen and magnesium concentration significantly depends on potassium management, resulting in the grain yield increase.

4. The ear leaf is a useful maize vegetative part to define an advanced symptoms of the crop nutritional status disturbance.

5. Any extension of N/K, N/Mg and N/Ca ratios due to effective potassium fertilizing system can be considered as the prerequisite of increasing yield of maize.

6. The DRIS is a useful procedure for evaluation of maize nutritional status at the beginning of flowering; in the studied case, corroborating the significant impact of potassium management on maize nutritional status.

7. The best growing conditions for maize are created on the medium K fertile soil, currently fertilized with potassium; these management system is a prerequisite of a high uptake K potential of maize.

REFERENCES

Campbell C.R., Plank C.O. 2000. *Reference sufficiency ranges field crops - corn.* In: C.R. Campbell (ed.) *Reference sufficiency ranges for plant analysis in the southern region of the United States.* Available online at: http://www.ncagr.gov/agronomi/saaesd/corn.htm (Verified 2010.12.30).

Elwali A.M.O., Gascho G.J., Summer ME.E. 1985. *Dris norms for 11 nutrients in corn leaves.* Agron. J., 77: 506-508.

Grzebisz W., Oertli J.J. 1994. *Evaluation of universal extractants for determining plant available potassium in intensively cultivated soils*. Comm. Plant Soil Anal., 24(11-12): 1295-1305.

Grzebisz W., Wrońska M., Diatta J.B., Dullin P. 2008*a*. *Effect of zinc application at early stages of maize growth on the patterns of nutrients and dry matter accumulation by canopy.* Part I. *Zinc uptake patterns and its redistribution among maize organs*. J. Elementol., 13(1): 17-28.

Grzebisz W., Wrońska M., Diatta J.B., Szczepaniak W. 2008*b*. *Effect of zinc application at early stages of maize growth on the patterns of nutrients and dry matter accumulation by canopy.* Part II. *Nitrogen uptake and dry matter accumulation patterns*. J. Elementol., 13(1): 29-39.

Grzebisz W., Baer A., Barłóg P., Szczepaniak W., Potarzycki J. 2010*a*. *Effect of nitrogen and potassium fertilizing systems on maize grain yield*. Fertilizers Fertilization. 40: 45-56.

Grzebisz W., Przygocka-Cyna K., Szczepaniak W., Diatta J.B., Potarzycki J. 2010*b*. *Magnesium as a nutritional tool of nitrogen efficient management - plant production and environment*. J. Elementol., 15(4):771-778.

GRZEBISZ W., GRANSEE A., SZCZEPANIAK W., DIATTA J.B. 2013. *The effects of potassium fertilization on water use-efficiency in crop plants*. J. Plant Nutr. Soil Sci., 176: 355-374.

GRZEBISZ W. 2013. *Crop response to magnesium fertilization as affected by nitrogen supply* Plant Soil, 368: 23-39.

JONES J.B., ECK H.V., VOSS R. 1990. *Plant analysis as an aid in fertilizing corn and grain sorghum.* In: *Soil testing and plant analysis*. R.L. WESTERMAN, (ed.) 3rd Soil Sci. Soc. Am. J., Book Ser. 3, Medison, WI: 521-527.

JONES R. J., SCHREIBER B. M.N., ROESSLER J.A. 1996. *Kernel sink capacity in maize: Genotypic and maternal regulation*. Crop Sci., 36: 301-306.

KRUCZEK A. 2005. *Phosphorus utilization from fertilizer and accumulation of mineral components in the initial stage of maize development*. Pol. J. Environ. Stud., 14(4): 467-475.

MALLARINO A.P., HIGASHI S.L. 2009. *Assessment of potassium supply for corn by analysis of plant parts*. Soil Sci. Soc. Am. J., 73: 2177-2183.

OOSTERHUIS D.M., LOKA D.A., RAPER T.B. 2013. *A Potassium and stress alleviation: Physiological functions and management of cotton*. J. Plant Nutr. Soil Sci., 176: 331-343.

OTEGUI M., BONHOMME R. 1998. *Grain yield components in maize.* I. *Ear growth and kernel set*. Field Crops Res., 56: 247-256.

PARRENT L.E. 2011. *Diagnosis oft he nutrient compostional space of fruit crops*. Rev. Bras. Frutic., Jaboticabal – SP, 3391: 321-334.

POTARZYCKI J. 2010. *Influence of balanced fertilization on nutritional status of maize at anthesis*. Fertilizers Fertilization, 39: 90-108.

POTARZYCKI J. 2011. *Effect of magnesium or zinc suplementation at the background of nitrogen rate on nitrogen management by maize canopy cultivated in monoculture.* Plant Soil Environ., 57: 19-25.

SCHULTE E.E., KELLING K.A. 1991. *Analysis: a diagnostic tool.* University of Wisconsin-Madison, NCH-46 Crop Fertilization, RR 4/91. Available online at: http://ces.purdue.edu/extmedia/NCH/NCH-46.html (Verified 2012.05. 25).

SOLTANPOUR P.N., MALAKOUTI M.J., RONAGHI A. 1995. *Comparison of diagnosis and recommendation integrated system and nutrient sufficiency range for corn*. Soil Sci. Soc. Am. J. 59: 133-139.

SUBEDI K., MA B. 2005. *Nitrogen uptake and partitioning in stay-green and leafy maize hybrids.* Crops Sci., 45: 740-747.

WALWORTH J.L., SUMMER M.E. 1987. *The Diagnosis and recommendation integrated system (DRIS)*. Adv. Soil Sci., 6: 149-188.

J. Elem. s. 549–566 DOI: 10.5601/jelem.2014.19.1.575

IMPACT OF INCREASING NITROGEN RATES ON THE COURSE OF THE NITROGEN CRITICAL CONCENTRATION CURVE DURING THE VEGETATIVE GROWTH OF WINTER WHEAT

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Abstract

Nitrogen is the dominant factor affecting the rate of wheat growth and yielding. Water supply during the critical stages of yield component formation is a factor limiting nitrogen use efficiency. A study dealing with this problem has been conducted, based on a long-term field experiment with four N levels (0, 60, 120, 180 kg N ha^{-1}) against the background of four systems of potassium management, including: medium and high K soil fertility levels without and with K fertilizer (MK-, MK+, HK-, HK+). The objective of the study was to evaluate the wheat nitrogen nutritional status during the vegetative period of wheat growth according to nitrogen concentrations in leaves and stems. The research was run in 2003, with severe deficit of water, and in 2004, under semi-dry water conditions. Grain yield of wheat responded to both experimental factors only in 2004. Nitrogen concentration in plant parts was dependent only on N doses, thus underlying good adaptation of wheat to the seasonal course of water supply. Nitrogen concentration in leaves followed the quadratic regression model. This type of response indicates an N saturation status, i.e. a non-limiting effect of this factor on plant growth. The pattern of N concentration in stems was in accordance with the linear regression model. This type of response indicates the N-limited growth due to an insufficient supply of nitrogen. The Critical Nitrogen Concentration (CNC) pattern showed significant adaptability of wheat to N fertilizer levels. The Nitrogen Nutrition Index, calculated from the CNC, can serve as an indicator of N dilution during the vegetative period of wheat growth.

Keywords: nitrogen concentration, plant organs, vegetation, dilution effect, wheat.

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WPŁYW WZRASTAJĄCYCH DAWEK AZOTU NA PRZEBIEG KRZYWEJ KRYTYCZNEJ KONCENTRACJI AZOTU W PSZENICY OZIMEJ W OKRESIE WZROSTU WEGETATYWNEGO

Abstrakt

Azot jest czynnikiem dominującym, kształtującym szybkość wzrostu pszenicy i plonowanie. Zaopatrzenie rośliny w wodę w krytycznych fazach formowania elementów struktury plonu jest głównym czynnikiem ograniczającym efektywność azotu. Problem ten badano w ramach wieloletniego doświadczenia statycznego, stosując 4 poziomy nawożenia azotem (0, 60, 120, 180 kg N ha^{-1}) na tle 4 systemów gospodarki potasem, w tym: o średnim i wysokim poziomie zasobności w przyswajalny azot oraz bez aplikacji potasu lub z bieżącą jego aplikacją (MK-, MK+, HK-, HK+). Celem badań była ocena stanu odżywienia pszenicy azotem w okresie wzrostu wegetatywnego. Badania prowadzono w 2003 r. o dużych niedoborach wody i 2004 r. – z niedoborami okresowymi. Plony pszenicy ozimej wykazały reakcję na oba czynniki doświadczalne tylko w 2004 roku. Koncentracja azotu w częściach rośliny zależała tylko od dawek azotu, co świadczy o dużej zdolności adaptacyjnej pszenicy do reżimu pogodowego w okresie wegetacji. Koncentracja azotu w liściach przebiegała zgodnie z kwadratowym modelem regresji. Taki typ reakcji na wzrastające dawki azotu wskazuje na stan wysycenia rośliny-organu azotem, co oznacza brak ograniczającego działania tego czynnika wzrostu. Natomiast model koncentracji azotu w źdźbłach był zgodny z modelem regresji liniowej. Ten model reakcji rośliny-organu wskazuje na ograniczenie wzrostu, wywołane niedostatecznym zaopatrzeniem w azot. Krzywa krytycznej koncentracji azotu (KKKN) wykazała bardzo dużą plastyczność reakcji pszenicy na dawki nawozowe azotu. Indeks odżywienia azotem (ION), obliczony na podstawie KKKN, może być zastosowany jako wskaźnik rozcieńczenia azotu w okresie wzrostu wegetatywnego pszenicy.

Słowa kluczowe: zawartość azotu, organy rośliny, wegetacja, efekt rozcieńczenia, pszenica.

INTRODUCTION

Nitrogen supply to growing plants, due to its strong impact on dry matter partitioning among plant organs, is considered as the main growth factor (Rubio et al., 2003). Nitrogen uptake rate depends on both its concentration in the soil solution, soil water content, and plant uptake potential as related to its nutritional status (Forde, Lorenzo 2001). The key challenge for agronomists is to reach during the crucial stages of yield component's formation the required range of nitrogen concentration. Nitrogen concentration in a plant during its life-cycle is not constant, changing in accordance to stage of growth, showing a specific dilution pattern for cereals (Faber 2004, Bartczak 2008), maize (Grzebisz et al., 2008). The principal reason for this phenomenon, is dry matter redistribution between metabolic, photosynthetic active parts, and structural -photosynthetic passive parts. The main reason for N dilution is a much faster growth rate of constitutional versus leaves during the vegetative period of the life-cycle. The concept of Critical Nitrogen Concentration (CNC) assumes that maximum biomass production in a defined environment requires an adequate N concentration in plant tissues (Lemaire et al. 2008). The general relationship between both components is described using the power function:

$$N_c = a\mathrm{DM}^{-b},$$

where:

N_c – critical nitrogen concentration (% DM),
DM – yield of dry matter (t ha^{-1}),
a, b – coefficients.

A specific role in each crop response to water and nitrogen stresses is attributed to potassium. Under ample-water condition, the mass-flow of nitrate-nitrogen towards the roots is the main route of plant requirement's covering. The hormone signaling cascade has been recently considered as the key factor changing the route of assimilates partitioning among plant organs in response to decline nitrogen-nitrate concentration in the soil solution on one side and the systemic response of aboveground parts on the other (Debaeke, Aboudrare 2004, Gonzalez-Dugo et al. 2010). Potassium is involved in plant response to water stress. This nutrient undergoes a constant circulation among plant parts, being responsible for new tissue's growth, including roots. Therefore, plants well supplied with potassium are capable to develop fresh roots, in turn exploring soil patches rich in water and nitrogen. Consequently, it is assumed that plant crops well-supplied with potassium are able to overcome to some extend a water stress due to increased uptake of soil nitrogen (Grzebisz et al. 2013).

The main objective of the study was to validate the concept of critical nitrogen concentration on the background of progressive nitrogen fertilizer rates and four systems of potassium fertilization in winter wheat during its vegetative growth.

MATERIALS AND METHODS

Study on winter wheat response to increasing nitrogen rates on the background of four potassium systems were carried out during two consecutive growing seasons 2002/03 and 2003/04 at Brody (Experimental Station of the Poznan University of Life Sciences). The long-term experimental trial was established in 1991 on a soil originated from a loamy sand underlined by a light loam soil, classified as Albic Luvisol. Three-factorial, split-block experiment, replicated four times, included following factors:

1. Soil potassium fertility level: medium, M and high, H;
2. Fresh potassium application: K- (without potassium) and K+ (100 kg K ha^{-1});
3. Four rates of nitrogen: 0, 60, 120 and 180 kg N ha^{-1}.

The tested systems of potassium fertilization, comprising two first factors are as follows: i) medium without fresh potassium application (acronym

MK-), ii) medium with fresh potassium application (MK+), iii) high without fresh potassium application (HK-), high with fresh potassium application (HK+).

Each year maize was a preceding crop for wheat. The size of the main plots was 53.4 m^2 and the individual plot of 13.35 m^2. The variety *Zyta* was sown in the last decade of September. Phosphorus and potassium were applied prior to sowing in rates adjusted to the soil test values and treatment. Phosphorus was applied in the form of triple super phosphate and potassium as potassium chloride. Nitrogen (ammonium saltpeter) was applied at equal rates of 60 kg N ha^{-1} in accordance to the experimental design, i.e., i) before Spring regrowth ii) at the end of tillering, iii) at the stage of the flag leaf appearance. At maturity, crops were harvested from the area of 8.4 m^2 using a plot combine harvester. Total grain yield was adjusted to 14% moisture content.

Plants for assessment of dry mater dynamics were sampled from an area of 1 m^2 in three consecutive stages of wheat growth according to the BBCH scale: 31, 37, and 59. At each sampling date, the harvested plant sample was partitioning, in accordance to stage of development, into subsamples of leaves, stems, and ears then dried (65°C). Finally, at each stage total and sub-sample dry matter per 1 m^2 was recorded.

The French and Schulz's approach of the water limited yield (WLY), modified by GRZEBISZ et al. (2013) into a graphical form, was applied to discriminate yield fraction dependent on the volume of transpired water, and those induced by potassium fertilizer. The algorithms for the water limited yield (WLY) calculation was as follows:

$$WLY = TE\,(R - \Sigma E_s) + WR,$$

where:

TE refers to the transpiration efficiency (TE = k/VPD;
k – biomass/transformation ratio;
VPD – vapor pressure deficit);
R – the sum of rainfall during the growth period;
E_s – the seasonal soil evaporation, equals to 110 mm;
WR – water reserves in the rooted soil volume at the beginning of growth of a particular crop.

Nitrogen concentration in all samples was determined by standard macro-Kjeldahl procedure. In experimental practice the critical nitrogen concentration, N_c, is obtained by plotting data on of empirically determined concentration N (% N in DM) in consecutive stages of plant crop growth *vs.* accumulated shoot biomass DM (t ha^{-1}). The existing relationship is classically described by a mononomial function of biomass DM:

$$N_c = a\mathrm{DM}^{-b},$$

where:

N_c – critical N concentration (% DM),
DM – dry matter yield (t ha^{-1}),
a and b – coefficients of equation.

The obtained N_c values are basic set of data for nitrogen nutrition index (NNI) calculation, which presents relative status of plant nitrogen (LEMAIRE, GASTAL 1997):

NNI = actual N concentration/critical N concentration

The obtained NNIs are expressed as the relative value. Therefore, the NNI index above 1.0 indicates non N-limiting plant crop canopy growth, whereas those below 1.0 reflect N deficiency.

The experimentally obtained data sets were subjected to conventional analysis of variance for the split-plot-plot design. The least significant difference values (LSD at $P \leq 0, 05$) were calculated to establish the significance of mean differences. The simple regression model was applied to determine some relationships between the studied plant characteristics.

RESULTS AND DISCUSSION

Growing conditions and water productivity

The study aimed at nitrogen impact on the seasonal course of critical nitrogen concentration in wheat plants were conducted in two consecutive seasons. Both years significantly differed in weather during spring vegetation (Table 1). The first, 2003, was dry. The whole sum of precipitation from

Table 1

Characteristics of meteorological conditions during spring vegetation of winter wheat*

Months	Sum of precipitation		Long-term average	Mean temperature		Long-term average
	2003	2004		2003	2004	
January	60.2	73.2	36.0	-1.6	-3.5	-1.9
February	74.0	32.4	29.8	-3.2	2.2	-0.8
March	19.9	20.9	38.0	3.4	5.1	2.7
April	21.1	23.3	38.6	8.2	10.0	7.6
May	20.1	44.3	56.2	16.0	13.6	12.9
June	35.0	58.8	66.5	19.8	16.3	16.2
July	96.7	59.6	78.7	19.6	17.3	17.7

* The Synoptic Station Brody

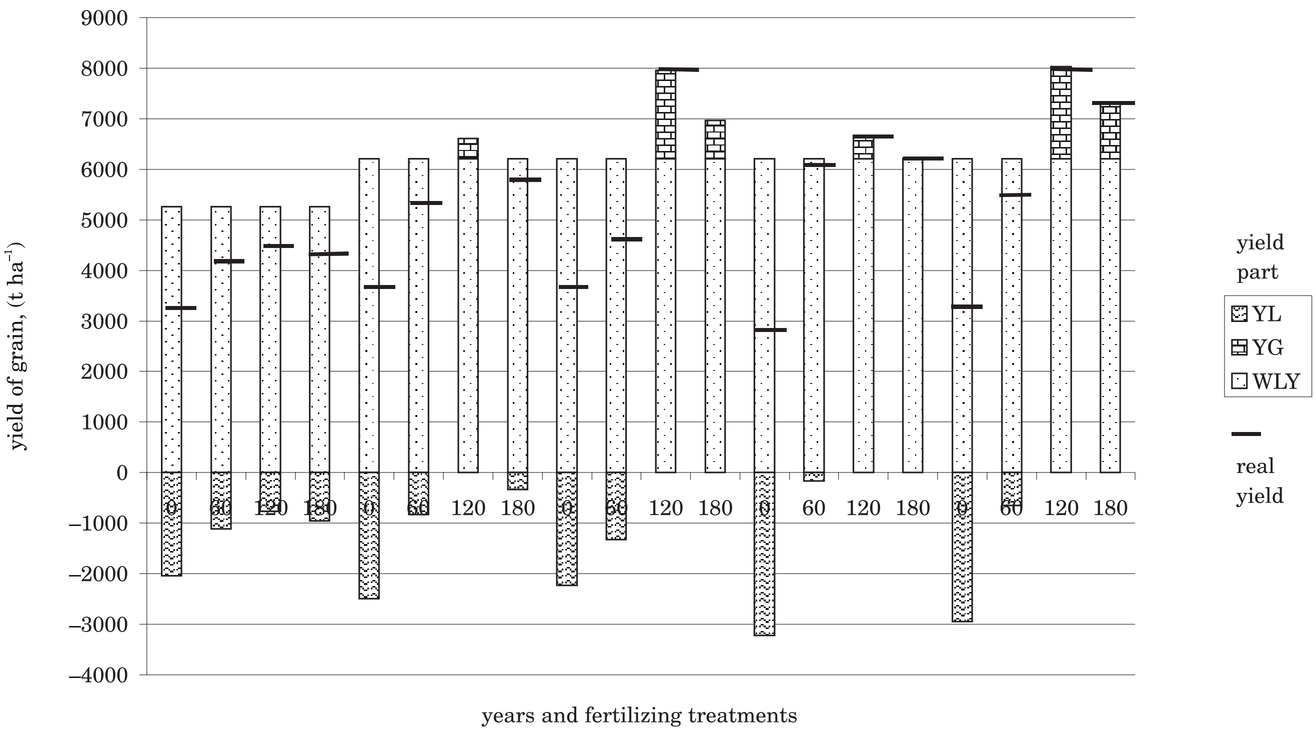

Fig. 1. Yield of winter wheat response to different growing condition in two distinct years –interpretation using the graphical French and Schulz's approach (Grzebisz et al. 2013): WLY – water limited yield, YG – yield gain, YL – yield loss

the third decade of March to the first decade of July amounted to 116.9 mm. In 2004, total sum of precipitation was slightly higher, amounting to 164.4 mm. The total sum of evapo-transpirated water amounted to 447 and 425 mm, in 2003 and 2004, respectively (calculation based on BROUWER, HEIBLOEM 1986*)*. In spite of relatively small differences in this factor, grain yield response to experimental treatments was year-to-year variable. In 2003, the theoretical yield, defined as Water Limited Yield (WLY) was calculated at the level of 5.258 t ha^{-1} and in 2004 at 6.204 t ha^{-1}. The real average yield of wheat in 2003 amounted to 3.2 t ha^{-1} in the control plot and 4.5 t ha^{-1} in the treatment fertilized with 120 kg N ha^{-1}. The harvested yield, in spite of response to fertilizer N, was much below the Water Limited Yield (WLY) – Figure 1. The yield gap due insufficient precipitation was not overcome, even creating favorable conditions for nutrients supply. In 2004, maximum yields were about 8 t ha^{-1}, above the WLY. The yield gain was due to significant impact of nitrogen and potassium on water productivity. In treatments without applied nitrogen, water productivity, as expected was below the WLY. A slightly higher yield loss was noted for high fertility soil. There has also been noted an inefficient water use in all plots fertilized with 60 kg N ha^{-1}. The optimum conditions for water productivity have revealed, irrespectively of K soil fertility level, in treatments with 120 kg N ha^{-1} provide a fresh application of fertilizer potassium (K+). These results corroborate the hypothesis formulated by GRZEBISZ et al. (2013) that only a mild-water stress a plant crop response positively to ample supply of potassium (high K soil fertility and/or K fresh application).

Dry matter yield of wheat in critical stages vegetative growth

Trends of dry matter yield have been evaluated in three crucial stages of wheat growth, i.e., 31, 37 and 59 in accordance to the BCCH scale. The initial one can be considered as a borderline between the exponential and linear biomass growth pattern (YIN et al. 2003). In general, it can be used to make the first evaluation of cereals nutritional status. The second begins the period of an ear growth, being crucial in the number of ears per unit area and spikelets per plant (ZERCHE, HECHT 1999). The third one, ending the heading phase, finishes the vegetative period. In the conducted study, patterns of leave's growth, as related to their biomass, were governed by two factors, i.e., the course of the weather during each of growth seasons and the rate of applied nitrogen (Table 2). Yield of leaves, irrespectively of the year, as recorded in BBCH 31 and 37, increased in accordance to progressing N rates. At the end of vegetative phase biomass of leaves (YL) followed the quadrate regression functions:

2003: $YL2003 = -0.0013N^2 + 0.488N + 56.59$ for $R^2 = 0.94$;

2004: $YL2004 = -0.0048N^2 + 1.453N + 9.96$ for $R^2 = 0.97$.

The presented patterns implicitly indicate on nitrogen availability during wheat vegetative growth as the main limiting factor for biomass of

Table 2

Statistical evaluation of main factors affecting biomass of leaves of winter wheat (g m^{-2})

Experimental factors	Level of factor	2003 (BBCH)			2004 (BBCH)		
		31	37	59	31	37	59
Soil fertility level for K**	M	84.03	80.4	76.55	146.5	152.8	167.2
	H	90.97	118.6	91.97	162.2	165.6	167.6
	LSD 0.05	n.s.*	n.s.	n.s.	n.s.	n.s.	n.s.
Potassium fertilizing***	K-	94.28	100.6	86.19	173.2	169.3	171.7
	K+	80.72	98.38	82.32	135.5	149.0	163.1
	LSD 0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N rates, (kg ha^{-1})	0	59.06	67.68	58.56	96.22	93.44	100.6
	60	115.9	104.0	75.22	212.5	164.8	156.0
	120	-	126.9	102.6	-	219.3	213.4
	180	-	-	100.6	-	-	200.0
	LSD 0.05	17.10	32.59	12.63	23.97	32.33	27.46

* non significant;
** M – medium, H – high soil K fertility level;
*** K- – potassium control, K+ – 100 kg K ha^{-1};

leaves. On average, wheat in 2003 produced only 50% of leaves yield as compared to 2004. In accordance to VOILLOT and DEVIENNE-BARRETT (1999) this drastic reduction can be explained by reduction in N supply from soil resources to growing wheat tissues. The authors of the cited paper showed that N remobilization from the 3rd uppermost leaf started at the end of 3-week lasting shortage of N supply. In the presented study the effect of potassium fertilizing systems was non-significant in both years. However, a positive trend of the high potassium fertility on biomass of leaves was observed during the vegetative period in 2003 and at BBCH 31 and 37 in 2004.

An analysis of dry matter yield of stems clearly documents the water stress negative impact on wheat growth during the period of ear growth, i.e., from BBCH 37 to 59. This conclusion is drowned based on biomass yield of stems in 2003 and 2004 at the end of the vegetative period. In 2003, it amounted to 300 g m^{-2}, but in 2004, it was three times higher, amounting to 900 g m^{-2}. The importance of this particular period for development of yield components is well described in literature. This huge difference implicitly indicates on reduction in the number of tillers in 2003. This conclusion is supported by BAQUE et al. (2006) and GRZEBISZ et. al. (2009) who showed for wheat, that the numbers of tillers are the first component affected by drought stress. Effect of increasing nitrogen rates (N) on the yield of stems (YS) followed the same pattern as found for leaves:

1) 2003: $YS = -0.006N^2 + 1.67N + 241.8$ for $R^2 = 0.96$;
2) 2004: $YS = -0.019N^2 + 5.61N + 642.5$ for $R^2 = 0.99$.

As a result of drought in 2003, yield of stems, averaged over years, was lower as compared to 2004, but the relative differences were much smaller. The same pattern of yield of ears (YE) to increasing N rates was found:

2003: $YE = -0.0023N^2 + 0.815N + 99.0$ for $R^2 = 0.96$;

2004: $YE = -0.0059N^2 + 1.786N + 146.6$ for $R^2 = 0.99$.

The course of both curves reveals also the fact, that efficiency of applied nitrogen increased both quantitatively and relatively much faster in 2004. Effect of the potassium system was low, revealing as a positive trend in the high potassium fertile soil. In both years, it was slightly stronger in early stages of wheat growth. It can be explained by plant's ability to take potassium from its non-exchangeable resources (Grzebisz, Oertli 1994, Kuchenbuch et al. 1986).

Nitrogen concentration in wheat parts

Nitrogen concentration in leaves is frequently used as an indicator of plant nutritional status. The observed differences in its plant characteristic were significant only for nitrogen treatments. It was observed, nitrogen concentration in leaves increased progressively with N rate. This trend was not significant only in 2004 at BBCH 59. Impact of seasons on this wheat attribute was quite specific. Water stressful conditions, as in 2003, significantly affected nitrogen concentration in leaves, which increased from BBCH 31 to BBCH 37, decreasing afterwards. However, in semi-dry 2004, the constant dilution trend was observed in 2004, even accelerating from BBCH 37 to 59. This phenomenon is inversely related to biomass of stems in consecutive stages of wheat growth, i.e., yields of structural parts of the wheat plant (Table 3). This morphometric process can be explained by two facts. The growing stem, including the ear is a big physiological sink for nitrogen. Its supply during the period of the ear growth is decisive both for assimilates production by leaves and the number of spikelets (Grzebisz 2013, Shearman et al. 2005, Zerche, Hecht 1999).

Nitrogen concentration in stems, averaged over all other treatments, was usually higher in the dry 2003, compared to the semi-dry 2004 (Table 4). It showed a declining trend during vegetative growth. In addition, its rate was much slower in the dry 2003 than in normal 2004. Nitrogen concentration in stems (SN) responded to increase N rates (N) following the linear regression model:

2003: $SN = 0.025N + 7.65$ for $R^2 = 0.96$

2004: $SN = 0.0127N + 7.21$ for $R^2 = 0.99$.

The rate of N concentration in stems in response to increasing N rates was twice as high in the dry 2003 compared to 2004. This attribute of developed equations clearly explains the higher yield of stems in 2004.

Nitrogen concentration of N in developed ears (NE), evaluated just before flowering, was almost the same in both years. However, it response to increasing nitrogen rates (N), was described by distinct regression models:

Table 3

Statistical evaluation of main factors affecting biomass of stems and ears of winter wheat (g m^{-2})

Experimental factors	Level of factor	2003 (BBCH)			2004 (BBCH)		
		stems		ears	stems		ears
		37	59	59	37	59	59
Soil fertility level for K**	M	199.1	300.0	139.1	281.9	904.4	232,1
	H	242.2	339.3	146.7	323.2	909.4	232.7
	LSD 0.05	n.s.*	n.s.	n.s.	28.3	n.s.	n.s.
Potassium fertilizing***	K-	222.2	327.7	143.9	305.7	921.1	227.1
	K+	219.1	311.6	141.9	299.4	892.7	237.7
	LSD 0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N rates, (kg ha^{-1})	0	151.8	246.0	101.5	173.8	634.6	148.7
	60	239.2	308.2	131.9	344.3	934.2	225.9
	120	270.9	371.8	170.6	389.4	1017.1	281.7
	180	-	352.6	167.6	-	1041.6	273.3
	LSD 0.05	39.36	39.99	18.63	75.68	170.7	46.97

* non significant,
** M – medium, H – high soil K fertility level,
*** K- – potassium control, K+ – 100 kg K ha^{-1};

Table 4

Statistical evaluation of main factors affecting nitrogen concentration in stems and ears of winter wheat (g kg^{-1} DM)

Experimental factors	Level of factor	2003 (BBCH)			2004 (BBCH)		
		stems		ears	stems		ears
		37	59	59	37	59	59
Soil fertility level for K**	M	13.08	10.03	18.58	13.41	8.466	18.37
	H	12.86	9.757	17.61	14.00	8.214	17.89
	LSD 0.05	n.s.	n.s.	0.832	n.s.	n.s.	n.s.
Potassium fertilizing***	K-	12.97	9.636	17.75	13.79	8.252	17.66
	K+	12.97	10.15	18.44	13.63	8.428	18.61
	LSD 0.05	n.s.	n.s.	n.s*	n.s.	n.s.	n.s.
N rates, (kg N ha^{-1})	0	11.11	7.882	16.59	12.12	7.187	15.56
	60	12.29	8.571	17.57	12.88	7.856	17.86
	120	15.51	10.98	18.64	16.12	8.949	19.69
	180	-	12.14	19.58	-	9.368	19.42
	LSD 0.05	1.327	0.593	1.198	1.476	1.257	1.653

* non significant,
** M – medium, H – high soil K fertility level,
*** K- – potassium control, K+ – 100 kg K ha^{-1};

2003: $NE = 0.0167N + 16.6$ for $R^2 = 1$;

2004: $NE = 0.0002N^2 + 0.056N + 15.43$ for $R^2 = 0.99$.

The first linear, implicitly indicates on nitrogen supply as the factor limiting nitrogen concentration in fully developed ears. In 2004, it revealed, as "the N saturation model," which underlined an ample supply of nitrogen to growing ears.

Critical nitrogen concentration course

The physiological term, the critical nitrogen concentration (N_c), defines the minimum concentration of N in the plant during its vegetative growth as a prerequisite of for the highest biomass. The course of N_c during the growing season is termed as the Critical Nitrogen Dilution Course (CNDC). It is developed by plotting pairs, comprising concentration of N_c (% DM) and respective biomass of aboveground plant parts (DM, t ha^{-1}). As presented in Tables 2-6 nitrogen fertilizer rate was the only factor significantly affected both plant biomass, and N concentration in wheat parts. Therefore, the CNDCs were calculated for each N treatment, based on all data for the responsive plot.

The N_c course during the vegetative period of wheat growth is well described by a power function. The key differences, resulting from the impact of fertilizer nitrogen rate, are attributed to both constants, i.e., "*a*" and "*b*". The first one informs about initial N concentration in wheat tissue, and the second one describes steepness of a curve over the course of vegetation, in

Table 5

Statistical evaluation of main factors affecting nitrogen concentration in leaves of winter wheat (g kg^{-1} DM)

Experimental factors	Level of factor	2003 (BBCH)			2004 (BBCH)		
		31	37	59	31	37	59
Soil fertility level for K**	M	27.70	29.67	25.89	22.26	21.39	12.04
	H	29.52	30.96	25.54	23.38	22.70	11.69
	LSD 0.05	1.527	n.s.	n.s.	n.s.	n.s.	n.s.
Potassium fertilizing***	K-	28.86	30.01	24.85	22.74	21.55	12.01
	K+	28.46	30.62	26.58	22.90	22.54	11.72
	LSD 0.05	n.s.*	n.s.	n.s.	n.s.	n.s.	n.s.
N rates, kg ha^{-1}	0	25.64	26.59	20.65	19.55	16.76	10.75
	60	31.58	29.13	22.71	26.08	20.54	11.79
	120	-	35.23	28.44	-	28.83	12.82
	180	-	-	31.05	-	-	12.10
	LSD 0.05	2.005	2.088	1.864	2.830	2.370	n.s.

* non significant,
** M – medium, H – high soil K fertility level,
*** K- – potassium control, K+ – 100 kg K ha^{-1};

Table 6

Statistical evaluation of main factors affecting nitrogen status in winter wheat at BBCH 31

Experimental factors	Level of factor	2003			2004		
		N (%DM)	N_c (% DM)	NNI	N (%DM)	N_c (% DM)	NNI
Soil fertility level for K**	M	2.77	3.40	0.84	2.23	2.84	0.79
	H	2.95	3.29	0.92	2.34	2.68	0.88
	LSD 0.05	n.s.*	n.s.	n.s.	n.s.	n.s.	n.s.
Potassium fertilizing***	K-	2.88	3.26	0.91	2.27	2.84	0.81
	K+	2.85	3.43	0.85	2.29	2.68	0.86
	LSD 0.05	n.s.	n.s.	n.s.	n.s.	0.13	n.s.
N rates, (kg ha^{-1})	0 60	2.56 3.16	3.70 2.99	0.70 1.06	1.96 2.61	2.48 3.03	0.79 0.88
	LSD 0.05	0.20	0.18	0.07	0.28	0.17	n.s.

* non significant,
** M – medium, H – high soil K fertility level,
*** K- – potassium control, K+ – 100 kg K ha^{-1};

fact, speed of N dilution. Therefore, in can be concluded that the developed NCDCs reflected fairly well the degree of N supply to wheat plants. Those grown in the N control plot were fully dependent on soil N resources. Consequently, the "*a*" constant was low, but the R^2 coefficient achieved the highest value, indicating a low N variability, in spite of different growth conditions (Figure 2*a*). In other three treatments, with increasing N rates, from 60 to 180 kg N ha^{-1}, the "*a*" coefficient was in the narrow range, from 3.04 to 3.12 (Figs. 2*b*-*d*). The obtained values of this coefficient are, in fact, low. The main differences between the published CNDC equations refer both to "*a*" and "*b*". With respect to the "*a*" coefficient it ranges from 4.64 (FABER 2004) to 5.07 as presented by (SZCZEPANIAK 2008) up to 5.35 as presented

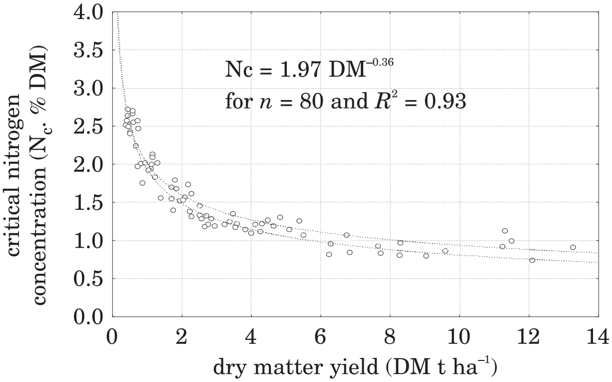

Fig. 2a. The relationships between dry matter yield and nitrogen concentration - nitrogenous control

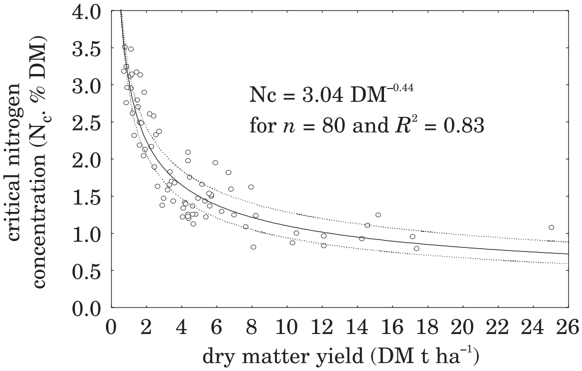

Fig. 2b. The relationships between dry matter yield and nitrogen concentration - plot with sub-optimal N rate: 60 kg N ha^{-1}

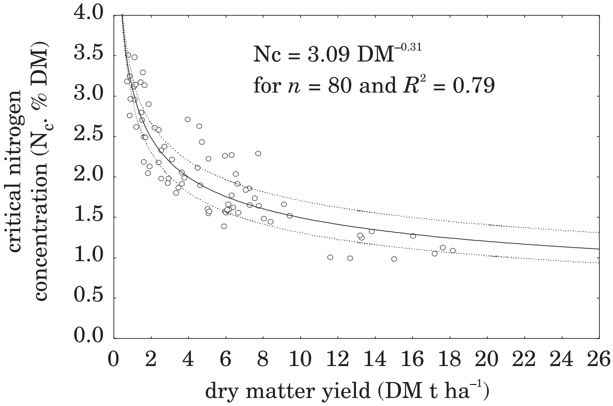

Fig. 2c. The relationships between dry matter yield and nitrogen concentration - plot with optimal N rate: 120 kg N ha^{-1}

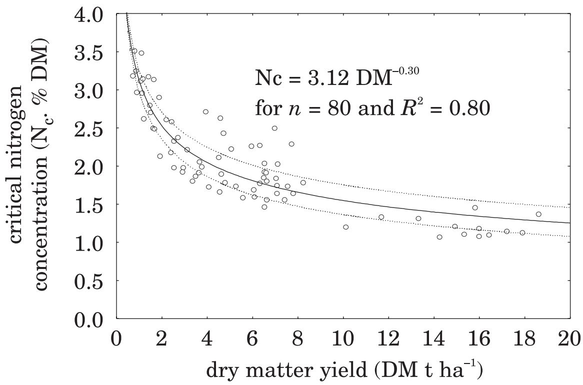

Fig. 2d. The relationships between dry matter yield and nitrogen concentration - plot with supra-optimal N rate: 180 kg N ha^{-1}

by (JUSTUS et al. 1994). The second coefficient “*b*” varied from -0.36 for the control plot through -0.44 for the 60 kg N ha^{-1} treatment to -0.30 for treatments with optimum N productivity. Based on these sets of data it can be concluded that the highest steepness of the NCDC the highest N dilution, indirectly underlying limited N supply. It was the key of attribute of the 60 kg N ha^{-1} treatment. For many years, the developed NCDC formula and its coefficients were considered as highly conservative (JUSTUS et al. 1994, LEMAIRE, GASTAL 1997). Eleven years later, LEMAIRE et al. (2008) added to this definition a part “..in a given environment,” indicating flexibility of the N_c to supply of nitrogen. It is worthy to mention that N supply does not depend only on N fertilizer rate. This study fully corroborates this opinion. The obtained “*a*” value of 3.1, averaged over-all N fertilized treatments, reflects N status of wheat producing yield of grain ranging from 4.0 to 8.0 t ha^{-1}, taking into account both dry 2003 and semi-dry 2004 years (Figure 1). In order to explain wheat canopy nutritional status a Nitrogen Nutrition Index (NNI) has been calculated. The NNI value of 1.0 or larger indicates non-limiting, whereas NNI below 1.0 correspond to N deficiency, i.e., N-limiting growth. The evaluation of wheat nutritional status has been conducted for consecutive stages of wheat growth, based on N_{c120} as the optimal. At the beginning of stem elongation (BBCH 31), the N_c values were both affected by N rate (N = 60 kg N ha^{-1}) and year. In 2003, plants grown on the control plot showed at BBCH 31 a very high value of N concentration of 3.7%, whereas in 2004, it reached only2.5% DM (Table 6). At the same time any impact on N_c (*ca* 3% DM) were found in treatments fertilized with 60 kg N ha^{-1}. This is the first indicator of N dilution, which was much faster

Table 7

Statistical evaluation of main factors affecting nitrogen status in winter wheat at BBCH 37

Experimental factors	Level of factor	2003			2004		
		N (%DM)	N_c (% DM)	NNI	N (%DM)	N_c (% DM)	NNI
Soil fertility level for K**	M	1.78	2.30	0.80	1.62	2.02	0.83
	H	1.87	2.13	0.90	1.70	1.98	0.89
	LSD 0.05	n.s.*	n.s.	n.s.	n.s.	n.s.	n.s.
Potassium fertilizing***	K-	1.81	2.22	0.85	1.66	1.98	0.87
	K+	1.84	2.21	0.85	1.66	2.02	0.85
	LSD 0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N rates, (kg ha^{-1})	0	1.58	2.45	0.65	1.38	2.31	0.59
	60	1.73	2.15	0.82	1.54	1.90	0.82
	120	2.16	2.04	1.08	2.07	1.79	1.16
	LSD 0.05	0.17	0.10	0.12	0.16	0.15	0.11

* non significant,
** M – medium, H – high soil K fertility level,
*** K- – potassium control, K+ – 100 kg K ha^{-1};

Table 8

Statistical evaluation of main factors affecting nitrogen status in winter wheat at BBCH 61

Experimental factors	Level of factor	2003			2004		
		%N	NC	NNI	%N	NC	NNI
Soil fertility level for K**	M	1.47	1.89	0.79	1.07	1.42	0.76
	H	1.43	1.82	0.80	1.04	1.43	0.74
	LSD 0.05	n.s.*	n.s.	n.s.	n.s.	n.s.	n.s.
Potassium fertilizing***	K-	1.41	1.84	0.78	1.04	1.41	0.75
	K+	1.49	1.87	0.81	1.07	1.43	0.76
	LSD 0.05	0.076	n.s.	n.s.	n.s.	n.s.	n.s.
N rates, (kg ha^{-1})	0 60	1.19 1.30	2.03 1.88	0.59 0.70	0.90 1.01	1.59 1.42	0.60 0.72
	120	1.58	1.75	0.91	1.16	1.34	0.86
	180	1.72	1.77	0.98	1.16	1.34	0.87
	LSD 0.05	0.07	0.08	0.06	0.10	0.08	0.09

* non significant,
** M – medium, H – high soil K fertility level,
*** K- – potassium control, K+ – 100 kg K ha^{-1};

in 2004 than in dry 2003. As a result, the NNI indices for this treatment were close to 1.0 in 2003, whereas below in 2004. In the second evaluated stage of wheat growth, i.e., BBCH 37, values of N_c were much lower as compared to the preceded stage and responded only to N rate.

Its values, in spite of different course of weather, were almost the same. This result can be explained by stabilization of canopy structure (see Tables 2 and 3). Patterns of N_c showed an opposite trend to increasing N rates, but the dilution effect was much faster in 2004 than 2003. Quite opposite trend was noted for NNI indices. They were in accordance to N rate, showing, however, a big impact of consecutive N rates. At the end of wheat vegetative growth, i.e., at BBCH 59, N_c values were slightly lower as compared to the BBCH 37, corroborating the declining trend over the course of vegetation.

The N dilution effect as presented in the previous stage was also pronounced. It was, however, much steeper in 2004 as compared to 2003. The opposite trend was observed for NNI indices, i.e., which increased in accordance to progressing N rates. However, it did not reach the optimum value, even in the plot with 180 kg N ha^{-1}.

CONCLUSIONS

1. Winter wheat response to freshly applied potassium fertilizer can reveal only under conditions of mild-water stress as found in 2004.

2. Nitrogen concentration decline in leaves during the ongoing wheat development is slower, i.e., conservative in dry, compared to semi-dry growth conditions, when undergoes dilution due to higher increase of dry matter yield.

3. Nitrogen concentration in leaves in response to increasing N fertilizer rates follows as a rule the quadrate regression level. In stems dominates the linear model, indicating the N-limited growth conditions.

4. The Critical Nitrogen Concentration pattern in wheat plants shows in response to increasing N rate a significant flexibility during the vegetative period of growth.

5. The Nitrogen Nutrition Index course during wheat growth and development could be used as an indicator of N dilution.

RERERENCES

Baque Md.A., Karim Md.A., Hamid A., Tetsuchi H. 2006. *Effects of fertilizer potassium on growth, yield nutrient uptake of wheat (Triticum aestivum) under water stress conditions*. South Pacific Stud., 27(1): 29-35.

Barczak B. 2008. *Contents and ratios of mineral components in winter barley biomass cultivated under conditions of different nitrogen fertilization.* J. Elementol., 13(3): 291-300.

Brouwer C., Heibloem M. 1986. *Irrigation water management: Irrigation water needs*. www.fao.org/docrep/s2022e/s2022e07.htm

Debaeke Ph., Aboudrare A. 2004.: *Adaptation of crop management to water-limited environments*. Europ. J. Agronomy, 21: 433-446.

Forde B., Loreznzo H. 2001. *The nutritional control of root development*. Plant Soil, 232: 51-68.

Faber A. 2004. *Validation of the critical nitrogen dilution curve for cereals*. Fragm. Agronom., 1(81): 29-41.

Gonzalez-Dugo V., Durand J-L., Gastal F. 2010. *Water deficit and nitrogen nutrition of crops. A review*. Agron. Sustain. Develop., 30: 529-544.

Grzebisz W., Oertli J.J. 1994. *Evaluation of universal extractants for determining plant available potassium in intensively cultivated soils*. Communications Plant Soil Anal., 24(11-12): 1295-1305.

Grzebisz W., Wrońska M., Diatta J.B., Szczepaniak W. 2008. *Effect of zinc application at early stages of maize growth on the patterns of nutrients and dry matter accumulation by canopy.* Part II. *Nitrogen uptake and dry matter accumulation patterns*. J. Elementol., 13(1): 29-39.

Grzebisz W., Musolf R., Szczepaniak W., Barłóg P. 2009. *Effect of artificially imposed water shortages against a background of potassium supply on dry matter, nitrogen and potassium accumulation by winter wheat*. Fertilizers Fertilization, 34: 40-52.

Grzebisz W., Gransee A., Szczepaniak W., Diatta J. 2013. *The effects of potassium fertilization on water use-efficiency in crop plants*. J. Plant Nutr. Soil Sci., 176: 355-374.

Kuchenbuch R., Classen N., Jungk A. 1986. *Potassium availability in relation to soil moisture.*

I. *Effect of soil moisture on potassium diffusion, root growth and potassium uptake of onion plants*. Plant Soil, 95: 221-231.

JUSTUS E., MARY B., MEYNARD J.M., MACHET J.M., THELIER-HUCHE L. 1994. *Determination of a critical dilution curve for winter wheat crops*. Ann. Bot., 74: 397-407.

LEMAIRE G., GASTAL F., 1997. *N uptake and distribution in plant canopies*. In: *Diagnosis of the nitrogen status in crops*. Ed. LEMAIRE G., Springer Verlang, 3-45.

LEMAIRE G., JEUFFROY M-H., GASTAL F. 2008. *Diagnosis tool for plant and crop N status in vegetative stage. Theory and practices for crop management*. Europ. J. Agronomy, 28: 614-624.

RUBIO G., ZHU J., LYNCH J. 2003. *A critical test of the prevailing theories of plant response to nutrient availability*. Am. J. Bot., 90(1): 143-152.

SZCZEPANIAK W. 2008. *The critical nitrogen concentration NCC concept as a tool to evaluate nitrogen nutritional status of winter wheat*. Fertilizer Fertilization, 32: 133-144.

SHEARMAN V., SYLVESTER-BRADLEY R., SCOTT R., FOULKES M. 2005. *Physiological processes associated with wheat progress in the UK*. Crop Sci., 45(1): 175-185.

VOILLOT M.O., DEVIENNE-BARRETT F. 1999. *Accumulation and remobilization of nitrogen in a vegetative winter wheat crop during or following nitrogen deficiency*. Ann. Bot., 83: 569-575.

ZERCHE S., HECHT R. 1999. *Nitrogen uptake of winter wheat during shoot elongation phase in relation to canopy high and shoot density*. Agribiol. Res., 52(3/4: 231-250.

YIN X., GOUDRIAN J., LANTINGA E., VOS J., SPIERTZ H. 2003. *A flexible sigmoid function of determinate growth.* Ann. Bot., 91: 361-371.

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IMPACT OF DIFFERENT FORMS OF NITROGEN FERTILIZER ON THE CONTENT AND UPTAKE OF MICROELEMENTS IN SORGHUM

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Abstract

The aims of the study were to determine the impact of various forms of nitrogen on the content and uptake of cooper, iron, manganese and zinc by sweet sorghum and to check what remains in the bagasse after extracting the plant juice.

In 201-2011, field experiments were conducted on sweet sorghum (cv. Sucrosorgo 304) fertilized with different forms of nitrogen (nitrate, ammonium and urea).

The fertilizer containing ammonia nitrogen markedly increased the amount of Mn in both years of the study and raised the amount of Zn in 2010. On average, significantly more Fe was found after fertilizing with nitrates (calcium nitrate), and more Zn was found after fertilizing with the form of ammonium (ammonium chloride). In 2010, there were significantly higher increments in the content of all the microelements. A lower content of microelements was found in the bagasse than in the whole plants. On average, throughout the whole study, a significant impact of the fertilization variants on the Zn content appeared. The highest uptake of Cu was found in plants which had been fertilized with nitrate and ammonium forms of nitrogen (ammonium nitrate), whereas Mn and Zn increased when fertilization included ammonium in the form of ammonium sulphate. After fertilizing with ammonium chloride, the uptake was slightly lower. On average for 2010-2011, the highest level of Cu, Mn and Zn occurred in the bagasse from plants that had been fertilized with ammonium chloride.

Keywords: sweet sorghum, nitrogen fertilization, microelements, biomass, bagasse.

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WPŁYW NAWOŻENIA RÓŻNYMI FORMAMI AZOTU NA ZAWARTOŚĆ I POBRANIE MIKROELEMENTÓW PRZEZ SORGO

Abstrakt

Celem badań było określenie wpływu różnych form azotu na zawartość i pobranie miedzi, żelaza, manganu i cynku przez sorgo cukrowe oraz powstałe po tłoczeniu soku wytłoki.

W doświadczeniu polowym w latach 2010-2011 uprawiano sorgo cukrowe (odmiana Sucrosorgo 304), które nawożono nawozami azotowymi zawierającymi różne formy azotu (amonową, azotanową oraz amidową).

Nawozy zawierające formę amonową azotu w obydwu latach badań istotnie wpłynęły na zwiększenie zawartości Mn, a w 2010 r. również Zn. Średnio istotnie więcej Fe stwierdzono w warunkach nawożenia formą azotanową (saletra wapniowa), a Zn – po nawożeniu formą amonową (chlorek amonu). W sorgu zebranym w 2010 r. oznaczono istotnie większą zawartość wszystkich badanych mikroelementów. W wytłokach stwierdzono mniejszą zawartość badanych mikroelementów niż w całych roślinach. Średnio w okresie badań istotny był wpływ stosowanych nawozów na zawartość Zn. Najwięcej Cu pobrały rośliny nawożone nawozem zawierającym obie formy azotu – azotanową i amonową (saletra amonowa), zaś Mn i Zn – formą amonową w postaci siarczanu amonu. Po nawożeniu chlorkiem amonu pobranie było nieznacznie mniejsze. Średnio w latach 2010-2011 największą ilość Cu, Mn i Zn stwierdzono w wytłokach z roślin nawożonych chlorkiem amonu.

Słowa kluczowe: sorgo cukrowe, nawożenie azotem, mikroelementy.

INTRODUCTION

Sweet sorghum belongs to the *Poacea*, family, thus it is a plant with the C_4 pathway of photosynthesis characterized by highly efficient photosynthesis and a better use of water and nitrogen than most plants with the C_3 pathway (Zhao et al. 2005). The plant is grown in semi-arid and arid regions of the world, mainly in areas unsuitable for corn (Kaczmarek et al. 2008). Although sorghum uses nitrogen more efficiently than most plants with the C_3 cycle, nitrogen is the main factor responsible for reduced yields (Young, Long, 2000).

The effect of nitrogen fertilization on plant yields is widely known. Among the three basic macroelements used in fertilization, the impact of nitrogen is the most immediate (Fageria, Moreira 2011). However, large doses of nitrogen raise production costs and affect the environment (Sainju et al. 2006, Marsalis et al. 2010). A way to control the adverse effects of fertilization on nature is to adjust the dose of a mineral fertilizer to the nutritional needs of a given crop and to slow down the mineral uptake by applying a fertilizer with a more controlled or slower nutrient release. Slow-acting fertilizers are characterized by a delay between the time of application and nutrient availability, as well as a slower release of nutrients into soil compared to conventional fertilizers (Korzeniowska 2009).

The level of microelements in the biomass depends mainly on the content of elements in the soil, the dose of fertilization, and the growth stage of plants (Bujanowicz et al. 2000). The antagonistic and synergistic effects of different microelements along with the soil condition can lead to a deficiency or excess in the concentration of elements in crop yields (Sposito 2008).

MATERIAL AND METHODS

Field experiments were set up according to the random block method. They were carried out in 2010 and 2011, in experimental fields at Pawlowice, which belong to the Department of Crop Production, Wroclaw University of Environmental and Life Sciences

The field trials, with four replicates, were established on sandy soil, which belonged to the Polish soil valuation class V, identified as rusty gley soil. Each plot covered 14.7 m^2 (7 m long and 2.1 m wide). Before sowing, mineral fertilizers were applied in the following doses: 30.5 kg P ha^{-1} in the form of triple superphosphate, 100 K ha^{-1} in the form of potassium salt, and 120 kg N ha^{-1} (in the form shown below). Various forms of nitrogen in nitrogen fertilizers were studied in the context of their impact on the content and uptake of microelements in sorghum plants and accumulation in bagasse, such as ammonium sulphate, ammonium chloride, calcium nitrate, ammonium nitrate, urea and slow-release urea under commercial name of Meister. For comparison of the effects of these fertilizers on the level of microelements, a control group was created (no nitrogen fertilizer). The study evaluated the Sucrosorgo 304 variety from Sorghum Partners LLC, which was sown in the first half of May, using 20 seeds with full germination ability per 1 m^2. Plants were harvested at the milky dough stage, ground and sampled for chemical analysis to determine the level of microelements. Next, some of the ground plant material was put into a press set at the pressure of under 30 bars to obtain plant juice for biotechnological purposes. Samples of the bagasse were collected, dried and ground for analyses of the content of microelements. From the plant material obtained the level of Cu, Fe, Mn and Zn was determined using the method of atomic absorption spectrometry after the mineralization of dry ash dissolved in a solution of 1 mol HNO_3 dm^{-3}. The results on the biomass yields and content of microelements were used to calculate the mineral uptake by sorghum plants and the accumulation of micronutrients in the bagasse of sorghum plants. The content of iron was excluded from analyses due to the fact that the laboratory equipment was made of stainless steel and iron.

Variance analysis was applied to the results with a Statistica 9 software package. Confidence intervals were tested with the Duncan's test at the significance level of (α = 0.05).

RESULTS AND DISCUSSION

Nitrogen fertilizers used in the study significantly influenced the content of Mn and Zn in sweet sorghum plants (Table 1). The fertilizer containing ammonium nitrogen significantly increased the level of Mn in both years and the level of Zn in 2010. The plants harvested in 2010 had significantly higher levels of all of the micronutrients. LEHMANN et al. (1999) reported that the content of Zn after fertilizing with ammonium sulphate was 24.1±4.9 mg kg^{-1} d.m., versus 22.4±8.5 mg kg^{-1} d.m. without nitrogen fertilization. For comparison, our research showed that the level of Zn was lower by 43.5 mg kg^{-1} d.m. (after fertilizing with ammonium sulphate) to 37.9 mg kg^{-1} d.m. (without nitrogen fertilization). In the research of PING et al. (2009), there was a higher concentration of Zn and Cu in the leaves than in the stalks of sweet sorghum plants. The stems of plants fertilized with ammonium sulphate had a lower content of Zn than after fertilization

Table 1

The content of microelements in sorghum plants at milk dough stage (mg kg^{-1} d.m.)

Year	Fertilizer type	Cu	Fe	Mn	Zn
2010	ammonium sulphate	3.263	61.46	56.15	75.81
	ammonium chloride	3.550	78.48	61.88	70.37
	calcium nitrate	3.175	79.01	30.96	62.87
	amonium nitrate	4.250	45.17	49.70	58.58
	urea	3.200	65.23	35.97	74.92
	slow-release urea -Meister	3.100	58.25	38.88	51.13
	control	3.575	73.68	33.40	51.75
LSD α = 0.05		n.s.	15.53	19.66	17.25
2011	ammonium sulphate	2.088	53.25	19.49	59.31
	ammonium chloride	2.225	53.76	16.26	66.13
	calcium nitrate	2.075	57.31	14.21	58.13
	amonium nitrate	2.025	48.53	11.84	49.63
	urea	2.250	55.93	12.45	38.25
	slow-release urea -Meister	2.063	53.00	10.85	31.69
	control	2.425	57.23	14.53	68.88
LSD α = 0.05		0.165	n.s.	4.59	16.04
Means from years 2010 and 2011	ammonium sulphate	2.675	57.36	37.81	67.56
	ammonium chloride	2.888	66.13	39.08	68.25
	calcium nitrate	2.625	68.16	22.59	60.50
	amonium nitrate	2.979	47.09	28.06	53.46
	urea	2.657	59.91	22.53	53.96
	slow-release urea -Meister	2.581	55.63	24.96	41.41
	control	3.000	65.45	23.96	60.31
LSD α = 0.05		n.s.	12.09	n.s.	14.88
2010	–	3.410	66.14	44.80	64.36
2011	–	2.144	53.91	14.21	51.93
LSD α = 0.05		0.254	6.19	6.31	8.25

with either of the two ammonium nitrate forms. The levels of Cu in leaves and stems were similar. MARCHIOL et al. (2007), after fertilizing sorghum with urea in a dose of 150 kg ha^{-1} reported 29.6 ± 6.3 mg Cu kg^{-1} d.m. and 55.5 ± 3.6 mg Zn kg^{-1} d.m. in thee stems, while the plants not fertilized with nitrogen had 28.6 ± 4.5 Cu and 86.4 ± 19 mg Zn kg^{-1} d.m., respectively.

The bagasse had a lower level of the micronutrients than the complete plant matter. In both years, there were significant differences between the types of nitrogen fertilizer and the resulting levels of Mn and Zn, and the level of Cu in 2010 (Table 2). The means from both years were significantly higher for Zn after fertilization with ammonium chloride (60.8 mg Zn kg^{-1} d.m.) and ammonium sulphate (51.8 mg Zn kg^{-1} d.m.) than with Meister (34.6 mg Zn kg^{-1} d.m.). The bagasse obtained from plants collected in 2010 had much higher concentrations of all the microelements. The difference in the level of manganese was particularly noteworthy.

Table 2

The content of microelements in sorghum bagasse (mg kg^{-1} d.m.)

Year	Fertilizer type	Cu	Fe	Mn	Zn
2010	ammonium sulphate	3.217	127.1	38.88	66.92
	ammonium chloride	3.513	65.94	49.20	63.38
	calcium nitrate	3.038	82.73	23.66	47.63
	amonium nitrate	3.400	64.95	33.90	46.75
	urea	2.725	63.89	37.74	55.06
	slow-release urea -Meister	2.717	67.12	29.20	43.42
	control	3.100	67.88	23.84	43.50
LSD $\alpha = 0.05$		0.389	n.s.	4.59	11.48
2011	ammonium sulphate	2.163	58.23	16.84	40.50
	ammonium chloride	2.188	59.46	13.64	58.25
	calcium nitrate	1.988	63.14	11.64	35.63
	amonium nitrate	1.325	53.23	9.188	32.44
	urea	2.050	61.39	11.08	31.25
	slow-release urea -Meister	2.038	100.2	9.113	28.00
	control	2.200	65.73	11.80	31.80
LSD $\alpha = 0.05$		n.s.	24.16	4.57	10.47
Means from years 2010 and 2011	ammonium sulphate	2.614	87.72	26.29	51.82
	ammonium chloride	2.850	62.70	31.42	60.81
	calcium nitrate	2.513	72.93	17.64	41.63
	amonium nitrate	2.017	57.13	17.43	37.21
	urea	2.388	62.64	24.41	43.26
	slow-release urea -Meister	2.329	86.02	17.72	34.61
	control	2.650	66.80	17.81	37.63
LSD $\alpha = 0.05$		n.s.	n.s.	n.s.	12.31
2010	means for fertilizers	3.086	78.13	34.64	54.44
2011		1.977	65.94	11.90	37.22
LSD $\alpha = 0.05$		0.235	n.s.	4.02	6.43

There are few studies on the content of microelements in the bagasse of sorghum. SESHAIAH et al. (2012) reported a higher content of copper (57.4 mg Cu kg^{-1} d.m.) and a similarly higher result level of zinc (48.8 mg Zn kg^{-1} d.m.).

Our study revealed a diverse impact of fertilization on the uptake of micronutrients in the plant biomass of sweet sorghum. Fertilizing with nitrogen as a nitrate (calcium nitrate) significantly increased the uptake of iron in both years (Table 3). In 2010, plants fertilized with ammonium nitrate had a much higher uptake of Cu, while plants fertilized with ammonium sulphate had a higher uptake of Zn and Mn. In 2011, sorghum fertilized with ammonium chloride had a much higher level of Zn, and plants fertilized with ammonium sulphate had a much higher level of Mn. The highest average level of Cu for both years 2010-2011 was determined in plants fertilized with ammonium nitrate; the highest level of Fe was in plants fertilized with calcium nitrate, and the highest levels of Mn and Zn occurred in plants fertilized with ammonium sulphate. There was a much higher uptake of

Table 3

The uptake of microelements by sorghum plants (g ha^{-1})

Year	Fertilizer type	Cu	Fe	Mn	Zn
2010	ammonium sulphate	39.47	743.8	679.3	916.1
	ammonium chloride	37.15	816.6	653.0	736.1
	calcium nitrate	35.74	890.3	348.2	709.4
	amonium nitrate	55.91	516.1	601.2	731.3
	urea	39.59	806.3	492.6	898.0
	slow-release urea -Meister	32.10	603.5	409.1	532.9
	control	20.53	423.1	191.8	297.2
LSD $\alpha = 0.05$		8.96	173.2	132.6	169.1
2011	ammonium sulphate	39.45	1006	368.9	1121.2
	ammonium chloride	40.71	982.7	297.2	1211.9
	calcium nitrate	37.92	1029	255.6	1050.9
	amonium nitrate	36.60	876.6	213.6	899.8
	urea	37.49	926.4	206.9	643.3
	slow-release urea -Meister	36.54	942.1	192.8	563.5
	control	33.68	794.9	201.8	956.7
LSD $\alpha = 0.05$		n.s.	126.4	55.8	186.3
Means for years 2010 and 2011	ammonium sulphate	39.46	874.9	524.1	1018.7
	ammonium chloride	38.93	899.6	475.1	974.0
	calcium nitrate	36.83	960.0	301.9	880.2
	amonium nitrate	46.26	696.4	407.4	815.5
	urea	38.54	866.3	349.7	770.7
	slow-release urea -Meister	34.32	772.8	300.9	547.7
	control	24.92	547.0	195.1	517.0
LSD $\alpha = 0.05$		5.86	141.9	124.0	172.2
2010	means for fertilizers	37.21	685.7	482.2	688.6
2011	means for fertilizers	37.78	947.8	252.7	918.3
LSD $\alpha = 0.05$		n.s.	71.9	60.8	103.2

Fe and Zn in the plant material from sorghum sown in 2011, and a higher uptake of Mn in 2010.

Ping et al. (2009) reported that the uptake of Zn in sorghum plants fertilized with ammonium nitrate ranged from 1.0 to 1.4 kg ha^{-1}, while fertilization with ammonium sulphate slightly decreased the uptake (1.0-1.2 kg ha^{-1}). The Zn uptake by plants with no nitrogen fertilization had a wider range (0.8-1.4 kg ha^{-1}), and the content of Cu ranged from 0.18 to 0.27 kg ha^{-1}. In the research of Marchiol et al. (2007), plants fertilized with urea had the uptake of 644 g ha^{-1} of Cu and 1223 g ha^{-1} of Zn, and plants with no nitrogen fertilization absorbed 148 g ha^{-1} of Zn. On the other hand, our research showed a much lower uptake of Cu than cited in other scientific research from various parts of the world. Our results were in the range of 20.5 to 55.9 g ha^{-1}, and available references report a range from 180 (Ping et al. 2009) to 644 g ha^{-1} (Marchiol et al. 2007)

Fertilization significantly affected the accumulation of micronutrients in bagasse (Table 4). In 2010, the highest increase concerned the level of Cu

Table 4

The accumulation of microelements in sorghum bagasse (g ha^{-1})

Year	Fertlizer type	Cu	Mn	Zn
	ammonium sulphate	36.99	546.1	835.1
	ammonium chloride	36.52	577.9	691.8
	calcium nitrate	33.15	293.0	593.8
2010	amonium nitrate	47.32	494.9	632.8
	urea	35.13	458.8	759.2
	slow-release urea -Meister	29.59	345.0	486.2
	control	19.67	168.2	280.6
LSD α = 0.05		6.63	77.5	118.7
	ammonium sulphate	36.01	311.0	865.8
	ammonium chloride	37.66	256.4	1070.0
	calcium nitrate	34.65	218.9	804.3
2011	amonium nitrate	28.45	177.2	698.6
	urea	33.47	182.6	546.7
	slow-release urea -Meister	33.31	163.8	488.4
	control	28.74	164.7	650.1
LSD α = 0.05		4.53	46.4	97.8
Means for years 2010 and 2011	ammonium sulphate	33.55	333.0	682.3
	ammonium chloride	35.25	359.2	787.8
	calcium nitrate	30.97	210.1	518.0
	amonium nitrate	29.52	264.7	515.9
	urea	30.05	291.7	535.3
	slow-release urea -Meister	28.57	207.9	426.9
	control	20.48	139.0	290.5
LSD α = 0.05		4.75	85.6	90.7
2010	means for fertilizers	30.89	341.8	534.2
2011		29.27	176.7	588.3
LSD α = 0.05		n.s.	40.9	n.s.

in the bagasse from plants fertilized with ammonium nitrate (47.3 g ha^{-1} of Cu), but in 2011 the highest Cu concentration in sorghum bagasse appeared after fertilization with ammonium chloride, ammonium sulphate and ammonium nitrate (37.7, 36.0, 34.7 g ha^{-1} of Cu respectively). The two-year average accumulation of Mn and Zn was significantly the highest after fertilization with the form of ammonium, either as ammonium sulphate or ammonium chloride.

CONCLUSIONS

1. Nitrogen fertilization in the form of ammonium resulted in a significantly higher average level of zinc in both the years of the study, whereas nitrogen used either as a nitrate or ammonium (ammonium nitrate) significantly reduced the iron content in sorghum plants relative to the control group.

2. Extracted juice showed the biggest decrease in the level of Zn 10.0-14.2 mg kg^{-1} d.m., and a somewhat smaller decrease in the level of Cu 0.17-0.33 mg kg^{-1} d.m.

3. The accumulation of micronutrients in bagasse was lower than in whole plants and the biggest difference was noted in the accumulation of Zn, which translated into a high level of this microelement in extracted sorghum juice.

4. Different forms of nitrogen fertilization had a significant impact on the uptake of microelements in the plant biomass of sorghum (highest uptake of Cu resulted from the application of ammonium nitrate, Fe – calcium nitrate, Mn and Zn – ammonium sulphate). The accumulation of all these microelements in sorghum bagasse was the highest after the application of ammonium chloride as a fertilizer.

REFERENCES

Bujanowicz B., Matras J., Klebaniuk R., Wojtasik J. 2000. *Lead and cadmium content in the leaves with tops grown in the neighborhood of the road with intensive traffic* Ann. UMCS, Sect. EE, 18(33): 257-262. (in Polish)

Fageria N.K., Moreira A. 2011. *The role of mineral nutrition on root growth of crop plants*. Adv. Agron. vol. 110: 251-331. Donald L.S. (ed.), Academic Press. DOI: 10.1016/B978-0-12-385531-2.00004-9

Kaczmarek S., Matysiak K., Krawczyk R. 2008. *Studies on chemical weed control in sorghum under Great Poland conditions*. Wyd. UP, Poznań., 233-234. (in Polish)

Korzeniowska J. 2009. *Progress in research on slow-release fertilizers*. Stud. Rap. IUNG – PIB, 18: 9-26. (in Polish)

Lehmann J., Feilner T., Gebauer G., Zech W. 1999. *Nitrogen uptake of sorghum (Sorghum bicolor* L.*) from tree mulch and mineral fertilizer under high leaching conditions estimated by nitrogen-15 enrichment*. Biol. Fertil. Soils., 30: 90-95. DOI: 10.1007/s003740050592

Marchiol L., Fellet G., Perosa D., Zerbi G. 2007. *Removal of trace metals by Sorghum bicolor and Helianthus annus in a site polluted by industrial wastes: a field experience.* Plant Physiol. Biochem., 45: 379-387. DOI: 10.1016/j.plaphy.2007.03.018

Marsalis M., Angadi S., Contreras-Govea F., 2010. *Dry matter yield and nutritive value of corn, forage sorghum, and BMR forage sorghum at different plant populations and nitrogen rates.* Field Crop. Res., 116: 52-57. DOI: 10.1016/j.fcr.2009.11.009

Ping Z., Wensheng S., Zhian L., Bin., Jintian L., Jingsong S. 2009. *Removal of metals by sorghum plants from contaminated land.* J. Environ. Sci., 21: 1432-1437. DOI: 10.1016/S1001-0742(08)62436-5

Sainju U., Whitehead W., Singh B., Wang S. 2006. *Tillage, cover crops, and nitrogen fertilization effects on soil nitrogen and sorghum yields.* Europ. J. Agron., 25: 372-382. DOI:10.1016/j.eja.2006.07.005

Seshaiah V., Reddy R., Nagalakshmi D., Rao S. 2012. *Evaluation of sweet sorghum (Sorghum bicolor (*L.*) moench) bagasse by chemical, in sacco and in vivo techniques in graded murrah buffalo bulls.* J. Vet. Adv., 2(8): 418-423.

Sposito G. 2008. *The chemistry of soils.* Chapter 1. *The chemical composition of soils*. Oxford University Press, 3-27.

Young K.J., Long S.P. 2000. *Crop ecosystem responses to climatic change: maize and sorghum*. In: *Climate change and global crop productivity*. Reddy K.R., Hodges H.F. (Eds.). CABI Publishing, Wallingford., 107-131.

Zhao G., Liu Y., Tian Y., Sun Y., Cao Y. 2010. *Preparation and properties of macromolecular slow-release fertilizer containing nitrogen, phosphorus and potassium*. J. Polym. Res., 17: 119-125. DOI 10.1007/s10965-009-9297-4

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EFFECT OF SELECTED DIVALENT CATIONS ON PROTEIN MOBILIZATION IN LENTIL (*LENS CULINARIS*) SPROUTS

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Abstract

The influence of calcium and magnesium on protein mobilization and non-protein nitrogen content in cotyledons of germinated lentil was studied.

Elicitation of metabolism in sprouts modifies the rate of protein mobilization and causes a subsequent increase in the non-protein nitrogen fraction in lentil cotyledons. Application of $MgCl_2$ proved to be the most beneficial, leading to the highest yield of non-protein nitrogen in cotyledons from 6-days-old lentil sprouts. Regardless of the type of elicitor, a decrease in the activity of proteolytic enzymes was observed in cotyledons from 3- and 4-day-old lentil sprouts. A significant correlation was found in sprouts elicited with $MgCl_2$ between the non-protein nitrogen content and activities of endo- and aminopeptidases.

The information provided by this study allows us to design conditions for lentil sprouting which will result in an enhanced level of potentially bioaccessible non-protein nitrogen (free amino acids and peptides).

Keywords: magnesium, calcium, protein mobilization, free amino acids, lentil, sprouting.

WPŁYW WYBRANYCH JONÓW DWUWARTOŚCIOWYCH NA MOBILIZACJĘ BIAŁEK W KIEŁKACH SOCZEWICY JADALNEJ (*LENS CULINARIS*)

Abstrakt

W pracy badano wpływ jonów wapnia i magnezu na szybkość mobilizacji białek i zawartość azotu niebiałkowego w kotyledonach kiełków soczewicy.

Elicytacja metabolizmu kiełków modyfikuje szybkość mobilizacji białek, powodując jednocześnie zwiększenie udziału frakcji azotu niebiałkowego w kotyledonach. Wykazano, że indukcja z użyciem roztworów $MgCl_2$ najefektywniej zwiększyła poziom azotu niebiałkowego w kotyledonach 6-dniowych kiełków. Bez względu na rodzaj elicytora, w kotyledonach 3- i 4-dniowych kiełków zaobserwowano spadek aktywności enzymów proteolitycznych. Dodatkowo, w kotyledonach indukowanych roztworami $MgCl_2$ określono wysoką dodatnią korelację między poziomem azotu niebiałkowego oraz aktywnością amino- i endoproteaz.

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Na podstawie wyników opracowano recepturę hodowli kiełków, które charakteryzują się zwiększonym poziomem potencjalnie biodostępnego azotu niebiałkowego (wolne aminokwasy i peptydy).

Słowa kluczowe: magnez, wapń, mobilizacja białek, wolne aminokwasy, soczewica, kiełkowanie.

INTRODUCTION

In recent years, food legumes have attracted much attention owing to their functional components and health promoting effects. The quality and potential bioactivity of low processed food such as sprouts is mainly determined by the quality of seeds and/or conditions of germination (Gawlik-Dziki et al. 2012, Świeca et al. 2012)

There are several reports about the effect of germination methods on the nutraceutical value of legumes, including, soybeans, mung beans or lentils (Zhao et al. 2005, Randhir, Shetty 2007). Most studies have been conducted using biotic and abiotic stresses and/or elicitors (Randhir Shetty 2007, Ghavidel Prakash 2007, Gawlik-Dziki et al. 2012). Elicitation of seedlings seems to be a useful technique used for improving the nutritional and nutraceutical potential of low-processed food.

Germination is one of the most common and effective processes for attaining better quality of legumes. During germination, the enhanced activity of proteases results in the immobilization of storage proteins (Muntz et al. 2001). Some proteases are known to require metals for their correct activity and/or are activated by metal ions (e.g. calcium- and calmodulin dependent proteases). Cytosolic calcium levels are drastically lowered during germination as a consequence of abscisic acid action (Khan 2010). There is some evidence that also Mg^{2+} may induce metabolic pathways involved in the mobilization of storage material and be responsible for the response to abiotic stress conditions (acts as an abiotic elicitor) (Shanker Venkateswarlu 2011). Additionally, calcium has been reported to inhibit Na^{+} uptake, thereby reducing its adverse effect on seed germination and stimulating the plant growth (Zehra 2012).

There is no information concerning the influence of calcium and magnesium on protein mobilization in germinated legumes, thus the objective of this study was to determine the influence of these cations on the content of protein and free amino acids at different germination stages of lentil. Additionally, an attempt was made to correlate the changes in free amino acid and protein fraction with the activity of proteases. The results obtained in this study could be valuable for designing conditions of lentil germination so as to develop functional food with an enhanced level of potentially bioaccessible non-protein nitrogen fraction (free amino acids and peptides).

MATERIAL AND METHODS

Seeds from the lentil cultivar Tina were purchased from the PNOS S.A. in Ozarów Mazowiecki, Poland. Seeds were sterilized in 1% (v/v) sodium hypochloride for 10 min, then drained and washed with distilled water until they reached neutral pH. They were placed in distilled water and soaked for 6 hours at 25°C. Seeds were dark germinated for 4 days in a growth chamber on Petri dishes (ϕ 125 mm) lined with absorbent paper. Seedlings were watered with 5 ml of Milli-Q water daily – control (Gawlik-Dziki, Świeca 2011). For the experiments, 10 mmol dm^{-3} $CaCl_2$, 100 mmol dm^{-3} $CaCl_2$, 10 mmol dm^{-3} $MgCl_2$ and 100 mmol dm^{-3} $MgCl_2$ were selected as inductors. All solutions were freshly prepared before each application. For treatments, 2-day-old seedlings were watered daily with the tested solutions. Sprout samples were collected gently, the embryonic axis and seed coats were removed and cotyledons were frozen rapidly to be kept in polyethylene bags at -20°C until analyses. For each treatment, three replicates were prepared.

Analytical methods

Soluble protein and non-protein nitrogen (peptides and free amino acid rich fraction) were measured using the methodology described by Periago et al. (1996). Approximately 25 cotyledons were extracted thrice with 25 ml of 0.02 mol dm^{-3} NaOH for 60 min and the three extracts were pooled. Insoluble material was removed by centrifugation at 8700 rcf for 20 min.

Soluble proteins were measured with the procedure described by Lowry (1951), with bovine albumin as a standard. After that, the supernatant was mixed with 20 ml of 30% TCA and the mixture was stirred for 15 min at 4°C. Protein was removed by centrifugation at 8700 rcf for 15 min. Non -protein nitrogen was assayed using the ninhydrin method (Suna 2006) and expressed as the leucine equivalent in mg g^{-1} f.m.

Extract preparation

Extracts for enzymatic assays were prepared from 25 cotyledons in each group. The cotyledons were homogenized in a mortar with 30 ml of 0.1 mol dm^{-3} TRIS HCl buffer pH 8 containing 150 mmol dm^{-3} NaCl and 10 mmol dm^{-3} β-ME. Samples were centrifuged at 8700 rcf for 20 min. The supernatants were used immediately to determine protease activity. All operations were carried out at 4°C.

Enzyme assays

Aminopeptidase activity was assayed using leucyl-*p*-nitroaniline (L-*p*NA) as substrate according to the method described by Ogiwara et al. (2005). Aminopeptidase activity was expressed in U per g of fresh weight (U g^{-1} f.m.).

One unit of enzyme activity is defined as an amount of enzyme required to release 1 µmol of pNA per in min. at the assay conditions.

The activity of endoprotease was assayed using hemoglobin as substrate and it was evaluated measuring peptide release by the increase in absorbance at 280 nm (ANSON 1938). Endoproteases were measures at 3 different pH using McIlvaine's buffers: acid (pH= 5.5), neutral (pH= 7) and alkaline (pH= 9). The endoprotease activity was expressed in U per g of fresh weight (U g^{-1}f.m.). One unit of enzyme activity is defined as an amount of enzyme catalyzing a change of 0.001 absorbance at the assay conditions.

Statistic

All experimental results were means ± S.D. of three parallel measurements. Two-way analysis of variance (Anova, the Tukey test) was used to compare groups within different elicitors. α values < 0.05 were regarded as a significant.

RESULTS AND DISSCUSION

The results regarding dormant seeds and sprouts before induction are summarized in Table 1. On germination, there was a statistically significant decrease in the soluble proteins content, which could be due to their being used as a source of energy by the sprouting process and/or intermediates for biosynthesis. This result agrees with an earlier report by RODRIGUEZ et al.

Table 1

Characteristics of dormant seeds and sprouts before elicitation

Specification	Dry seeds	Soaked	12-hours-old sprouts	24-hours-old sprouts
Proteins (mg g^{-1} f.m.)	132.35±5.29^{b}	120.57±4.82^{b}	106.45±4.26^{a}	104.21±4.17^{a}
Free amino acids and peptides rich-fraction (mg g^{-1} f.m.)	107.01±8.49^{a}	126.36±5.13^{b}	135.15±5.41^{b}	172.18±6.89^{c}
Aminopeptidase activity (U g^{-1} f.m.)	212.15±7.56^{c}	128.29±5.01^{b}	120.73±4.86^{ab}	117.06±3.78^{a}
Endoproteases activity at pH 5.5 (U g^{-1} f.m.)	79.49±3.18^{b}	67.85±2.71^{a}	93.23±3.73^{c}	94.14±3.77^{c}
Endoproteases activity at pH 7 (U g^{-1} f.m.)	104.78±4.69^{b}	82.25±2.87^{a}	101.74±4.07^{b}	106.44±4.26^{b}
Endoproteases activity at pH 9 (U g^{-1} f.m.)	111.65±2.45^{d}	82.43±3.12^{a}	99.91±3.08^{b}	103.07±4.12^{c}

Means followed by different letters are significantly different at α < 0.05. Each value represents the mean of 3 measurements (±SE).

(2008). Simultaneously, an increase in the non-protein fraction (peptides and amino acids) was observed. This coincides with the findings of ROZAN et al. (2001) and KUO et al. (2004).

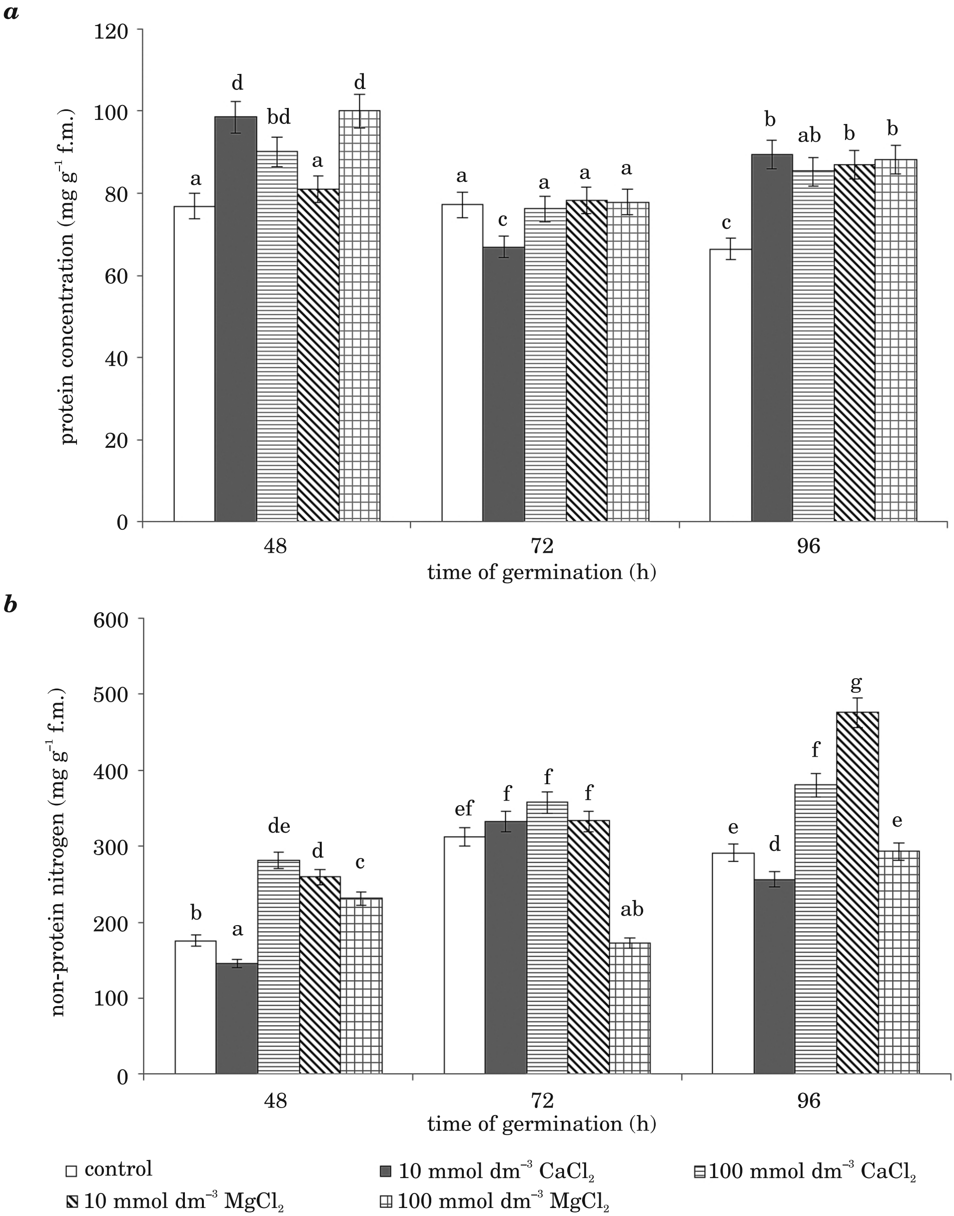

Fig. 1. Influence of $CaCl_2$ and $MgCl_2$ solutions on soluble protein (*a*) and non-protein nitrogen content (*b*)

Means followed by different letters are significantly different at $\alpha < 0.05$. Each value represents the mean of 3 measurements (±SE)

Table 2

Proteolytic activity in cotyledons of lentil sprouts elicited with $CaCl_2$ and $MgCl_2$ solutions

Specification	Day of germination (hours after induction)	Control	10 mmol dm^{-3} $CaCl_2$	100 mmol dm^{-3} $CaCl_2$	10 mmol dm^{-3} $MgCl_2$	100 mmol dm^{-3} $MgCl_2$
Aminopeptidase activity (U g^{-1} f.m.)	2 (24)	95.19 ± 3.81^{b}	87.84 ± 3.51^{ab}	101.84 ± 4.07^{bc}	92.13 ± 3.69^{b}	101.84 ± 4.07^{bc}
	3 (48)	111.07 ± 4.44^{bcd}	82.76 ± 3.31^{a}	97.89 ± 3.92^{b}	87.67 ± 3.51^{a}	97.89 ± 3.92^{b}
	4 (72)	105.39 ± 4.22^{bc}	108.39 ± 4.34^{c}	107.92 ± 4.32^{bc}	137.85 ± 5.51^{e}	124.03 ± 4.96^{de}
Endoprotease activity at pH 5.5 (U g^{-1} f.m.)	2 (24)	51.90 ± 2.08^{cd}	39.24 ± 1.57^{a}	46.05 ± 1.84^{b}	41.33 ± 1.65^{a}	46.05 ± 1.84^{b}
	3 (48)	60.67 ± 2.43^{d}	55.33 ± 2.21^{cd}	51.32 ± 2.05^{cd}	56.85 ± 2.27^{d}	51.32 ± 2.05^{c}
	4 (72)	88.82 ± 3.55^{e}	106.58 ± 4.26^{f}	92.86 ± 3.71^{e}	124.62 ± 4.98^{g}	106.72 ± 4.27^{f}
Endoprotease activity at pH 7 (U g^{-1} f.m.)	2 (24)	95.19 ± 3.81^{b}	87.84 ± 3.51^{ab}	101.84 ± 4.07^{bc}	92.13 ± 3.69^{b}	101.84 ± 4.07^{bc}
	3 (48)	111.07 ± 4.44^{c}	82.76 ± 3.31^{a}	97.89 ± 3.92^{b}	87.67 ± 3.51^{a}	97.89 ± 3.92^{b}
	4 (72)	105.39 ± 4.22^{bc}	108.39 ± 4.34^{c}	107.92 ± 4.32^{bc}	137.85 ± 5.51^{e}	124.03 ± 4.96^{d}
Endoprotease activity at pH 9 (U g^{-1} f.m.)	2 (24)	59.49 ± 2.38^{d}	43.67 ± 1.75^{ab}	52.63 ± 2.11^{c}	46.00 ± 1.84^{b}	40.53 ± 1.62^{a}
	3 (48)	49.33 ± 1.97^{b}	46.67 ± 1.87^{bc}	44.74 ± 1.79^{b}	47.95 ± 1.92^{bc}	44.74 ± 1.79^{b}
	4 (72)	82.89 ± 3.32^{e}	88.16 ± 3.53^{e}	88.31 ± 3.53^{e}	103.08 ± 4.12^{f}	101.49 ± 4.06^{f}

Means followed by different letters are significantly different at $\alpha < 0.05$. Each value represents the mean of 3 measurements (±SE).

In the control conditions, similar observations were made in the following days of germination. Considering the changes in the protein content in cotyledons from elicited sprouts, it could be seen that elicitation stopped the mobilization of proteins (Figure 1a). Three days after induction with ions, the level of soluble protein was still over 20% higher than in cotyledons from control sprouts. According to DOMASH et al. (2008), this may be linked to the plant's stress response, including production of stress proteins e.g. protease inhibitors. Paradoxically, the level of non-protein nitrogen in some cases was significantly elevated by induction with the analyzed abiotic elicitors. For instance, 24 hours after treatment with 100 mmol dm^{-3} $CaCl_2$, 10 mmol dm^{-3} $MgCl_2$ and 100 mmol dm^{-3} $MgCl_2$ the level of peptide and free amino acids was higher by about 60.20%, 47.57% and 31.39%, respectively. The highest elevation of non-protein nitrogen was observed in cotyledons from 4-day-old sprouts induced with 10 mmol dm^{-3} $MgCl_2$ (475.7 mg g^{-1} f.m.) – Figure 1b. The elevation of the non-protein nitrogen fraction was probably due to the proteolysis of damaged and dysfunctional proteins (membrane protein–lipid complexes, the nuclear matrix and other structures).

In comparison to the control sprouts, multiple proteolytic systems were activated only in cotyledons from 4-day-old sprouts elicited with $MgCl_2$ solutions. A significant increase occurred in the activity of aminopeptidase and endoprotease (Table 2). Statistical analysis of the Pearson's correlations showed that the non-nitrogen content was positively correlated with the activity of acidic endoproteases and aminopeptidases in control sprouts (Table 3). These findings agree with the ones presented by MUNTZ et al. (2001) on protein mobilization during germination of leguminous seeds. The correlation coefficients indicated that samples from $MgCl_2$ solutions treatment contained the highest levels of non-protein nitrogen. It may be speculated that the above conditions caused extensive damage to protein structures, and also that other classes of proteases are involved in protein cleavage. Other authors

Table 3

Relationships between soluble protein and non-protein nitrogen content and proteolytic activity

Specification	Control	10 mmol dm^{-3} $CaCl_2$	100 mmol dm^{-3} $CaCl_2$	10 mmol dm^{-3} $MgCl_2$	100 mmol dm^{-3} $MgCl_2$
N-PN /AP	0.98	-0.08	0.34	0.91	0.93
N-PN / EP 5.5	0.57	0.33	0.75	0.99	0.83
N-PN / EP 7	0.43	0.36	0.79	0.99	0.81
N-PN / EP 9	0.08	0.16	0.55	0.95	0.84
SP / AP	-0.14	0.42	0.55	0.97	0.10
SP / EP 5.5	-0.97	0.01	0.08	0.88	-0.12
SP / EP 7	-1.00	-0.02	0.02	0.87	-0.17
SP / EP 9	-0.96	0.18	0.35	0.94	-0.10

N-PN – non-protein nitrogen, AP – aminopeptidase activity, EP 5.5 – endoprotease activity at pH 5.5, EP 7 – endoprotease activity at pH 7, EP 9 – endoprotease activity at pH 9.

reported a key role of protease in protein metabolism during abiotic stresses (Antăo Malcata 2005, Palma et al. 2002).

Table 2 shows that the activity of proteases slightly decreased in the first days after induction. This effect was distinctly seen in cotyledons from 3-day-old sprouts, where activity of proteases was significantly decreased in comparison to control sprouts.

CONCLUSIONS

In the present research, it has been proven that elicitation with $CaCl_2$ and $MgCl_2$ solutions can be used to modify the rate of protein mobilization and may cause subsequent increase in the non-protein nitrogen fraction in cotyledons of germinated lentil.

1. Treatment with $MgCl_2$ solutions proved to be the most beneficial, leading to the highest yield of non-protein nitrogen in cotyledons from 6-day -old lentil sprouts.

2. Regardless of the type of elicitor, a decrease of the proteolytic activity was observed in cotyledons from 3- and 4-day-old lentil sprouts.

3. In sprouts elicited with $MgCl_2$ solutions, a significant correlation was found between the content of non-protein nitrogen content and activity of endo- and aminopeptidases.

The information provided by this study helps to design conditions for lentil sprouting which will result in production of food with an enhanced level of potentially bioaccessible non-protein nitrogen fraction (free amino acids and peptides).

REFERENCES

Anson M.L. 1938. *The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin*. J. Gen. Physiol., 22: 79-89.

Antăo C.M., Malcata X. 2005. *Plant serine proteases: biochemical, physiological and molecular features*. Plant Physiol. Biochem., 43(7): 637-650.

Domash V.I., Sharpio T.P., Zabreiko S.A., Sosnovskaya T.F. 2008. *Proteolytic enzymes and trypsin inhibitors of higher plants under stress conditions*. Russ. J. Bioorg. Chem., 34 (3): 318-322.

Gawlik-Dziki U., Świeca M. 2011. *Sprouts of selected plants as a source of bioavailable antioxidants and lipoxygenase inhibitors*. Ann. UMCS Lublin, Sect., DDD., 23, 3(17): 161-168.

Gawlik-Dziki U., Świeca M., Sugier D. 2012. *Enhancement of antioxidant abilities and the lipoxygenase and xanthine oxidase inhibitory activity of broccoli sprouts by biotic elicitors.* Acta Sci. Pol., Hort. Cult., 11(1): 13-25.

Ghavidel R.A., Prakash J. 2007. *The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seed*. LWT, 40: 1292-1299.

Khan S., Verma G., Sharma S. 2010. *A novel Ca^{2+}-activated protease from germinating Vigna radiata seeds andits role in storage protein mobilization*. J. Plant Physiol., 167: 855-861.

KUO Y-H., ROZAN P., LAMBEIN F., FRIAS J., VIDAL-VALVERDE C. 2004. *Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes*. Food Chem., 86: 537-545.

LOWRY O.H. 1951. *Protein measurement with the Folin phenol reagent*. J. Biol. Chem., 193: 265-275.

MUNTZ K., BELOZERSKY M.A., DUNAEVSKY Y.E., SCHLERETH A., TIEDEMANN J. 2001. *Stored proteinases and the initiation of storage protein mobilization in seeds during germination and seedling growth*. J. Exp. Bot., 52: 1741-1752.

OGIWARA N., AMANO T., SATOH M., SHIOI Y. 2005. *Leucine aminopeptidase from etiolated barley seedlings: characterization and partial purification of isoforms*. Plant Sci., 168: 575-581.

PALMA J.M., SANDALIO L.M., CORPAS F.J., ROMERO-PUERTAS M.C., MCCARTHY I., DEL RÍO L.A. 2002. *Plant proteases, protein degradation, and oxidative stress: role of peroxisomes.* Plant Physiol. Biochem., 40(6-8): 521-530.

PERIAGO M.J., ROS G., MARTÍNEZ C., RINCÓN F. 1996. *Variations of non-protein nitrogen in six Spanish legumes according to the extraction method used*. Food Res. Int., 29: 489-494.

RANDHIR R., SHETTY K. 2007. *Elicitation of the proline-linked pentose phosphate pathway metabolites and antioxidant enzyme response by ascorbic acid in dark germinated fava bean sprouts*. J. Food Biochem., 31: 485-508.

RODRÍGUEZA C., FRIAS J., VIDAL-VALVERDE C., HERNÁNDEZ A. 2008. *Correlations between some nitrogen fractions, lysine, histidine, tyrosine, and ornithine contents during the germination of peas, beans, and lentils.* Food Chem., 108(1): 245-252.

ROZAN P., KUO Y-H., LAMBEIN F. 2001. *Amino acids in seed and seedlings of genus Lens*. Phytochem., 58: 281-289.

SUNA S-W., LINA Y-C., WENGA Y-M., CHEN M-J. 2006. *Efficiency improvements on ninhydrin method for amino acid quantification*. J. Food Compos. Anal., 19: 112-117.

ŚWIECA M., GAWLIK-DZIKI U., KOWALCZYK D., ZŁOTEK U. 2012. *Impact of germination time and type of illumination on the antioxidant compounds and antioxidant capacity of Lens culinaris sprouts*. Sci. Hort., 140: 87-95.

ZEHRA A., GUL B., ANSARI R., KHAN M.A. 2012. *Role of calcium in alleviating effect of salinity on germination of Phragmites karka seeds*. S. Afr. J. Bot., 78: 122-128.

ZHAO J., DAVIS L.C., VERPOORTE R. 2005. *Elicitor signal transduction leading to production of plant secondary metabolites*. Biotechnol. Adv., 23(4): 283-333.

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CONTENT OF Na, K, Ca AND Mg IN COMPLEMENTARY INFANT FOOD

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Abstract

The aim of the study was to determine the content of selected mineral elements (sodium, potassium, calcium, magnesium) in fruit and vegetable desserts and dinner meals available on the Polish market designed for the nutrition of infants.

The research material consisted of commercial desserts and dinner meals for infants, purchased in Lublin and Warsaw (Poland) before their use-by date. Fifteen different types of desserts (in glass jars) and seventeen types of dinner meals (in glass jarshe t) from ten different manufacturers were tested. The tested baby foodstuffs were made in Poland, Germany, Slovakia, the Czech Republic and Switzerland. The content of Na, K, Ca and Mg was determined by means of the AAS flame technique on a Unicam 939 (AA Spectrometer Unicam) apparatus. The concentrations of Na, Ca and Mg in the analyzed baby foods did not exceed the norms. Assuming that a baby aged 9-12 months consumes one jar of dessert and one jar of dinner meal daily, he/she takes in 94 mg of Na (21% of the AI), more than 342 mg of K (45% of the AI), about 35 mg of Ca (7% of the AI) and above 41 mg of Mg (33% of the AI) which are included in the product.

Keywords: infant nutrition, fruit and vegetable food, desserts, dinner meals, macroelements.

ZAWARTOŚĆ Na, K, Ca I Mg W UZUPEŁNIAJĄCYCH PRODUKTACH SPOŻYWCZYCH PRZEZNACZONYCH DLA NIEMOWLĄT I MAŁYCH DZIECI

Abstrakt

Celem pracy było określenie zawartości wybranych składników mineralnych (sodu, potasu, wapnia, magnezu) w dostępnych na polskim rynku deserach owocowo-warzywnych oraz daniach obiadowych przeznaczonych do żywienia niemowląt.

Materiał do badań stanowiły gotowe desery oraz dania obiadowe przeznaczone dla niemowląt i małych dzieci, zakupione w sklepach spożywczych na terenie Lublina oraz Warszawy, w okresie ich przydatności do spożycia. Badano 15 różnych typów deserów (w słoikach) oraz 17 typów dań obiadowych (w słoikach). Badane produkty wyprodukowano w Polsce, Niemczech, Słowacji, Czechach oraz Szwajcarii. Zawartość Na, K, Ca i Mg

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oznaczono za pomocą płomieniowej techniki AAS w aparacie Unicam 939 (AA Spektrometr Unicam). Koncentracja Mg, Ca i Na w badanych deserach nie przekraczała dopuszczalnych norm. Zakładając, że niemowlę w wieku 9-12 miesięcy spożywa dziennie 1 słoik deseru oraz 1 słoik obiadku, przyjmuje wraz z tymi posiłkami 94 mg Na (21% AI), ponad 342 mg K (45% AI), ok. 35 mg Ca (7% AI) oraz prawie 41 mg Mg (33% AI).

Słowa kluczowe: żywienie niemowląt, owocowo-warzywne produkty dla niemowląt, desery, obiady, makroelementy.

INTRODUCTION

During the first 4–5 months of life, the baby's only food is mother's milk or a suitable milk substitute formula. Afterwards, it is recommended to enrich the diet with other food to ensure an adequate intake of minerals. Minerals are involved in many functions in the body, e.g. bone mineralization and enzymatic reactions. Infant nutrition often includes commercial products, industrially manufactured and targeted at this particular group of children. Such products, designed for infants and children up to 3 years of age, are regarded as foods for special nutritional purposes. Desserts are the first fruit meal in the child's diet.

Fruit and vegetables are an important source of sodium, potassium, calcium and magnesium in the baby's diet. Sodium is found in carrot, celery, parsley and table salt (Kunachowicz et al. 2005). Sodium helps regulate the functioning of nerves and muscular contraction; it also interacts with potassium for maintain extracellular fluid balance (Soetan et al. 2010). Carrot is an important source of potassium (Kunachowicz et al. 2005). Potassium activates a number of enzymatic reactions and participates in the transmission of neural impulses in the nervous and muscular systems; it also takes part in energy and carbohydrate transformations, systemic protein synthesis and amino acid transport in the cell (Shimoni 2005). Green vegetables, like broccoli or spinach, are among the best sources of calcium (Manson 2002). As well as being the major component of bones and teeth, calcium is a co-factor of numerous enzymes; calcium deficiency in children is manifested by rickets as well as a retarded growth (Manson 2002). Magnesium is a co-factor in a number of enzymatic reactions. Deficiency of Mg leads to anaemia, irritability, protein synthesis disorders and brittleness of the bones (Poleszak, Nowak 2006, Griffin et al. 2008). Green vegetables and legumes are an important source of magnesium (Kunachowicz et al. 2005).

The aim of the study was to determine the content of selected mineral elements (sodium, potassium, calcium, magnesium) in desserts and dinner means available on the Polish market and designed for the nutrition of infants.

MATERIAL AND METHODS

Food samples

The research material consisted of commercial fruit and vegetable desserts and dinner meals for infants, purchased in Lublin and Warsaw before their use-by date. Fifteen different types of desserts (in glass jars) and seventeen types of dinner mealls (in glass jar) from ten different manufacturers were tested. All were labelled as a "food for special purposes" (Tables 1, 2), some were additionally given the BIO symbol, which means that their components originated from certified organic farms, while two of the products

Table 1

Specification of baby desserts

Trade mark	Ingredients[c]	Annotation	Portion (g)	Country	Energy (kcal)	
					in 100 g*	in jar**
A-1	apple, pear, apple juice, wheat starch (gluten free), corn starch, vit. C	*a*	125	Poland	70	88
A-2	white grape juice, pear, plum, apricot, tapioca starch, rose hip, vit. C	*a*	125	Poland	69	86
B	apple, peach, grape juice, water, sugar, corn starch, vit. C	*a*	130	Poland	64	83
C-1	apple, apricot, vit. C	*a*	130	Poland	46	60
C-2	banana, pineapple juice, orange juice, apple, corn starch, lemon juice, vit. C	*a*	130	Poland	84	109
D-1	ecological yoghurt, water, apple, bilberry, sugar, carrot juice, vit. C	*a*, *b*	125	European Union	76	121
E-1	apple, apricot, yoghurt	*a*	190	Slovakia	59	77
E-2	apple, apricot	*a*	190	Slovakia	47	61
F-1	apple juice, apple, cherry	*a*, *b*	190	Germany	50	95
F-2	pear, apple	*a*, *b*	190	Germany	51	97
G-1	apple, banana, vit. C	*a*	125	Czech Republic	63	79
G-2	white grape juice, pear, plum, apricot, tapioca starch, rose hip, vit. C	*a*	125	Czech Republic	69	86
G-3	apple, rose hip, grape juice, wheat starch (gluten free), corn starch, vit. C	*a*	125	Czech Republic	64	80
H-1	banana, water, peach, rise flour, lemon juice, apple juice	*a*, *b*	190	Switzerland	63	120
H-2	water, apple, banana, pear juice concentrate, rice starch, apricot, lemon juice	*a*, *b*	190	Switzerland	65	124

a – food for special purposes, *b* – organic food, [c] – ISO 9001:2000, 14001:1996
* values as declared by the manufacturer, ** values calculated

Table 2

Specification of baby dinners

Trade mark	Ingredients[c]	Anno-tation	Portion, (g)	Country	Energy (kcal)	
					in 100 g *	in jar **
A-3	water, carrot, potato, rice, chicken meat, celery, soybean oil	*a*	125	Poland	48	60
C-3	spinach, potato, milk, water, cream	*a*, *b*	190	Poland	55	104
C-4	potato, carrot, water, chicken meat, corn oil, salt	*a*, *b*	190	Poland	56	106
C-5	carrot, water, potato, chicken meat, pea, corn starch, rape oil	*a*	190	Poland	61	116
C-6	carrot, tomato, pea, water, turkey, rice flour, onion, corn oil,	*a*, *b*	190	Poland	62	118
C-7	carrot, tomato, water, chicken meat, corn oil	*a*, *b*	190	Poland	53	101
D-2	water, potato, tomato, rice, chicken meat, onion, corn oil, salt, white pepper, caraway seed	*a*, *b*	220	European Union	69	152
D-3	tomato, carrot, pea, water, wheat pasta, chicken meat, wheat flour, corn oil, onion, salt	*a*, *b*	220	European Union	73	161
D-4	water, rice, carrot, turkey meat, corn oil, salt	*a*, *b*	220	European Union	72	158
H-3	broccoli, water, whole-meal rice flour, rice starch	*a*, *b*	190	Switzerland	34	65
H-4	carrot, potato, tomato, spinach, water, rice	*a*, *b*	220	Switzerland	34	75
H-5	water, potato, corn, beef meat, rice flour, vegetable oil	*a*, *b*	190	Switzerland	52	99
I-1	carrot, potato, spinach, parsnip, leek, water	*a*, *b*	190	Germany	24	46
I-2	carrot, pea, tomato, water, ham, whole-meal wheat pasta, sunflower oil, herbs	*a*, *b*	220	Germany	52	114
I-3	pumpkin, water, chicken meat, whole-meal rice flour, sunflower oil	*a*, *b*	190	Germany	74	141
I-4	carrot, potato, water, chicken meat, sunflower oil, herbs	*a*, *b*	190	Germany	55	104
J-1	carrot, water, rice, oil	*a*, *b*	125	Poland	45	56

The key under Table 1

were manufactured under the supervision of experts in baby nutrition and had the quality certificate ISO 9001:2000, 14001:1996. The tested baby foods were made in Poland, Germany, Slovakia, the Czech Republic and Switzerland. One manufacturer labelled the products as “Made in the European Union”.

Methods

The baby food samples were shaken manually before analysis. The content of Na, K, Ca and Mg was determined by means of the AAS flame technique on a Unicam 939 (AA Spectrometer Unicam) apparatus. Portions of approximately 10 g of the analyzed material were weighed out. The samples were dried at a temperature of 105°C for 48 hours and then mineralized in a zinc furnace at a temperature of 550°C for 16 hours. 10 ml of 6 N HCl was added to the burnt samples and the solution was filtered to measuring flasks and replenished with distilled water to the volume of 50 ml. The stock solution was used in the analyses, except for Mg and K, where the solution was diluted ten-fold.

All the chemical analyses were performed in two replications.

Calculations and statistical analysis

The content of Na, K, Ca and Mg in each of the analyzed products (n = 32) was converted into 1 g and peer container (a jar or a glass bottle). The size of a container was 125-190 g in the case of a dessert or 125-220 g for a dinner meal (Tables 1, 2). The calculation of % of the AI (adequate intake) of Na, K, Ca and Mg was based on the Polish recommendations (Jarosz, Bułhak-Jachymczyk 2008).

The results were subjected to statistical analysis. The Statistica 6.0 software was used to calculate the maximum, minimum and mean values, standard deviation (SD), standard error of the mean (SEM), and the median.

RESULTS

Table 3 presents the mean content of Na, K, Ca and Mg in the desserts and dinner meals, calculated per 1 g of the product and in one jar. One jar of a baby dessert contained an average of 15.11 mg of Na, 160.4 mg of K, 5.97 mg of Ca and 17.7 mg of Mg. A jar with a dinner meal contained on average 79.14 mg of Na, 181.7 mg of K, 28.62 mg of Ca and 23.3 mg of Mg. Assuming that a baby aged 9-12 months consumes one jar of dessert and one jar of dinner daily, he/she takes in 94 mg of Na (21% of the AI), more than 342 mg of K (45% of the AI), about 35 mg of Ca (7% of the AI) and above 41 mg of Mg (33% of the AI) which are included in the product (Table 3).

The highest content of sodium was found in the baby dinner meals (Table 3). All the baby dinners and only 13 desserts had labels informing that the product contained Na, and the amount of this element was given per 100 g of the product. Figures 1 and 2 present the declared and measured content of sodium in the examined desserts and dinners. The author's own studies revealed that only 3 desserts (A-1, G-1 and G-3) contained more sodium than declared by the manufacturer, although the differences were not significant.

Table 3

Results of baby food analyses and AI

Descriptive statistics	In 1 g of natural mass				In jar			
	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	Na (mg)	K (mg)	Ca (mg)	Mg (mg)
Baby desserts								
Maximum	0.22	2.59	0.22	0.31	41.8	486.4	27.5	58.9
Minimum	0.03	0.13	0.01	0.06	3.9	24.7	1.3	7.8
Mean	0.10	1.06	0.04	0.11	15.11	160.4	5.97	17.7
Median	0.09	1.02	0.02	0.09	11.5	127.5	3.8	11.4
SD	0.06	0.58	0.05	0.07	11.51	107.4	6.6	14.83
SEM	0.016	0.15	0.014	0.02	2.97	27.7	1.71	3.83
Baby dinners								
Maximum	1.11	1.93	0.34	0.18	224.2	366.7	64.60	39.6
Minimum	0.09	0.20	0.08	0.07	17.11	25.00	12.50	12.5
Mean	0.41	0.94	0.15	0.12	79.14	181.7	28.62	23.3
Median	0.21	0.95	0.12	0.11	44.00	180.5	24.70	22.8
SD	0.38	0.42	0.07	0.03	78.23	86.45	14.09	7.17
SEM	0.16	0.15	0.10	0.01	3.02	16.8	9.43	2.11
AI (6-12 months old children) acc. JAROSZ, BUŁHAK-JACHYMCZYK (2008)					370	400	400	70
% of AI *					21.39	45.44	7.15	33.29

SD – standard deviation, SEM – standard error of the means, AI – adequate intake
* assuming that a 12-month-old child consumes on average one jar of baby dessert and one jar of dinner daily

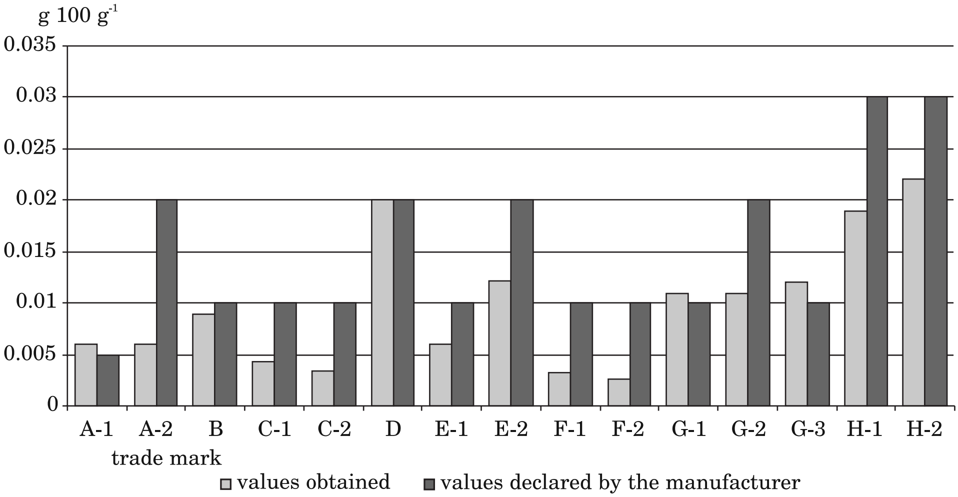

Fig. 1. Declared and measured content of sodium in examined baby desserts (g 100 g^{-1})

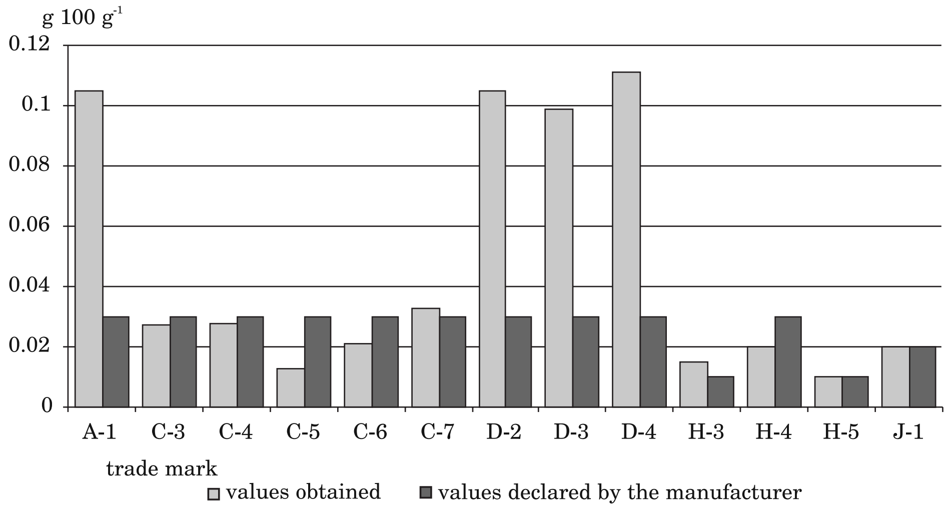

Fig. 2. Declared and measured content of sodium in examined baby dinner meals (g 100 g^{-1})

DISCUSSION

Processed nutritional products for infants are not very popular in Poland. Research has shown that only 22% of parents feed their children with such products on a regular basis (Winiarska-Mieczan, Gil 2007). The studies performed by these authors revealed that the most popular products are fruit and vegetable desserts, while dinner meals are fed far less often. Among desserts and fruit-vegetable purees, the most popular are those prepared from home-grown fruit, especially apples, as well as the products containing bananas (Górecka et al. 2007). Desserts are the first fruit meal in the child's diet. They contain pressed fruit and have delicate taste and smooth consistency, which makes them easy to swallow. Additionally, fruit desserts facilitate introduction of fruit into babies' diet and provide a valuable source of vitamins (Čižkova et al. 2009), as well as an important source of mineral elements.

Fruit and vegetable products typically contain insufficient amounts of Na and Ca; therefore infants must obtain these minerals from other sources. The author noticed that the average content of Na in baby desserts was 0.1 mg g^{-1}, while in dinners it was 0.41 mg g^{-1} of the product. The data available in the bibliography regarding the content of sodium in Polish fruit and/or vegetable products intended for infants revealed that the content of this mineral in juices reached 0.013-0.168 mg g^{-1} of the product (Winiarska-Mieczan, Nowak 2008). The study performed by Marzec et al. (2008) showed that the average content of sodium in fruit desserts was 0.069 mg g^{-1} of natural mass, whereas in fruit and vegetable juices it amounted to 0.072 mg g^{-1} of the product. The sodium content in Norwegian fruit puree products was similar to the Polish results, i.e. 0.029 mg g^{-1} on average (MELØ et al. 2008). According to Saudi Arabian studies, the content of sodium in fruit and vegetable pastes was 0.45 mg per 1 g of the product. A much better source of sodium for infants originates from cereals-based food (AL KHALIFA, AHMAD 2010, MELØ et al. 2008) and dinner meals (MELØ et al. 2008). The adequate daily sodium intake for babies up to 12 months of age in Poland is 370 mg (JAROSZ, BUŁHAK-JACHYMCZYK 2008). According to the regulation by the Polish Minister of Health regarding nutritional products of special use, sodium salt must not be added to dessert and pudding products prepared from fruit, except when such supplementation could be technologically justified (Journal of Laws 2007).

The author's own studies showed that the average content of Ca in the analysed baby food was 0.04 mg g^{-1} in desserts and 0.94 mg g^{-1} in dinners. According to MARZEC et al. (2009), the average content of calcium in baby fruit and fruit and vegetable desserts available in Poland is 0.071 mg g^{-1} of the product, while the mean content of this mineral in fruit and fruit and vegetable juices is nearly 0.087 mg in 1 g (MARZEC et al. 2009). Norwegian studies revealed that the mean content of calcium in fruit products was 0.1 mg in 1 g of the product (MELØ et al. 2008). The studies carried out in Saudi Arabia showed that the content of Ca in fruit and vegetable pastes intended for infants was 0.2 mg in 1 g (AL KHALIFA, AHMAD 2010). The richest sources of calcium for a baby are mother's breast milk, milk formula and cereal gruel. According to the European Union law (Commission Directive 2006), milk formulas intended for infants should contain 50-140 mg of Ca/100 kcal. In Polish, the adequate intake of calcium for babies up to 12 months of age is 400 mg (JAROSZ, BUŁHAK-JACHYMCZYK 2008).

The author found that the average content of K in baby desserts and dinners was about 1 mg g^{-1}, while the content of Mg was about 0.1 mg g^{-1} of these products. Other studies carried out in Poland showed that the level of potassium and magnesium in commercial baby food is high. According to MARZEC et al. (2008), the average content of potassium in baby soups is 1.32 mg g^{-1} of the product, in baby dinners – 1.61 mg g^{-1}. The study performed by WINIARSKA-MIECZAN and GIL (2002) revealed that vegetable and meat dinner meals contained approx. 0.4-0.6 mg of K per 1 g of the product. The concentration of Mg in baby dinners reached 0.076 mg – 0.105 mg g^{-1} of the product (MARZEC et al. 2007). The potassium and magnesium content in Norwegian commercial dinners was 1-2 mg and 0.07-0.11 mg g^{-1}, respectively (MELØ et al. 2008). The analyses carried out in Saudi Arabia showed that fruit and vegetable products contained as much as 0.69 mg of potassium and about 0.13 mg of magnesium per 1 g of the product (AL KHALIFA, AHMAD 2010). Cereal porridges and infant formulas are a much better source of K and Na for infants (MELØ et al. 2008).

CONCLUSION

The concentration of Na, Ca and Mg in the analyzed baby products did not exceed the acceptable norms (JAROSZ, BUŁHAK-JACHYMCZYK 2008). An excessive level of potassium was observed in nearly half of the studied desserts and dinners. Assuming that a baby aged 9-12 months consumes one jar of dessert and one jar of dinner daily, he/she takes in 94 mg of Na (21% of the AI), more than 342 mg of K (45% of the AI), about 35 mg of Ca (7% of the AI) and above 41 mg of Mg (33% of the AI) which are included in the product. Desserts and dinners are an important source of mineral elements in the baby's diet.

REFERENCES

AL KHALIFA A.S., AHMAD D. 2010. *Determination of key elements by ICP-OES in commercially available infant formulae and baby foods in Saudi Arabia.* Afr. J. Food Sci., 4(7): 464-468.

Commission Directive 2006/141/EC. Official Journal of the European Union.

ČIŽKOVA H., ŠEVČIK R., RAJCHL A., VOLDŘICH M. 2009. *Nutritional quality of commercial fruit baby food*. Czech J. Food Sci., 27: 134-137.

GÓRECKA D., SZCZEPANIAK B., SZYMANDERA-BUSZKA K., FLARCZYK E. 2007. *Popularity of processed foodstuffs for infants and small children among parents*. Acta Sci. Pol. Technol. Aliment., 6(4): 123-133.

GRIFFIN I.J., LYNCH M.F., HAWTHORNE K.M., CHEN Z., HAMZO M., ABRAMS S.A. 2008. *Magnesium retention in 12 to 48 month-old children*. J. Am. Coll. Nutr., 27: 349-355.

JAROSZ M., BUŁHAK-JACHYMCZYK B. (Ed.) 2008. *Norms of human nutrition: essential of prevention of obesity and non-infectious diseases.* PZWL, Warsaw.

Journal of Law 2007. The Official Journal of the Polish State, No 209, item 1518.

KUNACHOWICZ H., PRZYGODA B., NADOLNA I., IWANOW K. 2005. *Tables of nutritional value of food products and dishes.* PZWL, Warsaw.

MANSON P. 2002. *Calcium – an update*. Pharm. J., 268: 329-330.

MARZEC A., MARZEC Z., KIDAŁA J., ZARĘBA S. 2009. *Calcium and phosphorus content in some food products designed for nutrition of infants and small children.* Bromat. Chem. Toksykol., 3: 793-797.

MARZEC A., MARZEC Z., ZARĘBA S. 2007. *Magnesium, iron and manganese in some ford products designer for nutrition of infants*. Pol. J. Environ. Stud., 16(3A): 198-201.

MARZEC A., MARZEC Z., ZARĘBA S. 2008. *Natrium and potassium in some products designed for infant's nutrition.* Ann. UMCS, 1(9): 73-76.

MELØ R., GELLEIN K., EVJE L., SYVERSEN T. 2008. *Minerals and trace elements in commercial infant food.* Food Chem. Toxicol., 46(10): 3339-3342.

POLESZAK E., NOWAK G. 2006. *Magnesium in pathophysiology and therapy of affective disorders*. J. Elementol., 11(3): 389-397.

SHIMONI Y. 2005. *Dexamethasone and cardiac potassium currents in the diabetic rat*. Br. J. Pharmacol., 146(2): 280-287.

SOETAN K.O., OLAIYA C.O., OYEWOLE O.E., 2010. *The importance of mineral elements for humans, domestic animals and plants: a review.* Afr. J. Food Sci., 4(5): 200-222.

WINIARSKA-MIECZAN A., GIL G. 2007. *Evaluation of the infant's exposure to lead and cadmium in the ready-cooked food*. Bromat. Chem. Toksykol., 2: 137-144.

WINIARSKA-MIECZAN A., NOWAK K. 2008. *Determining the content of chosen minerals in fruit-vegetable baby juices.* J. Elementol., 13(3): 433-442.

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CHEMICAL COMPOSITION OF SPRING BARLEY *(HORDEUM VULGARE* L.) GRAIN CULTIVATED IN VARIOUS TILLAGE SYSTEMS

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Abstract

The study evaluated the chemical composition of spring barley grain sown in 3 tillage systems (main plots): a) conventional tillage (CT) – shallow ploughing and harrowing after harvest of the previous crop, ploughing in the autumn; b) reduced tillage (RT) – only cultivator after harvest of the previous crop, and c) herbicide tillage (HT) – only glyphosate (360 g L^{-1}) after harvest of the previous crop. In the springtime, a cultivation set composed of a cultivator, a string roller and a harrow was used on all the plots. The second experimental factor was barley cultivar (subplots): 1) husked Tocada, and 2) naked-grain Rastik. The depth of tillage varied according to the intended purpose: shallow ploughing to 10-12 cm, autumn ploughing to 25-30 cm, and cultivator tillage to 10-15 cm. The soil under the experiment was Chalk Rendzina with the texture of sandy loam, rich in available phosphorus and potassium and slightly alkaline. The study demonstrated that the content of total protein and crude fiber in the grain depended only on a barley cultivar. In turn, the content of macro- and microelements was affected by both factors: the cultivar and tillage system. Herbicide tillage (HT) was shown to reduce the content of phosphorus (P), potassium (K), calcium (Ca), iron (Fe) and copper (Cu) in barley grain, compared to conventional tillage (CT). Simultaneously, it raised the phytate-P content of grain compared to reduced tillage (RT). The content of phytate-P was significantly higher in cv. Tocada than in cv. Rustik.

Keywords: spring barley, protein, starch, crude fiber, P-phytate, mineral composition, tillage system.

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SKŁAD CHEMICZNY ZIARNA JĘCZMIENIA JAREGO *(HORDEUM VULGARE* L.) W RÓŻNYCH SYSTEMACH UPRAWY ROLI

Abstrakt

W badaniach oceniano skład chemiczny ziarna jęczmienia jarego wysiewnego w 3 systemach uprawy roli: a) tradycyjnym (CT) – podorywka i bronowanie po zbiorze przedplonu oraz orka wykonana jesienią; b) uproszczonym (RT) – tylko kultywator po zbiorze przedplonu; c) herbicydowym (HT) – tylko glifosat (360 g L^{-1}) po zbiorze przedplonu. Drugim czynnikiem doświadczenia były odmiany jęcznienia: 1) Tocada o ziarnie oplewionym, 2) Rastik o ziarnie nieoplewionym. Zabiegi uprawowe wykonano na określoną głębokość: podorywka 10-12 cm, orka jesienna 25-30 cm, kultywatorowanie 10-15 cm. Wykazano, że zawartość białka ogółem i włókna surowego w ziarnie zależały jedynie od odmiany, natomiast zawartość makro- i mikroelementów – od odmiany i systemu uprawy roli. Herbicydowa uprawa roli (HT) wpływała na zmniejszenie w ziarnie jęczmienia zawartości fosforu (P), potasu (K), wapnia (Ca) oraz żelaza (Fe) i miedzi (Cu), w stosunku do uprawy tradycyjnej (CT). Jednocześnie uprawa HT wpływała na zwiększenie zawartości P-fitynowego w ziarnie, w stosunku do RT. Odmiana Tocada zawierała znacznie więcej P-fitynowego niż odmiana Rastik.

Słowa kluczowe: jęczmień jary, białko, skrobia, włókno surowe, P-fitynowy, skład mineralny, uprawa roli.

INTRODUCTION

The implementation of modern tools and machines for crop cultivation as well as plant protection chemicals has resulted in a variety of innovative solutions in plant cultivation, resulting in the development of new tillage systems. In practice, however, these solutions are not always optimal because their effectiveness depends on the interaction of many natural and economic factors on a farm (Gruber et al. 2012). As reported by Morris et al. (2010), in conventional and no-till systems, the yield of plants is a function of many interacting factors, whose effects are hardly predictable. Nonetheless, in general the no-till system produces slightly lower yields of crops than the conventional system. According to De Vita et al. (2007), zero tillage is more effective than the conventional system on arid soils as it reduces the evaporation of water from soil, thus ensuring better moisture availability to plants. Also López-Bellido et al. (1996) report that crop yielding in the no-till system decreases as the sum of precipitations increases. It is mostly cereals that are sown under various modifications of the zero tillage system, mainly because of the dominant acreage of cereal crops and their good adaptability to agronomic conditions. One of the key cereals is barley, whose grain is widespread in human nutrition (flakes, groats) and in animal feeds. The chemical composition and quality of barley grain change under the combined effects of cultivar-specific traits, weather conditions and agricultural practice (Ruibal-Mendieta et al. 2005, Zeb et al. 2006). Studies by Khan and Zeb (2007) and Chowdhry et al. (1995) demonstrated significant differences in the chemical composition of wheat grains caused by various weather conditions and agronomic treatments. According to Morris et al. (2009), the content of ash in wheat grain is affected more by the weather during its maturation than by its genotype. Also, Kraska (2011) and Woźniak and Makarski (2012) determined a higher ash content in the grain of wheat sown in the ploughless rather than in the conventional (ploughing) system. In turn, according to Araus et al. (1998), the highest content of minerals was assayed in grain exposed to drought at the early stage of maturation. As reported by Paris and Gavazzi (1972) and Eser et al. (1997), the chemical composition of grain depends on the soil type and fertilization. As shown by Morris et al. (2009), habitat conditions and cultivar-specific preferences also affect the content of protein and gluten in wheat grain. In a study by Peltonen and Virtanen (1994), high doses of nitrogen increased the protein content in grain, whilst in the research by Woźniak (2013) the protein content of grain was modified by tillage systems, with more protein determined in the grain harvested from the herbicide than from the conventional (ploughing) system.

The goal was to evaluate the effect of tillage systems on the chemical composition of grain of two spring barley cultivars.

MATERIAL AND METHODS

A field experiment with different tillage systems was carried out in 2010-2012 at the Experimental Farm in Uhrusk (51°18′12″N, 23°36′50″E), Poland. It was established with the method of randomized sub-blocks in three replications, where tillage systems were the main experimental factor (*main plots*) and barley cultivars were the second experimental factor (*subplots)*. The blocks 8 m x 75 m in size were divided into 3 sub-blocks, and each of the sub-blocks was further divided into 2 plots. Three tillage systems: a) conventional (CT), b) reduced (RT), and c) herbicide (HT); and two barley cultivars: 1) husked Tocada and 2) naked-grain Rastik, were evaluated. The analyzed cultivars are classified in the Common Catalogue of Varieties of Agricultural Plant Species (EU 2007) and designed for feedstuff purposes. Conventional tillage (CT) included shallow ploughing and harrowing after previous crop (pea) and pre-winter ploughing. Reduced tillage (RT) included only field cultivation after harvest of the previous crop, and herbicide tillage (HT) consisted of an application of glyphosate (360 g L^{-1}) – 4 L ha^{-1} after harvest of the preceding crop. In the springtime, a cultivation set composed of a cultivator, a string roller and a harrow was used on all the plots. The depth of tillage varied according to intended purpose: shallow ploughing to a depth of 10-12 cm, autumn ploughing to 25-30 cm and cultivator tillage to 10-15 cm.

The soil under the experimental area was Chalk Rendzina with the texture of sandy loam, rich in available phosphorus (214 mg P kg^{-1}) and potassium (237 mg K kg^{-1}) and slightly alkaline $pH_{(KCl)}$ = 7.2. The total N content was 1.03 g kg^{-1} and organic C equalled 7.60 g kg^{-1}. According to the classification by the IUSS Working Group WRB (2006), this soil was identified as Rendzic Phaeozem.

Barley was sown in the first decade of April, at a seed density of 320 seeds m^{-2}. Mineral fertilization was as follows: 70 kg N ha^{-1}, 26 kg P ha^{-1} and 83 kg K ha^{-1}. The herbicide (a.s. mecoprop + MCPA + dicamba) was applied in a dose of 1.5 L ha^{-1} at the tillering stage (22-23 on the Zadoks scale (Zadoks et al. 1974)).

Determinations of the content of mineral components in wheat grain were conducted after dry mineralization of the samples at 600°C. The resultant ash was dissolved in 5 cm^3 of 6M HCl, then replenished to the volume of 50 cm^3 with redistilled water. Measurements were carried out by Absorption Spectrometry with excitation in acetylene-air flame on a UNICAM 939 apparatus. Phytate-phosphorus was extracted from the ground sample with 5% TCA for 60 min. Next, the extract was centrifuged for 10 min at 3000 rpm. Phytate-P in the supernatant was determined with the spectrophotometric method (λ = 500 nm) using Wade reagent (0.3 g $FeCl_3$ $6H_2O$ + 3.0 g sulfosalicylic acid in 1.0 L) (Latta, Skin 1980, Dragičević et al. 2011). Nitrogen in barley grain was determined with the Kjeldahl's method and converted into total protein (N · 6.25). The starch content was assayed by shaking the grain samples with TRIS buffer (pH=9.2) until complete solubilization of protein. The remaining precipitate was hot-dissolved in water. Starch was determined spectrophotometrically (λ = 660 nm) in the form of a complex with iodine. Crude fiber was determined using a Fibertec TM 2010 system for dietary fiber assay in food according to AOAC, AACC and AOCS standards. The method was based on the producer's application called FOSS TECATOR.

The results were processed statistically using analysis of variance (Anova), while the significance of differences between mean values was evaluated with the Tukey's HSD test, $P < 0.05$.

RESULTS AND DISCUSSION

The tillage system appeared to have no effect on the protein content in grain, which depended only on a barley cultivar (Table 1). Grain of the hull-less cultivar Rastik contained significantly more protein than grain of the husked cultivar Tocada. Similar observations were made for crude fiber content in grain, which was influenced only by a barley cultivar, being twice as high in husked Tocada as in naked-grain Rastik. Also the variance components (F-Value) indicate that values of the above parameters are cultivar-specific rather than influenced by tillage system (Table 2). In contrast, the content of starch in grain was the same in both barley cultivars but differentiated by the tillage systems. The grain originating from HT was characterized by a higher starch content than the CT grain. The quality and

Table 1

Content of total protein, starch and crude fiber in spring barley grain

Cultivars (C)	Tillage systems (TS)			Mean
	CT*	RT	HT	
Total protein (%)				
Tocada	8.5	8.4	8.3	8.4
Rastik	12.7	12.6	12.7	12.7
Mean	10.6	10.5	10.5	-
** $HSD_{0.05}$ for TS – ns; C – 0.23; TS · C – ns				
Starch (%)				
Tocada	59.6	60.3	61.0	60.3
Rastik	60.0	60.1	60.9	60.3
Mean	59.8	60.2	61.0	-
** $HSD_{0.05}$ for TS – 0.87; C – ns; TS · C – ns				
Crude fiber (%)				
Tocada	4.4	4.4	4.5	4.4
Rastik	2.1	2.2	2.2	2.2
Mean	3.3	3.3	3.3	-
$HSD_{0.05}$ for TS – ns; C – 0.19; TS · C – ns				

* CT – conventional tillage, RT – reduced tillage, HT – herbicide tillage,
** $HSD_{0.05}$ – honestly significant difference, ns – not significant, $P < 0.05$;

Table 2

F-Value for total protein, starch and crude fiber in spring barley grain, $P < 0.05$

Effects	Total protein	Starch	Crude fiber
	F-Value		
TS*	0.19	6.38	0.20
C**	2343.9	0.62	1083.7
TS · C	0.46	0.90	0.11

* TS – tillage systems, ** C – cultivars;

chemical composition of grain are determined by the interaction of cultivar-specific, habitat and agrotechnical factors (RUIBAL-MENDIETA et al. 2005, ZEB et al. 2006). This implies that grain of the same cultivar grown under various agronomic, climatic, soil and technical conditions may differ in levels of mineral and organic compounds (protein, starch or dietary fiber). Of similar opinion are KHAN and ZEB (2007), MORRIS et al. (2009), KRASKA (2011) as well as WOŹNIAK and MAKARSKI (2013). In our experiment, the content of protein and crude fiber depended largely on a barley cultivar, whereas the starch content was determined by a tillage system. Woźniak (2013) showed no effect of tillage system on one wheat cultivar and its significant impact on another one. Nonetheless, both wheat cultivars contained highly diverse protein content in grain in particular years.

The content of phosphorus (P) was significantly higher in grain from RT and CT plots than in the grain from the HT system (Table 3), as well as in grain of cv. Rastik compared to cv. Tocada. The analysis of variance components (*F*-Value) demonstrates that the phosphorus (P) content in grain

Table 3

Content of macroelements and phytate-P in spring barley grain

Cultivars (C)	Tillage systems (TS)			Mean
	CT*	RT	HT	
P (g kg^{-1} d.m.)				
Tocada	3.19	3.20	3.23	3.21
Rastik	3.94	3.97	3.50	3.80
Mean	3.57	3.59	3.37	-
** $HSD_{0.05}$ for TS – 0.19; C – 0.16; TS · C – 0.28				
Phytate-P (g kg^{-1} d.m.)				
Tocada	1.82	1.69	1.93	1.81
Rastik	1.49	1.56	1.52	1.52
Mean	1.66	1.62	1.72	-
$HSD_{0.05}$ for TS – 0.08; C – 0.06; TS · C – 0.11				
K (g kg^{-1} d.m.)				
Tocada	3.21	3.63	3.12	3.32
Rastik	3.41	3.30	2.91	3.21
Mean	3.31	3.47	3.02	-
$HSD_{0.05}$ for TS – 0.14; C – ns; TS · C – 0.20				
Mg (g kg^{-1} d.m.)				
Tocada	0.96	1.01	0.94	0.97
Rastik	0.95	0.90	0.89	0.91
Mean	0.95	0.96	0.92	-
$HSD_{0.05}$ for TS – ns; C – 0.04; TS · C – ns				
Ca (g kg^{-1} d.m.)				
Tocada	0.56	0.38	0.30	0.41
Rastik	0.32	0.30	0.37	0.33
Mean	0.44	0.34	0.33	-
$HSD_{0.05}$ for TS – 0.07; C – 0.05; TS · C – 0.09				

* CT – conventional tillage, RT – reduced tillage, HT – herbicide tillage,
** $HSD_{0.05}$ – honestly significant difference, ns – not significant, $P < 0.05$;

Table 4

F-Value for mineral composition of spring barley grain, $P < 0.05$

Effects	P	Phytate-P	K	Mg	Ca	Fe	Zn	Cu	Mn
	F-Value								
TS*	5.36	6.24	36.05	1.19	11.48	476.21	137.80	22.97	0.10
C**	94.01	150.71	6.58	6.64	16.01	79.15	711.40	37.84	0.29
TS x Y	7.37	12.98	13.32	1.98	18.18	323.61	157.27	20.10	0.34

* TS – tillage systems, ** C – cultivars;

depended more on cultivar-specific traits than on a tillage system (Table 4). In turn, the content of phytate-P was significantly higher in the HT than in the RT system. The grain of cv. Tocada contained significantly more phytate-P than the grain of cv. Rastik. The tillage system was found to affect potassium (K) in grain, with the highest concentrations of this element noted in grain from RT plots, followed by CT plots, and the lowest ones in grain from HT plots. The content of magnesium (Mg) depended only on a barley cultivar and was higher in the grain of cv. Tocada than in the grain of cv. Rastik. Finally, the content of calcium (Ca) was higher in grain from the CT system than from the RT and HT systems, and in the grain of cv. Tocada than cv. Rastik. The evaluation of variance components indicates that the Ca content of grain depended to a similar extent on the cultivar-specific traits and tillage systems.

The content of iron (Fe) was higher in grain from the CT system compared to the RT and HT systems (Table 5). A higher Fe content was determined in the grain of cv. Tocada than cv. Rastik. Based on the *F*-Value, it may be concluded that the Fe content was more dependent on a cultivar than on a tillage system. The content of zinc (Zn) was also higher in grain from CT plots, lower in grain from HT plots and the lowest in grain from RT system. The grain of cv. Tocada contained 3-fold more Zn than cv. Rastik. In turn, the *F*-Value indicates that the Zn content of grain was affected more by a cultivar than by a tillage system. Also, the content of copper (Cu) was significantly higher in grain from CT plots than in grain from the RT and HT systems. The grain of cv. Tocada contained more Cu compared to the grain of cv. Rastik. In turn, the content of manganese (Mn) in grain remained unaffected by the experimental factors.

The grain of the two barley cultivars differed in the content of minerals. This trait was also differentiated by the tillage systems. This confirms the results reported by Kraska (2011) or Woźniak and Makarski (2012), where the mineral composition of grain depended on a tillage system. In our experiment, the HT system resulted in a lower content of phosphorus (P), potassium (K), calcium (Ca), iron (Fe) and copper (Cu) and in an increased content of phytate-P in grain compared to the CT or RT system. Similar observations were made by Jackowska and Borkowska (2002) or by Paris and Gavazzi (1972). In the research by Woźniak and Makarski (2012), grain

Table 5

Content of microelements in spring barley grain

Cultivars (C)	Tillage systems (TS)			Mean
	CT*	RT	HT	
Fe (mg kg^{-1} d.m.)				
Tocada	115.00	45.22	28.32	62.85
Rastik	55.45	52.16	45.31	50.97
Mean	85.22	48.69	36.82	-
** $HSD_{0.05}$ for TS – 4.36; C – 3.56; TS · C – 6.16				
Zn (mg kg^{-1} d.m.)				
Tocada	55.41	15.42	42.10	37.64
Rastik	13.20	13.41	7.98	11.53
Mean	34.31	14.41	25.04	-
$HSD_{0.05}$ for TS – 3.20; C – 2.61; TS · C – 4.52				
Cu (mg kg^{-1} d.m.)				
Tocada	10.61	6.49	5.48	7.53
Rastik	5.54	5.42	5.36	5.44
Mean	8.08	5.96	5.42	-
$HSD_{0.05}$ for TS – 1.11; C – 0.90; TS · C – 1.56				
Mn (mg kg^{-1} d.m.)				
Tocada	14.29	15.26	14.56	14.70
Rastik	14.39	14.49	15.00	14.63
Mean	14.34	14.88	14.78	-
$HSD_{0.05}$ for TS – ns; C – ns; TS · C – ns				

* CT – conventional tillage, RT – reduced tillage, HT – herbicide tillage,
** $HSD_{0.05}$ – honestly significant difference, ns – not significant, $P < 0.05$;

originating from the no-till system was characterized by more total ash, zinc (Zn) and copper (Cu) but less potassium (K), magnesium (Mg) and manganese (Mn). Higher concentrations of macroelements in grain can be explained by the fact that these elements are better accessible to plants by being able to penetrate more easily into deeper soil strata, especially in intensively scarified (aerated) soil in the ploughing system. The diversified content of elements in grain may also be due to various conditions of soil hydration in the tested tillage systems. The mineral uptake by plants from very dry soil is considerably reduced than from well-hydrated soil (WOŹNIAK, MAKARSKI 2012). Especially interesting seems to be the iron (Fe) content in the grain from the CT system compared to the RT and HT systems. Also KRASKA (2011) demonstrated changes in the mineral composition of wheat grain harvested from different plots. The grain harvested after red clover had a higher content of iron (Fe) than the grain harvested after other preceding crops. In our experiment, this may be explained by the easy and rapid mineralization of organic matter in the ploughing system, which means good release to soil nitrogen compounds, which are available to plants. In turn JACKOWSKA and BORKOWSKA (2002) report that a high nitrogen content in soil reduces the availability of some microelements to plants, thereby decreasing their content in grain.

CONCLUSIONS

1. The tillage systems had no significant effect on the content of total protein and crude fiber in barley grain, which were observed to depend only on a barley cultivar.

2. The herbicide tillage system (HT) compared to the conventional tillage system (CT) decreased the content of phosphorus (P), potassium (K), calcium (Ca), iron (Fe) and copper (Cu) while raising the phytate-P content in barley grain.

REFERENCES

ARAUS J.L., AMARO T., CASADESUS J., ASBATI A., NACHIT M.N. 1998. *Relationship between ash content, carbon isotope discrimination and yield in drain wheat*. Aust. J. Agric. Res., 25(6): 835-843.

CHOWDHRY M.A., RAMZAN M., ALAM K., KHALIQ I. 1995. *Correlation analysis for grain yield and quality traits in wheat*. J. Agric. Res., 33(2): 71-74.

DE VITA P., DI PAOLO E., FECONDO G., DI FONZO N., PISANTE M. 2007. *No-tillage and conventional tillage effects on durum wheat yield, grain quality, and soil moisture content in Southern Italy*. Soil Till. Res., 92: 69-78.

DRAGIČEVIĆ V.D., SREDOJEVIĆ S.D., PERIĆ V.A., NIŠAVIĆ A.R., SREBRIĆ M.B. 2011. *Validation study of a rapid colorimetric method for the determination of phytic acid and inorganic phosphorus from seeds*. Acta Period. Technol., 42: 11-21.

ESER D., ADAK M.S., BIESANTZ A. 1997. *Effect of fallow, winter lentil, nitrogen fertilization and different Tillage on wheat yield in the dry farming areas of Central Anatolia*. Turk. J. Agric. For., 23: 567-576.

European Union 2007. *Common Catalogue of Varieties of Agricultural Plant Species*. Official Journal C 304 A/01.

GRUBER S., PEKRUN C., MÖHRING J., CLAUPEIN W. 2012. *Long-term yield and weed response to conservation and stubble tillage in SW Germany*. Soil Till. Res., 121: 49-56.

IUSS Working Group WRB 2006. *World reference base for soil resources 2006*. World Soil Resources Reports No. 103: FAO, Rome.

JACKOWSKA I., BORKOWSKA H. 2002. *The influence of nitrogen fertilization on the content of trace elements in grain of same winter wheat cultivars*. Ann. UMCS, Sect. E, 57: 87-91.

KHAN I., ZEB A. 2007. *Nutrition composition of Pakistan wheat varieties*. J. Zhejiang Univ. Sci., B 8(8): 555-559.

KRASKA P. 2011. *Content of some elements in grain of spring wheat cv. Zebra depending on soil tillage systems and catch crops*. J. Elem., 16(3): 407-419. DOI: 10.5601/jelem.2011.16.3.06.

LATTA M., SKIN M. 1980. *A simple and rapid colorimetric method for phytate determination*. J. Agr. Food Chem., 28: 1313-1315.

LÓPEZ-BELLIDO L., FUENTES M., CASTILLO J.E., LÓPEZ-GARRIDO F.J., FERNÁNDEZ E.J. 1996. *Long-term tillage, crop rotation, and nitrogen fertilizer effects on wheat yield under rainfed Mediterranean conditions*. Agron J., 88: 783-791.

MORRIS C.F., LI SHUOBI, KING G.E., ENGLE D.A., BURNS J.W., ROSS A.S. 2009. *A comprehensive genotype and environment assessment of wheat grain ash content in Oregon and Washington: analysis of variation*. Cereal Chem., 86(3): 307-312.

MORRIS N.L., MILLER P.C.H., ORSON J.H., FROUD-WILLIAMS R.J. 2010. *The adoption of non-inversion tillage systems in the United Kingdom and the agronomic impact on soil, crops and the environment* – A review. Soil Till. Res., 108: 1-15.

PARIS P., GAVAZZI C. 1972. *The ash content of durum wheat in northern Italy*. Food Sci. Technol. Abstracts Tecnica Molitoria, 23(22): 709-728.

PELTONEN J., VIRTANEN A. 1994. *Effect of nitrogen fertilization on the physicochemical and functional properties of bread wheat*. Cereal Chem., 62: 427-430.

RUIBAL-MENDIETA N.L., DELACROIX D.L., MIGNOLET J.M.P., MARQUES C., ROZENBERG R., PETITJEAN G., HABIB-JIWAN J.L., MEURENS M., QEENTIN-LECLERCO J., DELZENNE N.M., LARONDELLE Y. 2005. *Spelt (Triticum aestivum ssp. spelta) as a source of breadmaking flours and bran naturally enriched in oleic acid and minerals but not phytic acid*. J. Agric. Food. Chem., 53: 2751-2759.

WOŹNIAK *A. 2013. The effect of tillage systems on yield and quality of durum wheat cultivars*. Tur. J. Agric. For., 37(2): 133-138. DOI:10.3906/tar-1201-53.

WOŹNIAK A., MAKARSKI B. 2012. *Content of minerals in grain of spring wheat cv. Koksa depending on cultivation conditions*. J. Elem., 17(3): 517-523. DOI: 10.5601/jelem.2012.17.3.13.

WOŹNIAK A., MAKARSKI B. 2013. *Contents of minerals, total protein and wet gluten in grain of spring wheat depending on cropping systems*. J. Elem., 18(3): 297-305. DOI: 10.5601/jelem.2013.18.2.09.

ZADOKS J.C., CHANG T.T., KONZAK C.F. 1974. *A decimal code for the growth stages of cereals*. Weed Res., 14: 415-421.

ZEB A., ALI Z., AHMAD T., ABDUMANON A. 2006. *Physicochemical characteristics of wheat varieties growing in the same and different ecological regions of Pakistan*. Pak. J. Biol. Sci., 9(9): 1823-1828.

J. Elem. s. 607–616

DOI: 10.5601/jelem.2014.19.2.642

REVIEW PAPERS

ZINC IN MEDICINE AND TREATMENT

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Abstract

Zinc is an essential and the second most abundant trace element in humans. It is critical for the growth, development and differentiation of cells, as well as for RNA transcription, DNA synthesis, cell division and cell activation. Zinc deficiency affects mainly functions of the immune system, but other consequences include inferior sperm activity, skin lesions, growth retardation, impaired wound healing, anemia and gastrointestinal disorders. Zinc supplementation protects against the hepatotoxic effects of alcohol, enhances the transport of water and electrolytes across the intestinal mucosa and improves immune and anti-inflammatory responses. Zinc is also known as an essential mineral for normal mobilization of vitamin A from the liver to the plasma. Besides, it increases the promoter response to 1,25-dihydroxyvitamin D in osteoblasts. On the other hand, excessive amounts of free zinc in tissues are toxic and accelerated zinc accumulation of zinc is a potent killer of neurons and glial cells.

Over 300 signaling molecules and transcription factors contain zinc as a cofactor. Free zinc in immune and tumor cells is regulated by 14 distinct zinc importers (ZIP) and transporters. An elevated amount of zinc transporters LIV-1, a subfamily of ZIP zinc transporters, appears in estrogen receptor–positive breast cancer and has been used as a reliable breast cancer marker. However, the fact that malignant cells are unable to accumulate zinc is an important factor in the development and progression of malignancy of prostate cancer.

Keywords: zinc deficiency, innate and adaptive immunity, neurodegenerative diseases, breast cancer, prostate cancer.

CYNK W MEDYCYNIE I LECZNICTWIE

Abstrakt

Cynk to drugi pod względem ilości w organizmie mikroelement niezbędny dla ludzi. Jest konieczny do wzrostu, rozwoju i podziału komórek, ich różnicowania i aktywacji, a także do syntezy DNA i transkrypcji RNA. Niedobór cynku wpływa głównie na funkcjonowa-

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nie układu immunologicznego, ale i na aktywność plemników, zmiany patologiczne na skórze, opóźnienie wzrostu, nieprawidłowe gojenie ran, anemię i zaburzenia układu pokarmowego. Suplementacja cynku chroni przed hepatotoksycznością alkoholu, poprawia transport wody i elektrolitów w śluzówce jelit, polepsza zarówno działanie układu odpornościowego, jak i odpowiedź przeciwzapalną organizmu. Cynk jest niezbędny w mobilizacji witaminy A z wątroby do osocza i zmniejsza odpowiedź promotora 1,25-dihydroxywitaminy D w osteoblastach. Nadmiar wolnego cynku jest toksyczny dla tkanek, a także dla neuronów i komórek glejowych.

Cynk jest kofaktorem ponad 300 mediatorów i czynników transkrypcyjnych. Jego aktywność w komórkach układu immunologicznego i nowotworowych jest regulowana przez 14 odmiennych cynkowych transporterów (ZIP). Zwiększone ilości transportera LIV1, należącego do rodziny transporterów cynkowych, odnotowano w receptorze estrogenowym raka piersi, i dlatego jest używany jako marker nowotworowy. Z kolei niezdolność komórek nowotworowych do akumulacji cynku jest ważnym czynnikiem w rozwoju i postępie choroby nowotworowej raka prostaty.

Słowa kluczowe: niedobór cynku, odporność wrodzona i nabyta, choroby neurodegeneracyjne, rak piersi, rak prostaty.

INTRODUCTION

Zinc was first mentioned in ancient Egypt, in the Smith Papyrus from 2000 BC. The use of zinc in medicinal skin cream which it described continued through the Roman times until our day (JAE-YONG KOH 2010). Zinc is the second most abundant trace metal, with 2-4 grams in humans. Normal blood plasma zinc levels range from 0.7 mg l^{-1} to 1.3 mg l^{-1} (PASTERNAK et al. 2010). It is an essential trace element, critical for the cell growth, development and differentiation, RNA transcription, DNA synthesis, cell division and cell activation. Zinc deficiency is harmful to embryogenesis and early childhood development, deteriorating mainly the immune system (PAKASI et al. 2010). Besides, zinc affects sperm activity, which conditions reproduction (BARRIER-BATTUT et al. 2002). Excess free zinc in tissues is toxic (JAE-YONG KOH 2010). In the periodic table of elements, zinc belongs to group IIb, which comprises two toxic metals: cadmium and mercury. Nevertheless, zinc is considered to be relatively non-toxic to humans (PLUM et al. 2010).

Zinc is essential in members of all enzyme classes, including over 300 signaling molecules and transcription factors (PAKASI et al. 2010). An optimal nucleic acid and protein metabolism, as well as a proper cell growth, division and functioning require sufficient availability of zinc (PLUM et al. 2010). Zinc is also an ionic signal. Zn^{2+} enters cells through gated channels, and moves among various organelles and storage depots within cells, where it modifies protein function by binding to and detaching from zinc-dependent proteins (PLUM et al. 2010).

Free zinc in immune and tumor cells is regulated by 14 distinct zinc importers (ZIP) and transporters (ZNT1-8). Zinc deficiency induces cell death *via* apoptosis or necrosis, while sufficient zinc levels allow for the maintenance of autophagy. Cancer cells have disturbed zinc importers and frequently increased zinc levels, which help them to survive (PAKASI et al. 2010).

Zinc is taken up primarily in the proximal small intestine, and depends heavily on ZIP4. Once transported by enterocytes and into blood, zinc binds to albumin, transferrin, α-2 macroglobulin and immunoglobulin G. Then, the element is transported to the liver, where it is stored in hepatocytes until being released back into blood to bind to carrier molecules again and to travel to tissues, where its intake will be regulated by zinc import and transport proteins. Red meat is a primary source of zinc for people. Nuts, fruit, whole grain bread, dairy products and fortified breakfast cereals are other zinc supplies. Oysters have the highest zinc quantity per serving among any common foodstuff (JOHN et al. 2010). Zinc is also known as an essential mineral for normal mobilization of vitamin A from the liver to plasma (PAKASI et al. 2010). Zinc supplementation protects against the hepatotoxic effects of alcohol (MARET, KRĘŻEL 2007).

Zinc deficiencies occur due to malabsorption syndromes and other gastrointestinal disorders, chronic liver and renal diseases, sickle cell disease, excessive alcohol intake, malignancy, cystic fibrosis, pancreatic insufficiency, rheumatoid arthritis and other chronic conditions – Figure 1 (JOHN et al. 2010). Besides, severe zinc deficiency is characterized by skin lesions, growth retardation, impaired wound healing, anemia, mental retardation and impaired visual and immunological function, which were observed even in mild zinc deficiency (HAASE, RINK 2009).

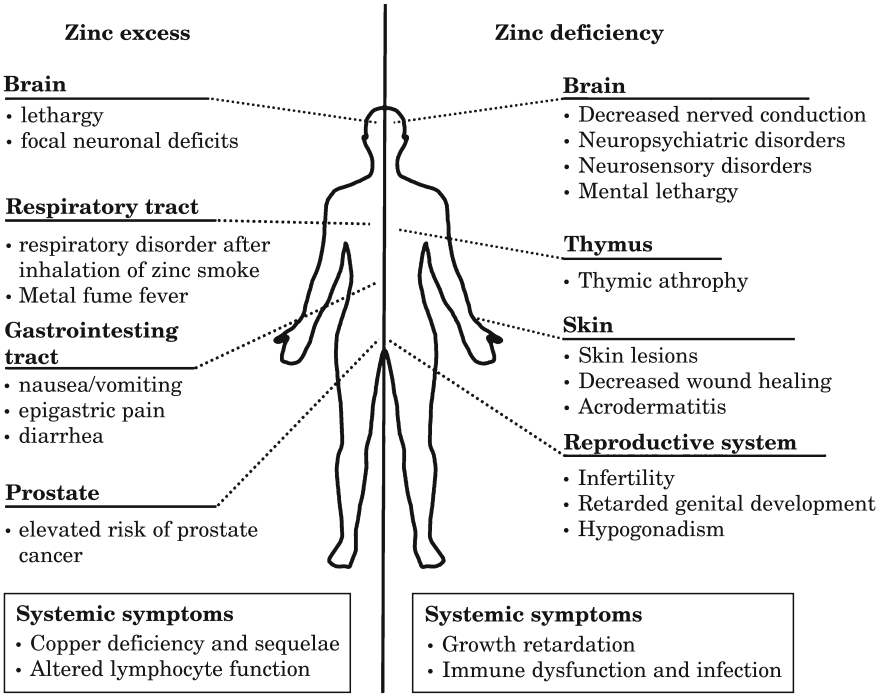

Fig. 1. Comparison of the effects of zinc intoxication *versus* deficiency (PLUM et al. 2010)

ZINC DEFICIENCES

Zinc supplementation in pregnant women

3.5 million deaths each year are caused by maternal and child malnutrition. There was a micronutrient supplementation trial conducted on 4998 pregnant women in rural Nepal from 1999 to 2001. The effect of zinc supplementation on child growth and body composition at 6–8 years of age was examined. The results showed that child growth may benefit from antenatal zinc supplementation, especially in areas where zinc deficiency is common (Steward et al. 2009).

Anemia

There were some studies in Mexico, where children less than 2 years old with several micronutrient deficiencies were examined. The most widely used indicators were plasma or serum zinc concentration. The results showed that zinc deficiency may stop children from attaining the full growth and development potential. One out five children had anemia. Low serum zinc concentrations were found in one out of three children living in urban areas and in one out of ten living in rural areas (Duque et al. 2007).

Acute diarrhoea in children

In 2003, diarrhoea was estimated to account for 18% of the reported 10.6 million deaths of children aged less than five years (Winch et al. 2008). In developing countries, each year, almost 2.5 million child deaths are caused by diarrhoea, 35% of which are due to acute diarrhoea. The importance of zinc in the pathophysiology of acute diarrhoea is prominent in higher daily fecal losses of these elements during acute diarrhoeal episodes. Fortunately, zinc supplementation improves the transport of water and electrolytes across the intestinal mucosa, preventing villous atrophy and improving immunity (Patel et al. 2009). Besides, zinc can contribute to a reduced application of antimicrobials, inappropriate for children, in the treatment of childhood diarrhoea through a replacement effect (Winch et al. 2008).

The impact of zinc supplementation for acute diarrhoea has a beneficial effect by shortening the duration of diarrhoea (Patel et al. 2009)

Rickets in children

Zinc plays a significant role in bone maturation, especially in the early stages of mineralization. The vitamin D receptor contains 2 zinc-finger domains. Zinc increases the promoter response to 1,25-dihydroxyvitamin D in osteoblasts. It has been suggested that zinc deficiency might predispose children to rickets, due to calcium deficiency which limits bone mineralization, especially in children with very low calcium intakes (Thacher et al. 2009).

Innate and adaptive immunity

Zinc deficiency affects multiple aspects of innate and adaptive immunity. As a result, people suffer from thymic atrophy, altered thymic hormones and lymphopenia (John et al. 2010). Thymic artrophy and high susceptibility to bacterial, fungal and viral infection are observed in patients suffering from *Acrodermatitis enteropathica*, which is a rare autosomal recessive inheritable disease. The zinc-specific malabsorption syndrome is induced by a mutation of the gene responsible for the intestinal zinc transport protein hZip4. Fortunately, all symptoms can be reversed by nutritional supplementation of zinc (Haase, Rink 2009). Zinc deficiency is associated with the rapid progression of HIV. Zinc acetate has been shown to be effective in the long-term treatment of Wilson disease because of its ability to compete with copper for binding sites (Saper , Rash 2009). Zinc deficiency also plays a role in the immunosenescence of the elderly (Haase, Rink 2009). Zinc-deficient patients had severe immune dysfunction, mainly affecting T helper cells (Parasad, 2008). During zinc deficiency, changes in the gene expression for cytokines, DNA repair enzymes, zinc transporters and signaling molecules are observed. Besides, oral zinc supplementation improves immunity and anti-inflammatory responses, which suggests that zinc is critical for normal immune cellular functions. Zinc deficiency disturbs proliferation of lymphocytes. Thymulin, a hormone secreted by thymic epithelial cells, requires zinc as a cofactor. It exists in plasma in the zinc-bound, active form and the zinc-free, inactive form. For this reason, zinc is essential for the differentiation and functioning of T cells. Furthermore, a decrease in the number of T cells is caused by zinc deficiency, which depressed production of Th1 cell cytokines, IFN-g, IL-2 and tumor necrosis factor (TNF)-α, which play a major role in tumor suppression. Zinc deficiency reduces the lytic activity of natural killer cells, impairs NKT cell cytotoxicity and immune signaling, impacts the neuro-endocrine-immune pathway and alters production of cytokines in mast cells. Zinc supplementation improves NK cell functions. Zinc homeostasis is mediated by metallothioneins (MTs), small cysteine-rich proteins that bind zinc. Tumor cells are mainly affected by zinc through its regulatory role in gene expression and cell survival (John et al. 2010).

Pulmonary tuberculosis patients

A clinical trial conducted in Indonesia showed that patients suffering from severe tuberculosis, when given zinc supplementation, had their sputum conversion time significantly reduced and experienced other significant health benefits (Pakasi et al. 2010).

Positive effect on skin

Nanostructures which include inorganic physical UV filters such as titanium dioxide (TiO_2) and zinc oxide (ZnO) are widely used in cosmetic dermatology. They can be found in sunscreens, in which they appear in particles sized between 60 to 200 nm in order to obtain transparent emulsion. Miniaturization of these minerals increases both their transparency and filtering capacity because of a higher reflective index (MORGANTI 2010).

It has been reported that some alopecia areata patients have zinc deficiency. Zinc supplementation has a positive effect on such patients and could serve as an adjuvant therapy (HOON et al. 2009). Delayed wound healing is associated with zinc deficiency. It has been noticed that zinc supplementation activates the nuclear factor-kappa B (NF□B), expression of pro-inflammatory cytokines (interleukin-1b and tumor necrosis factor-a) and neutrophil infiltration, which play a significant role in wound healing (BASAVARAJ et al. 2010).

ZINC IN ACUTE BRAIN INJURY

Zinc accumulation as a cause of neuronal death

Accelerated accumulation of zinc is a potent killer of neurons and glial cells. In 1986, some scientists demonstrated that a brief (15 min) exposure to 300-600 µM zinc resulted in extensive neuronal death in cortical cell culture. The fact that zinc was cytotoxic suggested that it might play a major role in neuronal injury (JAE-YONG KOH 2010).

ZINC IN NEURODEGENRATIVE DISEASES

Alzheimer disease

Alzheimer disease (AD) is characterized by the loss of cortical neurons and progressive deterioration of cognitive function, memory and self-care (JAE-YONG KOH 2010). In a healthy person, a normal amount of interstitial zinc is needed for the degradation of Aβ by zinc-dependent proteinases, which prevents its accumulation in the interstitium. Metalloproteinases are present in cerebrospinal fluid (CSF) for example neprilysin, insulin degrading enzyme (IDE) and matrix metalloproteinases (MMP2 and 3) (STROZYK et al. 2009). During Alzheimer disease, the accumulation of amyloid-β (Aβ) protein, which is linked to Zn^{2+}, neurofibrillary tangles (NFTs) and neurophil threads in the neocortex is observed (JAE-YONG KOH 2010). A high concentration of zinc (1 mM) has been found within amyloid plaques (STROZYK et al. 2009). Free Zn^{2+} in the extracellular fluid induces amyloid deposition. Transient hypoperfusion, head trauma or local paroxysmal neuronal firing lead to an increase in extracellular zinc levels, which might induce the binding of zinc to Aβ (JAE-YONG KOH 2010).

Parkinson disease (PD)

Zinc concentration was significantly lower in severe PD compared to controls. The data revealed an imbalance of inter-element relations and suggested some disturbance in the homeostasis of elements during the progression of PD (Muralidhar, Ponnuswamy 2004)

Amyotrophic Lateral Sclerosis (ALS)

Some abnormalities of zinc-metalloproteins have implicated zinc in the pathophysiology of ALS (Lou Gehrig disease). The familial form of ALS in man is accompanied by mutations in metalloenzyme Cu-Zn-superoxide dismutase (SOD). (Jae-Yong Koh 2010).

Cerebral ischemia

In cerebral ischemia, zinc is able to function both as a signaling mediator and neurotoxin. Both neurotoxic and neuroprotective capabilities were noticed. The earliest research provided indirect evidence for the toxic translocation of zinc from presynaptic neurons into selective postsynaptic neurons. On the other hand, during a brief period of global ischemia, intracellular zinc accumulation in vulnerable CA1 pyramidal hippocampal neurons preceded degeneration, which could be prevented by intracerebroventricular administration of zinc-chelator, ethylenediaminetetraacetic acid (EDTA) saturated with calcium (Ca-EDTA). The administration of extracellular zinc chelator prevents intracellular zinc accumulation, which precedes neuronal degeneration. Increased intracellular zinc levels during ischemia serve as a critical mediator of neuronal death. Equal doses of zinc chloride (ZnCl2), PP (protoporphyrin) and ZnPP (zinc protoporphyrin) were found to reduce the size of lesions, but only ZnPP and PP were detected to improve the health of a patient with ischemic brain edema. That suggests that in comparison to protoporphyrin, zinc ions provide neuroprotection *via* mechanisms other than reducing brain edema. Zinc supplementation could provide neuroprotection to the CA1 hippocampal subfield during global ischemia in the gerbil. (Galasso, Dyck, 2007)

Implications for Diseases with Particular Emphasis on Diabetes

Zinc is involved in many aspects of insulin metabolism and signaling. In type 2 diabetes, oxidative stress disturbs both zinc metabolism and MT levels, in which the proper control of zinc availability is essential for normal functions. This type of diabetes is associated with the polymorphism of the pancreatic β-cell-specific zinc transporter ZnT-8 (SLC30A8). ZnT-8 provides zinc for insulin maturation or storage in these cells. Zinc supplementation in animals and humans improves antidiabetogenic and insulinomimetic properties.

As a result, zinc-containing drugs are being synthesized and tested. Because cardiovascular diseases are a major cause of mortality in type 2 diabetes, major studies focus on the diabetic heart. There were some investigations in which the level of free zinc increases from 520 pM in normal to 870 pM in diabetic rat cardiomyocytes. However, activities of metallothionein and reduced glutathione and other enzymes of the antioxidant defense decreased (Maret, Krężel 2007).

Breast cancer

Increased amounts of zinc transporter LIV-1 (SLC39A6) are present in estrogen receptor–positive breast cancer and in tumors that spread to lymph nodes. The LIV-1 subfamily of ZIP zinc transporters consists of nine human sequences. It is a highly conserved group of eight transmembrane domain proteins, which are situated on the plasma membrane and which are responsible for zinc transport into cells. LIV-1 has been used as a reliable marker of luminal A type clinical breast cancer (Taylor et al. 2007).

Prostate cancer

Zinc accumulated in the epithelial cells of a prostate is essential to production and secretion of citrate. The production of citrate and its secretion into prostatic fluid is important for reproduction. In human prostate cancer, zinc downregulates HIF-1a protein levels. The lost ability of the malignant cells to accumulate zinc and citrate is an important factor in the development and progression of the malignancy of prostate cancer (Pasternak et al. 2011). This process is the result of decreased expression of specific zinc uptake transporters and glioblastoma cells under hypoxia. Research into the molecular mechanisms involved showed that zinc induced HIF-1a proteasomal degradation, which was prevented by treatment with proteasomal inhibitor MG132. Besides, zinc could be useful as an inhibitor of HIF-1a in human tumors to repress important pathways involved in tumor progression. Furthermore, it could be used in anticancer therapies (Nardinocchi et al. 2010). Zinc may modulate the IGF-1 metabolism in relation to carcinogenesis. It was observed that an optimal prostate zinc concentration plays a protective role against cancer (Prasad et al. 2010).

CONCLUSIONS

This paper discusses both excess and deficiency of zinc. Zinc is toxic to neurons and glial cells if its accumulated becomes accelerated. On the other hand, an optimal level of zinc in humans is necessary for the proper functioning of our bodies. Zinc supplementation has a positive effect on the skin and immune system. It also helps to prevent or treat many diseases such as anemia, rickets, diarrhoea, prostate cancer and pulmonary tuberculosis. Besides, the level of zinc transporter LIV1 could be a reliable marker of breast cancer. Moreover, zinc-containing drugs are tested on diabetic patients because of their anti-diabetogenic and insulinomimetic properties.

REFERENCES

Barrier- Battuth., Delajarraunde et al. 2002. *Calcium, magnesium, cooper and zinc in seminal plasma of fertile stallions, and their relationship with semen freezability*. Theriogen., 58: 229-232.

Basavaraj K.H., Seemanthini C., Rashmi R. 2010. *Diet in dermatology: present perspectives*. Ind. J. Dermatol., 55(3): 205-210.

Galasso S.L., Dyck R.H. 2007. *The role of zinc in cerebral ischemia*. Mol Med., 13(7-8): 380-387.

Haase H., Rink L. 2009. *The immune system and the impact of zinc during aging*. Immun. Ageing., 12:6-9.

Hoon P., Chul Woo K., M.D., Sang Seok K., Chun Wook P., 2009. *The therapeutic effect and the changed serum zinc level after zinc supplementation in Alopecia areata patients who had a low serum zinc level*. Ann Dermatol., 21(2): 142-146.

Jae-Yong Koh, 2010. *Endogenous zinc in neurological diseases*. J. Clin. Neurol., 30: 6(2): 109.

John E., Lascow T., Buchser W.J. et al. 2010. *Zinc in innate and adaptive tumor immunity*. J. Transl. Med., 8:118.

Maretl W., Krężel A. 2007. *Cellular zinc and redox buffering capacity of metallothionein/thionein in health and disease*. Mol. Med., 13(7-8): 371-375.

Morganti P. 2010. *Use and potential of nanotechnology in cosmetic dermatology*. Clin. Cosmet. Investig Dermatol., 3: 5-13.

Muralidhar L.H., Ponnuswamy S. 2004. *Serum trace element levels and the complexity of inter-element relations in patients with Parkinson's diseases*. J. Trace Elem. Med. Biol., 18(2): 163-171.

Nardinocci L., Pantisano V., Puca R. 2010. *Zinc downregulates HIF-1a and inhibits its activity in tumor cells in vitro and in vivo*. PLoS ONE, 5(12): 15048.

Pakasi T.A., Karyadi E., Made Desy Suratih N. et al. 2010. *Zinc and vitamin A supplementation fails to reduce sputum conversion time in severely malnourished pulmonary tuberculosis patients in Indonesia*. Nutr. J., 9:41.

Pasternak K., Horecka A., Kocot J. 2010. *Biochemistry of zinc*. Ann. UMCS Sect. DDD, 23(1): 19-26.

Patel A., Dibley M.J., Mamtannil M. et al. 2009. *Zinc and copper supplementation in acute diarrhea in children: a double-blind randomized controlled trial*. BMC Med., 10: 7:22.

Plum L.M., Rink L., Haase H. 2010. *The essential toxin: impact of zinc on human health*. Int. J. Environ. Res. Public Health, 7: 1342-1365.

Prasad A. S. 2008. *Zinc in human health effect of zinc on immune cells*. Mol. Med., 14(5): 353-357.

Prasad A. S., Mukhtar H. Beck F. W. J., 2010. *Dietary zinc and prostate cancer in the TRAMP Mouse Model*. J. Med. Food., 13(1): 70-76.

Saper R. B., Rash R. 2009. *Zinc: an essential micronutrient*. Am. Fam. Physician., 1; 79(9): 768

Steward C.P., Christian P., Leqlerq S.C. et al. 2009. *Antenatal supplementation with folic acid + iron + zinc improves linear growth and reduces peripheral adiposity in* school-age *children in rural Nepal*. J. Clin. Nutr., 90: 132-40.

STROŻYK D., LAUNER L.J., ADLARD P.A. et al. 2009. *Zinc and copper modulate alzheimer Aβ levels in human cerebrospinal fluid.* Neurobiol. Aging., 30(7): 1069-1077.

TAYLOR K.M., MORGAN H.E., SMART K. et al. 2007. *The emerging role of the LIV-1 subfamily of zinc transporters in breast cancer.* Mol. Med., 13(7-8): 396-406.

THACHER T.D., ALIU O., GRIFFIN I.J. 2009. *Meals and dephytinization affect calcium and zinc absorption in Nigerian children with rickets*. J. Nutr., 139: 926-932.

WINCH P.J., GILROY K.E., FISCHER WALKER C.L. 2008. *Effect of HIV/AIDS and malaria on the context for introduction of zinc treatment and low-osmolarity ORS for Childhood Diarrhoea.* J. Health Popul. Nutr., 26(1): 1-11.

XIMENA D., FLORES-HERNANDEZ S., FLORES-HUERTA S. et al., 2007. *Prevalence of anemia and deficiency of iron, folic acid, and zinc in children younger than 2 years of age who use the health services provided by the Mexican Social Security Institute.* Public Health, 7:345. DOI:10.1186/1471-2458-7-345

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MAGNESIUM – AN IMPORTANT COMPONENT OF HIGH-ENERGY COMPOSITIONS

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Abstract

Magnesium is a widely used component in high-energy compositions. Mixtures containing this metal can be found in show and military pyrotechnics, rocket propellants and various explosive masses. Magnesium containing compositions have high combustion temperature, which allows one to achieve the desired special effect. Two important stages in designing new high-energy mixtures, i.e. compatibility of substances and optimal composition, were described. The calculations were based on mixtures containing magnesium. In line with the standard STANAG 4147, using differential scanning calorimetry, compatibilities of mixtures of magnesium with octogen (HMX) and magnesium with hekzaazahekzanitroizowurzitane (CL-20) were examined. Magnesium is compatible with these nitroamines. An optimal composition which ensures the maximum combustion temperature and specific impulse was determined using the calculation programme isp2001. The optimum composition of the Mg : HMX composition burns at a lower temperature than the Mg : CL-20 mixture. The combustion temperature was 3493K for the former mixture and 3807K for the latter one. The specific impulse determined for both compositions was 273s. The specific impulse was established for mixtures with different shares of magnesium. The mixture containing in octogen reached the maximum specific impulse at 5% Mg, while the mixture containing CL-20 reached the highest specific impulse at 15% of this metal.

The dependence of the specific impulse of rocket propellant containing polybutadiene with terminal hydroxyl groups (HTPB), ammonium perchlorate and magnesium was examined. The maximum value of the impulse increases with a decreasing amount of the binder. When another binder such as for poly(glycidyl azide) (GAP) was used, a reverse relationship was observed. The specific impulse increased with an increased binder content.

The influence of various oxidants on the combustion temperature of pyrotechnic mixtures was defined. The highest combustion temperature was achieved for compositions with the magnesium content in the range of 20 to 45%. The effect on combustion temperature of the oxidants polytetrafluoroethylene, potassium chlorate and iron oxide was compared.

Keywords: magnesium, application, combustion, calculations, pyrotechnics.

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MAGNEZ – ISTOTNY SKŁADNIK MIESZANIN WYSOKOENERGETYCZNYCH

Abstrakt

Magnez jest szeroko stosowanym składnikiem w mieszaninach wysokoenergetycznych. Układy zawierające ten metal znaleźć można w pirotechnice widowiskowej i wojskowej, w paliwach rakietowych oraz licznych mieszaninach wybuchowych. Mieszaniny zawierające magnez mają wysoką temperaturę palenia, co umożliwia osiągnięcie żądanego efektu specjalnego. Na przykładzie mas zawierających magnez przedstawiono dwa istotne etapy występujące podczas opracowywania nowych mieszanin wysokoenergetycznych, takie jak oznaczenie kompatybilności składników i określanie optymalnego składu mieszaniny. Zgodnie z normą STANAG 4147, z wykorzystaniem różnicowej kalorymetrii skaningowej zbadano kompatybilność magnezu z oktogenem (HMX) i z heksaazaheksanitroizowurcytanem (CL-20). Magnez jest kompatybilny z tymi nitroaminami. Wykorzystując program ISP2001, wyznaczono optymalny skład mieszanin umożliwiający osiągnięcie maksymalnej temperatury palenia lub impulsu właściwego. Mieszanina Mg : HMX o optymalnym składzie spala się w niższej temperaturze niż mieszanina Mg : CL-20. W przypadku pierwszej z wymienionych mieszanin temperatura spalania to 3493K, natomiast drugiej 3807K. Wyznaczony dla obydwu mieszanin impuls właściwy osiągnął tę samą wartość równą 273s, przy różnej zawartości magnezu. Mieszanina zawierająca w swoim składzie oktogen maksymalną wartość impulsu osiągnęła w przypadku 5% zawartości Mg, natomiast zawierająca CL-20 – 15% zawartości metalu.

Zbadano zależność impulsu właściwego od składu paliwa rakietowego zawierającego polibutadien z końcowymi grupami hydroksylowymi (HTPB), chloran (VII) amonu i magnez. Maksymalna wartość impulsu zwiększa się wraz ze zmniejszeniem zawartości lepiszcza. Po zmianie lepiszcza na poliazydek glicydylu (GAP) zaobserwowano odwrotną zależność. Maksymalny impuls właściwy wzrastał, gdy rosła zawartość lepiszcza w paliwie.

Określono wpływ różnych utleniaczy na temperaturę palenia mieszanin pirotechnicznych. Najwyższe temperatury spalania uzyskano w przypadku mieszanin o zawartości magnezu od 20 do 45%. Porównywano wpływ na temperaturę spalania następujących utleniaczy: politetrafluoroetylenu, chloranu(VII) potasu oraz tlenku żelaza.

Słowa kluczowe: magnez, zastosowanie, spalanie, obliczenia, pirotechnika.

INTRODUCTION

Magnesium is an essential miroelement for live organisms for a number of reasons. Above all, it participates in many metabolic processes. Besides, it is found in the cellular nucleus, whose functions it sustains. Moreover, owing to its high biochemical activity, magnesium is a valuable metallic co-enzyme in over 300 reactions of phosphate transfer (Baker 2002, Pasternak et al. 2010). Magnesium is also an important ingredient of high-energy mixtures containing an oxidant and a reductant. An oxidant contained in mixture facilitates its combustion without access to air oxygen, which means that the desired special effect is possible. Magnesium containing compositions have found a broad range of applications in show and military pyrotechnics, in rocket propellants and explosive mixtures. The MTV compositions, made of magnesium, polytetrafluoroethylene (PTEE) and Viton, are popular in military pyrotechnics. While burning these mixtures reach high temperature and generate solid reaction products. Thus, they can be successfully used in igniter systems to blow up powder loads (Gocmez 1999). The intensity of infrared radiation in a narrow range of wavelengths means that MTV compositions can be used in decoy flares and in thermal traps. MTV compositions are also used in tracers, where they make a projectile trajectory visible (Koch 2002). It is possible to achieve more intensive emission of infrared radiation by replacing PTEE with carbon polyflouride – CF_x (Koch 2005). CF_x reacts with magnesium and other metals in a highly exothermic autocatalytic reaction (Cudziło et al. 2007). Compositions of magnesium with PTEE and Viton present several valuable characteristics, such as low hygroscopicity, easy processing (pelleting, pressing), weak dependence of combustion rate on pressure or temperature, and burning stability at low pressure and temperature. Besides, these mixtures are safe while making and storing (Boskovic, Negoicic 2009). In military pyrotechnics, magnesium containing compositions are used, for example, in red (Zagidullinovich 2012) and orange (Korobkov et al. 2010) flare masses, in firecrackers (Yueming 2003) and in Bengal fireworks (Lyadov, Kuznetsov 1997).

In order to improve the stability of pyrotechnic compositions, magnesium and aluminium alloys are often used (Lyadov, Kuznetsov 1997, Yueming 2003, Zagidullinovich et al. 2012), e.g. composed of 54% of magnesium by weight. Such alloy is an intermetallic compound Al_3Mg_4, which – compared to magnesium - is less reactive and more fragile, which makes it is easier to be ground (Szydłowska 1957). Another method which enhances the stability of magnesium is the modification of its surface (Smith 2009).

Magnesium is used in solid heterogeneous rocket propellants, especially in amateur applications (http://rakiety.pomorze.pl/Stronadomowa/atakna10km.htm, http://www.nakka-rocketry.net/propel.html). In some situations, the presence of magnesium in fuel is undesirable because while burning it will emit large amounts of combustion products. To eliminate the trail following a rocket, metals and ammonium perchlorate (AP) should be replaced with other substances (Książczak et al. 2005). The metal is substituted by high-energy compounds, such as hexogen (Flanagan 1994), whereas AP is replaced with ecological oxidants, e.g. ammonium dinitroamide (Gołofit et al. 2013).

The purpose of this study, carried out on two compositions with magnesium, has been to demonstrate two essential stages which must be proceeded through while designing new high-energy compositions: determination of the compatibility of components and an optimal composition of a new mixture.

DISCUSSION

The first step when elaborating new, effective and stable high-energy compositions is to analyze the compatibility of components. Compatibility is achieved when there is no negative influence of one mixture component on the chemical stability of the other component. The norm regulating compatibility investigations is the STANAG 4147 (NATO STANAG 4147MMS 1992). One of the applicable methods is the comparison of the decomposition peak maximum temperature (T_{max}) of individual substances and their mixtures, determined by differential scanning calorimetry (DSC) or differential thermic analysis (DTA). Non-compatible compositions are mixtures for which the T_{max} of decomposition is by 20K more than the T_{max} of the less stable component. If the said difference is within the range of 4 to 20 K, then the composition possesses an unsure compatibility. A mixture whose decomposition T_{max} is no less than 4 K below the T_{max} of the less stable component is said to be a compatible composition.

Investigations were performed to determine the compatibility of magnesium with such nitroamides as octogen (HMX) and hekzaazahekzanitroizowurzitane (CL-20). Such compositions can be used in thermobaric explosives (CUDZIŁO et al. 1996, CHAN et al. 2004). Multi-component products with magnesium and nitroamides can also be used as rocket propellants (http://www.starmolecule.com/faq/rocket-fuel/). DSC determinations were accomplished on samples of components weighing 1 mg each and their mixtures weighing 2 mg. Analyses were made in the range of temperatures from 430 to 600 K, at a temperature increase rate of 2K min^{-1}, in aluminium vessels fitted with a hole. Figure 1 shows results of DSC analyses for magnesium (solid line), octogen (broken line) and their 1 : 1 composition (dotted line).

As expected, in the above temperature range, no exothermic peak associated with the oxygenation of a sample was observed for magnesium samples. The maximum decomposition peak appears at 554K for HMX, and at 556K for its mixture with magnesium. Hence, the two components are compatible. Also, the compatibility of magnesium with CL-20 was determined, with the results illustrated in Figure 2.

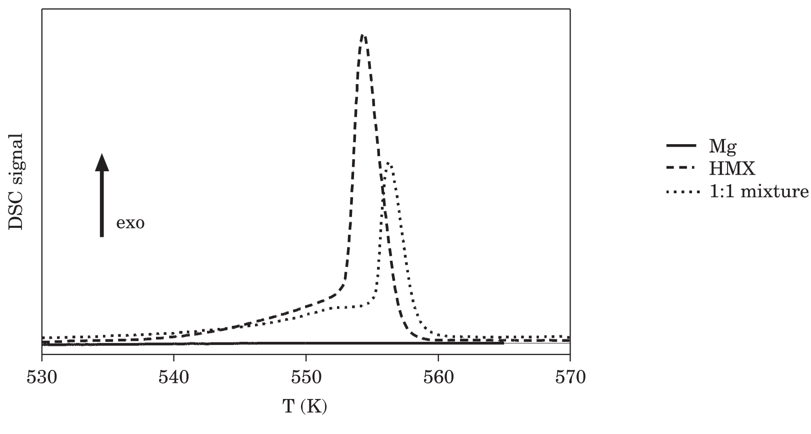

Fig. 1. DSC curves for decomposition of octogen, magnesium and their mixture in a 1:1 ratio by weight

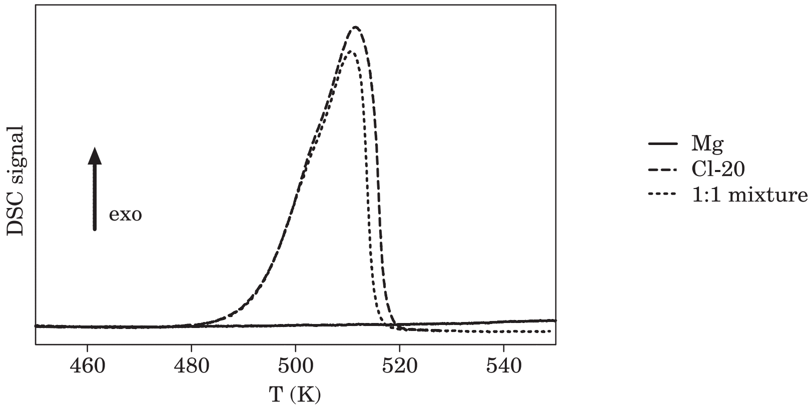

Fig. 2. DSC curves for decomposition of CL-20, magnesium and their mixture in a 1:1 ratio by weight

The maximum decomposition peak temperature for CL-20 was 511K, and for its composition with magnesium it decreased to 510K. Thus, magnesium was compatible with CL-20, same as it was with HMX.

Having determined the compatibility, it is then crucial to design such a composition of the mixture which would ensure the maximum special effect. For compositions used in decoy flares or in thermal traps, one of the significant parameters is the combustion temperature; in mixtures used as rocket fuel, it is their specific impulse (SINGH et al.1988), which characterizes the fuel efficiency. These two parameters were determined with the isp2001 software (http://www.dunnspace.com/isp.htm). For analyses on rocket propellant components, it was assumed that the pressure in the engine chamber was 70 atm and the gas expansion was up to 1 atm. Figure 3 depicts the effect of the magnesium content on specific impulse and combustion temperature in the analyzed compositions.

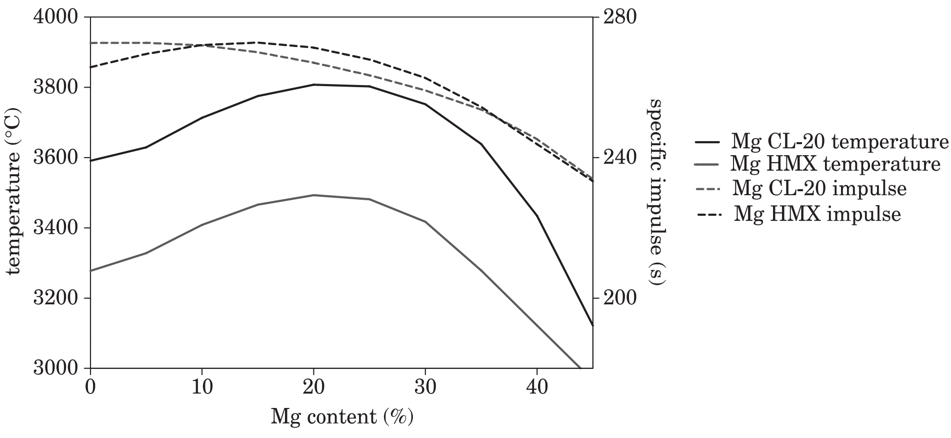

Fig. 3. Dependence of combustion temperature and specific impulse on the magnesium content in mixtures with octogen (HMX) and CL-20

In both cases, the highest combustion temperature was achieved for compositions containing about 25% of magnesium. The maximum combustion temperature was 3807K for the Mg and CL-20 mixture, and 3493K for the mixture of the same metal with HMX. The specific impulse of the magnesium and octogen composition was the highest (273s) at the 15% share of magnesium. The highest value of the specific impulse for compositions of magnesium with CL-20 was 273s, and it was obtained at a 5% contribution of Mg. Should we wish to achieve the highest combustion temperature, then the right composition is 25% Mg and 75% CL-20. In turn, if our aim is the maximum specific impulse, then the most appropriate mixtures are the ones composed of 5% Mg and 95% Cl-20 or 15% Mg and 85% HMX. Considering the costs of raw materials, the mixture with octogen is better. If it is more important to diminish the trail following a projected missile, then the composition with CL-20 is a better choice.

Heterogenic rocket fuels contain a binder . Below we discuss the results of our calculations for different compositions of fuel containing magnesium. The effect of magnesium on the specific impulse of fuel containing hydroxyl-terminated polybutadiene (HTBP), ammonium perchlorate and magnesium is illustrated in Figure 4.

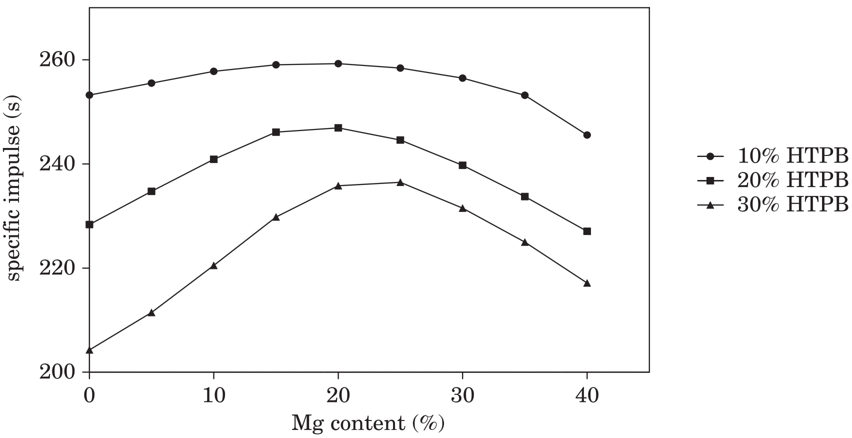

Fig. 4. Dependence of specific impulse on the composition of rocket propellant with HTPB, ammonium chlorate (VII) and magnesium

The specific impulse of a HTPB, AP and Mg composition containing 10% of the binder changes from 253s for the mixture without magnesium to 246s for the one with 40% of Mg, reaching the maximum of 259 s at 20% share of HTPB. For a composition containing 20% and 30% of the binder, the specific impulse changes from 228 and 204s in mixtures without magnesium to 227 and 217s, respectively, in compositions with 40% of magnesium up to the highest values of 245 and 236s in compositions with 20% and 25% of Mg, respectively. The specific impulse of compositions containing HTPB, AP and

Mg increases as the percentage of the binder decreases, and the maximum specific impulse at any tested share of HTBP can be noticed when the share of magnesium is 20 to 25%.

The influence of magnesium concentrations on the specific impulse of rocket fuel containing glycidyl azide polymer (GAP), ammonium perchlorate (AP) or magnesium is illustrated in Figure 5.

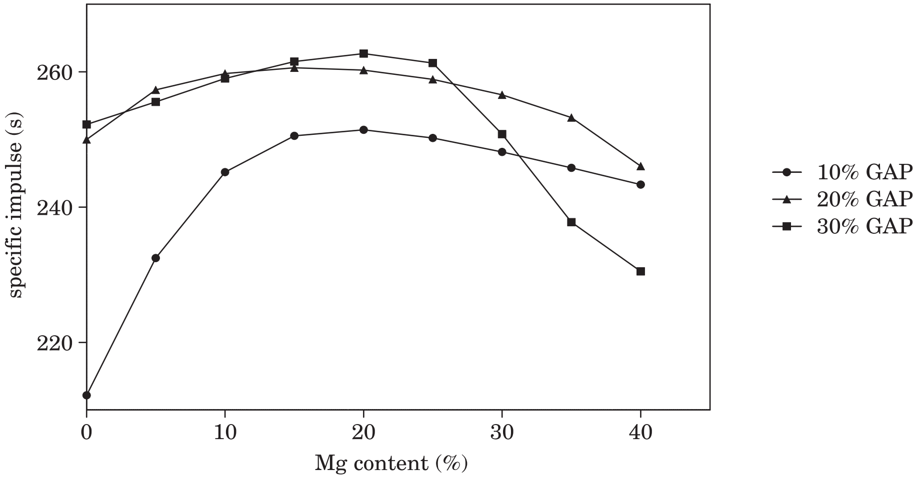

Fig. 5. Dependence of specific impulse on the composition of rocket propellants with GAP, ammonium chlorate (VII) and magnesium

The specific impulse of a composition of GAP, AP, Mg containing 10% of the binder changes from 212s in the mixture without magnesium to 243s in the mixture with 40% of Mg, reaching the maximum value of 251s at 20% share of GAP. For the composition containing 20 and 30% of the binder , the specific impulse changes respectively to 250 and 252s in mixtures without magnesium and 246 and 230s in mixtures containing 40% of Mg, reaching the highest values of 261 and 263s at 15 and 20% magnesium content, respectively. The specific impulse of compositions containing GAP, AP and Mg increases as the content of the binder increases, and at any given content of GAP, the maximum value of the specific impulse can be observed at the 15 to 20% share of magnesium.

Despite large differences in the properties of GAP and HTPB, similar values of the specific impulse were obtained for both agents, namely 263 for GAP and 259s for HTPB. The maximum values of the specific impulse for both polymers occurred at a similar content of magnesium.

Magnesium can also be used in pyrotechnic compositions used by the army and by civilians. In order to achieve the maximum special effect, it is extremely important to add appropriate amounts of magnesium to high-energy compositions. The effect of magnesium on the achieved pyrotechnic effect has been demonstrated based on calculations aided by a software programme package isp2001 [http://www.dunnspace.com/isp.htm].

The temperature of adiabatic combustion of the analyzed compositions used in the show and military pyrotechnics has been determined. This part of the study is worth underlining because we can observe the influence of oxygen on the value of combustion temperature (GRANSDEN, TAYLOR 2007). Figure 6 shows the dependence of the combustion temperature of pyrotechnic compositions on the content of magnesium. Calculations were made for mixtures containing from 5 to 50% magnesium by weight, assuming that the mixtures were burned at atmospheric pressure.

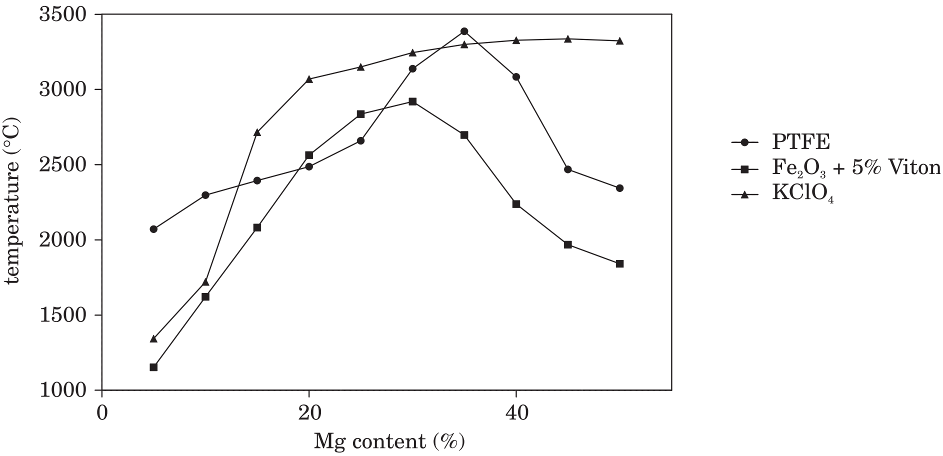

Fig. 6. Dependence of combustion temperature of pyrotechnic compositions containing polytetrafluoroethylene (PTFE), potassium chlorate (VII) or iron oxide as an oxidant with 5% Vitone

The combustion temperature of a mixture which contains politetrafluoroetylen (PTFE) and an oxidant changes from 2072K at the 5% magnesium content to 2344K at 50%, and reaches the maximum value at a 35% share of the reducer. These values coincide with literature data (DE YONG, SMIT 1991, KUWAHARA et al. 1997). The calculations for the compositions with iron oxide were carried out on the assumption that the fluoridated polymer Viton made up 5% of the whole content. The combustion temperature of such a mixture changes from 1153K at a 5% magnesium share to 1841K when magnesium constitutes 50% of the composition, reaching the maximum value of 2920K at a 30% share of the reducer. A composition of magnesium and potassium chlorate (VII) presents the combustion temperature of 1344K at a 5% share of the reductant to 3336K when the content of the metal is 45%. The combustion temperature of all mixtures increases when the content of magnesium increases, reaching its highest value at a 30 to 45% content of the metal. The combustion temperature of mixtures which contain polytetrafluoroethylene or iron oxide as an oxidant reaches the highest value within a low range of changes in the magnesium content. A composition of potassium chlorate (VIII) and magnesium has a high combustion temperature when the content of the oxidant varies from 20 to 50%.

SUMMARY

Magnesium is an essential component of high-energy compositions with civil and military applications. It is for example broadly used in show pyrotechnics, in thermobaric mixtures and in rocket propellants. Magnesium-containing mixtures have high combustion temperature, which enables the user to achieve the desired special effect. The actual effect depends on the percentage of magnesium in a composition. Thus, when designing new compositions, the early stages of work can be supported by calculation programmes, such as ISP2001 (http://www.dunnspace.com/isp.htm). Another aspect to keep in mind is the compatibility of components in a mixture. The determinations carried out in the current study prove that magnesium is compatible with HMX and CL-20. The calculations imply that it is possible to attain an approximately same specific impulse for both compositions, which would equal 273s. The maximum special effect is just one of the criteria taken to select components of new high-energy masses. Other criteria, e.g. price, safe application, simplicity of manufacture, trailing ability, stability, etc., help to select the most suitable ingredients for new high-energy compositions.

The specific impulse of rocket propellant containing polybutadiene with terminal hydroxyl groups (HTPB), ammonium perchlorate and magnesium increases as the content of a given binder decreases. When another binder, such as poly(glycidyl azide) (GAP) was used, a reverse dependence was observed. The maximum specific impulse increases as the amount of GAP increases. Through the comparison of combustion temperatures of the following oxidants: polytetrafluoroethylene, potassium chlorate (VII) and iron oxide, an optimum content of magnesium was set at 20 to 45%, depending on an oxidant.

Some limitation to the use of magnesium in high-energy compositions is due to its reactivity. This is the reason why it is often used in the form of an alloy with aluminium, such as Al_3Mg_4. Another solution is to modify the surface of magnesium.

REFERENCES

Baker S.B., Worthley L.I.G. 2002. *The essentials of calcium, magnesium and phosphate metabolism:* Part I. *Physiology.* Crit. Care Resuscit., 4: 301-306.

Boskovic G., Negoicic D. 2009. *Propellant pyrotechnic mixtures based on polytetrafluoroethylene (PTFE).* Sci. Tech. Rev., 59(1): 70-76.

CHAN M., MEYERS G. 2004. *Advanced thermobaric explosive compositions*. United States Patent, 6,955,732 B1.

Chemical compatibility of ammunition components with explosives and propellants (non nuclear applications). NATO STANAG 4147MMS (Edition1). 1992.

CUDZIŁO S., SZALA M., HUCZKO A., BYSTRZEJEWSKI M. 2007. *Combustion reactions of poly(carbon monofluoride), (CF)n, with different reductants and characterization of the products*. Propell Explos Pyrot., 32(2): 149-154.

CUDZIŁO S., TRZCIŃSKI W., MARANDA A. 1996. *Detonation behaviour of HMX-based explosives containing magnesium and polytetrafluoroethylen*. Energetic Materials-Technology, Manufacturing and Processing. Federal Republic of Germany.

DE YONG L. V., SMIT K. J. 1991. *A theoretical study of the combustion of magnesium/teflon/viton pyrotechnic composition*. MRL Technical Report MRL-TR-91-25.

FLANAGAN J. E., GRAY J. C. 1994. Patent EP 0608488.

GOCMEZ A., YILMAZ G. A., PEKEL F. 1999. *Development of MTV compositions as igniter for HTPB/AP based composite propellants.* Propell Explos Pyrot., 24: 65-69.

GOLOFIT T., MAKSIMOWSKI P., BIERNACKI A. 2013. *Optimization of potassium dinitramide preparation.* Propell Explos Pyrot., 38(2): 261-265,

GRANSDEN J. I., TAYLOR M. J. 2007. *Study of confined pyrotechnic compositions for medium/large calibre gun igniter applications.* Propell Explos Pyrot., 32(6): 435-446.

KOCH E. Ch. 2002. *Metal-fluorocarbon-pyrolants:* III. *Development and application of magnesium/teflon/viton (MTV).* Propell Explos Pyrot., 27: 262-266.

KOCH E. Ch. 2005. *Metal/fluorocarbon pyrolants:* VI. *Combustion behaviour and radiation properties of magnesium/poly(carbon monofluoride) pyrolant*. Propell Explos. Pyrot., 30: 209-215.

KOROBKOV A. M. et al. 2010. *Amber light pyrotechnic composition.* Patent RU2394802.

KSIĄŻCZAK A., MAKSIMOWSKI P., GOŁOFIT T. 2005. *Low trail and ecological rocket propellants*. Probl. Tech. Uzbr., 95(2): 133-141. (in Polish)

KUWAHARA T., MATSUO S., SHINOZAKI N. 1997. *Combustion and sensitivity characteristics of Mg/TF pyrolants.* Propell Explos Pyrot., 22: 198-202.

LYADOV V., KUZNETSOV P. 1997. *Composition for color-flame Bengal candle.* Patent RU2087456

PASTERNAK K., KOCOT J., HORECKA. 2010. *Biochemistry of magnesium*. J. Elementol., 15(3): 601-616.

SINGH H., SOMAYAJULU M.R., BHASKAR RAO R. 1988. *Selection of an igniter system for magnesium-based solid fuel rich.* Propell Explos Pyrot., 13: 52-54.

SMITH P. 2009. *Surface-modified magnesium powders for use in pyrotechnic composition.* Patent US2009025841 (A1).

SZYDŁOWSKA A. 1957. *Pyrotechnics Foundations*. Wyd. MON, Warszawa. (in Polish)

YUEMING Z. 2003. *Safety fireworks*. Patent CN1438467 (A).

ZAGIDULLINOVICH A.N., VALENTINOVICH J.V., SERGEEVICH R.M., IVANOVICH S.A. 2012. *Pyrotechnic composition for red signalling light.* Patent RU2466119.

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