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## ORIGINAL PAPERS

## LEVEL OF MAGNESIUM IN TISSUES AND ORGANS OF FRESHWATER FISH\*

## Ewa Brucka-Jastrzębska, Dorota Kawczuga

Chair of Physiology University of Szczecin

#### Abstract

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

A total of 125 fish comprised 25 individuals of each species, aged 6, 12, 18 and 24 months. The fish were cultured in privately owned fish breeding ponds (Western Pomerania, Poland). Tissue and organ samples were wet mineralised in concentrated HNO $_3$  in a CEM MDS 2000 microwave oven. Magnesium concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP-MS) in a Jobin Yvon type JY-24 apparatus. The research had an approval of the Polish Local Ethics Committee no 9/05. The magnesium concentration in the tissues and organs ranged from 26.3÷174.2 mg kg $^{-1}$  w.w. The lowest Mg concentration was found in the gills of rainbow trout (26.3±5.4 mg kg $^{-1}$  w.w.), and the highest – in the liver of rainbow trout (174.2±27.6 mg kg $^{-1}$  w.w.). The magnesium concentrations were also significantly affected by the type of feed.

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

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## POZIOM MAGNEZU W TKANKACH I NARZĄDACH RYB SŁODKOWODNYCH

## Abstrakt

W procesie rozwoju organizmów zasadniczą rolę odgrywają warunki hodowli. Ryby słodkowodne charakteryzują się dużym zróżnicowaniem pod względem miejsca bytowania w toni wodnej, warunków hydrochemicznych i rodzaju pożywienia.

Celem pracy było zbadanie wpływu wieku ryb, warunków termicznych i zanieczyszczeń antropogenicznych na poziom magnezu w tkankach (krew) i narządach (skrzela, wątroba, nerki i mięśnie grzbietowe) pięciu gatunków ryb: karpia (Common carp L.), pstrąga tęczowego (Oncorhynchus mykiss Walbaum), jesiotra syberyjskiego (Acipenser baeri Brandt), szczupaka pospolitego (Esox lucius L.) i amura białego (Ctenopharyngodon idella Valenciennes). Badania przeprowadzono w 6., 12., 18. i 24. miesiącu życia. Ryby do badań pobierano z prywatnego ośrodka hodowlanego na Pomorzu Zachodnim (Polska). Próbki tkanek i narządów zmineralizowano na mokro w stężonym HNO3 w piecu mikrofalowym CEM MDS 2000. Mg oznaczono z użyciem emisyjnej spektrometrii atomowej w plazmie indukcyjnie sprzężonej (ICP-AES) w aparacie Jobin Yvon typ JY-24. Na przeprowadzenie badań uzyskano zgodę (nr 9/05) Lokalnej Komisji ds. Etyki. Poziom Mg w tkankach i narządach ryb kształtował się w zakresie 26.3±174.2 mg kg<sup>-1</sup> w.w. Najniższy poziom Mg oznaczono w skrzelach pstrąga tęczowego (26.3±7.4 mg kg<sup>-1</sup> w.w.), a najwyższy w jego wątrobie (174.2±27.6 mg kg<sup>-1</sup> w.w.). Można wnioskować, że stężenie magnezu były zależne od rodzaju spożywanej paszy.

Słowa kluczowe: karp, pstrag tęczowy, jesiotr syberyjski, szczupak, amur biały, magnez.

## INTRODUCTION

Magnesium as a metal itself was first obtained in England in 1808, by Sir Humphry Davy, who performed electrolysis of a mixture of magnesia and mercury oxide. Antoine Bussy prepared magnesium in a consistent form in 1831. Davy's first suggestion for the name was magnium, but now the name magnesium is used. Because of some important interaction between phosphate and magnesium ions, the latter are essential to the basic nucleic acid chemistry of life and are crucial for all cells of all known living organisms. Over 300 enzymes require the presence of magnesium ions for their catalytic action, including all enzymes utilizing or synthesizing ATP, or those that use other nucleotides to synthesize DNA and RNA (Floor 2006). Magnesium compounds are used medicinally as common laxatives, antacids and in a number of situations where stabilization of abnormal nerve excitation and blood vessel spasm is required. High solubility of magnesium ions in water helps ensure that it is the third most abundant element dissolved in seawater. Magnesium ions are sour in taste, and in low concentrations help to impart natural tartness to fresh mineral waters. The free element (metal) is not found naturally on Earth. The free metal burns with a characteristic brilliant white light, making it a useful ingredient in flares. The metal is now mainly obtained by electrolysis of magnesium salts obtained from brine.

Breeding conditions, e.g. feeding, have a significant effect on the development of fish. In the growth and development of both terrestrial and aquatic animals, culture conditions play a key role. A variety of chemicals occur in the natural environment, most of which, however, do not penetrate inside living organisms in significant amounts despite being in direct contact. Due to human actions, man-made pressure on the environment is increasing. It can interfere, for instance, in the homeostasis of aquatic environments, which may cause soem disturbance in the fish body balance.

During the evolution, countless relationships have developed between organisms and their environment. When these relationships are disrupted by altered environmental conditions, diseases or even death of organisms may occur. Elements of the environment such as water, air and food deliver essential components for organisms, but at the same time they may be sources of xenobiotics and harmful substances, which can impair life functions of organisms.

The control of levels of micro- and macroelements in fish organisms is a significant diagnostic research tool, as it shows the physiological condition of organisms. Concentration of some mineral elements in fish depends mainly on the culture and water type in which the fish are bred, as well as the season of the year and the feeds the fish receive. All the elements affect the homeostatic behavior of fish, which may vary due to the excess or deficiency of any of the factors. Their surplus or shortage may lead to serious disorders of the biochemical processes, which can result in many diseases.

The aim of this study has been to evaluate the effect of culture conditions and culture site on levels of the selected macroelement (Mg) in five species of freshwater fish: rainbow trout (Oncorhynchus mykiss Walbaum), common carp (Cyprinus carpio L.), Siberian sturgeon (Acipenser baeri Brandt), northern pike (Esox lucius L.) and grass carp (Ctenopharyngodon idella Valenciennes). The study encompassed tissues (blood) and organs (kidney, liver, gills and dorsal muscles) in fish.

## MATERIAL AND METHODS

The study involved 125 individuals of freshwater fish reared in commercial fish farms in West Pomeranian Province, Poland. The fish were represented by 25 individuals of each of the five species: rainbow trout, common carp, Siberian sturgeon, northern pike and grass carp. The research had an approval of the Polish Local Ethics Committee no 9/05.

The fish aged from 6 to 24 months, weighed from 147.8 to 985.4 g and measured from 18.4 to 39.5 cm (total length). The fish were collected four times: in April (6 month – spring), October (12 month – autumn), April (18 month – spring) and October (24 month – autumn).

The fish were fed Aller Aqua pelleted feeds (Table 1), each species with an appropriate feed type. All the fish feed products were produced by extrusion. The fish feed must cover the basic metabolism of the fish and ensure healthy growth. In order to meet these requirements, the fish feed composition must fulfill all requirements for nutrients, vitamins (A, E and  $D_3$ ) and minerals. The daily food ration was  $3.4\pm0.8~g$  per kg fish. The fish were fed twice a day.

 ${\it Table \ 1}$  Composition of Aller Aqua feed for individual species of freshwater fish

			Examined fis	sh	
Feed parameters	common carp	rainbow trout	Siberian sturgeon	northern pike	grass carp
Name of feed	Aller classic	Aller 576	Aller M/L	Aller M/L	Aller classic
Composition					
Size of feed (mm)	5.0	M/L	M/L	M/L	5.0
Protein (%)	35.0	42.0	45.0	45.0	35.0
Fat (%)	9.0	30.0	15.0	15.0	9.0
Carbohydrates (%)	43.0	14.0	21.0	21.0	43.0
Ash (%)	7.0	7.5	8.0	8.0	7.0
Fiber (%)	5.0	1.0	2.5	2.5	5.0
All-out energy (gross energy) (Kcal MJ <sup>-1</sup> )	4325/18.1	5823/2.3	4924/20.5	4924/20.5	4325/18.1
Digestible energy (Kcal MJ <sup>-1</sup> )	3353/14.0	4833/20.2	38887/16.2	38887/16.2	3353/14.0
Nitrogen (d.m.* %)	5.2	7.1	7.9	7.9	5.2
Phosphorus (d.m. %)	1.3	7.0	1.2	1.2	1.3
Energy in dry matter (Kcal MJ <sup>-1</sup> )	4701/19.6	6162/25.7	5381/022.5	5381/022.5	4701/19.6

<sup>\*</sup> d.m. - dry mass

Table 2 presents chemical and biochemical parameters of water in which the fish were kept. Fish behaviour and appearance were recorded. Intravital examination involved observation of fish behaviour, assessment of rearing conditions, as well as evaluation of the quality and general appearance of fish skin, fins, eyes and gills. *Post mortem* examination involved autopsy to verify if there were any anatomical or pathological changes in internal organs.

For chemical analysis, samples of tissues (blood) and organs (skin, liver, kidney, dorsal muscles) were collected from each fish. The collected material was stored at -20°C. Prior to analysis, 1-g subsamples of organs and tissues, weighed to the nearest 0.001 g, were wet mineralised in 3  $\rm cm^3$  HNO $_3$  in a CEM MDS 2000 microwave oven. The solutions obtained were quantita-

 $\label{thm:condition} \mbox{Table 2}$  Hydrochemical parameters at the fish farm 30 km from Szczecin

		cocis at the ns			
	April/ Spring	October/ Autumn	April/ Spring	October/ Autumn	Statistically
Water parameters		month of	fish life		significant differences
	6	12	18	24	$P \le 0.01$
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	
Temperature (°C)	11.20 ± 2.88	6.80 ± 3.40	13.80 ± 3.40	7.85 ± 4.24	a, b, c
pН	$7.78 \pm 0.55$	$7.38 \pm 0.95$	$7.48 \pm 0.95$	$7.68 \pm 0.91$	-
Dissolved oxygen (mg dm <sup>-3</sup> )	7.81 ± 0.35	$7.98 \pm 0.63$	7.94 ± 0.55	$7.33 \pm 0.73$	-
Oxygen saturation (%)	78.21 ± 2.50	79.51 ± 3.48	78.79 ± 4.28	76.55 ± 4.41	-
Alkalinity (mmol dm <sup>-3</sup> )	1.75 ± 0.80	1.69 ± 0.88	1.65 ± 0.68	1.78 ± 0.78	-
Water hardness (mg dm <sup>-3</sup> )	8.25 ± 1.08	7.25 ± 1.18	7.75 ± 1.58	7.36 ± 1.35	-
ChOD (mg dm <sup>-3</sup> )	1.67 ± 1.32	1.71 ± 1.09	1.66 ± 1.12	1.62 ± 1.19	-
BOD (mg dm <sup>-3</sup> )	4.34 ± 1.22	4.61 ± 1.33	4.81 ± 1.32	4.75 ± 1.13	-
NH <sub>4</sub> +-N (mg dm <sup>-3</sup> )	$1.18 \pm 0.75$	$1.34 \pm 0.48$	1.26 ± 0.29	1.39 ± 0.33	-
NO <sub>3</sub> <sup>-</sup> -N (mg dm <sup>-3</sup> )	7.41 ± 1.05	6.11 ± 1.15	$6.78 \pm 0.55$	6.12 ± 1.45	a, d
NO <sub>2</sub> N (mg dm <sup>-3</sup> )	$0.68 \pm 0.16$	$0.48 \pm 0.36$	$0.56 \pm 0.26$	$0.52 \pm 0.58$	a
PO <sub>4</sub> <sup>3-</sup> -P (mg dm <sup>-3</sup> )	$0.15 \pm 0.07$	$0.14 \pm 0.05$	$0.13 \pm 0.03$	$0.16 \pm 0.03$	-
Ca <sup>2+</sup> (mg dm <sup>-3</sup> )	66.51 ± 4.25	59.51 ± 3.75	69.31 ± 2.79	55.54 ± 3.25	c, d
Cd <sup>2+</sup> (mg dm <sup>-3</sup> )	0.02 ± 0.01	0.01 ± 0.01	$0.02 \pm 0.01$	0.02 ± 0.01	-
Pb <sup>2+</sup> (mg dm <sup>-3</sup> )	$0.03 \pm 0.05$	$0.03 \pm 0.07$	$0.03 \pm 0.07$	$0.04 \pm 0.057$	-
Mg <sup>2+</sup> (mg dm <sup>-3</sup> )	16.33 ± 4.05	15.23 ± 3.33	17.44 ± 4.47	13.79 ± 3.41	c

a – statistically significant differences in the water parameter between the 6<sup>th</sup> and 12<sup>th</sup> month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter between the 12<sup>th</sup> and 18<sup>th</sup> month of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter between the 18th and 24th month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the 6<sup>th</sup> and 24<sup>th</sup> month of fish life ( $P \le 0.01$ ).

tively transferred to polyethylene vials and brought up to 25 g with deionised water. Magnesium was determined with inductively coupled plasma atomic emission spectrometry (ICP-AES) in a JY-24 Jobin Yvon apparatus. Tissue concentrations of metals were reported as mg kg $^{-1}$  wet weight (mg kg $^{-1}$  w.w.).

The results were subjected to statistical treatment with Statistica 6.0 software. Analyses of variance (ANOVA) were performed at the significance levels of P = 0.01.

## RESULTS AND DISCUSSION

The growth in industrial and agricultural production has resulted in an increasing number of systems impacted by contaminants present in wastewater releases. For example, heavy metals (Cd, Pb, Cu, Zn) are known to accumulate in organs of fish (BÁLINT et al. 1997). These metals pollute aquatic and terrestrial ecosystems, adversely affecting the environment and inhabiting organisms. High concentration of metals in fish tissues can lead to a redox reaction, generating free radicals, especially reactive oxygen species (Dautremepuits et al. 2002). These highly reactive compounds may induce tissue alternations and change some physiological responses of fish (Paris--Palacios et al. 2000, Varanka et al. 2001). Magnesium plays a regulatory role in prooxidant and antioxidant processes (Lopez-Torres et al. 1993, Ozmen et al. 2004). Aquatic organisms are more sensitive to exposure and toxicity compared to terrestrial ones, including mammals, and in this respect they may provide experimental data for evaluation of subtle effects of oxidative stress, mutagenicity and other adverse effects of pollutants (Valavanidis et al. 2006). Moreover, the water parameters can affect water organisms, for example the influence of high temperature on aquatic biocenosis manifests itself as an increase in biological production rate and also shorter lifecycles of aquatic organisms, which die in large numbers due to the lack of synchronisation with climate rhythms. This results in the accumulation of organic matter and an increase in biological oxygen demand, along with a decrease in oxygen solubility and availability.

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

Intravital and *post mortem* examination showed no changes in fish behaviour, as well as in their external and internal appearance. Comparison of water parameters (Table 2) revealed only slight differences between the dates of taking samples.

The research has shown that Mg concentration changed during the growth of fish. The average magnesium content in blood ranged from 87.7 to  $168.2~\rm mg~kg^{-1}$  w.w. (Table 3). The highest magnesium levels were detect-

 $\label{eq:Table 3} \text{Mg level in blood of five freshwater fish species aged 6, 12, 18 and 24 months}$ 

		Mg (mg k	g-1 w.w.)		Statistically
Eigh ann aige		month of	f fish life		significant
Fish species	6	12	18	24	differences $P \le 0.01$
	mean ± SD	mean ± SD	mean ± SD	mean $\pm$ SD	F 20.01
Common carp	103.9 ± 18.3	163.6 ± 35.1	126.2 ± 23.5	161.3 ± 23.9	a, b, c, d
Rainbow trout	118.3 ± 35.4	163.5 ± 25.3	165.6 ± 35.1	168.2± 24.4	a, d
Siberian sturgeon	88.5 ± 26.2	144.5 ± 26.3	110.8 ± 32.9	141.5 ± 34.2	a, b, c, d
Northern pike	85.3 ± 24.5	91.5 ± 18.6	97.1 ± 21.7	89.1 ± 25.4	ns
Grass carp	105.4 ± 27.6	99.4 ± 27.4	98.5 ± 25.1	104.3 ± 24.1	ns

a – statistically significant differences in the water parameter between the  $6^{\rm th}$  and  $12^{\rm th}$  month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter between the  $12^{\rm th}$  and  $18^{\rm th}$  month of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter between the  $18^{\rm th}$  and  $24^{\rm th}$  month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the  $6^{\rm th}$  and  $24^{\rm th}$  month of fish life ( $P \le 0.01$ ); ns - no significant differences

ed in blood of rainbow trout (168.2±24.4 mg kg<sup>-1</sup> w.w.). The lowest magnesium levels were found in blood of northern pike (85.3±24.5 mg kg<sup>-1</sup> w.w.). Magnesium was distributed in fish blood according to the following pattern of decreasing concentrations: northern pike > grass carp > Siberian sturgeon > common carp > rainbow trout. Statistically significant differences were also detected in the blood Mg levels during the growth of common carp and rainbow trout (Table 3).

The average Mg content in gills ranged from 26.3÷91.2 mg kg<sup>-1</sup> w.w. (Table 4). The highest Mg levels were detected in gills of grass carp (91.2±12.4 mg kg<sup>-1</sup> w.w.) and the lowest ones were found in gills of rainbow trout (26.3±5.4 mg kg<sup>-1</sup> w.w.). Magnesium was distributed in fish gills according to the following pattern of decreasing concentrations: rainbow trout > Siberian sturgeon > northern pike > common carp > grass carp. Statistically significant differences were also found in Mg levels in gills during the growth of common carp, rainbow trout and Siberian sturgeon (Table 4).

The average Mg content in liver ranged from 35.3÷174.2 mg kg<sup>-1</sup> w.w. (Table 5). The highest Mg levels were detected in liver of rainbow trout (174.2±27.6 mg kg<sup>-1</sup> w.w.). The lowest Mg levels were found in liver of grass carp (35.3±9.5 mg kg<sup>-1</sup> w.w.). Magnesium was distributed in fish liver according to the following pattern of decreasing concentrations: grass carp > northern pike > Siberian sturgeon > rainbow trout > common carp. We also found statistically significant differences in the liver Mg concentrations during the growth of rainbow trout, Siberian sturgeon and northern pike (Table 5).

		Mg (mg k	kg <sup>-1</sup> w.w.)		Statistically
Eigh massing		month of	f fish life		significant
Fish species	6	12	18	24	differences $P \le 0.01$
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	F≤0.01
Common carp	43.6 ± 11.5	72.6 ± 24.4	62.2 ± 13.5	74.3 ± 11.9	a, b, c, d
Rainbow trout	26.3 ±5.4	41.5 ± 18.3	43.6 ± 22.1	62.2 ± 17.4	a, c, d
Siberian sturgeon	43.2 ± 13.2	51.3 ± 12.3	47.8 ± 11.9	58.3 ± 13.2	d
Northern pike	64.3 ± 17.5	68.5 ± 6.6	67.4 ± 11.3	59.8 ± 19.4	ns
Grass carp	85.5 ± 17.3	91.2 ± 12.1	78.3 ± 15.4	84.6 ± 17.8	ns

a – statistically significant differences in the water parameter between the  $6^{\rm th}$  and  $12^{\rm th}$  month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter between the  $12^{\rm th}$  and  $18^{\rm th}$  month of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter between the  $18^{\rm th}$  and  $24^{\rm th}$  month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the  $6^{\rm th}$  and  $24^{\rm th}$  month of fish life ( $P \le 0.01$ ); ns – no significant differences

		Mg (mg k			Statistically significant
Fish species	6	12	18	24	differences $P \le 0.01$
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	F≤0.01
Common carp	144.6± 35.6	161.5 ± 45.2	154.6 ± 23.2	158.1 ± 26.4	ns
Rainbow trout	102.1 ±31.8	169.6 ± 18.5	103.1 ± 38.1	174.2 ± 27.6	a, b, c, d
Siberian sturgeon	41.3 ± 7.9	114.2 ± 18.1	61.2 ± 28.2	102.8 ± 28.8	a, b, c, d
Northern pike	65.5 ± 21.3	63.7 ± 16.6	79.6 ± 17.7	89.3 ± 19.6	b, d
Grass carp	35.3 ± 9.5	46.1 ± 8.4	48.5 ± 18.3	43.4 ± 18.8	ns

a – statistically significant differences in the water parameter among the 6 and 12 month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter among the 12 and 18 of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter among the 18 and 24 of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter among the 6 and 24 of fish life ( $P \le 0.01$ ); ns – no significant differences

The average Mg content in kidneys ranged from 66.1÷168.7 mg kg<sup>-1</sup> w.w. (Table 6). The lowest Mg levels were detected in kidneys of Siberian sturgeon (66.1±17.9 mg kg<sup>-1</sup> w.w.). The highest Mg levels were found in kidneys of northern pike (168.7±32.6 mg kg<sup>-1</sup> w.w.). Magnesium was distributed in fish kidneys according to the following pattern of decreasing concen-

 $$\operatorname{Table}$\ 6$$  Mg level in kidney of five freshwater fish species aged 6, 12, 18 and 24 months

		Mg (mg k	g-1 w.w.)		Statistically
Figh appoing		month of	f fish life		significant
Fish species	6	12	18	24	differences $P \le 0.01$
	mean ± SD	mean ± SD	mean ± SD	mean $\pm$ SD	F 50.01
Common carp	$73.6 \pm 25.6$	141.8 ± 25.2	65.3 ± 38.6	145.3 ± 34.4	a, b, c, d
Rainbow trout	83.4 ± 22.9	147.3 ± 37.9	87.4 ± 21.7	110.3 ± 31.4	a, b, c, d
Siberian sturgeon	66.1 ± 17.9	74.1 ± 21.8	73.2 ± 23.2	68.8 ± 15.2	ns
Northern pike	155.3 ± 31.7	168.7 ± 32.6	163.5 ± 26.6	151.9 ± 26.4	ns
Grass carp	85.4 ± 24.6	91.2 ± 16.7	93.7 ± 25.4	128.9 ± 28.8	c, d

a – statistically significant differences in the water parameter between the  $6^{\rm th}$  and  $12^{\rm th}$  month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter between the  $12^{\rm th}$  and  $18^{\rm th}$  month of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter between the  $18^{\rm th}$  and  $24^{\rm th}$  month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the  $6^{\rm th}$  and  $24^{\rm th}$  month of fish life ( $P \le 0.01$ ); ns – no significant difference

trations: Siberian sturgeon > grass carp > common carp > rainbow trout > northern pike. Statistically significant differences were also found in Mg levels in kidneys during the growth of common carp, rainbow trout and grass carp (Table 6).

The average Mg content in dorsal muscles ranged from  $46.6 \div 143.1$  mg kg<sup>-1</sup> w.w. (Table 7). The highest Mg levels were detected in dorsal muscles of northern pike ( $143.1 \pm 28.4$  mg kg<sup>-1</sup> w.w.). The lowest Mg levels were found in dorsal muscles of common carp ( $46.6 \pm 8.9$  mg kg<sup>-1</sup> w.w.). Magnesium was distributed in fish dorsal muscles according to the following pattern of decreasing concentrations: common carp > Siberian sturgeon > grass carp > rainbow trout > northern pike. Statistically significant differences were also found in Mg levels in dorsal muscles during the growth of common carp, rainbow trout and Siberian sturgeon (Table 7).

It was found that the breeding site significantly affected the Mg concentration in the tissues and organs among individual freshwater fish species. The magnesium concentration were also significantly affected by the type of feed. All the applied pellet feeds were different in the concentration of fat (9-15%) and protein (35-45%) – Table 1. It was noticed that in common carp and grass carp fed Aller Aqua Aller classic pellet pasture, or in northern pike and Siberian sturgeon fed Aller Aqua Aller M/L, the Mg concentrations were higher compared to rainbow trout fed Aller Aqua Aller 576 pellet pasture. The results allow us to state that the culture site, culture conditions and the feeding type have a significant effect on the Mg level in tissues and organs of the examined fish.

		Mg (mg k	kg <sup>-1</sup> w.w.)		Statistically
Figh anguing		month of	f fish life		significant
Fish species	6	12	18	24	differences $P \le 0.01$
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	F 50.01
Common carp	85.4± 24.1	57.6 ± 15.5	46.6 ± 8.9	82.6 ± 21.7	a, c
Rainbow trout	87.2 ±33.5	61.3 ± 14.6	99.3 ± 27.2	59.5 ± 25.4	a, b, c, d
Siberian sturgeon	63.6 ± 16.2	89.7 ± 24.4	47.2 ± 15.2	46.7 ± 14.8	a, b, d
Northern pike	125.7 ± 32.7	138.1 ± 24.6	138.3 ± 24.7	143.1 ± 28.4	ns
Grass carp	72.4 ± 24.3	68.7 ± 27.3	71.5 ± 24.7	69.5 ± 11.3	ns

a – statistically significant differences in the water parameter between the  $6^{\rm th}$  and  $12^{\rm th}$  month of fish life ( $P \le 0.01$ ); b – statistically significant differences in the water parameter between the  $12^{\rm th}$  and  $18^{\rm th}$  month of fish life ( $P \le 0.01$ ); c – statistically significant differences in the water parameter between the  $18^{\rm th}$  and  $24^{\rm th}$  month of fish life ( $P \le 0.01$ ); d – statistically significant differences in the water parameter between the  $6^{\rm th}$  and  $24^{\rm th}$  month of fish life ( $P \le 0.01$ ); ns – no significant differences

Many authors reported considerably higher levels of this element in muscles of freshwater and marine fish (Pujin et al. 1990, Kargin 1996, Gro-SHEVA et al. 2000, JURKIEWICZ-KARNAKOWSKA 2001). Magnesium is weakly accumulated in fish tissues, as it is retained by gills, where the metal is deposited in large amounts (WITESKA 2003). This may be explained by the fact that Mg penetrates to blood less easily than other metals (Ni, Cd, Pb). Levels of some bioelements in fish bodies depend on culture methods, season of the year, feed quality and water chemistry. All these factors together influence the physiological condition of fish, which can be disturbed by excess or deficiency of minerals. Surplus or shortage of minerals may seriously disturb biochemical processes and upset internal homeostasis, leading in consequence to various diseases. Tacon (1992) reported that disorders occurred in organisms of various fish species due to deficiency or excess of macroelements, which were caused by avitaminosis or poisoning and improper nutrition. It is therefore important to monitor levels of macroelements in fish organisms and tissues.

Among the examined freshwater fish species, statistically significant differences in the levels of the macroelement were observed. The analysed bioelement (Mg), which by some is regarded as one of the most important macronutrients, was reported to accumulate in excess during diseases caused by viral or bacterial conditions, and during increased activity of hepatocytes (Pouramahad, O'Brien 2000, Lushchak et al. 2005). Levels of magnesium recorded in this study were not high (Tables 3, 4, 5, 6, 7) and remained within the normal range for salmonids (Salmonidae) and cyprinids (Cyprinidae).

For the sturgeon family (*Acipenseridae*), northern pike (*Esox lucius* L.) and grass carp (*Ctenopharyngodon idella* Valenciennes), an accurate normal range of magnesium remains undetermined.

In our research, we have observed that magnesium concentration in fish blood of common carp and rainbow trout (Table 3), in gills of common carp, rainbow trout and Siberian surgeon (Table 4), in liver of rainbow trout and northern pike (Table 5) in kidney of grass carp (Table 6) and in muscles of northern pike decreased with the age of fish. Another observation was that the magnesium concentration in kidneys of northern pike increased (Table 6).

Oikari et al. (1985) have shown that an infusion of magnesium salt into the body cavity of freshwater-adapted fish (rainbow trout) affects the magnesium concentration in plasma. Magnesium could either be reabsorbed or secreted in control freshwater-adapted trout, apparently as a function of nutritional status. Fish could switch from reabsorption to secretion in response to magnesium loading. It is suggested that freshwater fish eliminate excess dietary magnesium renally (Oikari et al. 1985). Variability of metal concentrations in freshwater fish must be seen from a wider perspective of other variables such as habitat, seasonal variations, age of fish, Fulton's condition factor and individual ability for metal uptake (Allen 1993, Canli, Atli 2003, Lopez-Torres et al. 1993, Martinez-Alvarez et al. 2005, Ritola et al. 2002, Svobodova et al. 1997).

Fish are characterized by species-specific and seasonal changeability of macroelements. Stosik and Deptula (2000) found changes in magnesium concentrations due to the season of the year and the change in the level of lymphocytes in the examined fish. According to these authors, the above changes resulted from a close relationship of the season and the solar exposure. In many other research projects, it has been shown that Mg levels were different depending on the temperature, season, sex, feeding type and culture type. (Thomas et al. 1999). We have found that feeding common carp, Siberian sturgeon and rainbow trout Aller Aqua pellet pasture affected the concentration of the analyzed element. The results have confirmed that the content of magnesium was within the physiological reference ranges of fish. The differences found in the bio—element levels resulted from individual and seasonal variability typical of fish. Due to their environmental requirements, fish may be regarded as indicators, which supply information on the degree of pollution of the aquatic environment.

## CONCLUSIONS

- 1. The culture site was found to have statistically significant influence on the magnesium concentrations in organs and tissues in the examined freshwater fish species.
- 2. We have found that feeding common carp, Siberian sturgeon, rainbow trout, northern pike and grass carp (Aller Aqua pellet pasture) affected the concentration of the analyzed element.
- 3. The differences in the concentration of magnesium are a result of individual species differences.
- 4. Mg concentration in the analyzed organs and tissues of freshwater fish were significantly varied.

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# ANALYSIS OF SERUM COPPER AND ZINC CONCENTRATION AMONG EXCESS BODY MASS PERSONS DUE TO THEIR AGE

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#### Abstract

Metabolic alterations in physiological aging may depend on oxidative-antioxidative balance and biomineral status. The aim was to analyze concentrations of serum copper (Cu) and zinc (Zn) among excess body mass persons due to their age. Material: 72 healthy Caucasians, divided into 3 groups: AGE1, AGE2, AGE3 (30-45, 46-60, 61-75 years old respectively), with body mass index (BMI) ≥25 kg m<sup>-2</sup> were qualified for oral glucose tolerance test (OGTT) and fasting (G0') and 2-hours (G120') glycemia were determined (enzymatically). Type 2 diabetes mellitus was excluded. Concentration of serum Cu and Zn (AAS), insulin (ELISA) and plasma lipids: total cholesterol, high density lipoproteins cholesterol, triacyloglyceroles (enzymatically) were measured in fasting samples. Low density lipoproteins cholesterol was obtained using Friedewald formula. Insulin Resistance ratios and Cu/Zn ratio were calculated. Results: No differences concerning BMI, waist, diastolic blood pressure, lipids and insulin concentrations and insulin resistance ratios were observed. Increasing G0', G120' and systolic blood pressure from group 1 to 3 (p=0.01, p=0.01, p=0.04 respectively) were found. AGE2 group had the lowest Zn concentration 10.67±3.37 µmol dm<sup>-3</sup>, (p=0.002) and the highest Cu/Zn ratio 1.73±0.64 (p=0.0003). We calculated negative correlations Zn and SBP (R=-0.45, p=0.04), Zn and DBP (R=-0.46, p=0.04), Zn and G 120' (R=-0.45, p=0.03)in the oldest group. Conclusions: Different zinc concentration while aging may occur, and may imply different metabolic usage of the biominerals, especially in obese patients otherwise healthy subjects.

Key words: aging, trace elements, zinc, cooper.

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# ANALIZA STĘŻEŃ CYNKU I MIEDZI W SUROWICY OSÓB ZE ZWIĘKSZONĄ MASĄ CIAŁA W ZALEŻNOŚCI OD WIEKU

## Abstrakt

W procesie starzenia obserwuje się rozwój zaburzeń metabolicznych, w których dyskutuje się udział stanu równowagi oksydacyjno-antyoksydacyjnej oraz biopierwiastków: cynku i miedzi. Celem badań była ocena stężenia cynku i miedzi w surowicy osób o zwiększonej masie ciała w zależności od ich wieku. W badanich uczestniczyły 72 osoby rasy białej o zwiększonym BMI ≥25 kg m<sup>-2</sup>; przeprowadzono test doustnego obciążenia glukozą (OGTT), mierząc glikemię na czczo (G0') i po 2 h (G120') enzymatycznie. Wykluczono osoby z cukrzycą typu 2. Na czczo oznaczono stężenie cynku i miedzi (AAS), insuliny (ELISA) w surowicy oraz stężenie parametrów lipidowych: cholesterolu całkowitego, cholesterolu frakcji HDL, triacylogliceroli (enzymatycznie). Cholesterol frakcji LDL wyznaczono wzorem Friedewalda. Obliczono wskaźniki insulinooporności oraz stosunek Cu/Zn. Badano grupy w zależności od wieku: AGE1, AGE2, AGE3 (odpowiednio: 30-45, 46-60, 61-75 lat). Stwierdzono, że grupy nie różniły się BMI, obwodem pasa, rozkurczowym ciśnieniem tętniczym, profilem lipidowym, stężeniem insuliny oraz wskaźników insulionoporności. Analiza grup 1-3 wykazała wzrastające stężenie G0', G120' i ciśnienie skurczowe (SBP), odpowiednio p=0.01; p=0.01; p=0.04 oraz różnice w stężeniu Zn (p=0.002) i Cu/Zn (p=0.0003), z najniższymi wartościami Zn 10,67±3,37 µmol dm<sup>-3</sup> i najwyższym Cu/Zn 1,73±0,64 - u AGE2; u AGE3 zaobserwowano ujemne korelacje dla Zn i SBP (R=-0,45; p=0,04), Zn i DBP (R=-0.46; p=0.04), Zn i G120' (R=-0.45; p=0.03). Dysproporcja stężeń Zn między grupą w średnim a podeszłym wieku może być tłumaczona różnymi sposobami wykorzystania Zn w obliczu zaburzeń metabolicznych.

Słowa kluczowe: starzenie się, pierwiastki śladowe, miedź, cynk.

## INTRODUCTION

Owing to modern medicine, the population of elderly people has been growing. Increased body mass resulting in overweight or obesity and different metabolic alterations may occur while aging. Studies concerning obesity, hypertension, dyslipidemia and hyperglycemia are currently discussed (Ceriello 2008, Hsueh et al. 2010, Su et al. 2008).

The oxidative-antioxidative status may play an important role in the development of obesity-related disorders when we age. Copper and zinc are essential cofactors involved in it. There are necessary for normal functions of antioxidant, hematological, vascular, and neurological systems. It is known that overweight and obese individuals are predisposed to lower blood concentrations of vitamins and minerals compared to people with normal body weight (Singh et al. 1998, Marreiro et al. 2006).

Elderly people are more prone to insulin-resistance than middle-aged individuals. Starting from the age of 30, plasma glucose concentration rises: fasting glycemia increase about 1-2 mg cm $^{-3}$  and postprandial glycemia about 2-4 mg cm $^{-3}$  in every 10 years (Winger, Hornick 1996).

There is very little knowledge about trace elements such as zinc or copper or biomechanisms causing aging and finally death. Studies on longevity and metabolic factors contributing to healthy aging are observed. Alterations in the bioavailability of essential trace elements i.e. zinc and copper, which are components of certain enzymes and nucleoproteins, may lead to some tissues and systems dysregulations. During physiological aging, the zinc status declines (Mocchegiani et al. 2006) while plasma copper increases (Milne, Johnson 1993).

It is difficult to assess whether the changes in the trace element status are associated with chronic disease or aging. Studies on the biomineral status in humans and the balance of trace elements may combine all pathological mechanisms involved and explain obesity-related problems while aging.

The aim of this study has been to analyze concentration of serum copper (Cu) and zinc (Zn) among obese persons due to their age.

## MATERIAL AND METHODS

The study was performed in the Department of Clinical Biochemistry and Laboratory Medicine, Chair of Chemistry and Clinical Biochemistry of Poznan University of Medical Sciences, under the permission from the local ethics group in accordance with the Declaration of Helsinki of 1975 for Human Research, and the study protocol was approved by the Bioethics Committee of Poznan University of Medical Sciences in Poznan, Poland.

Subjects and Settings: Healthy Caucasians, 72 persons from the Poznan metropolitan area, with no acute disease or severe chronic disorder, were assessed. The following exclusion criteria were complied: coronary arterial disease, history of diabetes, neoplastic diseases, inflammatory diseases, previous therapy, use of antioxidant drugs, alcohol use, smoking and electrocardiograph findings specific for myocardial ischaemia. Subjects were divided into 3 age-groups, such as AGE1: 0-45 years old (n=24, males=12, females=12), AGE2: 46-60 years old (n=24, males=12, females=12) and AGE3: 61-75 years old (n=24, males=12, females=12). Blood sampling and biochemical analysis: Blood was collected by venous arm puncture. All studied persons were qualified for an oral glucose tolerance test (OGTT) according the WHO recommendation (World Health Organization, 1999). The newly diagnosed type 2 diabetic patients were excluded. Glucose and lipids assay: The concentration of plasma glucose at 0 minutes (fasting) and 120 minutes (postprandial) of the 75-g OGTT, and plasma lipids: total cholesterol (T-C), high density lipoprotein cholesterol (HDL-C), triacyloglyceroles (TAG) were evaluated enzymatically using a bioMerieux reagent kit (France) and a UV-160A Shimadzu spectrophotometer (Japan). Low density lipoprotein cholesterol (LDL-C) was obtained using Friedewald formula. Insulin assay: Fasting serum insulin was measured by the ELISA method (BioSource, Belgium) on a microplater reader (Sunrise Tecan, Switzerland). Insulin resistance, IR ratio – IR = {Ins/G0' (mg cm $^{-3}$ )} and Homeostatic Model Assessment for Insulin Resistance, HOMA-IR = {G0' (mmol dm $^{-3}$ )\*Ins}/22,5 were calculated. *Trace elements assay:* Serum copper and zinc concentrations were determined in duplicate by flame atomic absorption spectrometry (Zeiss AAS-3, Germany). The reference sera level 1 and level 2 (Randox, United Kingdom) were used for monitoring the accuracy of the determinations. The Cu/Zn ratio was established.

Statistical analysis: Statistica (version 6.0) for Windows was used for statistical analysis. The normality of value distribution was checked by the Shapiro-Wilk test. The non-parametric Kruskal Wallis followed by the Man-Whitney U test were applied to assess the significance of differences between the groups. In order to determine the relation between Cu or Zn and the other factors, Pearson linear correlation was used. A p < 0.05 was considered statistically significant. The results are expressed as a mean +/- standard deviations (SD) and a median, given in round brackets.

## RESULTS

Among the 72 studied persons, 24 were young adults aged  $39 \pm 4$  (41), 24 were middle-aged 52 ± 4 (51) years old and 24 were elderly people aged 65 ± 6 (64). The baseline characteristics and clinical parameters of the agegroups 1-3 are presented in Table 1. There were no differences concerning the BMI, waist, diastolic blood pressure, lipids and insulin concentrations as well as IR and HOMA-IR ratios in investigated groups. The Kruskal-Wallis test followed by the Man-Whitney U test showed increasing fasting (G0') and postprandial (G120') glycemia and systolic blood pressure (SBP) from group 1 to 3 (p=0.01, p=0.01, p=0.04, respectively). In the youngest group (AGE1), positive correlation between age and G0' (R=0.43, p=0.034) was observed. In the middle-aged group (AGE2), positive correlation between age and BMI (R=0.37, p=0.036), age and SBP (R=0.35, p=0.046) and negative correlation between Zn and waist (R=-0.36, p=0.05) were calculated. In the last group of elderly patients (AGE 3) negative correlations between Zn and SBP (R=-0.45, p=0.044), Zn and DBP (R=-0.46, p=0.042), Zn and G120' (R=-0.45, p=0.026) were observed (all relationships in Table 2). We did not observe any differences in cooper concentration among the age-groups. In AGE2 group, the lowest zinc concentration:  $10.67\pm3,37$  µmol dm<sup>-3</sup> (p=0.002) and the highest Cu/Zn ratio: 1.73±0.64 (p=0.0003) were noticed (Figures 1 and 2).

Table 1

Baseline characteristics and clinical parameters of the age subgroups

Parameters	$AGE1 \\ n=24$	$\begin{array}{c} \text{AGE2} \\ n = 24 \end{array}$	AGE3 $n=24$	Mann-Whitney U test
Age (years)	$39.0 \pm 4.0 \ (41.0)$	$52.0 \pm 4.0 (51.0)$	$65.0 \pm 6.0 (64.0)$	
$BMI~(kg~m^{-2})$	$28.7 \pm 4.0 \ (28.6)$	$30.1 \pm 4.6 (29.8)$	$28.0 \pm 4.0 (28.0)$	
Waist (cm)	$97.4 \pm 12.9 (99.0)$	$99.7 \pm 15.5 (99.0)$	$98.4 \pm 13.0 \ (98.0)$	
SBP (mm Hg)	$123.0 \pm 18.0 (120.0)^*$	$135.0 \pm 14.0 (135.0)$ *	$142.0 \pm 25.0 (140.0)$	* p=0.022
DBP (mm Hg)	$80.0 \pm 12.0 (80.0)$	$84.0 \pm 10.0 (81.0)$	$88.0 \pm 15.0 \ (88.0)$	
G0' (mmol dm <sup>-3</sup> )	$5.374 \pm 0.676 \ (5.252)$		$5.885 \pm 0.605 (5.830)$	
G120' (mmol dm <sup>-3</sup> )	$6.545 \pm 1.793 \ (6.352)$	$6.325 \pm 1.815 (5.995) \#$	7.865 ± 1.925 (7.920)#	# p=0.004
$\operatorname{Ins}\left(\mathrm{mU}\mathrm{dm}^{-3}\right)$	$10.6 \pm 5.4 (10.0)$	$13.0 \pm 10.6 (10.0)$	$13.5 \pm 12.7 (8.7)$	
IR	$0.106 \pm 0.045 \ (0.099)$	$0.128 \pm 0.105 \ (0.097)$	$0.125 \pm 0.114 \; (0.075)$	
HOMA-IR	$2.67 \pm 1.58 \ (2.31)$	$3.29 \pm 2.73 (2.44)$	$3.63 \pm 3.57 (2.20)$	
$T-C \text{ (mmol dm}^{-3}\text{)}$	$5.772 \pm 0.988 \ (5.382)$	$6.188 \pm 1.326 \ (6.084)$	$5.980 \pm 1.118 \ (6.032)$	
$TAG (mmol dm^{-3})$	$1.727 \pm 1.072 \ (1.399)$	$1.908 \pm 1.479 \ (1.387)$	$1.614 \pm 0.768 (1.434)$	
HDL-C (mmol dm <sup>-3</sup> )	$1.349 \pm 0.372 (1.313)$	$1.295 \pm 0.455 (1.131)$	$1.347 \pm 0.416 (1.326)$	
LDL-C (mmol dm <sup>-3</sup> )	$3.614 \pm 0.728 \ (3.640)$	$4.004 \pm 0.936 (3.952)$	$3.900 \pm 0.910 (3.770)$	
$Cu^{2+}$ (µmol dm <sup>-3</sup> )	$16.36 \pm 2.42 \; (16.13)$	$16.70 \pm 1.86 (16.66)$	$16.46 \pm 2.35 (16.53)$	
$\mathrm{Zn^{2+}}(\mu\mathrm{mol}\;\mathrm{dm^{-3}})$	$13.52 \pm 2.56 (13.42)^{*}$	$10.67 \pm 3.37 (10.40)$ *#	$12.73 \pm 2.58 (12.66) \#$	$^*p=0.0023$ # $p=0.025$
Cu/Zn	1.28 ± 0.47 (1.19)*	$1.74 \pm 0.64 (1.55)$ *#	$1.34 \pm 0.30 (1.32) \#$	* p=0.0004 # p=0.006

# - mark which parameters/groups differ significantly

 $\label{eq:Table 2} {\it Correlations in 1-3 age groups}$ 

NS

NS

R=-0.46, p=0.042

R=-0.45, p=0.026

Correlations	Age 1	Age 2	Age 3	
Age and G0'	R=0.43, p=0.034	NS	NS	
Age and BMI	NS	R=0.37, p=0.036	NS	
Age and SBP	NS	R=0.35, p=0.046	NS	
Zn and waist	NS	R=-0.36, p=0.05	NS	
Zn and SBP	NS	NS	R=-0.45, p=0.044	

NS

NS

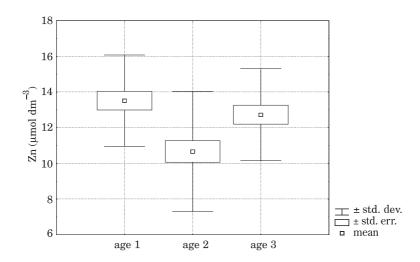


Fig. 1. Comparison of the groups – Zn concentration. Kruskal-Wallis ANOVA test, p=0.02

## DISCUSSION

Zn and DBP

Zn and G120'

The age-related changes in body mass depend on reduction in muscle mass and water volume (30-40%) alongside increased fat mass. Additionally, biochemical imbalance such as glucose and lipid concentrations, is observed. Therefore, it is difficult to decide whether obesity could be attributed to aging processes or to metabolic disorders, including changes in trace elements.

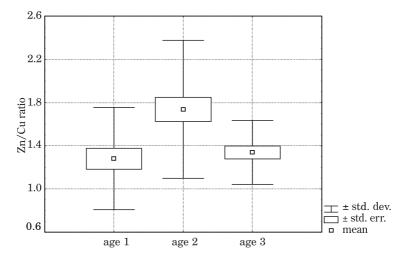


Fig. 2. Comparison of the groups – Cu/Zn ratio. Kruskal-Wallis ANOVA test, p=0.0003

The status of zinc and cooper is broadly discussed in the context of aging (Hotz et al. 2003, Morris 2006, Belbraouet 2007, Lam 2008). In most of these studies, zinc deficiency is defined as a serum concentration below 10.7 µmol dm<sup>-3</sup>. Holtz et al. (2003) observed that serum zinc concentration tends to increase into the third decade of age and decline afterwards. Ravaglia et al. (2000) investigated the oldest-old (octogenarians) and found that serum zinc significantly decreased in this population group compared to elderly (over 60 years old) and younger populations. Our study on an obese population showed the lowest zinc level and the highest Cu/Zn ratio in the middle-aged group (10.67±3.37 µmol dm<sup>-3</sup> and 1.73±0.64, respectively) while in the younger subjects and the elderly group, zinc concentration was higher than the deficiency status.

Ghayour-Mobarhan et al. (2008) showed significant differences in the copper and zinc status in patients with dyslipidemia, with or without established coronary arterial disease, compared with control subjects, arguing that differences in the serum zinc decrease and copper increase may be related to inflammation. The groups we examined Our did not differ in the lipid profile, but the total cholesterol, LDL-cholesterol and triacylglyceroles were the highest in the middle-aged group. Thus, the zinc deficiency in observed AGE2 group could be associated with disturbed lipids.

In a study run by Belbraouet et al. (2007), elderly hospitalized patients were at a higher risk of zinc than copper. We investigated obese but otherwise healthy subjects and confirmed Belbraouet's observations that the zinc status decreases and copper does not change in obese patients while they age.

## CONCLUSIONS

Essential trace elements, zinc and cooper, are involved in many biochemical processes and their plasma concentrations may depends on the dietary, age and health status. While we age, changes in the plasma zinc concentration could imply different metabolic usage of the biominerals, especially in obese but otherwise healthy subjects. Thus, investigations on trace elements may help to prevent and treat diseases, thus extending people's lifespan.

## Acnowledgement

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No conflict of interest was declared with relation to this work.

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# IMPACT OF WATER POLLUTION ON ACCUMULATION OF MAGNESIUM AND CALCIUM BY STRATIOTES ALOIDES L.

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## Abstract

Water soldier is a plant growing all across lowlands in Poland, in eutrophic reservoirs of still and slowly flowing water. Because this macrophyte is used as a fertilizer or a component in fodder for cattle or pigs, the purpose of this paper was to determine the content of magnesium and calcium in water soldier depending on pollution of water with selected heavy metals.

The study used water and plant samples collected from the natural environment and a hydroponic experiment (6 variants of water pollution with heavy metals and two terms of plants' exposure). Concentrations of magnesium and calcium in the mineralized samples were measured in three cycles with the atomic absorption spectrometry method on a spectrometer Solaar S AA. Water soldiers (from the hydroponic experiment) contained 5.911 g Mg kg<sup>-1</sup> d.w. and 16.32 g Ca kg<sup>-1</sup> d.w. It was determined that both addition of a heavy metal and the exposure time had a statistically significant effect on concentrations of magnesium and calcium in water soldier, as well as on their concentration in water. Except the control and regardless the exposure time, most magnesium remained in those water soldier specimens that had been exposed to iron and cadmium. The smallest amounts of magnesium in a plant were observed when water had been enriched with ions of zinc and cooper. After 6 weeks of exposing the plants to the metals, the content of magnesium was 12% lower than after 3 weeks. On the other hand, the smallest amount of calcium in water soldier was recorded for specimens exposed to iron and cadmium, while the largest amount was found in those grown with an addition of zinc and in the control group. The drop in calcium content measured on the second term, compared to the first one, was 6.119 g kg<sup>-1</sup> d.w. Pollution of water with heavy metals has a negative effect on development of water soldier and on accumulation of calcium and magnesium. The reduced con-

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tent of calcium and magnesium in plants collected from waters polluted with heavy metals will affect the value of water soldier both as a fertilizer and a fodder additive.

Key words: magnesium, calcium, Stratiotes aloides, water.

# WPŁYW ZANIECZYSZCZENIA WÓD NA AKUMULACJĘ MAGNEZU I WAPNIA PRZEZ STRATIOTES ALOIDES L.

#### Abstrakt

Osoka aloesowata występuje na całym niżu na terenie Polski w zeutrofizowanych zbiornikach wód stojących i powoli płynących. Ze względu na stosowanie tego makrofitu do użyźniania gleb i jako komponentu pasz dla bydła i świń celem pracy było określenie zawartości magnezu i wapnia w osoce aloesowatej w zależności od zanieczyszczenia wód wybranymi metalami ciężkimi.

W badaniach wykorzystano próbki wód i roślin pobrane ze środowiska naturalnego i z doświadczenia hydroponicznego (6 wariantów skażania wód i 2 czasy ekspozycji roślin na działanie jonów metali ciężkich). Pomiary zawartości magnezu i wapnia w zmineralizowanych próbkach wykonano techniką ASA w 3 powtórzeniach.

Wykorzystana w doświadczeniu hydroponicznym osoka aloesowata zawierała 5,911 g Mg kg<sup>-1</sup> s.m. i 16,32 g Ca kg<sup>-1</sup> s.m. Na podstawie analizy statystycznej ustalono, że zarówno dodatek metalu ciężkiego, jak i czas ekspozycji miały istotny statystycznie wpływ na zawartość Mg i Ca w osoce aloesowatej i stężenie tych pierwiastków w wodzie. Najwięcej Mg, poza kontrolą, niezależnie od czasu ekspozycji, pozostało w osobnikach osoki aloesowatej poddanych działaniu Fe i Cd. Najmniejsze ilości Mg w roślinie stwierdzono po dodatku do wody jonów Zn i Cu. Po 6 tygodniach ekspozycji roślin na działanie metali zawartość Mg była o 12% mniejsza niż po 3 tygodniach. Najmniejszą zawartość Ca w osoce aloesowatej zanotowano u osobników poddanych działaniu Fe i Cd, a największą po dodatku Zn i w kontroli. Spadek zawartości Ca w II terminie w stosunku do I wyniósł 6,119 g kg<sup>-1</sup> s.m. Zanieczyszczenie wód metalami ciężkimi ma niekorzystny wpływ na rozwój osoki aloesowatej oraz kumulację Ca i Mg. Zmniejszona zawartość Ca i Mg w roślinach pobranych zanieczyszczonych metalami ciężkimi wód obniży wartość zarówno uzyskanego nawozu, jak i dodatku paszowego.

Słowa kluczowe: magnez, wapń, Stratiotes aloides, woda.

## INTRODUCTION

Pollution of surface waters with compounds of nitrogen, phosphorus and heavy metals affects the presence and development of different kinds of hydrophytes (Smolders et al. 1996, 2000, 2003, Maleva et al. 2004). Water soldier colonizes both eutrophic and mesotrophic reservoirs of still and slowly flowing water. Apart from Poland, where it occurs over its entire lowlands (Szmeja 2006, Tarkowska-Kukuryk 2006), it is found in continental waters on the whole European territory of Russia, in the Caucasus, in Scandinavia, within the basins of the Baltic and Mediterranean Seas and the European part of the Atlantic Ocean basin, in Western Siberia and Northern Kaza-

khstan (Roelofs 1991, Efremov, Sviridenko 2008). In some European countries, water soldier is protected by law (Sculthorpe 1967, Brammer 1979), while in the countries where this species covers large areas of water reservoirs (Suutari et al. 2009) it is used as a fertilizer and a component in fodder for cattle or pigs (Cook, Urmi-König 1983)

The purpose of this paper was to determine the content of magnesium and calcium in water soldier, depending on pollution of water with selected heavy metals.

## MATERIAL AND METHODS

The samples of water and plants, used for chemical determinations, were collected from the natural environment and from a hydroponic experiment. In 2008, in the Drawskie Lake District Protected Landscape Area and in Swidwie Nature Reserve, nine habitats of water soldier were established, from which samples of water and plants were collected on 11 August. In the same year, a hydroponic experiment was run to grow water soldier in solutions of NPK with addition of heavy metals. On 20 June, water soldier specimens used for that experiment were collected from a ditch encircling Lake Swidwie and transported to a laboratory, where they underwent cleaning and selection procedures. Next, they were grouped into pairs Stratiotes aloides (a mature and a young specimen), which did not differ in their masses and represented similar development stages, and placed in 14 vases containing 8 liters of distilled water each. In order to satisfy nutritional needs of the plants, the vases were fed with solutions of biogenic compounds, namely:  $NH_4NO_3$ ,  $K_2HPO_4$ ,  $KNO_3$ . Concentration of NPK compounds was 0.92 mg  $NH_4^+$   $dm^{-3}$ ,  $2.2 \text{ mg PO}_4^{3-}$   $dm^{-3}$ ,  $2.76 \text{ mg NO}_3^ dm^{-3}$  and  $2.56 \text{ mg K}^+$   $dm^{-3}$ . After one week, the water in the vases where the water soldier specimens were growing was supplemented with solutions of such heavy metals as:  $\operatorname{CuCl}_2 \cdot 2\operatorname{H}_2\operatorname{O}, \ \operatorname{ZnSO}_4 \cdot 7\operatorname{H}_2\operatorname{O}, \ \operatorname{Pb}(\operatorname{CH}_3\operatorname{COO})_2 \cdot 3\operatorname{H}_2\operatorname{O}, \ \operatorname{MnSO}_4 \cdot \operatorname{H}_2\operatorname{O}, \ \operatorname{FeCl}_3,$ 3CdSO<sub>4</sub>·8H<sub>2</sub>O. In the vases, the concentration of the heavy metals was: 12.3 mg Fe<sup>2</sup>dm<sup>-3</sup>, 6.08 mg Mn dm<sup>-3</sup>, 12.1 mg Zn dm<sup>-3</sup>, 0.6 mg Cu dm<sup>-3</sup>, 3.04 mg Pb dm<sup>-3</sup> and 0.05 mg Cd dm<sup>-3</sup>. In the experiment, the control sample was a vase with two specimens of Stratiotes aloides, growing in a solution of biogenic compounds without any addition of heavy metals. The plants were exposed to the solution of heavy metals for 3 and 6 weeks (terms 1 and 2). For further tests, water and plant samples were collected from the vases on two dates, i.e. on 21 July and 11 August, and from the natural environment - on 11 August 2008. All the collected plant samples were dried up and ground. The water samples were subjected to mineralization in HNO<sub>3</sub>, while the plant samples were mineralized in heat, in a mixture of the acids HNO<sub>3</sub> and HClO<sub>4</sub>. The content of magnesium and calcium

in the mineralized samples was measured with the atomic absorption spectrometry method on a spectrometer Solaar S AA in three cycles.

Additionally, in the water solutions where the *Stratiotes aloides* specimens had grown, both in the natural environment and the hydroponic experiment, the concentrations of N-NO $_3$ , N-NH $_4$  and orthophosphates(V) were determined. To determine compounds of nitrogen and phosphorus in water, colorimetric methods were applied in compliance with the Polish standards. The measurements were made in a spectrophotometer Spekol 11. In the water samples, by means of electro-analytical methods, percentage of waterborne oxygen, pH and electrolytic conductivity were determined. For measuring physical qualities, an oxygen meter (a micro-processor unit HI 9145), a laboratory pH-meter (CP-411 with a temperature probe, for liquids, with an electrode EPS-1) and a conductometer (inoLab Coud 730 with an electrode TetraCon 325) were used.

The test results were processed statistically, based on two-factor ANOVA. Significance of differences between the mean values was determined with Tukey's test, at  $\alpha$ =0.05.

## RESULTS AND DISCUSSION

In polluted aquatic environments, water plants can absorb nutrients and heavy metals both from water and ground sediments. Most of the metals affect the microelements critical for maintenance of life of aquatic organisms and display toxic effects only when their concentration available to an organism exceeds the value necessary to satisfy its nutritional requirements. Copper, iron, zinc, manganese, cobalt and selenium are all very important for metabolism. On the other hand, exposure of aquatic organisms to high concentrations of the same elements may strongly affect their development. Other metals such as lead, cadmium and mercury, do not play any significant role in life cycles of organisms; nevertheless, they can cause damage to an organism's tissues if they are present in the environment in toxic concentrations. Reactions of plants to heavy metals depend on the individual sensitivity of each plant, intensity of the stress (duration, concentration) and the form the metal is available in. The toxic effect of absorption of the heavy metals results from their high concentration in cells. By making bonds with functional groups of proteins and polynucleotides, heavy metals upset functions of membranes in the photosynthetic and mitochondrial transport of electrons and inactivate many enzymes involved in regulating the basic cellular metabolism. As a result, these phenomena lead to reduction of the cell energy balance, disturb absorption of minerals, impair growth and development of plants, or even cause their death (Gruca-Królikowska, Wacławek 2006).

In our hydroponic experiment, we observed that water soldier absorbed heavy metals. After three weeks of water soldier plants' exposure to heavy metals, a decrease in masses of the specimens in question, as a result of their negative influence on the plant development, was recorded. After another three weeks, decomposition of older leaves of the plants was seen analogously in the control group (Table 1).

 $\label{eq:Table 1} \mbox{Table 1}$  Percentage loss of mass of water soldier (%)

Addition of heavy metal	Loss mass (%)						
	control	Pb	Cd	Fe	Zn	Cu	Mn
I term	6.25	28.4	23.5	50.5	11.3	45.5	62.7
II term	54.2	40.6	52.2	49.9	59.1	68.3	68.9

In order to determine whether the decomposition of external leaves of water soldier had to be attributed to the heavy metals alone, comparative analysis was performed for natural habitat conditions of *Stratiotes aloides* and the conditions in the hydroponic experiment. The analysis was based on physical and chemical parameters of the water reservoirs where water soldier had grown (Table 2).

Table 2
Physicochemical parameters of water

Parameters		Natural reservoirs		Hydroponic experiment	
		mean	SD	mean	SD
Physical	pH	7.05	0.24	7.23	0.42
	$electrolytic \ conductivity \ (\mu S \ cm^{-1})$	503	208	258	106
	$O_2$ (%)	72.7	13.6	49.1	21.1
Chemical	$\text{N-NO}_3~(\text{mg dm}^{-3})$	0.099	0.014	0.216	0.084
	$\text{N-NH}_4~(\text{mg dm}^{-3})$	0.079	0.078	0.129	0.165
	PO <sub>4</sub> <sup>3-</sup> (mg dm <sup>-3</sup> )	0.466	0.631	0.106	0.065

The natural water pH was close to neutral, whereas a slightly higher pH value was determined in the hydroponic experiment (Table 3). According to the research conducted by Renman (1989), water soldier grows in waters with pH between 5.64 do 7.50. However, a study conducted by Obolewski et al. (2009) in the area of the River Lyna oxbow lakes showed that water soldier populated waters with pH from 7.6 to 8.49.

In their work, Pindel and Woźniak (1998) proved that Stratiotes aloides grew in waters where electrolytic conductivity equaled 240  $\mu S$  cm<sup>-1</sup> and was similar to the conditions in the hydroponic experiment. On the other hand, in the tested waters collected from the natural environment, the value of this parameter was more than double. Nevertheless, Obolewski et al. (2009) inform that in the area of the River Lyna oxbow lakes water soldier occurred in waters with electrolytic conductivity within the range from 364 to 471  $\mu S$  cm<sup>-1</sup>.

In the analyzed waters, collected from the natural environment, the content of water-borne oxygen was much higher than in the water from the hydroponic experiment. The drop in the value of this parameter proves consumption of oxygen in bio-chemical processes resulting from decomposition of dead leaves of water soldier.

In respect of its presence in plant tissues, nitrogen is the fourth most abundant element, after carbon, oxygen and hydrogen. Nitrogen participates in almost all bio-chemical reactions taking place in cells, and its shortage frequently hampers formation of new tissues. The main forms of N available to plants include the ions  $\mathrm{NO_3}^-$  i  $\mathrm{NH_4}^+$  (Sakakibara et al. 2006). According to Tarkowska-Kukuryk (2006), water soldier grows in waters where concentration of N-NO3 remained within the range from 0.03 to 0.24 mg dm<sup>-3</sup>, and N-NH4 from 0.19 to 0.27 mg dm<sup>-3</sup>. Similar concentrations of N-NO3 and N-NH4 were observed in waters collected from the natural environment, as well as from the hydroponic experiment.

Phosphorus is absorbed by plants generally in an oxidized form:  $\rm H_2PO_4^{-1}$  or  $\rm HPO_4^{2-}$ , and is a macronutrient playing a critical role in both catabolism and anabolism. It is also involved in the mechanism of transporting organic compounds and inorganic ions by cellular membranes. When there is a deficit of phosphorus, the intensity of various metabolic transformations, especially anabolic ones, slows down, leading to a slower growth of plants (Schachtman et al. 1998). According to the results obtained by Obolewski et al. (2009), Stratiotes aloides dwells in waters with even higher concentration of orthophosphates(V) (0.63-1.31 mg dm $^{-3}$ ) than we found in the water samples collected from natural reservoirs or particularly from the hydroponic experiment. The decreased concentration of orthophosphates(V) in the hydroponic experiment from 2.20 to 0.1 mg dm $^{-3}$  may indicate that water soldier absorbs large quantities of phosphorus from the aquatic environment.

When comparing the *Stratiotes aloides* habitat conditions in its natural environment to those in the hydroponic experiment, one may conclude that a decrease in the mass and decomposition of older leaves of water soldier specimens resulted from the negative influence of heavy metals on the development of plants and, probably, from the shortage of phosphorus. Those factors led also to the accumulation of magnesium and calcium in the plants concerned (Table 3, Figure 1). Many researchers point to the fact that water soldier dwells in waters with high concentration of calcium. It has been

 ${\it Table 3}$  Mean content of magnesium and calcium in water soldier and concentration of these elements in water depending on the addition of a heavy metal and the exposure time

Treatment		of elemen	tent ats in lant <sup>-1</sup> d.w.)	Concentration of elements in water (mg dm <sup>-3</sup> )		
		Ca	Mg	Ca	Mg	
	control	11.21	5.293	6.161	5.976	
	Pb	3.50	4.915	4.781	3.634	
	Cd	9.404	5.074	1.694	4.063	
Addition of heavy metal (D)	Fe	1.831	5.628	0.114	1.367	
of neavy metal (D)	Zn	12.450	2.773	6.604	1.522	
	Cu	8.271	3.050	7.088	1.402	
	Mn	1.921	4.344	0.045	0.976	
F (C)	I	10.00	4.712	4.039	3.319	
Exposure time (C)	II	3.881	4.167	3.528	2.092	
	D	0.074	0.052	0.047	0.029	
$\mathrm{LSD}_{0.05}$	С	0.214	0.152	0.137	0.083	
	DC	0.349	0.248	0.224	0.135	

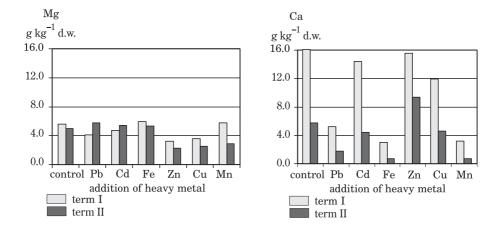


Fig. 1. Content of magnesium and calcium in water soldier from the hydroponic experiment

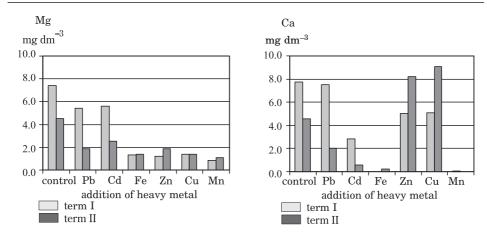


Fig. 2. Concentration of magnesium and calcium in water from hydroponic experiment

proven that calcium is accumulated by plants until September and during intensive photosynthesis the surplus of this element precipitates in the form of carbonates on surfaces of submerged leaves (Cook, Urmi-König 1983, Bram-MER, WETZEL 1984, RENMAN 1989, KRÓLIKOWSKA 1997, SZMEJA 2006). On average, the water soldier collected from the natural reservoirs accumulated 6.837 g Mg kg<sup>-1</sup> d.w. and 23.28 g Ca kg<sup>-1</sup> d.w. at a concentration of magnesium in water 1.756 mg dm<sup>-3</sup> and calcium 52.42 mg dm<sup>-3</sup>. According to the results obtained by Obolewski et al. (2009), water soldier grows in waters with a much higher concentration of ions of this element (8.90-12.90 mg Mg dm<sup>-3</sup>). In plants, magnesium occurs in large quantities in chloroplasts and ribosomes. It is a very important element, which participates in activating enzymes involved in processes of photosynthetic and oxidative phosphorylation, glycolysis, the citric acid cycle, synthesis of multi-molecular building and spare materials and in transformation of nitric compounds. With the transpiration current, calcium is transported to the top of the plant, and because of its low mobility it is accumulated mainly in older leaves. Any surplus of calcium, which can have a negative effect, is excluded from metabolic transformations and bound with oxalic acid in the form of crystals. As a potassium antagonist, calcium causes lower elasticity of cellular walls and membranes, particularly in ageing cells (Pasternak et al. 2010).

The water soldiers grown in the hydroponic experiment contained 5.911 g Mg kg<sup>-1</sup> d.w. and 16.32 g Ca kg<sup>-1</sup> d.w. In the course of the tests, it was determined that both an addition of a heavy metal and the exposure time had a statistically significant effect on concentrations of magnesium and calcium in water soldier, as well as on their concentration in water (Table 3). Except the control and regardless the exposure time, most magnesium remained in those water soldier specimens that had been exposed to iron and

cadmium. The smallest amounts of magnesium in a plant were observed where water had been enriched with ions of zinc and copper. After 6 weeks of exposing the plants to the metals, the content of magnesium was 12% lower than after 3 weeks. On the other hand, the smallest amount of calcium in water soldier was recorded in specimens exposed to iron and cadmium, while the largest amount was found in those with an addition of zinc and in the control group. The drop in calcium content on the second term, compared to the first term, was  $6.119~{\rm g~kg^{-1}}$  d.w.

Apart from the control group, the highest content of calcium in the plant material was observed on the 1<sup>st</sup> term in the variant enriched with zinc and cadmium, whereas the lowest was seen in the one supplemented with iron and manganese. On the 2<sup>nd</sup> term, apart from the control group, the highest content was recorded for the copper and cadmium variant, while the lowest content of that element was observed in the variant with the addition of iron and manganese (0.667 g Ca kg<sup>-1</sup> d.w. and 0.685 g Ca kg<sup>-1</sup> d.w.). The values of the results obtained from the hydroponic experiment are much lower than those obtained from the analysis of the plant material collected from the natural environment. Accelerated by the presence of heavy metals, decomposition of older leaves of water soldier resulted in the fact that our determinations of calcium accumulation concerned generally younger leaves, which contained smaller quantities of this element.

As a result of the accelerated atrophy and decomposition of the oldest water soldier leaves, ions of calcium and magnesium appeared in the water solutions (Figure 2).

On the 2<sup>nd</sup> term, a slight growth in the magnesium content was observed in the water supplemented with manganese and zinc, while a drop was seen in the control group and the lead and cadmium variant. On the 1<sup>st</sup> term, concentrations of magnesium in water remained within the range from 0.860 to 7.432 mg Mg dm<sup>-3</sup>, whereas on the 2<sup>nd</sup> term, this range was from 1.097 to 4.513 mg Mg dm<sup>-3</sup>. The results obtained from the hydroponic experiment exceed the limits stated by Obolewski et al. (2009), as well as those achieved in our own tests in the natural environment.

When analyzing calcium concentration in water samples, we found that it distinctly decreased on the 2<sup>nd</sup> term of measuring this element both in the control group and in the variant enriched with zinc, copper and manganese, which can be a sign that calcium, freed from older leaves, is very well absorbed by younger ones. The results obtained from the vase experiment regarding the calcium content in water are much lower than those obtained from the water samples collected from the natural environment.

Because of the high content of biogenic elements, water soldier is sometimes used as a fertilizer or compost. In some countries, e.g. Finland, the plant is used as fodder for cattle and pigs. Calcium and magnesium accumulated by the plant are exceptionally valuable elements. Fertilizers enriched with these elements can contribute to reduction of soil acidification and,

when introduced as an additive to the fodder, they can lower the risk of occurrence of such diseases as rickets, hypocalcaemia, hypomagnesaemia and allergies in cattle and pigs. Considering the increasing pollution of waters with heavy metals, one must admit that introduction of water soldiers to the nutritional chain of plants (soil fertilization) and animals (fodder additives) will not only contribute to economic utilization of *Stratiotes aloides*, but also to dispersion of heavy metals. However, a lower content of calcium and magnesium in the plants collected from waters polluted with heavy metals depresses the value of water soldier as a fertilizer or a fodder additive.

#### CONCLUSIONS

- 1. Pollution of water with heavy metals has a negative effect on development of water soldier and on accumulation of calcium and magnesium.
- 2. The reduced content of calcium and magnesium in plants collected from waters polluted with heavy metals will affect the value of water soldier both as a fertilizer and a fodder additive.

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# EFFECT OF INSECTICIDES ON PHOSPHORUS AND POTASSIUM CONTENT IN TUBERS OF THREE POTATO CULTIVARS

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#### Abstract

Potato tubers were examined in a field experiment conducted over the years 2004--2006 and arranged as a split-plot design with three replicates. Two factors were studied: I - three mid-early cultivars of edible potato: Wiking, Mors, Zagiel; II - six methods of Colorado beetle control: control (without application of chemicals), Actara 25 WG (0.08 kg ha<sup>-1</sup>), Regent 200 SC (0.1 dm<sup>3</sup> ha<sup>-1</sup>), Calypso 480 SC (0.05 dm<sup>3</sup> ha<sup>-1</sup>), Calypso 480 SC (0.075 dm<sup>3</sup> ha<sup>-1</sup>) and Calypso 480 SC (0.1 dm<sup>3</sup> ha<sup>-1</sup>). Potassium content was determined in the dry matter of potato tubers following wet mineralization and using atomic absorption spectrophotometry (AAS). Phosphorus was determined by means of the photometric method. The objective of the study was to examine the effect of insecticides used to control Colorado beetle on phosphorus and potassium content in potato tubers. Most potassium was found in the tubers of Mors -27.19 g kg<sup>-1</sup> on average, significantly less in Wiking -25.14 g kg<sup>-1</sup> on average, and the least in Zagiel -23.67 g kg<sup>-1</sup> on average. The highest and lowest phosphorus concentrations were determined in, respectively, Mars (3.490 g kg<sup>-1</sup> on average) and Wiking (2.910 g kg<sup>-1</sup> on average). Application of insecticides resulted in a significant increase in potassium and phosphorus content in tubers, which ranged from an average of 0.15 to 0.61 g kg<sup>-1</sup> for potassium and 0.059 to 0.118 g kg<sup>-1</sup> for phosphorus, compared with the control. Our analysis of the influence of atmospheric conditions on phosphorus content in potato tubers demonstrated that the highest phosphorus levels, 3.563 g kg-1 on average, were determined in 2006, which was characterized by the highest precipitation and temperature. The lowest potassium accumulation in potato tubers was recorded in 2004,  $2.897~\rm g~kg^{-1}$  on average.

Key words: insecticides, cultivars, phosphorus, potassium.

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#### WPŁYW INSEKTYCYDÓW NA ZAWARTOŚCI FOSFORU I POTASU W BULWACH TRZECH ODMIAN ZIEMNIAKA

#### Abstrakt

Materiał badawczy stanowiły bulwy ziemniaka pochodzące z doświadczenia polowego przeprowadzonego w latach 2004-2006 założonego metodą losowanych podbloków (split-plot) w trzech powtórzeniach. Badano w nim dwa czynniki: I - trzy średnio wczesne odmiany ziemniaka jadalnego: Wiking, Mors, Żagiel, II - sześć sposobów zwalczania stonki ziemniaczanej: 1) obiekt kontrolny – bez ochrony chemicznej, 2) Actara 25 WG (0,08 kg ha<sup>-1</sup>), 3) Regent 200 SC (0,1 dm<sup>3</sup> ha<sup>-1</sup>), 4) Calypso 480 SC (0,05 dm<sup>3</sup> ha<sup>-1</sup>), 5) Calypso 480 SC (0,075 dm<sup>3</sup> ha<sup>-1</sup>), 6) Calypso 480 SC (0,1 dm<sup>3</sup> ha<sup>-1</sup>). Zawartość potasu oznaczono w suchej masie bulw ziemniaka po mokrej mineralizacji, metodą absorpcyjnej spektrofotometrii atomowej (ASA). Fosfor oznaczono metoda fotometryczna. Celem badań było określenie wpływu insektycydów do zwalczania stonki ziemniaczanej na zawartość fosforu i potasu w bulwach ziemniaka. Najwięcej potasu zawierały bulwy odmiany Mors – średnio 27,19 g kg<sup>-1</sup>, istotnie mniej Wiking – średnio 25,14 g kg<sup>-1</sup>, a najmniej odmiana Żagiel – średnio 23,67 g kg<sup>-1</sup>. Natomiast najwięcej fosforu zawierała odmiana Mors – średnio 3,490 g kg<sup>-1</sup>, a najmniej Wiking – średnio 2,910 g kg<sup>-1</sup>. W wyniku stosowania insektycydów odnotowano istotne zwiększenie ilości potasu w bulwach w porównaniu z obiektem kontrolnym - średnio od 0.15 do 0.61 g kg<sup>-1</sup> oraz zawartości fosforu – średnio od 0.059 do 0.118 g kg<sup>-1</sup>. Analizując wpływ warunków atmosferycznych na zawartość fosforu w bulwach ziemniaka, stwierdzono, że najwięcej zawierały go ziemniaki uprawiane w 2006 r. – średnio 3,563 g kg<sup>-1</sup>, w którym zanotowano najwyższe opady i temperatury. Najmniej tego pierwiastka bulwy kumulowały w 2004 r. – średnio 2,897 g kg $^{-1}$ .

Słowa kluczowe: insektycydy, odmiany, fosfor, potas.

#### INTRODUCTION

Potatoes are a very popular crop plant in Poland and their consumption remains high. As the mineral content of potato tubers is approximately 1%, they may be a significant source of nutrients and essential elements for the human body, although they can also contain harmful elements (SZTEKE et. al. 2006).

Studies by Wichrowska et al. (2009) have indicated that consumption of about 300 g potatoes provides the body with 48.6% of potassium and 25.1% of phosphorus.

The mineral content of potato tubers is predominantly influenced by the genotype and weather conditions. Moreover, it can also be affected by agrotechnological practices, fertilization, soil tillage and plant protection agents in particular (CZEKAŁA, GŁADYSIAK 1995, KRASKA 2002, KOZERA et al. 2006, SAWICKA, KUŚ, 2002 WICHROWSKA et al. 2009).

There is not much information in the available literature on the influence of insecticides on macronutrients in potato tubers. Hence, the objec-

tive of this field study was to determine the effect of an application of insecticides to control the Colorado beetle on the phosphorus and potassium content in edible potato tubers.

#### MATERIAL AND METHODS

We examined tubers harvested in a field experiment conducted over the years 2004-2006 at Zawady Experimental Farm, owned by the former University of Podlasie (University of Natural Sciences and Humanities). The experimental design was a split-plot arrangement of treatments with three replicates.

Two factors were examined in the experiment:

 $factor\ I-three\ mid-early\ edible\ potato\ varieties:\ Wiking,\ Mors,\ Zagiel;$ 

factor II – six methods of Colorado beetle control:

- 1) control no application of chemicals,
- 2) Actara 25 WG at the rate of  $0.08 \text{ kg ha}^{-1}$ .
- 3) Regent 200 SC at the rate of 0.1 dm<sup>3</sup> ha<sup>-1</sup>,
- 4) Calypso 480 SC at the rate of  $0.05 \text{ dm}^3 \text{ ha}^{-1}$ .
- 5) Calypso 480 SC at the rate of  $0.075 \text{ dm}^3 \text{ ha}^{-1}$ ,
- 6) Calypso 480 SC at the rate of  $0.1 \text{ dm}^3 \text{ ha}^{-1}$ .

Potassium content was determined in the dry matter of potato tubers following wet mineralization and using atomic absorption spectrophotometry (AAS). Phosphorus was determined by means of the photometric method.

The results were statistically analysed by means of variance analysis. The F Fisher-Snedecor test was used to detect the significance of each source of variation and mean separation between variables was obtained by Tukev's Least Significant Difference test at the significance level of p=0.05.

Air temperatures and precipitation during the potato growing seasons varied in individual years (Table 1). In 2004, the total rainfall was 320.9 mm with the highest precipitation occurring in May – 97.0 mm. September was the driest month as it received only 19.5 mm rain. The average monthly air temperatures in 2004 varied and ranged from 8.0 to 18.9°C.

In the growing season of 2005, alternating months of extremely dry and very wet weather appeared. Average air temperatures in all the months of the potato growing season varied relative to the long-term means.

The highest rainfall, 358.6 mm, was recorded in 2006. Substantial fluctuations of precipitation were observed during the summer months, which are critical to potato growth and yielding.

Table 1
Characteristics of weather conditions during potato vegetation seasons (RSD Zawady)

Years			Mor	nths			Apr-Sept		
iears	Apr	May	June	July	Aug	Sept	Apr-Sept		
Rainfalls (mm)									
2004	35.9	97.0	52.8	49.0	66.7	19.5	320.9		
2005	12.3	64.7	44.1	86.5	45.4	15.8	268.8		
2006	29.8	39.6	24.0	16.2	228.1	20.9	358.6		
Multiyear average	52.3	50.0	68.2	45.7	66.8	60.7	343.7		
		Air tei	mperature	(oC)					
2004	8.0	11.7	15.5	17.5	18.9	13.0	14.1		
2005	8.7	13.0	15.9	20.2	17.5	15.0	15.0		
2006	8.4	13.6	17.2	22.3	18.0	15.4	15.8		
Multiyear average	7.7	10.0	16.1	19.3	18.0	13.0	14.0		

#### RESULTS AND DISCUSSION

The results of our study demonstrated that potassium and phosphorus accumulation in potato tubers significantly depended on the cultivar, Colorado beetle control method and years (Tables 2, 3).

Of the examined cultivars, the highest potassium content was determined in the tubers of Mors, on average 27.19 g kg<sup>-1</sup>. Significantly less potassium was found in Wiking (25.14 g kg<sup>-1</sup> on average) and the least in cv. Żagiel tubers (23.67 g kg<sup>-1</sup> on average). Most phosphorus was accumulated in the tubers of cv. Mars (3.490 g kg<sup>-1</sup>, on average) and the least in cv. Wiking (2.910 g kg<sup>-1</sup> on average). It agrees with the results reported by Kołodziejczyk and Szmigiel (2005), Mazurczyk (1994), Wichrowska et al. (2009) as well as Zarzecka and Mystkowska (2004), who demonstrated significant influence of cultivars on potassium and phosphorus content in potato tubers.

Application of insecticides was followed by an average increase of 0.15 to  $0.61~\rm g~kg^{-1}$  in tuber potassium content compared with the content in the tubers of non-controlled potatoes (Table 2). A significant increase in potassium content was found in tubers harvested from all the "insecticidal" treatments, apart from the Actara 25 WG-sprayed plots.

Compared with the control content, the plant protection agents applied to control Colorado beetle significantly increased the phosphorus content of potato tubers by an average of 0.059 to 0.118 g kg $^{-1}$  (Table 3). The lowest increase in phosphorus content, by 0.059 g kg $^{-1}$ , was recorded in the treatment where Calypso 480 SC had been applied at the rate of 0.05 dm $^3$  ha $^{-1}$ 

 ${\rm Table} \ 2$  Content of potassium in the dry matter in potato tubers (g  ${\rm kg}^{-1}$  )

Potato beetle control		Years			Cultivars		Mean		
methods	2004	2005	2006	Wiking	Mors	Żagiel	Mean		
1. Control treatment	26.05	24.46	24.54	24.76	27.03	23.24	25.01		
2. Actara 25 WG 0.08 kg ha <sup>-1</sup>	26.27	24.53	24.69	24.80	27.14	23.54	25.16		
3. Regent 200 SC 0.1 dm <sup>3</sup> ha <sup>-1</sup>	26.26	24.64	25.29	25.08	27.38	23.73	25.39		
4. Calypso 480 SC 0.05 dm <sup>3</sup> ha <sup>-1</sup>	26.50	24.48	25.12	25.13	27.10	23.86	25.37		
5. Calypso 480 SC 0.075 dm <sup>3</sup> ha <sup>-1</sup>	26.50	24.63	25.28	25.47	27.22	23.71	25.46		
6. Calypso 480 SC 0.1 dm <sup>3</sup> ha <sup>-1</sup>	26.81	24.65	25.41	25.61	27.29	23.96	25.62		
Mean	26.39	24.56	25.05	25.14	27.19	23.67	-		
$\begin{array}{c} \operatorname{LSD}_{0.05} \text{ for: years} \\ \operatorname{cultivars} \\ \text{methods of controlling the potato beetle} \end{array}$									

 $\label{eq:Table 3}$  Content of phosphorus in the dry matter in potato tubers (g kg $^{-1}$ )

Potato beetle control		Years			Cultivars		Mean	
methods	2004	2005	2006	Wiking	Mors	Żagiel	Mean	
1. Control treatment	2.803	2.992	3.552	2.847	3.408	3.093	3.116	
2. Actara 25 WG 0.08 kg ha <sup>-1</sup>	2.888	3.133	3.565	2.914	3.488	3.184	3.195	
3. Regent 200 SC 0.1 dm <sup>3</sup> ha <sup>-1</sup>	2.934	3.125	3.574	2.902	3.533	3.193	3.211	
4. Calypso 480 SC 0.05 dm <sup>3</sup> ha <sup>-1</sup>	2.878	3.089	3.559	2.896	3.495	3.154	3.175	
5. Calypso 480 SC 0.075 dm <sup>3</sup> ha <sup>-1</sup>	2.908	3.122	3.574	2.905	3.505	3.194	3.201	
6. Calypso 480 SC 0.1 dm <sup>3</sup> ha <sup>-1</sup>	2.971	3.150	3.582	2.995	3.511	3.198	3.234	
Mean	2.897	3.102	3.563	2.910	3.490	3.169	-	

whereas the highest increase (an average of 0.118 g kg<sup>-1</sup>) was found following an application of the same insecticide but at the rate of 0.1 dm<sup>3</sup> ha<sup>-1</sup>. Similar results have been reported by Kraska and Palys (2005). They applied insecticides with the following active ingredients: deltamethrin, bensultap and acetamiprid, and found an increase in potassium and phosphorus content of, respectively 2.4 and 0.3 g kg<sup>-1</sup> in the dry matter of potato roots, compared with the control. By contrast, Prośba-Białczyk et al. (2002) Zchukova et al. (1992) found no changes in potassium and phosphorus concentrations due to application of plant protection agents. According to Leszczyński (2002), application of insecticides and fungicides in most cases does not result in significant changes in chemical composition of potato tubers.

Studies by Kołodziejczyk and Szmigiel (2005) revealed that potassium content in potato tubers was significantly influenced by weather conditions. The highest potassium concentration was determined in the tubers of potato whose growing season was characterized by lower temperatures and higher rainfall, which was confirmed in the present study, as the highest potassium content (26.39 g kg<sup>-1</sup>, on average) was found in the tubers of potatoes cultivated in 2004 and the lowest – in the dry 2005 (Table 2). On the other hand, studies by Wadas et al. (2007) demonstrated that more potassium was accumulated in tubers in the years characterized by lower total precipitation during the stage of tuber initiation.

Our analysis of the impact of atmospheric conditions on phosphorus content in potato tubers indicated that most phosphorus, 3.563 g kg $^{-1}$  on average, was accumulated in the tubers of potato grown in 2006, when the highest precipitation was accompanied by the highest temperatures (Table 3). Least phosphorus, 2.897 g kg $^{-1}$  on average, was accumulated in the tubers in 2004, which coincides with the findings by Zarzecka and Mystkowska (2004). Klikocka (2001) and Mazurczyk and Lis (2001) found that weather conditions during the growing season had no significant influence on phosphorus content in potato tubers.

#### CONCLUSIONS

- 1. The insecticides applied to control the Colorado beetle increased potassium content in potato tubers compared with the control.
- 2. The highest phosphorus content was determined in the tubers of potato harvested from the plots sprayed with the following insecticides: Regent 200 SC and Calypso 480 SC, at the respective rates of 0.1 and  $0.1 \, \mathrm{dm}^3 \, \mathrm{ha}^{-1}$ .
- 3. In the experiment discussed, phosphorus and potassium content in potato tubers was largely determined by cultivars and weather conditions in the individual years.

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### QUALITY OF FRUIT CHERRY, PEACH AND PLUM CULTIVATED UNDER DIFERENT WATER AND FERTILIZATION REGIMES

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#### Abstract

In 2003–2005, a two-factors field experiment was set up on sandy soil of low useful water retention. The aim of the study has been to determine the effect of irrigation and mineral fertilization on the quality of some species of stone fruit trees. The treatments were designed by the split-plot method in 7 replications with cherry, 5 replications with peach, and 4 replications with plum trees. Swards between the trees and herbicide fallows in the rows of trees were maintained. Two factors were considered: subcrown irrigation and mineral fertilization. The mineral fertilization comprised the following variants: 0 NPK –no fertilization, 1 NPK – 130 kg NPK ha<sup>-1</sup>, 2 NPK – 260 kg NPK ha<sup>-1</sup> for cherry and plum trees, and 0 NPK –no fertilization, 1 NPK – 150 kg NPK ha<sup>-1</sup>, 2 NPK – 300 kg NPK ha<sup>-1</sup> for peach. Irrigation and fertilization differentiated concentrations of macro- and micronutrients in fruits of cherry, peach and plum trees. Irrigation resulted in a higher content of sugars and vitamin C in the dry matter of peaches and a lower concentration of sugars in cherries. A higher dose of mineral fertilizers depressed the dry matter content in fruit of plum trees and the concentration of sugars in the fresh matter of cherries.

Key words: irrigation, fertilization, mineral compounds, sugar, vitamin C, acidity.

# JAKOŚĆ OWOCÓW WIŚNI, BRZOSKWINI I ŚLIWY UPRAWIANYCH W ZRÓŻNICOWANYCH WARUNKACH WODNYCH I NAWOZOWYCH

#### Abstrakt

Doświadczenia polowe przeprowadzono na glebie lekkiej o małej retencji wody użytecznej. Badano wpływ nawadniania i nawożenia NPK na jakość owoców wiśni, brzoskwini

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i śliwy. Wszystkie doświadczenia założono metodą losowanych podbloków w układzie zależnym (ang. split-plot), w 7 powtórzeniach w doświadczeniu z wiśnią Łutówka, 5 – z brzoskwinią Redhaven i 4 – w doświadczeniu ze śliwą Cacanska Rana. Czynnikiem badanym było nawadnianie podkoronowe oraz nawożenie mineralne. W uprawie wiśni i śliwy zastosowano następujące dawki: 0 NPK – bez nawożenia, 1 NPK – 130 kg NPK ha<sup>-1</sup> (40+30+60), 2 NPK – 260 kg NPK ha<sup>-1</sup> (80+60+120). W uprawie brzoskwini: 0 NPK – bez nawożenia, 1 NPK – 150 kg NPK ha<sup>-1</sup> (40+30+60), 2 NPK – 300 kg NPK ha<sup>-1</sup> (80+60+120). Nawadnianie i nawożenie mineralne różnicowało zawartość makro- i mikroskładników oraz azotanów w owocach wiśni, brzoskwini i śliwy. Zastosowane nawadnianie wpłynęło istotnie na zawartość cukrów i witaminy C w suchej masie brzoskwini oraz koncentrację cukrów w owocach wiśni. Wysoka dawka nawozów przyczyniła się do zmniejszenia się istotnie zawartości suchej masy w owocach śliwy oraz koncentracji cukrów ogółem w świeżej masie owoców wiśni.

Słowa kluczowe: nawadnianie, nawożenie, składniki mineralne, cukier, witamina C, kwasowość.

#### INTRODUCTION

The quantity and biological quality of fruit tree yields depend on both irrigation and fertilization (RZEKANOWSKI, ROLBIECKI 1996, OSTROWSKA, OCHMIAN 2003, PODSIADŁO et al. 2005, INTRIGLIOLO, CASTEL 2006). Production of large quantities of fruit is not the key point. It is more important to maintain high quality of fruit along with its abundant yield (MORGAŚ, SZYMCZAK 2007). Concentrations of mineral components in orchard plants are very important for both agrotechnology and human nutrition. Their inadequate levels or disproportionate ratios often cause a reduction in yields and a decrease in their quality (Koszański et al. 2006). The aim of the present study has been to determine the effect of subcrown irrigation and mineral fertilization on the amount of some macro- and micronutrients as well as the amount of nitrates, sugars, vitamin C and acidity of cherry, peach and plum fruits.

#### MATERIAL AND METHODS

In 2003-2005, three two-factor field experiments were set up at the Agricultural Experimental Station in Lipnik near Stargard Szczeciński. The field experiments were carried out on soil classified as a good rye complex soil and, in respect of soil cultivation, as light soil of small useful water retention. The experiments were designed with the method of random subblocks in a dependent system of split-plots, in seven replications with cv. Łutówka cherry, five with cv. Redhaven peach and four with cv. Cacanska Rana plum trees. The trees were planted in the following spacing: cherry trees  $-4 \times 2$  m, peach trees  $-3.5 \times 3$  m, plum trees  $-4.5 \times 4$  m. Swards between the trees and herbicide fallows in the rows of trees were maintained.

The first experimental factor consisted of subcrown irrigation, minisprinkling: without irrigation; irrigated plots. Mineral fertilization made up the second factor. The following fertilizer doses were applied in the cultivation of cherry and plum trees: no fertilization, 1 NPK - 130 kg NPK ha<sup>-1</sup> (40+30+60), 2 NPK – 260 kg NPK ha<sup>-1</sup> (80+60+120); in the cultivation of peach trees: no fertilization, 1 NPK - 150 kg NPK ha<sup>-1</sup> (40+30+60), 2 NPK - 300 kg NPK ha<sup>-1</sup> (80+60+120). Nitrogen fertilizers were applied in early spring, before the vegetation started, whereas phosphorous and potassium fertilizers were introduced according to agronomic recommendations. Irrigation was applied according to the indications of a tensiometer at the water potential of soil 0.01 MPa. For the irrigation, a subcrown system was used, in which water was distributed through Hadar mini-sprinklers. One mini-sprinkler was set for each tree. Water doses supplied under the tested stone fruit trees varied in individual periods of vegetation from 0.22 to 0.68 m<sup>3</sup> tree<sup>-1</sup> (21.7 to 61.3 mm ha<sup>-1</sup>), depending on monthly precipitation totals in each year. The following chemical elements were determined annually: nitrogen – with Kiejdahl's method, potassium and calcium – with the photometric method, phosphorus - with colorimetric method; magnesium, iron and zinc - with atomic absorption spectrometry (AAS), nitrates - potentiometrically, sugar – with Luff-Schoorl's method, acidity – potentiometrically, and vitamin C – by spectrometry. The results were processed statistically using the analysis of variance for multiannual experiments on treatments means from individual experiments with reconstruction errors. The significance of differences was assessed at  $LSD_{0.05}$  by Tukey's test. Analysis of correlation for the features which were significantly differentiated by the interaction of experimental factors was also carried out.

#### RESULTS AND DISCUSSION

Production of high quality of fruit relies on good agronomic technology, protecting the fruit — bearing plants and providing them with required nutrients and water. Water deficits during the period of intensive growth of shoots and fruit has negative effect on both quantity and quality of yields. Apart from a large volume of yield, it is also important to obtain yields of good biological value, which determines the usefulness of fruit for consumption and processing (Krawiec 2000, Lipecki 2001). Research on the influence of irrigation on the content of macro- and micronutrients in fruit of trees shows it has differentiated effect on the quality of fruit. Increased, decreased or unchanged An increase and a decrease or no changes concentration of nutrients in the fruit growing on irrigated trees—have been observed (Podsiade et al. 2009). The results of our own research show a decrease in the concentration of nitrogen in fruits of all three kinds of fruit trees but it

was only in peaches that the drop was significant drop (4%). On the irrigated plots, lower concentrations of nitrates in fruits of cherry and plum trees, magnesium in cherries, iron and zinc in cherries, zinc in peaches and iron in plums were also observed (Table 1). Calcium is a nutrient which greatly affects the quality of fruit. Its deficiency causes cracking of cherries and plums. Irrigation rose the calcium concentration in fruits of cherry trees but lowered it in fruits of peach and plum trees. In our earlier study (Podsiadle et al. 2009), it was shown that irrigation did not cause any change

 $\begin{tabular}{ll} Table 1 \\ Content of mineral compounds in the dry matter of cherry, peach and plum fruit \\ (means for 2003-2005) \\ \end{tabular}$ 

Treatm	ent	N	P	K	Ca	Mg	Fe	Zn	N-NO <sub>3</sub> -		
			(g	kg <sup>-1</sup> d.n	n.)		(m	g kg <sup>-1</sup> d	.m.)		
				Cherry							
Irrigation	О	11.5	2.25	12.8	0.65	0.90	130.0	9.50	98.3		
	W	11.3	2.36	13.4	0.70	0.61	117.8	8.44	74.9		
Fertilization	0 NPK	11.2	2.14	12.5	0.72	0.77	141.7	9.08	91.6		
	1 NPK	11.2	2.37	13.3	0.56	0.66	106.7	10.5	66.6		
	2 NPK	11.9	2.41	13.5	0.74	0.84	123.3	7.33	101.8		
$NIR_{0.05}$	O W	n.s. n.s.	n.s. n.s.	n.s. n.s.	n.s.	n.s. n.s.	n.s. 18.0	n.s. n.s.	n.s. n.s.		
	Peach										
Irrigation	О	9.20	2.12	12.0	1.42	0.76	87.7	14.1	18.4		
	W	8.84	2.19	12.7	1.26	0.77	88.9	12.9	18.4		
Fertilization	0 NPK	8.79	2.08	12.5	1.41	0.77	93.3	13.7	17.9		
	1 NPK	8.97	2.32	12.7	1.51	0.79	91.7	12.8	18.8		
	2 NPK	9.29	2.08	11.8	1.11	0.74	80.0	14.0	18.7		
$\mathrm{NIR}_{0.05}$	O W	0.33 n.s.	n.s. n.s.	n.s. n.s.	n.s.	n.s. n.s.	n.s.	n.s. n.s.	n.s. n.s.		
				Plum				•			
Irrigation	О	9.29	1.87	11.7	0.61	0.61	103.3	6.33	67.0		
	W	9.13	2.01	12.0	0.51	0.61	78.9	6.94	60.8		
Fertilization	0 NPK	8.76	1.95	11.5	0.55	0.60	101.7	6.92	62.8		
	1 NPK	9.45	1.93	12.2	0.48	0.61	88.3	6.83	61.8		
	2 NPK	9.43	1.93	11.9	0.65	0.62	83.3	6.17	67.2		
$LSD_{0.05}$	O W	n.s. n.s.	n.s. n.s.	n.s. n.s.	n.s.	n.s. n.s.	n.s.	n.s. n.s.	n.s. n.s.		

 $n.s.-not\ significant$ 

in the amount of calcium in cherries. The present results of analyses of fruits have demonstrated that the complementary irrigation differentiated the content of sugars, vitamin C and acids (Tables 2-4). Significant effects of this treatment, such as an increased content of sugars (by 5%) in the dry matter of peach and vitamin C (by 43%) in the fresh matter of peach and a decrease in the concentration of sugars in the dry matter of cherry (by 3%) were also observed. The results reported by RZEKANOWSKI, ROLBIECKI (1996) show a decrease in the level of dry matter and in the content of vitamin C in the fruit of irrigated apple trees. These authors noticed that irrigation also depressed the content of sugars in cv. Spartan apple tree fruits but caused no significant differences in this respect in apples grown on other apple trees.

Table 2 Content of dry matter, sugar, vitamin C and acidity in cherry fruit (means from 2003-2005)

Treatment		Dry matter (g 100 g <sup>-1</sup> f.m.)	Sugar (% f.m.)	Sugar (% d.m.)	Vitamin C (mg 100 g <sup>-1</sup> f.m.)	Acidity (g 100 g <sup>-1</sup> f.m.)		
Irrigation	O	0.13	7.64	58.9	4.58	1.12		
	W	0.13	7.34	57.0	4.51	1.16		
Fertilization	0 NPK	0.13	7.76	57.6	4.44	1.19		
	2 NPK	0.12	7.22	58.3	4.64	1.09		
$LSD_{0.05}$	O	n.s.	n.s.	0.19	n.s.	n.s.		
	W	n.s.	0.05	n.s.	n.s.	n.s.		

n.s. - not significant

Table 3
Content of dry matter, sugar, vitamin C and acidity in peach fruit (mean from 2003-2005)

Treatm	Treatment		Sugar (% f.m.)	Sugar (% d.m.)	Vitamin C (mg 100 g <sup>-1</sup> f.m.)	Acidity (g 100 g <sup>-1</sup> f.m.)
Irrigation	O	0.12	7.14	59.3	4.21	0.46
	W	0.12	7.51	62.4	6.00	0.49
Fertilization	0 NPK	0.12	7.20	61.1	4.94	0.49
	2 NPK	0.13	7.45	60.6	5.27	0.45
$LSD_{0.05}$	O	n.s.	n.s.	0.31	0.18	n.s.
	W	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. - not significant

Mineral fertilization, applied in this experiment, caused an increase in the content of nitrogen in the fruits of all the kinds of trees, potassium and phosphorus in fruit of cherry trees and potassium in fruit of plum trees (Table 1). Concentration of phosphorus in fruits of peach and plum trees, affected by fertilizing, ranged within an approximately same level in all the

Table 4 Content of dry matter, sugar, vitamin C and acidity in plum fruit (means from 2003-2005)

Treatm	Treatment		Sugar (% f.m.)	Sugar (% d.m.)	Vitamin C (mg 100 g <sup>-1</sup> f.m.)	Acidity (g 100 g <sup>-1</sup> f.m.)
Irrigation	O	0.15	7.33	49.8	1.06	1.39
	W	0.13	6.49	48.6	1.37	1.40
Fertilization	0 NPK	0.15	7.29	49.8	1.20	1.37
	2 NPK	0.13	6.53	48.6	1.23	1.42
$LSD_{0.05}$	O	n.s.	n.s.	n.s.	n.s.	n.s.
	W	0.02	n.s.	n.s.	n.s.	n.s.

n.s. - not significant

fertilizing combinations (Table 1). These results are partly confirmed by the study on cherry trees conducted by Podsiadło et al. (2009), who observed that increasing doses of mineral fertilizers were accompanied by an increasing content of nitrogen and potassium in cherries, whereas the concentration of phosphorus did not change. In the present study, high mineral fertilizing was also found to be favourable to storing nitrates in fruits of all the fruit trees (Table 1). A similar relationship was reported by Chelpiński et al. (2009), who reported an increase in the amount of nitrates in peaches after application of multi-component fertilizers. In this experiment, fertilizing increased the content of calcium and magnesium in fruits of cherry and plum trees but decreased it in peaches. Intensive mineral fertilizing also modified concentration of micronutrients in the fruits, but only in cherries a significant effect of this treatment appeared such as a 13% decrease in iron. Mineral fertilizing had no significant influence on the content of dry matter of cherries and peaches, which ranged within an approximately same level. A high dose of fertilizers decreased significantly the dry matter content in fruit of plum trees (by 3%) and also the concentration of total sugars in the fresh matter of fruit of cherry trees (by 4%). The experiments run by SZWEDO et al. (2000), Švag•Dys, Viškelis (2002) as well as our own studies showed no significant influence of mineral fertilizing on the content of sugars in the dry matter, vitamin C and acidity in fruits of the examined kinds of fruit trees.

Additionally, in the analyzed material, positive, highly significant correlation between the content of potassium and calcium and the concentration of sugars in the fresh matter of fruit of plum trees was observed. Moreover, a highly significant positive relationship between the concentration of nitrogen and the content of sugars in the dry matter of fruit of peach trees was recorded (Table 5). The results are not confirmed by Ben (1997), who noted that the concentration of nitrogen and calcium showed negative correlation with the content of sugars, and the relationship between the content of phosphorus, potassium and magnesium and the concentration of sugars was non-significant.

Table 5

Correlation between conc	entrations of macronutrients in fruit (x) of cherry,
peach and plum trees versus the	content of sugar in the fresh and dry matter of cherries,
	peaches and plums (y)

Specification Y	X	X Macronutrient								
Sugar total	1	N		P	К		Ca		Mg	
Cherry in fresh matter	0.13 0.43	-0.58 <b>0.82</b> *	-0.49 -0.16	-0.93 0.53	-0.96 -0.78	-0.89 0.19	0.00 0.00	-0.87 0.49	0.45 0.66	-0.87 0.67
Peach in fresh matter in dry matter	-0.24 -0.61	<b>0.82</b> 0.13	-0.59 -0.47	-0.13 0.13	0.36 0.70	-0.02 -0.28	-0.21 0.45	-0.63 -0.13	0.78 0.13	-0.63 -0.13
Plum in fresh matter in dry matter	-0.18 0.22	-0.72 -0.72	-0.89 -0.69	-0.14 0.51	-0.63 -0.15	<b>0.95</b> 0.26	0.44 0.47	<b>0.98</b> 0.14	-0.84 -0.82	-0.54 -0.78

<sup>\*(</sup>figures in bold) significant

#### CONCLUSIONS

- 1. Irrigation results in the differentiation of the content of macro- and micronutrients in fruits of cherry, beach and plum trees. In fruit of peach trees, decreased nitrogen content and a lower concentration of nitrates in fruits of cherry and plum trees were observed. Irrigation led to increased content of sugars and vitamin C in the dry matter of fruit of peach trees and decreased concentration of sugars in fruit of cherry trees.
- 2. Mineral fertilization decreased the content of iron in fruit of cherry trees. In the fertilized treatments, an increase in the concentration of nitrogen was observed in fruits of all the fruit trees. A high dose of fertilizers significantly decreased the content of dry matter in fruit of plum trees and total concentration of sugars in the fresh matter of fruit of cherry trees.
- 3. The strongest relationships between the content of potassium and calcium and the concentration of sugars in the fresh matter were noticed in fruit of plum trees. A highly significant positive relationship was also observed between the concentration of nitrogen and the content of sugars in the dry matter of cherries. No differences were recorded between the content of nitrogen and the concentration of sugars in the fresh matter of fruit of peach trees.

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## A COMPARISON OF THE EFFICIENCY OF ORGANIC AND MINERAL IRON COMPOUNDS IN THE GREENHOUSE CULTIVATION OF LETTUCE

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#### Abstract

In 2007-2008, pot experiments were conducted on cv. Michalina head lettuce grown in an unheated greenhouse in spring. The aim of the study was to compare organic and mineral iron compounds as well as their rates in terms of the efficiency of their effect on yield of lettuce and iron content in lettuce leaves. The experimental factors included: 1) sources of iron, i.e. 2 mineral – iron(II) sulfate and iron (III) sulfate, and 3 organic – iron (III) citrate, iron (II) gluconate and iron chelate Fe (III) DTPA, 2) level of iron, i.e. 6 levels – 20 (control), 45, 70, 95, 120 and 220 mg Fe dm<sup>-3</sup> substrate.

Lettuce was grown in 6 dm $^3$  containers. Limed highmoor peat, enriched with macroand micronutrients to meet requirements of lettuce, was used as a substrate. Each experiment included 26 combinations comprising 4 replications each. A container with 4 lettuce plants constituted one replication. Lettuce was harvested at the consumption stage. Concentrations of iron, copper, manganese and zinc were determined in plant material by ASA after wet mineralization in a mixture of acids  $HNO_3$  and  $HClO_4$  at a 3:1 ratio (v:v).

The application of iron(III) sulfate, iron(II) sulfate, iron citrate or iron gluconate ranging from 45 to 220 mg Fe dm<sup>-3</sup> and Librel Fe-DTPA chelate ranging from 45 to 120 mg Fe dm<sup>-3</sup> did not have any significant effect on the yield of lettuce. Librel Fe-DTPA applied at a rate of 220 mg Fe dm<sup>-3</sup> resulted in a reduction of yield. The highest Fe content (irrespectively of the applied Fe rates) in lettuce leaves was recorded after the application of Librel Fe DTPA, while the lowest one – after iron gluconate was used. When analyzing the effect of Fe levels, irrespectively of the used iron compounds, the lowest Fe content in leaves was observed in the control, growing at the levels of 45 and 70 mg Fe dm<sup>-3</sup>, as well as at 90 and 120 mg Fe dm<sup>-3</sup> and reaching its peak after the application of 220 mg Fe dm<sup>-3</sup>.

 $Key\ words:\ iron\ compounds,\ lettuce,\ yielding,\ micronutrients.$ 

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# PORÓWNANIE SKUTECZNOŚCI ORGANICZNYCH I MINERALNYCH ZWIĄZKÓW ŻELAZA W UPRAWIE SAŁATY W SZKLARNI

#### Abstrakt

W latach 2007-2008 wiosną w nieogrzewanej szklarni przeprowadzono doświadczenia wazonowe z sałatą głowiastą odmiany Michalina. Celem pracy było porównanie organicznych i mineralnych związków żelaza, a także ich dawek pod kątem skuteczności działania na plon sałaty oraz zawartość żelaza w jej liściach. Czynnikami doświadczeń były: 1) źródło żelaza, tj. 2 mineralne – siarczan żelaza (II) i siarczan żelaza (III) oraz 3 organiczne – cytrynian żelaza (III), glukonian żelaza (II) i chelat żelaza Fe (III) DTPA; 2) poziom żelaza, tj. 6 poziomów – 20 (kontrola), 45, 70, 95, 120, 220 mg Fe dm<sup>-3</sup> podłoża.

Sałatę uprawiano w pojemnikach o objętości 6 dm<sup>3</sup>. Jako podłoże zastosowano torf wysoki zwapnowany i wzbogacony w makro- i mikroskładniki zgodnie z wymaganiami sałaty.

Każde doświadczenie obejmowało 26 kombinacji składających się z 4 powtórzeń. Powtórzenie stanowił pojemnik z 4 roślinami sałaty. Zbiór sałaty przeprowadzono w fazie dojrzałości konsumpcyjnej. W materiale roślinnym oznaczono zawartość żelaza, miedzi, manganu i cynku metodą ASA po mineralizacji na mokro w mieszaninie kwasów  $\mathrm{HNO}_3$  i  $\mathrm{HClO}_4$  w stosunku 3:1 (v:v).

Zastosowanie siarczanu żelaza (III), siarczanu żelaza (II), cytrynianu żelaza lub glukonianu żelaza w zakresie 45-220 mg Fe dm<sup>-3</sup> oraz chelatu Librel Fe-DTPA w zakresie 45-120 mg Fe dm<sup>-3</sup> nie miało istotnego wpływu na plon sałaty. Librel Fe-DTPA zastosowany na poziomie 220 mg Fe dm<sup>-3</sup> spowodował zmniejszenie plonu. Największą zawartość Fe (niezależnie od dawki Fe) w liściach sałaty stwierdzono po zastosowaniu Librel Fe DTPA, a najmniejszą po zastosowaniu glukonianu żelaza. Analizując wpływ poziomów Fe, niezależnie od związków żelaza, stwierdzono najmniejszą zawartość Fe w liściach w próbie kontrolnej, większą po zastosowaniu 45 i 70 mg Fe dm<sup>-3</sup>, jeszcze większą w przypadku 90 i 120 mg Fe dm<sup>-3</sup> i największą po zastosowaniu 220 mg Fe dm<sup>-3</sup>.

Słowa kluczowe: związki żelaza, sałata, plonowanie, mikroskładniki.

#### INTRODUCTION

Iron deficiency is associated with the incidence of anaemia, which affects approx. 500 million people worldwide. Groups at the highest risk include children and women at the child-bearing age (Thompson 2007). Iron deficits in food occur despite the fact that its total content in soils is generally quite high (on average 2%). Plant chlorosis caused by deficit of available iron is common worldwide, particularly on calcareous soils and in arid and semi-arid regions (Ruszkowska, Wojcieska-Wyskupajtys 1996, Lucena 2003).

The iron requirements in Europe and Poland are satisfied much more often than in the Third World countries. However, the amount of iron consumed with typical diets by different population groups in Poland is frequently lower that the recommended level. Golcz and Dłubak (1998) as well as Bosiacki and Tyksiński (2009) indicated low content of iron in vegetables sold in the city of Poznań. Studies on a variety of suitable sources of iron for plants and their rates need to be conducted.

The aim of the study has been to compare organic and mineral iron compounds as well as their rates in terms of their efficiency on the yield of lettuce and on the iron content in its leaves.

#### MATERIAL AND METHODS

In 2007-2008, pot experiments were conducted on cv. Michalina head lettuce grown in an unheated greenhouse in spring. The experimental factors included:

#### A) iron compounds:

- mineral iron (III) sulfate  $\rm Fe_2(SO_4)_3 \cdot H_2O$  and iron (II) sulfate  $\rm FeSO_4 \cdot 7H_2O;$
- organic iron (III) citrate  $C_6H_5O_7Fe\cdot H_2O$ , iron (II) gluconate  $C_{12}H_{22}FeO_{24}$  and iron (III) chelate Librel Fe-DTPA,

#### B) level of iron:

- 6 levels – the control (20), 45, 70, 95, 120 and 220 mg Fe dm<sup>-3</sup> substrate.

Each year of the experiment, on 21 March, lettuce seeds were sown in a single-seed system to multiple trays with cups size of 4x4 cm filled with highmoor peat (Lithuania), limed at 5 g dm<sup>-3</sup>  $\rm CaCO_3$  and enriched with Azofoska at 1.5 g dm<sup>-3</sup>. Lettuce seedlings were transplanted to containers of 6 dm<sup>-3</sup> filled with appropriately prepared substrate (11 April). Each combination included 4 replications, with a container with four lettuce plants constituting one replication. The control was the same for the entire experiment, i.e. fertilization with macro- and micronutrients except iron.

The substrate was prepared from highmoor peat, which was limed based on the neutralization curve to pH in  $\rm H_2O=6.3$ . After liming, sufficient concentrations of Ca -2045, Mg -160, S-SO $_4-25$  mg dm $^{-3}$  were found in the substrate, therefore they were not supplemented. The other nutrients in the substrate were supplemented to the following levels (in mg dm $^{-3}$ ): N -180, P -140, K -220, Zn -20, Mn -20, Cu -5, B -1 and Mo -1. Iron was applied according to the assumptions adopted for the experiment, considering its initial content in limed peat of 20 mg Fe dm $^{-3}$ .

While growing, plants were watered to 70% water capacity, which was measured using Wahnschaffe cylinders. On 16 May 2007 and 13 May 2008, lettuce was harvested. Plants were cut, weighed, dried in a convection dryer and homogenized. Concentrations of iron, manganese, zinc and copper were determined in the plant material by ASA after wet mineralization in a mixture of acids  $\mathrm{HNO_3}$  and  $\mathrm{HClO_4}$  at a 3:1 ratio (v:v). The results of fresh weight of lettuce heads and content of micronutrients in plants were analyzed statistically. After significant differences had been identified, means were clustered according to the Newman Keuls test at the significance level  $\alpha$ =0.05.

#### RESULTS AND DISCUSSION

Mean results from the 2-year experiment indicate that the average weight of lettuce heads did not differ significantly after the application of iron in the form of  $\text{Fe}_2(\text{SO}_4)_3 \cdot \text{H}_2\text{O}$ ,  $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ ,  $\text{C}_6 \text{H}_5 \text{O}_7 \text{Fe} \cdot \text{H}_2 \text{O}$  or  $\text{C}_{12} \text{H}_{22} \text{FeO}_{24}$ at rates ranging from 45 to 220 mg Fe dm<sup>-3</sup> and in the form of Librel Fe-DTPA at rates ranging from 45 to 120 mgFe dm<sup>-3</sup> (Table 1). It was only the highest level of the Librel Fe-DTPA chelate (220 mg Fe dm<sup>-3</sup>) that resulted in a significant reduction of lettuce yield. At this rate of the chelate, the yield of lettuce was lower, also in comparison to the yield harvested in the control combination. Tyksiński (1992), when growing lettuce on peat, observed that yields of lettuce did not differ at iron rates ranging from 0 to 900 mg Fe dm<sup>-3</sup> applied in the form of iron (II) sulfate. In turn, Tyksiński and Komosa (2007), after an application of 125 mg Fe dm<sup>-3</sup> in the form of chelate Fe-DTPA, stated a significant reduction of lettuce yield in relation to a rate of 75 mg Fe dm<sup>-3</sup>. Moreover, in combinations of 100 and 125 mg Fe dm<sup>-3</sup>, brown necrotic spots appeared on leaves. These authors suggested that such a response of plants was not an effect of excess iron, but rather of the carrier used in the production of the chelate.

Table 1 The effect of iron compounds on the yield of lettuce fresh matter (g container $^{-1}$ ). Mean values for the experiments in 2007 and 2008

Fe		Fe le	vel (mg d	m <sup>-3</sup> subst	rate)		
compounds	20 (control)	45	70	95	120	220	$\overline{x}$
$\mathrm{Fe_2(SO_4)_3}\!\cdot\!\mathrm{H_2O}$	496.5 b	517.4 b	495.1 b	491.5 b	500.5 b	468.9 b	495.0 b
$\mathrm{FeSO_4}\!\cdot\!7\mathrm{H_2O}$	496.5 b	498.5 b	492.6 b	488.8 b	536.4 b	516.9 b	504.9 b
$\mathrm{C_6H_5O_7}\mathrm{Fe}\!\cdot\!\mathrm{H_2O}$	496.5 b	523.4 b	536.8 b	536.9 b	511.9 b	517.0 b	520.4 b
$\mathrm{C}_{12}\mathrm{H}_{22}\mathrm{FeO}_{24}$	496.5 b	522.1 b	517.3 b	546.6 b	539.4 b	491.3 b	518.9 b
Librel Fe-DTPA	496.5 b	523.9 b	515.6 b	458.3 b	459.5 b	237.0 a	448.5 a
$\overline{x}$	496.5 b	517.1 b	511.5 b	504.4 b	509.5 b	446.2 a	

Means followed by same letters are not significantly different at  $\alpha = 0.05$ .

When analyzing the iron content in lettuce, it was found to be dependent on the applied compound and the level of iron (Table 2). The biggest amounts of iron were found in plants fertilized with Librel Fe-DTPA, while the lowest amounts were found in the case of iron gluconate. In turn, when iron was supplied in the form of iron (III) sulfate, lettuce had a significantly higher content of iron in comparison to that fertilized with iron (II) sulfate and iron citrate. Irrespective of the source of iron, over the entire range

 $\label{eq:Table 2} Table \ 2$  The iron content in lettuce leaves (mg  $\ kg^{-1}\ d.\ m.).$  Mean values for the in experiments 2007 and 2008

Fe	Fe level (mg dm <sup>-3</sup> substrate)						
compounds	20 (control)	45	70	95	120	220	$\overline{x}$
$\mathrm{Fe_2(SO_4)_3}\!\cdot\!\mathrm{H_2O}$	149.9 ab	179.9 <i>b-e</i>	184.8 <i>b-е</i>	207.5 de	203.0 с-е	216.6 e	190.3 $c$
$\mathrm{FeSO_4}\!\cdot\!7\mathrm{H_2O}$	149.9 ab	159.7 a-d	152.2~ab	176.0 b-е	172.0 a-d	170.5 a-d	163.4 b
$\mathrm{C_6H_5O_7~Fe}\cdot\mathrm{H_2O}$	149.9 ab	164.1 <i>a-d</i>	162.0 <i>a-d</i>	162.7 a-d	151.9 ab	166.9 <i>a-d</i>	159.6 b
$\mathrm{C_{12}H_{22}FeO_{24}}$	149.9 ab	146.3~ab	147.2~ab	154.1 <i>a-c</i>	$122.2 \ a$	136.7 ab	142.8 a
Librel Fe-DTPA	149.9 ab	169.1 <i>a-d</i>	181.3 <i>b-e</i>	262.6 f	283.9 f	391.2 g	239.6 d
$\overline{x}$	149.9 a	163.8 b	165.5 b	192.6 c	186.6 c	216.4 d	

Means followed by same letters are not significantly different at  $\alpha = 0.05$ .

of fertilization treatments, the content of iron in lettuce was significantly higher than in the control combination. Its highest content was recorded after the introduction of 220 mg Fe dm<sup>-3</sup> to the substrate. Lettuce grown in a substrate with 45 and 70 mg Fe dm<sup>-3</sup> contained similar levels of iron and significantly lower ones than in a substrate with 95 and 120 mg Fe dm<sup>-3</sup>. It needs to be stressed that after the application of Librel Fe-DTPA chelate within the range of 95-220 mg Fe dm<sup>-3</sup>, a significantly higher content of iron appeared in lettuce in comparison with the other experimental objects. The efficiency of Fe chelation by DTPA and citric acid was investigated by Hoffman et al. (2004). Very good chelation efficiency was observed for DTPA. In a study by Tyksiński and Komosa (2007), after the application of Fe-DTPA chelate at rates of 100-125 mg Fe dm<sup>-3</sup>, lettuce showed symptoms of iron excess in the form of brown necrotic spots on leaves or marbled chlorosis. Such plant response was not found in this study.

Iron has a significant effect on the volume and quality of yield; however, it creates considerable problems due to it being readily transformed into forms hardly available to plants (Garcia-Mina et al. 2003, Chohura et al. 2007). Wreesman (1996) stated that the application of chelate forms is of primary importance in the limitation of iron immobilization, as they are characterized by good water solubility and low dissociation constant.

The content of manganese in lettuce depending on the source and level of iron in the substrate is given in Table 3. Lettuce fertilized with iron (III) sulfate contained significantly more manganese than that fertilized with the other iron compounds. The lowest manganese content was recorded in lettuce grown on a substrate with chelate Librel Fe-DTPA. Manganese content in plants decreased with the increasing levels of iron in the substrate, although the reduction was significant (except for 45 mg Fe dm<sup>-3</sup>) only in

Table 3

The manganese content in lettuce leaves (mg kg <sup>-1</sup> d. m.).
Mean values for the experiments in 2007 and 2008

Fe	Fe level (mg dm <sup>-3</sup> substrate)						
compounds	20 (control)	45	70	95	120	220	$\overline{x}$
$\mathrm{Fe_2(SO_4)_3}\!\cdot\!\mathrm{H_2O}$	308.4 <i>b-d</i>	326.9~cd	330.3~cd	339.8~cd	306.4 <i>b-d</i>	351.6 d	327.2 c
$\rm FeSO_4 \cdot 7H_2O$	308.4 <i>b-d</i>	313.4 <i>b-d</i>	289.8 b-d	275.6 b-d	281.9 b-d	285.2 b-d	292.4 b
$\mathrm{C_6H_5O_7}$ Fe $\cdot\mathrm{H_2O}$	308.4 <i>b-d</i>	329.9~cd	288.4 b-d	291.6 b-d	266.3 b-d	257.9 b-d	290.4 b
$\mathrm{C_{12}H_{22}FeO_{24}}$	308.4 <i>b-d</i>	327.9~cd	292.9 b-d	270.8 b-d	$246.0\ bc$	$228.5 \ b$	279.1 b
Librel Fe-DTPA	308.4 b-d	155.5 a	152.5 a	137.4 a	139.3 a	101.8 a	165.8 a
$\overline{x}$	308.4 c	290.7 bc	270.8 ab	263.1 ab	247.9 a	244.9 a	

Means followed by same letters are not significantly different at  $\alpha = 0.05$ .

comparison to the control combination. A similar dependence between manganese and iron in studies on lettuce was reported by Tyksiński (1993).

The content of zinc, similarly to manganese, was the highest in lettuce fertilized with iron (III) sulfate, while the lowest one was found in lettuce fertilized with chelate Librel Fe-DTPA (Table 4). Plants fertilized with iron (II) sulfate were better nourished with zinc than those fertilized with iron citrate or iron gluconate. The level of iron ranging from 70 to 220 mg Fe dm<sup>-3</sup> substrate in relation to the level of 45 mg Fe dm<sup>-3</sup>, as well as the control combination (20 mg Fe dm<sup>-3</sup>) caused a significant reduction of the zinc content in plants. The content of zinc in lettuce grown after the application of 70 mg Fe dm<sup>-3</sup> was significantly bigger only in relation to the treatment including 120 mg Fe dm<sup>-3</sup>. The determined zinc content fell within the range considered by Tyksiński (1992) as optimal for lettuce.

Different iron fertilization rates had an effect on the nutritional status of lettuce in copper (Table 5). Irrespectively of the level of iron in the substrate, the mean copper content in plants was the highest when iron was applied in the form of Librel Fe-DTPA chelate. Lettuce fertilized with iron (II) sulfate, iron citrate or iron gluconate contained less copper than that fertilized with iron (III) sulfate. With the iron content in the substrate ranging from 70 to 95 mg Fe dm<sup>-3</sup>, the content of copper in plants was similar to that recorded in the control. After the application of 45 mg Fe dm<sup>-3</sup> substrate, the content of copper in lettuce was significantly lower, while at 120 and 220 mg Fe dm<sup>-3</sup> it was significantly higher. A similar increase in the content of copper under the influence of increasing levels of iron in the substrate was found in investigations on lettuce conducted by Tyksiński and Komosa (2008), while Anchondo et al. (2001) showed an opposite dependence in leaves and fruits of chili pepper grown in a hydroponic system.

Table 4

The zinc content in lettuce leaves (mg  $kg^{-1} d. m.$ ). Mean values for the experiments in 2007 and 2008

Fe	Fe level (mg dm <sup>-3</sup> substrate)						
compounds	20 (control)	45	70	95	120	220	$\overline{x}$
$\mathrm{Fe_2(SO_4)_3}\!\cdot\!\mathrm{H_2O}$	150.3 <i>f-h</i>	188.8 i	188.8 i	185.9 i	162.2 g-i	190.2 i	177.7 e
$\rm FeSO_4 \cdot 7H_2O$	150.3 <i>f-h</i>	178.8 <i>h-i</i>	160.1 g-i	161.2 g-h	149.9 <i>f-h</i>	152.6 <i>f-h</i>	$158.8 \ d$
$\mathrm{C_6H_5O_7}$ Fe $\cdot\mathrm{H_2O}$	150.3 <i>f-h</i>	167.4 g-i	149.9 <i>f-h</i>	141.6 e-g	127.6 <i>d-f</i>	123.5 <i>d-f</i>	143.4~c
$\mathrm{C}_{12}\mathrm{H}_{22}\mathrm{FeO}_{24}$	150.3 <i>f-h</i>	161.0 g-i	122.7 d-f	124.9 <i>d-f</i>	113.9 с-е	104.6 <i>b-d</i>	129.6 b
Librel Fe-DTPA	150.3 <i>f-h</i>	94.5 a-c	80.1 ab	75.9 a	89.7 a-c	110.8~cd	100.2 a
$\overline{x}$	150.3 c	158.1 c	140.3 b	137.9~ab	128.7 a	136.3 ab	

Means followed by same letters are not significantly different at  $\alpha = 0.05$ .

Table 5 The copper content in lettuce leaves (mg kg $^{-1}$  d. m.). Mean values for the experiments in 2007 and 2008

Fe	Fe level (mg dm <sup>-3</sup> substrate)						
compounds	20 (control)	45	70	95	120	220	$\overline{x}$
$\mathrm{Fe_2(SO_4)_3}\!\cdot\!\mathrm{H_2O}$	9.9 <i>a-e</i>	9.5 а-е	9.6 а-е	12.8 e-i	12.7 e-i	14.2 hi	11.4 b
$\mathrm{FeSO_4}\!\cdot\!7\mathrm{H_2O}$	9.9 <i>a-e</i>	7.5 a-c	9.5 a-e	10.7 a-h	10.9 <i>b-h</i>	11.3 <i>c-i</i>	9.9 a
$\mathrm{C_6H_5O_7}\mathrm{Fe}\!\cdot\!\mathrm{H_2O}$	9.9 <i>a-e</i>	7.3 a-c	10.1 <i>a-f</i>	6.7~a	10.1 <i>a-f</i>	10. 4 a-h	9.1~a
$\mathrm{C_{12}H_{22}FeO_{24}}$	9.9 <i>a-e</i>	7.3 a-c	8.5 a-d	6.9~ab	10.3 a-g	10.1 <i>a-f</i>	8.9 a
Librel Fe-DTPA	9.9 а-е	11.9 <i>d-i</i>	11.7 <i>d-i</i>	14.1 g-i	14.0 <i>f-i</i>	14.8 i	12.8 c
$\overline{x}$	9.9 b	8.7 a	9.9 b	10.2 b	11.6 c	12.17 c	

Means followed by same letters are not significantly different at  $\alpha = 0.05$ .

Differences in the content of iron as well as manganese, zinc and copper under the influence of fertilization with  ${\rm FeSO_4}$  and Librel Fe-DTPA chelate are confirmed in a study by YLIVAINIO et al. (2004) with  ${\rm FeSO_4}$  and the chelates Fe-ETPA, Fe-EDDS and Fe-EDDHA.

In view of the iron nutrition status of lettuce, fertilization with Librel Fe-DTPA chelate at 45-120 mg Fe dm<sup>-3</sup> is recommendable. This level provides the highest iron content in lettuce, similar yields and an optimal copper content. Reduced concentrations of zinc and manganese may be increased by fertilization with these microelements.

#### CONCLUSIONS

- 1. Application of iron (III) sulfate, iron (II) sulfate, iron citrate or iron gluconate ranging from 45 to 220 mg Fe dm<sup>-3</sup>, or Librel Fe-DTPA chelate within the range from 45 to 120 mg Fe dm<sup>-3</sup> did not have any significant effect on yields of lettuce. Librel Fe-DTPA applied at 220 mg Fe dm<sup>-3</sup> resulted in reduced yields.
- 2. The highest Fe content (irrespectively of Fe rates) was found after the application of Librel Fe-DTPA chelate, while the lowest one after the application of iron gluconate.
- 3. Irrespectively of the applied iron compounds, the highest increase in the content of iron in lettuce in relation to the control was found at 220 mg Fe dm<sup>-3</sup> substrate.

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# EFFECT OF NITROGEN, PHOSPHORUS AND POTASSIUM FERTILIZATION ON THE CONTENT OF MACROELEMENTS IN FRUITS OF AUBERGINE (SOLANUM MELONGENA L.) GROWN ON ORGANIC SUBSTRATES

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#### Abstract

A plant growing experiment was conducted in 2003-2004 on the aubergine cultivars Epic  $F_1$  and Solara  $F_1$  grown in an unheated polyethylene tunnel greenhouse at the Experimental Station in Marcelin, the University of Life Sciences in Poznań. The seedlings were planted on 15 May on beds at a 0.5x0.5 m spacing, i.e. 4 plants m<sup>-2</sup>, in 6 dm³ cylinders filled with substrate limed to p $H_{H_{2O}}$  = 6.5, which consisted of: 1) highmoor peat from Lithuania, 2) pine bark from the pulp plant Zakłady Celulozowe + fen peat from Biskupice near Poznań (v : v = 1 : 1). The basic fertilization, i.e. pre-vegetation fertilization and top dressing with macronutrients, based on an analysis of the substrate was applied, using the universal method in 0.03 M CH<sub>3</sub>COOH. The fertilization was designed to achieve the following substrate abundance levels: L (N – 300, P – 265, K – 500 mg dm<sup>-3</sup>), S (N – 400, P – 350, K – 665 mg dm<sup>-3</sup>), H (N – 500, P – 440, K – 830 mg dm<sup>-3</sup>), while maintaining the N : P : K ratio as 1 : 0.9 : 1.7.

The aim of this study has been to determine the effect of nitrogen, phosphorus and potassium on the content of macronutrients in fruits of two aubergine cultivars grown on organic substrates. The fruits were harvested in the first decade of August and underwent chemical analyses in order to determine concentrations of macroelement. For determination of the total forms of nutrients, the fruit samples were subjected to wet mineralization and the following nutrients were assayed: N, P, K, Ca, Mg. Significant differences between

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the cultivars were found only in the mean concentration of potassium, (Epic  $\rm F_1-26.65~g~K~kg^{-1}~d.m$ , Solara  $\rm F_1-29.72~g~K~kg^{-1}~d.m$ ) while the concentrations of nitrogen and calcium were significantly different between the substrate variants. No effect of the fertilization level on the mean content of nitrogen, phosphorus, potassium, magnesium or sulfur was found in aubergine fruits.

Key words: aubergine, fruits, macronutrients.

# WPŁYW NAWOŻENIA AZOTEM, FOSFOREM I POTASEM NA ZAWARTOŚĆ MAKROELEMENTÓW W OWOCACH OBERŻYNY (SOLANUM MELONGENA L.) UPRAWIANEJ W PODŁOŻACH ORGANICZNYCH

#### Abstrakt

W latach 2003–2004 przeprowadzono doświadczenia wegetacyjne z uprawą oberżyny odm. Epic  $F_1$  i Solara  $F_1$  w nieogrzewanym tunelu foliowym, w Stacji Doświadczalnej Marcelin Uniwersytetu Przyrodniczego w Poznaniu. Rośliny sadzono 15 maja na zagonach w rozstawie 0,5 x 0,5 m, tj. 4 rośliny m<sup>-2</sup>, w cylindrach o objętości 6 dm<sup>3</sup> wypełnionych podłożem zwapnowanym do p $H_{H2O}$  = 6,5. Skład podłoża to: 1) torf wysoki z Litwy, 2) kora sosnowa z Zakładów Celulozowych + torf niski z Biskupic k. Poznania (v : v = 1 : 1). Nawożenie podstawowe – przedwegetacyjne i pogłówne makroskładnikami oparte na analizie podłoża wykonanej metodą uniwersalną w 0,03 M CH<sub>3</sub>COOH – ustalono do założonych poziomów: N (N – 300, P – 265, K – 500 mg dm<sup>-3</sup>), S (N – 400, P – 350, K – 665 mg dm<sup>-3</sup>),  $W~({\rm N}-500,~{\rm P}-440,~{\rm K}-830~{\rm mg~dm^{-3}})$  z zachowaniem proporcji makroskładników N : P : K = 1:0,9:1,7. Celem pracy było określenie wpływu nawożenia azotem, fosforem i potasem na zawartość makroelementów w owocach 2 odmian oberżyny uprawianej w podłożach organicznych. Owoce do analiz chemicznych na zawartość makroelementów zebrano w I dekadzie sierpnia, poddano mineralizacji i oznaczono zawartość: N, P, K, Ca, Mg. Większą zawartość azotu oznaczono w owocach roślin uprawianych w torfie wysokim (13,69 g N kg<sup>-1</sup> s.m.) w porównaniu z uprawianymi w mieszaninie kory z torfem (11,65 g N kg-1 s.m.). Istotne różnice w średniej zawartości składnika, porównując odmiany, stwierdzono tylko w zawartości potasu w owocach (Epic  $F_1$  – 26,65 g K kg $^{-1}$  s.m, Solara  $F_1$  –  $29{,}72~{\rm g~K~kg^{-1}~s.m}),$  natomiast analizując rodzaj podłoża – w przypadku azotu i wapnia. Nie stwierdzono wpływu poziomu nawożenia na średnią zawartość azotu, fosforu, potasu, magnezu i siarki w owocach oberżyny.

Słowa kluczowe: oberżyna, owoce, makroelementy.

#### INTRODUCTION

Aubergine fruits are valued for their taste and dietary properties. Fleshy, low-calorie (15-24 kcal) aubergines are not a significant source of vitamins (small amounts of vitamins B, PP, L-ascorbic acid) but they are rich in minerals. Aubergines possess a highly advantageous composition of minerals, i.e. much potassium, calcium, iron and phosphorus but little sodium (Cebula 1996). 100 g of fresh aubergine fruit contains 220 mg potassium, which covers 10% of daily allowance for that nutrient (Hermann 1996). Moreover, aubergine fruits contain chlorine (52 mg%), phosphorus (up to 47 mg%), sulfur

(44 mg%), similar amounts of calcium and magnesium (from 11 to 18 mg%), as well as iron, manganese, copper and zinc (Hermann 1996).

The aim of this study has been to determine the effect of nitrogen, phosphorus and potassium on the content of macroelements in fruits of 2 cultivars of aubergine grown on organic substrates.

#### MATERIAL AND METHODS

A plant-growing experiment was conducted in 2003-2004 on the aubergine cultivars Epic  $F_1$  and Solara  $F_1$  grown in an unheated polyethylene tunnel greenhouse at the Experimental Station in Marcelin, the Poznań University of Life Sciences. The seedlings were planted on 15 May on beds at a 0.5 x 0.5 m spacing, i.e. 4 plants  $\rm m^{-2}$ , in 6 dm³ cylinders filled with substrate limed to  $\rm pH_{H2O}=6.5$ , which consisted of: 1) highmoor peat from Lithuania, 2) pine bark from the pulp plant Zakłady Celulozowe + fen peat from Biskupice near Poznań (v : v = 1 : 1). Basic fertilization, i.e. prevegetation fertilization and top dressing with macronutrients, based on analysis of the substrate, was applied using the universal method in 0.03 M CH\_3COOH. The fertilization pattern was designed to achieve substrate abundance levels: low (N), standard (S) and high (W), while maintaining the N : P : K ratio at 1 : 0.9 : 1.7 (Table 1). The other macro- and micronutrients constituted the background of the experiment.

 $\begin{tabular}{l} Table 1 \\ Nutrient levels in the pre-vegetation and top dressing fertilization \\ of aubergine \\ \end{tabular}$ 

or ausorgine							
	Fertilization						
Nutrient	pre-vegetation top dress			ing			
	${ m mg~dm^{-3}}$						
	LSH	L	S	H			
N	350	300	400	500			
P	310	265	350	440			
K	580	500	665	830			
Ca	1500 - 2000						

The top dressing treatment was performed 3 times at 4-week intervals. Deficiencies of nitrogen, phosphorus and potassium were supplemented to the established levels. Mineral fertilizers  $\mathrm{NH_4NO_3}$ ,  $\mathrm{KH_2PO_4}$  and  $\mathrm{K_2SO_4}$  were applied in the experiment.

All agricultural measures were performed following the current recommendations for auberginecultivation. Fruits were picked several times at the picking maturity stage. Fruits for chemical determination of concentrations of macronutrients were harvested in the first decade of August. In order to determine the total forms of nutrients, fruit samples were subjected to wet mineralization in:

- sulfosalicylic acid, using sodium thiosulfate as a reducer and a selenium mixture as a catalyst – in order to determine nitrogen,
- sulfuric acid in the presence of an oxidant  $H_2O_2$  in order to determine the other macronutrients (except nitrogen).

After mineralization of the plant material, the following determinations were performed:

- total N by distillation according to Kjeldahl in a Parnas-Wagner apparatus,
- P by colorimetry with ammonia molibdate (according to Schillak),
- K, Ca by flame photometry,
- Mg by atomic absorption spectrometry (AAS).
- S-by nephelometry with BaCl<sub>2</sub>.

The results of the chemical analyses were analyzed statistically using Duncan's test at the significance  $\acute{a}=0.05$ .

#### RESULTS AND DISCUSSION

Fruits of both aubergine cultivars contained varied contents of macroelements depending on the substrate and fertilization levels. Higher concentrations of nitrogen were found in fruits of plants grown on highmoor peat (13.69 g N kg $^{-1}$  d.m.) in comparison to those grown on a mixture of bark and peat (11.65 g N kg $^{-1}$  d.m.). Fruits from the highmoor peat variant contained more nitrogen at the low and high fertilization levels in comparison to the standard level. However, no significant differences were found in the mean nitrogen content in fruits of both cultivars at different fertilization levels. The results concerning the nitrogen content in fruits of both aubergine cultivars were markedly lower than those reported by Michaelojć and Buczkowska (2008, 2009), who studied the effect of nitrogen fertilizer, the method of plant habit formation as well as the rate and type of potassium fertilizer and obtained results close to  $\pm$  20 g N kg $^{-1}$  d.m.

In aubergine fruits, more phosphorus was determined than found by Michałojć, Buczkowska (2008, 2009). The posphorus content in fruits of both cultivars grown in the mixed substrate increased with an increasing level of fertilization, but the differences were not significant. Similarly, no significant differences between cultivars or fertilization levels were recorded in the phosphorus content in fruits.

Table 2

Mean content of macronutrients in aubergine fruits depending on the cultivar, substrate and fertilization level in 2003-2004.

G 1	G 1	Fertiliza-		Nutrie	ent solutio	on (g kg <sup>-1</sup>	d.m.)	
Cultivar	Substrate	tion level	N	P	K	Ca	Mg	S 1.87 1.80 1.70 1.75 1.60 1.80 1.65 1.77 1.82 1.75 1.92 1.90 1.75 a 1.80 a 1.77 a 1.78 a
		L	14.40	5.02	28.47	0.90	1.80	1.87
	highmoor peat	S	12.17	4.82	25.62	0.70	1.62	1.80
Enio E		Н	14.32	4.45	25.57	0.80	1.40	1.70
Epic F <sub>1</sub>		L	10.20	3.92	26.10	1.10	1.50	1.75
	pine bark + low - moor peat	S	10.05	4.30	27.02	0.90	1.55	1.60
	P	Н	11.10	4.32	27.10	0.97	1.57	1.80
		L	13.50	4.47	28.12	0.92	1.47	1.65
	highmoor peat	S	12.57	4.60	28.65	0.82	1.45	1.77
Solovo F		Н	15.17	5.00	29.37	1.12	1.50	1.82
Solara F <sub>1</sub>		L	12.25	4.22	30.70	1.22	1.62	1.75
	pine bark + low - moor peat	S	12.80	4.50	29.42	1.02	1.47	1.92
	P	Н	13.52	5.07	32.07	1.00	1.47	1.90
I	Mean for cultivar		12.04 <i>a</i> 13.30 <i>a</i>	4.47 a 4.64 a	26.65 <i>a</i> 29.72 <i>b</i>	$0.89 \ a \ 1.02 \ a$	1.57 a 1.50 a	
M	Mean for substrate			4.72 a 4.39 a	27.63 a 28.73 a	$0.87 \ a \ 1.03 \ b$	1.54 a 1.53 a	
Mean for fertilization level			$12.58 \ a$ $11.90 \ a$ $13.53 \ a$	4.41 <i>a</i> 4.55 <i>a</i> 4.71 <i>a</i>	28.35 <i>a</i> 27.68 <i>a</i> 28.53 <i>a</i>	$1.03 \ b$ $0.86 \ a$ $0.97 \ ab$	1.60 a 1.52 a 1.48 a	1.75 a 1.77 a 1.80 a

Means marked by the same letters do not differ significantly at the level of  $\alpha$ = 0.05 L, S, H – fertilization level: L – low, S – standard, H – high

The highest potassium content was found in fruits of cv. Epic  $F_1$  grown on peat at the low fertilization level (28.47 g K kg $^{-1}$  d.m.) and in fruits of cv. Solara  $F_1$  grown on the mixed substrate of lowmoor peat and bark at the high fertilization level (32.07 g K kg $^{-1}$  d.m.). Moreover, significant differences were shown in the potassium content between cultivars (26.65 g K kg $^{-1}$  d.m. Epic  $F_1$ , 29.72 g kg $^{-1}$  d.m. Solara  $F_1$ ). These results were similar to the ones reported by Michałojć and Buczkowska (2008, 2009).

Aubergine fruits contained from 0.70 g Ca kg<sup>-1</sup> d.m. to 1.22 g Ca kg<sup>-1</sup> d.m. The mean calcium concentrations did not differ significantly. However, a significant effect of the substrate on the mean calcium content was observed in aubergine fruits. At the low fertilization level aubergine fruits accumulated the highest amounts of calcium.

In this study, no effect of cultivar, substrate or fertilization level was found on the mean content of magnesium and sulfur in aubergine fruits. Different concentrations of macronutrients in fruits, despite statistically significant differences, are confirmed by ZORNOZA et al. (1995), who showed that genetic variation of cultivars has some effect on the differentiated uptake, translocation and accumulation of anions and cations in plants. According to KANAHAMA (1994), the nutrients which are translocated the fastest from vegetative organs to fruits include phosphorus and nitrogen; slower transport was found for potassium, while magnesium and calcium were the slowest nutrients.

#### CONCLUSIONS

- 1. Significant differences between the cultivars were found only in the mean concentration of potassium, while the concentrations of nitrogen and calcium were significantly different between the substrate variants.
- 2. Higher mean amounts of nitrogen were determined in fruits of plants grown on highmoor peat in comparison to the mixed substrate.
- 3. No effect of the fertilization level on the mean concentrations of nitrogen, phosphorus, potassium, magnesium or sulfur was found in aubergine fruits.

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## EFFECT OF LAND USE OF FIELDS EXCLUDED FROM CULTIVATION ON SOIL CONTENT OF AVAILABLE NUTRIENTS

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#### Abstract

Fallowing farmland is one of the ways of keeping soil excluded from agricultural production. A fallow field is not cultivated or cropped for many years. Proper management of fallow land can bring meaurable profits for both the producer and the soil environment. The purpose of this study has been to evaluate the abundance of soil in avialable forms of phosphorus, potassium and magnesium on farmland excluded from production. In 1996, an experiment was run, comprising five treatments: a bare fallow field, a field turfed with fodder galega (Galega orientalis Lam.), a classical fallow field, a fallow field covered with fodder galega (Galega orientalis Lam.) with awnless bromegrass (Bromus intermis) and a fallow field sown with awnless bromegrass (Bromus intermis). The only agritechnical treatment performed periodically was mechanical weeding of the bare fallow. The plant biomass grown on plant-covered treatments remained on field every year. Plant material was sampled only for tests. Once the growing season finished, soil samples were taken from each treatment in four replicates from the 0-25 cm and 25-50 cm deep layers. The soil samples were assayed in order to determine concentrations of available forms of phsophorus, potassium and magnesium using generally applicable analytical methods. In both layers of soil (0-25 and 25-50 cm deep), significantly more P, K and Mg available to plants were found in soil turfed with fodder galega. It was also observed that the concentration of phosphorus in the subsoil from this treatment was significantly higher in 2007 than in 2000. Strong correlation was determined between the uptake of phosphorus by plants and the concentration of its bioavailable form in soil in the following year. Keeping soil as a bare field as well as leaving natural plants for several years resulted in the biggest depletion of bioavailable forms of macronutrients in soil.

Key words: soil, fallow land, bare field, available nutrients, P, K, Mg.

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# WPŁYW ZAGOSPODAROWANIA GRUNTÓW WYŁĄCZONYCH Z UPRAWY NA ZAWARTOŚĆ SKŁADNIKÓW PRZYSWAJALNYCH W GLEBIE

#### Abstrakt

Odłogowanie gruntów jest jednym ze sposobów utrzymania gleb wyłączonych z produkcji. Pole odłogowane nie jest uprawiane i obsiewane przez wiele lat. Właściwe postępowanie z tego typu gruntami może przynieść wymierne korzyści zarówno dla producenta, jak i środowiska glebowego. Celem badań była próba oceny stanu zasobności gleb w przyswajalne formy fosforu, potasu oraz magnezu w gruntach wyłączonych z produkcji. W 1996 r. założono doświadczenie, które obejmowało 5 obiektów: ugór czarny, obiekt zadarniony rutwicą wschodnią (Galega orientalis Lam.), odłóg klasyczny, obiekt pokryty mieszanką rutwicy wschodniej (Galega orientalis Lam.) ze stokłosą bezostną (Bromus intermis) i obiekt obsiany stokłosa bezostna (Bromus intermis). Jedynym zabiegiem agrotechnicznym wykonywanym okresowo było mechaniczne odchwaszczanie czarnego ugoru. Biomasa roślinna wyrosła na obiektach zadarnionych pozostawała corocznie na polu. Materiał roślinny pobierano jedynie do badań. Po zakończeniu wegetacji pobierano próbki glebowe z każdego obiektu w 4 powtórzeniach z warstwy 0-25 cm i warstwy 25-50 cm, w których oznaczono zawartość przyswajalnych form fosforu, potasu i magnezu ogólnie dostępnymi metodami analitycznymi. W niniejszej pracy zaprezentowano wyniki badań przeprowadzonych w latach 2000-2007. W badaniach udowodniono, że sposób odłogowania i ugorowania gleby istotnie wpływał na zawartość przyswajalnych form fosforu, potasu i magnezu. W obydwu warstwach gleby (0-25 cm i 25-50 cm) istotnie najwięcej P, K i Mg przyswajalnego znajdowało się w glebie obsianej rutwicą wschodnią. W 2007 r. stwierdzono również istotny wzrost koncentracji fosforu na tym obiekcie w podglebiu, w porównaniu z 2000 rokiem. Określono silną zależność między pobraniem fosforu przez rośliny a zawartościa przyswajalnej formy w glebie w roku następnym. Utrzymywanie gleby w czarnym ugorze, a także kilkuletnie pozostawienie roślinności naturalnej przyczyniło się w największym stopniu do zubożenia gleby w dostępne formy makroelementów.

Słowa kluczowe: gleba, odłóg, ugór, składniki przyswajalne P, K, Mg.

#### INTRODUCTION

Maintaining proper concentration of nutrients in bioavailable forms is one of the conditions shaping soil fertility (Kościk, Kalita 2000, Łętkowska, Straczyńska 2001). It can be assumed that while farmland is kept fallow or bare, the soil fertility is unimportant. This approach, however, is highly irresponsible. If agricultural production is to be restored on fallow land, proper abundance in nutrients is a necessary condition for obtaining high yields of demanded quality in the future. Should any of the nutrients required by crops be deficient, restoring the soil's necessary fertility would involve additional costs of fertilisation. The practice of fallowing land most often includes discontinuation of mineral fertilisation, which means that the input of nutrients to soil stops. Therefore, it is highly recommended to check the fertility of soil under fallow fields. According to Niedzwiecki et al. (1999), concentrations of bioavailable nutrients in soil under fallow fields is not variable. A study reported by Wójcikowska-Kapusta et al. (2003) demonstrated

that fallow soil, similarly to cropped land, was very low and low in available phosphorus and potassium, and the way it was managed did not affect the concentration of total and available forms of these nutrients in deeper layers. These authors determined significantly higher concentrations of available forms of phosphorus in humus horizons of cropped or fallow soils than in the other soil genetic horizons. Other researchers observed considerably weaker leaching of phosphorus from fallow land turfed with grass (ULČN, MATTSSON 2003).

The purpose of the present study has been to evalutae the abundance of soils in available forms of phosphorus, potassium and magnesium under fields excluded from agricultural production.

#### MATERIAL AND METHODS

A large area experiment was established in 1996 on a field in Knopin (the commune of Dobre Miasto, the Province of Warmia and Mazury). The experiment was set up on a field which belonged to a private farmer. Prior to the experiment, all the land at that farm had been cultivated and in 1996 it was cropped with winter oat. The field set out for the experiment was turned into fallow land. The experimental field was divied into five treatments, each covering 1,600 m<sup>2</sup>:

- 1) bare land.
- 2) fodder galega (Galega orientalis Lam.),
- 3) classical fallow,
- 4) fodder galega (*Galega orientalis* Lam.) with awnless bromegrass (*Bromus inermis*),
- 5) awnless bromegrass (*Bromus inermis*).

Prior to the establishment of the experiment, the soil was limed (3 t CaO ha<sup>-1</sup>) and supplied with phsophorus and potassium fertilisers in the amounts corresponding to 26.1 kg P ha<sup>-1</sup> and 74.7 kg K ha<sup>-1</sup>. In late April of 1997, under the optimum temperature and humidity conditions, seeds of the test plants were sown on fields in treatments 2, 4 and 5. Seeds of fodder galega, scarified and inoculated with *Rihsobium galeage*, were sown. In the treatment with single-species seeding, 30 kg ha<sup>-1</sup> were sown. When fodder galega was mixed with awnless bromegrass, 40 kg ha<sup>-1</sup> (30 kg of fodder galega and 10 kg of awnless bromegrass of cv. 'Brudzyńska') were sown. The mixture of bromegrass and white clover of cv. Romena was composed of 10 kg of seeds of bromegrass and 5 kg of seeds of white clover (treatment 5). The first treatment was maintained as a bare field and, as a need arose, it was loosened and simultaneously weeded. In the following years of the trials, no fertilisation or any other chemical treatments were performed. Samples for analyses were taken from plant-covered treatments while the

whole remaining biomass was left on fields. Before the trials began, 4 soil pits were made in order to obtain detailed characterictis of the soil. The humus horizon characterised by the particle size distribution of light loam reached down to 30 cm over the whole field. Soil under the whole experimental field was classified as good wheat complex, class II according to the Polish soil classification system. The abundance of the humus horizon in plant-available nutrients was within: 71.9-119.2 mg P kg $^{-1}$ , 196.2-223.0 mg K kg $^{-1}$  and 69.1-85.1 mg Mg kg $^{-1}$  of soil. The content of organic carbon in this soil layer ranged from 9.50 to 12.03 g kg $^{-1}$  and that of total nitrogen – from 1.08 to 1.24 g kg $^{-1}$ .

The results of the study presented in this article come from 2000-2007. Every year after the growing season, soil samples were collected from each treatment in four replicates from the layers of 0-25 cm and 25-50 cm deep. The soil material was brought to the air-dry state, after which it was crushed and passed through a 1 mm mesh sieve. The ready samples underwent the following chemical analyses: available magnesium was determined with Schachtschabel's method and available phosphorus and potassium were assayed with Egner-Riehm's method. The results were subjected to analysis of variance for a two-factor experiment, using the software application Statistica v. 7.0.

#### RESULTS AND DISCUSSION

The authors' own study has demonstrated that the way farmland is kept fallow has strong influence on the concentration of avialable phosphorus in the 0-25 cm deep soil layer (Table 1), The highest abundance of soil in plantavailable forms of this element, from the first to the last year of the experiment, was maintained in soil cropped with fodder galega. Significantly less available phopphorus was present in soil under the other treatments. It should be emphasised that soil cropped with the mixture of fodder galega and awnless bromegrass, although poorer in phosphorus than soil covered exclusively with the papilionaceous plant, maintained a significantly higher concentration of this element compared to the classical or bare fallow field. Significant differences obtained for the interaction between the tested factors imply that the ways of keeping land idle have a varied influence on the amounts of available phosphorus in soil. Under the effect of fodder galega, a distinct tendency appeared for the soil concentration of available phosphorus to increase. When fallow land had been sown with awnless bromegrass for many years, soil became demonstrably poorer in available P. In soil under bare fallow, the quantity of available phosphorus also declined, but the decreasing tendency was weaker in later years. The references concerning this issue provide us with contradictory information. Dzienia et al. (1997), for example, report that the concentration of this nutrient in fallow land

 $\label{thm:concentration} Table \ 1$  Concentration of available P in soil in mg kg $^{-1}$  (0-25 cm and 25-50 cm deep layer)

The atom and				Ye	ar				A
Treatment	2000	2001	2002	2003	2004	2005	2006	2007	Average
Bare field	<b>79.95</b> 71.55	<b>75.60</b> 69.95	<b>73.90</b> 70.42	<b>74.50</b> 68.81	<b>75.15</b> 67.03	<b>76.23</b> 68.42	<b>74.51</b> 67.44	73.81 66.92	<b>75.45</b> 68.82
Fodder galega	<b>107.2</b> 72.51	<b>106.0</b> 66.46	113.0 73.23	112.0 78.85	<b>116.5</b> 78.03	<b>119.0</b> 68.89	118.1 69.04	119.2 75.20	113.9 72.78
Classical fallow	<b>73.05</b> 72.05	<b>72.25</b> 71.10	<b>75.63</b> 72.35	<b>76.45</b> 71.15	<b>78.80</b> 69.40	<b>76.36</b> 71.42	<b>61.48</b> 68.48	<b>74.32</b> 68.45	<b>73.54</b> 70.55
Fodder galega+brome grass	<b>89.00</b> 69.50	<b>87.50</b> 64.69	<b>81.45</b> 68.25	<b>79.01</b> 68.45	<b>78.83</b> 73.45	<b>81.45</b> 67.55	<b>82.25</b> 68.55	<b>81.92</b> 69.35	<b>82.68</b> 68.72
Bromegrass	<b>81.80</b> 62.51	<b>72.50</b> 61.24	<b>67.85</b> 60.41	<b>67.76</b> 62.45	<b>67.75</b> 63.60	<b>72.15</b> 65.24	<b>69.41</b> 64.99	<b>74.22</b> 62.81	<b>71.68</b> 62.91
Average	<b>86.20</b> 69.62	<b>82.77</b> 66.69	<b>82.37</b> 68.93	<b>81.94</b> 69.94	<b>83.41</b> 70.30	<b>85.04</b> 68.30	<b>81.15</b> 67.70	<b>84.69</b> 68.55	-
	0-25	5 cm					25-50 cr	n	
$LSD_{0.05}$ for years 0.028 $LSD_{0.05}$ for treatments 0.023				$LSD_{0.05}$ for years 0.011 $LSD_{0.05}$ for treatments 0.008					
$LSD_{0.05}$ for int	eraction	0.064		LS	$\mathrm{SD}_{0.05}$ fo	r intera	ction	0.025	

increases. A completely different opinion is expressed by Wojnowska et al. (2003) or Phiri et al. (2001), who report that land fallowing leads to considerable depletion of available phosphorus from soil resources. In turn, the results of the experiments presented by Wojcikowska-Kapusta et al. (2003) indicate that the way farmland is kept idle has no influence on the soil concentration of plant-available forms of phosphorus.

The abundance of the subsoil (25-50 cm deep) in available phsophorus was also modified by the way the land was kept fallow (Table 1). Analogously to the surface layer, significantly more available phosphorus was determined in soil cropped with fodder galega. In addition, significantly more phosphorus available to plants in the subsoil under fodder galega was observed in 2004 than in 2007. Maintaining a bare or a classical fallow field resulted in a similar decline in the concentration of bioavailable phosphorus. These results correspond to the data reported by Ulčn and Mattisson (2003), who observed much smaller losses of this element from a similar soil horizon under grass. Noteworthy is the fact that plant cover, depending on its type, can maintain or even raise the resources of plant-available forms of phosphorus not only in the arable soil layer but also in the soil horizon directly underneath.

Our own tests did not confirm the results obtained by the above authors. On the contrary, it can be claimed that the way land is kept fallow has a dominant effect on modification of bioavailable forms of phorphorus. It should be added, however, that the concentration of this element was high or very high during the whole experiment. This, perhaps, was the reason why the results were different from the references. Another fact which seems to support this conclusion is a very strong dependence between the uptake of phosphorus by plants and the concentration of the bioavailable form of this element in the following year (Figure 1).

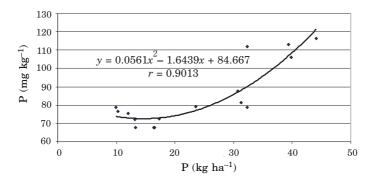


Fig. 1. Dependence of the concentration of available P in soil in mg kg $^{-1}$  on the amount of the element accumulated in plant biomass in kg ha $^{-1}$ 

The abundance of soil (0-25 cm deep) in available potassium was also dependent on the type of fallowing (Table 2). Significantly more bioavailable potassium was maintained in soil under fodder galega, classical fallow and bare field than in soil under bromegrass and significantly less of this element was found in soil under the mixture of fodder galega and awnless bromegrass. The results suggest that the concentration of bioavialable potassium in the consecutive years was strongly depressed in soil from all the treatments. However, considering the fact that both species grown in the experiment demand much potassium and are highly productive plants, it can be claimed that much of the available potassium was temporarily acummulated in organic matter. After some time, it returns to the plant-available form, therefore the decrease in the concentration of potassium in plantcovered treatments need not be considered solely as a loss. An equally unfavourable effect of land fallowing on the concentration of available potassium in soil has been reported by Wojnowska et al. (2003), Łekowska and STRĄCZYŃSKA (2001), NIED•WIECKI et al. (1998), DZIENIA et al. (1997). Similarly to phosphorus, Wójcikowska-Kapusta et al. (20030) claim that land fallowing has no effect on the soil abundance in this nutrient. Amounts of substances leached from soil are directly dependent on water management and primarily on amounts of precipitation, which shape the extent of migration of ele-

 $\label{eq:Table 2} Table~2$  Concentration of available K in soil in mg kg $^{-1}$  (0-25 cm and 25-50 cm deep layer

The atmosph				Ye	ar				A
Treatment	2000	2001	2002	2003	2004	2005	2006	2007	Average
Bare field	<b>206.6</b> 128.0	<b>209.3</b> 122.2	<b>209.7</b> 125.1	<b>207.7</b> 124.3	<b>191.5</b> 125.3	<b>199.2</b> 116.2	<b>190.5</b> 120.4	<b>194.9</b> 116.0	<b>201.2</b> 122.2
Fodder galega	<b>222.9</b> 125.3	<b>202.4</b> 123.3	<b>204.2</b> 122.5	<b>207.6</b> 115.5	<b>208.5</b> 122.2	<b>220.7</b> 98.30	<b>221.1</b> 99.60	<b>222.2</b> 121.5	<b>213.7</b> 116.0
Classical fallow	<b>211.3</b> 115.5	<b>201.5</b> 118.5	<b>209.2</b> 110.2	<b>204.9</b> 114.2	<b>207.5</b> 126.1	<b>210.5</b> 103.0	<b>195.1</b> 99.60	<b>207.3</b> 114.4	205.9 112.7
Fodder galega+brome grass	<b>195.9</b> 116.0	186.8 120.5	<b>189.6</b> 111.2	184.6 105.2	<b>183.5</b> 114.2	<b>195.8</b> 103.0	<b>182.6</b> 107.9	198.8 111.1	189.7 111.1
Bromegrass	<b>208.9</b> 119.0	<b>200.5</b> 115.2	<b>202.4</b> 108.2	<b>203.8</b> 112.3	<b>182.5</b> 104.3	<b>201.5</b> 103.9	<b>195.1</b> 99.60	<b>202.5</b> 103.8	<b>199.7</b> 108.3
Average	<b>209.1</b> 120.8	<b>200.1</b> 119.9	<b>203.0</b> 115.4	<b>201.7</b> 114.3	<b>194.7</b> 118.4	<b>205.5</b> 104.9	196.9 105.4	<b>205.1</b> 113.4	
	0-25	5 cm					25-50 cm	n	
LSD <sub>0.05</sub> for ye	LSD $_{0.05}$ for years 0.207			L	$SD_{0.05}$ f	or years		0.049	
0.00	$LSD_{0.05}$ for treatments 0.164 $LSD_{0.05}$ for treatments 0.463 $LSD_{0.05}$ for interaction 0.411								

ments (Koc et al. 1999). Depressing the concentration of available potassium in soil under the bare fallow could have been due to its increased leaching. It should be remembered that this element accumulated in organic matter produced each year in all the treatments except the bare field remained on field. Potassium accumulated in biomass was therefore returned to soil and because it is not arrested in permanent organic bonds, it quickly becomes available to plants. Thus, it seems that even the losses in potassium demonstrated in our study in soil under plant cover could be smaller than the amounts determined by chemical analyses.

Similar relationships in terms of available potassium as in the surface layer of soil occurred in the 25-50 cm deep layer (Table 2). Statistical calculations proved that the way of keeping farmland fallow significantly modified the concentration of bioavailable K in the soil horizon under humus. Fluctuations recorded in our study were evidently smaller than in the surface soil layer. Differentiation in the abundance of the subsoil in this nutrient depending on years proved to be non-significant. However, it depended to a large extent on the way farmland was excluded from agricultural use. The best way of land fallowing, which prevented loss of ptassium, was by sowing a field with fodder galega. It is possible that the highest capacity of galega to take up potassium contributed to a more effective mobilisation of this

nutrient. Other possible causes could be translocation of potassium from deeper horizons (the root system) and upper layers (leaching).

The most profound loss in soil abundance appeared in the treatment with awnless bromegrass. Good utilisation of nutrients from less dissolvable compounds and consequently their mobilisation by fodder galega may have resulted in larger accumulation of bioavailable magnesium in soil under this plant or under its mixture with awnless bromegrass (Table 3). In the soil from the other treatments, concentrations of available magnesium decreased in the following years of the trials. A similar opinion is expressed by Blecharczyk et al. (1985), Dzienia et al. (1997), Wojnowska et al. (2003). In contrast, Malicki and Podstawka-Chmielewska (1998) report that discontinuation of farming can contribute to an increase in the concentration of available magnesium in soil. Finally, Baran et al. (2001) concluded that land fallowing did not change the soil abudance in this nutrient.

Based on the results of soil analyses, it can be assumed that there was some translocation of available magnesium from the 0-25 cm to the subsoil (Table 3). This effect was demonstrably more evident under the classical fallow field and the field overgrown with awnless bromegrass. In turn, the decreased abundance of the upper soil layer as well as the subsoil under the bare field may suggest that magnesium was translocated even deeper into

Table 3 Concentration of available Mg in soil in mg kg $^{-1}$  (0-25 cm and 25-50 cm deep layer

TD 4				Ye	ar				A
Treatment	2000	2001	2002	2003	2004	2005	2006	2007	Average
Bare field	<b>71.73</b> 78.75	<b>70.60</b> 74.25	<b>71.62</b> 72.12	<b>68.13</b> 73.51	<b>66.93</b> 69.00	<b>66.00</b> 72.00	<b>68.10</b> 68.50	<b>65.00</b> 71.00	<b>68.51</b> 72.39
Fodder galega	<b>80.00</b> 81.50	<b>78.00</b> 83.20	<b>79.25</b> 84.51	<b>81.98</b> 87.24	<b>85.50</b> 93.75	<b>81.00</b> 86.00	<b>84.00</b> 82.00	<b>86.00</b> 88.22	<b>81.97</b> 85.80
Classical fallow	<b>79.50</b> 86.50	<b>74.75</b> 95.51	<b>72.36</b> 94.25	<b>74.86</b> 98.51	<b>74.87</b> 104.0	<b>73.05</b> 96.00	<b>69.00</b> 95.00	<b>70.50</b> 100.0	<b>73.61</b> 96.22
Fodder galega+brome grass	<b>83.03</b> 85.50	<b>82.55</b> 84.25	<b>83.35</b> 89.42	<b>86.15</b> 94.52	<b>83.85</b> 97.75	<b>77.00</b> 94.00	<b>83.00</b> 96.00	<b>73.00</b> 95.50	<b>81.49</b> 92.12
Bromegrass	<b>68.75</b> 82.50	<b>66.63</b> 84.58	<b>63.50</b> 83.25	<b>63.50</b> 86.24	<b>64.25</b> 88.00	<b>51.00</b> 87.00	<b>68.00</b> 86.00	<b>50.00</b> 84.00	<b>61.95</b> 85.20
Average	<b>76.60</b> 82.95	<b>74.51</b> 84.36	<b>74.02</b> 84.71	<b>74.92</b> 88.00	<b>75.08</b> 90.50	<b>69.61</b> 87.00	<b>74.42</b> 85.50	<b>68.90</b> 87.74	
	0-25	5 cm					25-50 cm	n	
$ \begin{array}{ c c c c } LSD_{0.05} \ \text{for years} & 0.045 \\ LSD_{0.05} \ \text{for treatments} & 0.036 \\ \end{array} $			$LSD_{0.05}$ for years 0.113 $LSD_{0.05}$ for treatments 0.089				0.089		
$LSD_{0.05}$ for int	eraction	0.101		Là	$5D_{0.05}$ for	or intera	ction	0.253	

the soil. The statistical calculations confirmed the significant effect of both the way land was kept fallow and the duration of land fallowing on the concentration of available magnesium. Decreased concentration of this nutrient was observed only under the bare field. Lack of plant cover resulted in a 19% decline in the concentration of plant-available forms of this element in the 25-50 cm deep soil layer compared to grass-covered fields. The presence of plant cover had positive influence on retaining available magnesium in the subsoil.

Time-related fluctuations in the soil abundance in available magnesium may be related to the amount of Mg accumulated in plants growing on fallow land (Figure 2). Strong correlation was found between soil abundance in available magnesium and the amount of the accumulated element in plants left on field in the previous year.

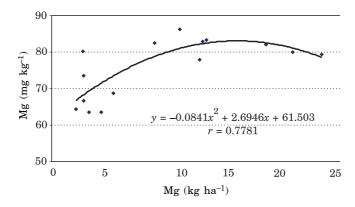


Fig. 2. Dependence of the concentration of available Mg in soil in mg kg<sup>-1</sup> on the amount of the element accumulated in plant biomass in kg ha<sup>-1</sup>

#### CONCLUSIONS

- 1. Keeping soil excluded from agricultural production for many years as a fallow field turfed with fodder galega may increase the availability of phosphorus, potassium and magnesium in the 0-25 and 25-50 cm deep soil horizons.
- 2. A mixture of fodder galega and awnless bromegrass shows a similar effect on available magnesium.
- 3. A bare fallow field and the classical fallow do not protect soil from losing plant-available forms of P, K and Mg.

4. Soil abundance in available phosphorus and magnesium when a field has been kept fallow for many years and plants have remained on field after harvest depends on the amount of these nutrients accumulated in biomass.

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# FOLIAR NUTRITION OF POPPY PLANTS (PAPAVER SOMNIFERUM L.) WITH SELENIUM AND THE EFFECT ON ITS CONTENT IN SEEDS\*

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#### Abstract

Selenium is a trace element which in small amounts is necessary for human and animal nutrition. In a living organism, it helps a number of antioxidant enzymes to function normally. In many parts of the world, including the Central European region, its content in agricultural products is very low. Attempts are therefore made to increase its content and cover human requirements with biologically valuable products by incorporating selenium into the system of plant nutrition. In a vegetation trial established in 2008 and 2009, we explored the effect of foliar applications of Se(IV) on yields and on its content in seeds and the uptake of selenium by a poppy (Papaver somniferum L.) stand. Selenium was applied at a rate of 300 g ha-1 during the stage of the end of elongation growth and after the fall of blossoms. Poppy yields were significantly influenced by the weather in the experimental years. In the dry year of 2009, poppy production was 40.6% lower than in 2008. Selenium application at the end of elongation growth reduced poppy seed yields by an average of 11.5%. Late supplementary nutrition at the stage after blossom fall also reduced yields (by 11.8%). Owing to the effect of extra-root Se nutrition, the content of selenium in poppy seeds increased highly significantly from 139 µg kg<sup>-1</sup> to 757 µg kg<sup>-1</sup> of seeds. Also the uptake of selenium by the poppy stand was significant and after foliar application it increased 4.8 times.

Key words: selenium, poppy, foliar application, yield of seeds, quality of seeds.

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## NAWOŻENIE DOLISTNE MAKU (PAPAVER SOMNIFERUM L.) SELENEM I WPŁYW NA ZAWARTOŚĆ SELENU W ZIARNIE MAKU

#### Abstrakt

Selen to pierwiastek śladowy, który w niewielkich ilościach jest niezbędny w żywieniu ludzi i zwierząt. W żywym organizmie selen wspomaga prawidłowe działanie wielu enzymów antyoksydacyjnych. W wielu rejonach świata, łącznie z obszarem Europy Środkowej, zawartość selenu w produktach rolnych jest niewielka. W związku z tym podejmowane są starania, by zwiększyć jego zawartość przez wprowadzenie selenu do systemu nawożenia roślin uprawnych, by pokryć zapotrzebowanie na ten pierwiastek w diecie dzięki biologicznie wartościowym produktom.

W doświadczeniu wegetacyjnym przeprowadzonym w latach 2008 i 2009 badano wpływ dolistnego nawożenia Se(IV) na plonowanie maku (*Papaver somniferum* L.) oraz na zawartość selenu w ziarnie maku i pobór tego pierwiastka przez rośliny. Selen zastosowano w dawce 300 g ha<sup>-1</sup> pod koniec fazy wzrostu łodygi oraz w czasie przekwitania. Plony maku były silnie uzależnione od warunków pogodowych w czasie doświadczenia. W suchym 2009 r. plon maku był o 40,6% niższy niż w 2008 r. Zastosowanie selenu pod koniec fazy wzrostu łodygi ograniczyło plon maku o średnio 11,5%. Późniejsze zastosowanie nawożenia selenem, w fazie przekwitania, także zmniejszyło plony (o 11,8%). Dzięki dodatkowemu dożywieniu korzeni selenem zawartość Se w ziarnie maku wzrosła istotnie ze 139 µg kg<sup>-1</sup> do 757 µg kg<sup>-1</sup>. Pobór selenu przez łan maku także był istotny, i wzrósł 4,8-krotnie po nawożeniu dolistnym tym pierwiastkiem.

Słowa kluczowe: selen, mak, nawożenie dolistne, plon ziarna, jakość ziarna.

#### INTRODUCTION

Selenium is classified into a group of micronutrients which appear in plants in the form of a number of allotropic variants, like sulphur (Terry 2000). In human and animal organisms, it ensures a number of metabolic functions. Its deficiency damages the enzyme systems which protect cells from oxidation stress and in humans it strengthens the defence system of the organism (Combs, Gray 1998, Broome 2002, Arthur 2003).

Plant products lack Se, particularly in the Central European region. Therefore, efforts are made to increase its content and to cover human demands with biologically valuable products by incorporating selenium into the system of plant nutrition (SMRKOLJ, STIBILJ 2004, GERM et al. 2007, OźOLT et al. 2008). The recommended daily rate of selenium ranges around 40 µg in women and 50 µg in men (Alexander 2005) or – according to the National Health and Medical Research Council – from 50 to 200 µg per individual/day (Pfannhauser 1994). It is administered in the form of inorganic compounds and is more difficult to utilise than biologically bound selenium in plants.

Although there are many reference sources dealing with foliar applications of Se to various plant species, such as potatoes (Poggi et al. 2000, Cuderman et al. 2008), rice (Fang et al 2008, Liu and Gu 2009), soybean (Yang et al. 2002, Martinez et al. 2009), leguminous plants and grasses (Hu et al.

2009, Hambuckers et al. 2008), or various vegetable species (Smrkolj et al. 2005, Slejkovec, Goessler 2005, Kapolna et al. 2009, Rios et al. 2010), relatively little is known about the effect of selenium application on the growth and development of poppy grown as foodstuff.

#### MATERIAL AND METHODS

In 2008 and 2009, in small-plot field experiments, we monitored the effect of foliar applications of Se(IV) on yields and quality of poppy seeds. In 2008, an experiment was conducted in Morkovice (49°14»48.53"N, 17°12»19.61"E), and in 2009 it was set up in Žabčice (49°0»42.32"N, 16°36»9.98"E). The experiment was established according to the design given in Table 1. The basic data about the agrochemical soil composition are given in Table 2.

 $\label{eq:Table 1} \ensuremath{\text{Table 1}}$  Design of the experiment

Variant no.	Treatments of fertilization	Dose of Se (g ha <sup>-1</sup> )	Source of Se (IV)	Time of application
1	control	0	-	-
2	Se1	300	${ m Na_2SeO_3}$	end of elongation growth
3	Se2*	300	${ m Na_2SeO_3}$	after flowering

<sup>\*</sup>only in 2009

Table 2
Agrochemical characteristics of the soil

Year pH	nH/CaCl	Content of nutrients in mg $kg^{-1}$ DM soil (Mehlich 3)							
	pH/CaCl <sub>2</sub>	$N_{\min}$	P	K	Ca	Mg	S <sub>water-sol</sub> .		
2008	6.2	15.0	91	254	2672	244	29.0		
2009	6.2	5.0	66	179	4477	313	10.6		

An Accord seeding machine was used for sowing spring poppy, variety Major, on 15 February 2008 and 24 March 2009. The seeding rate in both years was 1.5 kg ha $^{-1}$ , inter-row spacing 0.2 m. The size of the experimental plot was 15 m $^2$  (2008) and 10 m $^2$  (2009) and each treatment was repeated 4 times. Nitrogen was applied in both years at a rate of 60 kg ha $^{-1}$ . Pests and fungi were controlled according to their occurrence in the respective years of the experiment. Poppy was harvested in the stage of full maturity on 5 August 2008 and 10 August 2009. Table 3 shows the average monthly temperatures and sums of precipitation in the experimental years.

The content of selenium was determined in the seeds after wet mineralisation using an atomic absorption spectrophotometer (AAS).

The results were assessed using the programme Statistica 7.1 CZ by applying variance analysis (ANOVA) followed by Fisher's test at a 95% and 99% level of significance (P<0.05, P<0.01).

#### RESULTS AND DISCUSSION

Tables 4 and 5 show that poppy seed yields in the experimental years were significantly (P<0.01) affected by the weather. While the average yield in 2008 reached 1.781 t of poppy seeds per ha, in 2009 the yield was 40.6% lower (1.059 t ha<sup>-1</sup>). The cause was dry and warmer than average April and the first half of May in 2009 (Table 3), which inhibited growth of the aerial plant mass and resulted in reduced seed production.

 $\label{eq:Table 3} Table \ 3$  Course of weather conditions in 2008 (Morkovice) and in 2009 (Šabčice)

	Sum of precipitation (mm)				Tempera	Žabčice       2009     LTA       -3.3     -2.0       0.4     0.2       5.0     4.3       14.5     9.6       15.6     14.6       17.3     17.7		
Month	Mork	covice	Žab	čice	Mork	covice	Žak	očice
	2008	LTA	2009	LTA	2008	LTA	2009	LTA
January	11.3	27.0	20	24.8	2.42	-2.2	-3.3	-2.0
February	3.1	25.0	57.6	24.9	3.52	-0.7	0.4	0.2
March	48.4	31.0	78.1	23.9	4.23	3.7	5.0	4.3
April	39.4	42.0	3.6	33.2	9.33	8.7	14.5	9.6
May	68.0	65.0	42.4	62.8	14.37	14.2	15.6	14.6
June	77.9	74.0	114.7	68.6	19.00	16.9	17.3	17.7
July	58.4	78.0	74.0	57.1	19.26	18.8	20.7	19.3
August	44.3	78.0	29.6	54.3	19.74	17.8	21.1	18.6

LTA - long term average

The application of Se in the individual years of the experiment at a rate of 300 g per ha significantly (P<0.05) reduced the yields of poppy seed. Table 4 shows the production of poppy seeds in 2008 reduced by 7.1% compared to the control. In 2009, the production of poppy seeds showed a similar trend; the application of selenium during the stage of the end of elongation growth reduced seed yields by 18.3% (Table 5). In literature, the information about the effect of Se on growth and production of oil plants is contradictory. In their experiment, Banuelos et al. (1997) discovered that yields of rape were lower due to a high supply of Se in the soil (40 mg kg $^{-1}$ 

Table 4

Table 5

b

F	Effect of Se application on the poppy yield in 2008							
	Yield (t $ha^{-1}$ ) ± SE	Rel. (%)	P<0.05					
	1.770 ± 0.029	100.0	а					

92.9

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

Variant of fertilization Control Se1

Variants with identical letters express statistically non-significant differences.

 $1.645 \pm 0.022$ 

Effect of Se application on the poppy yield in 2009

Variant of fertilization	Yield (t $ha^{-1}$ ) ± SE	Rel. (%)	P<0.05
Control	$1.165 \pm 0.015$	100.0	a
Se1	$0.952 \pm 0.049$	81.7	b
Se2	1.028 ± 0.075	88.2	ab

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

Variants with identical letters express statistically non-significant differences.

of soil). Likewise Ruiz et al. (2007) reported inhibited growth of sunflowers after the application of selenium, which appeared as reduced yield of ach enes. In contrast, Torshin et al. (1994) and Dadnia et al. (2008) reported that soil and foliar applications of selenium had a positive effect on seed and achene yields in oil plants. In our experiment, late supplementary fertilisation with selenium during the stage after the fall of blossoms (var. Se2) also reduced yields (by 11.8%). However, the reduction in poppy production was not significant (P < 0.05).

Based on average yields in the experimental years (Table 6), it was evident that poppy seed yield was reduced by 11.5% due to the application of 300 g Se per ha during the end stage of elongation growth as compared to the treatment where selenium had not been applied, but the reduction was not statistically significant (P < 0.05).

Table 6 Average effect of Se application on the poppy yield

Variant of fertilization	Yield (t $ha^{-1}$ ) ± SE	Rel. (%)	P<0.05	
Control	$1.468 \pm 0.115$	100.0	a	
Se1	1.299 ± 0.133	88.5	a	

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

Variants with identical letters express statistically non-significant differences.

Due to Se application to plants at the end of elongation growth, the content of selenium in poppy seeds increased statistically highly significantly (P<0.01) – Figure 1, i.e. from 139 µg kg<sup>-1</sup> to 757 µg kg<sup>-1</sup> of seeds, which means a nearly 5.5-fold growth compared to the treatment not fertilised with selenium. Many references report (Gupta, MacLeod 1999, Grant et al. 2007, Dadnia et al. 2008, Lyons et al. 2009, Dadnia et al. 2009) a multiple growth in the selenium content in seeds of various plants as a result of its soil or foliar application. In our experiment, the uptake of selenium per unit of area increased almost five times due to supplementary fertilisation (Table 7).

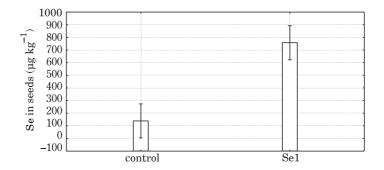


Fig. 1. Average content of Se in poppy seeds ( $\mu$ g kg<sup>-1</sup>). Error bars present Fisher P<0.05

 $\label{eq:Table 7} \mbox{Table 7}$  Selenium uptake by poppy seeds (mg  $\mbox{ha}^{-1}$ )

Variant of fertilization	Se uptake (mg ha <sup>-1</sup> )	Rel. (%)	P<0.05
Control	204.1	100.0	a
Se1	983.3	481.9	b

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

Variants with identical letters express statistically non-significant differences.

#### CONCLUSIONS

In respect of the experimental years, the reduction of poppy seed production was more marked in 2009, when the poppy plants were stressed by the dry and warm weather at the beginning of vegetation. Foliar application of Se at the end of elongation growth at a rate of 300 g ha<sup>-1</sup> reduced poppy seed yields by 11.5% as an average of the experimental years. The reduction in seed yields (by 11.8%) after the application of selenium in the period

after the fall of blossoms was not statistically significant. The amount of selenium in seeds and its uptake in poppy yields owing to foliar application increased 5.4 and 4.8 times, respectively.

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# EFFECT OF DIFFERENT SULFUR DOSES AND FORMS ON CHANGES IN THE MINERAL NITROGEN CONTENT OF SOIL

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#### Abstract

Nitrogen, in particular nitrate nitrogen, is a mobile nutrient that can be readily dispersed in soil, thus causing environmental pollution. Sulfur may have a beneficial effect on nitrogen transformations in soil and in plants. The objective of this study was to determine the effect of sulfur fertilization on the dynamics of changes in mineral nitrogen  $(N-NH_4^+$  and  $N-NO_3^-)$  content of soil samples collected each year in the spring and fall at a depth of 0-40 and 40-80 cm. A three-year field experiment was conducted in Byszwałd near Lubawa. The soil had acidic reaction (pH  $_{\rm 1\ mol\ KCl\ dm^{-3}}$  of 5.30) and contained the following concentrations of mineral nutrients: mineral nitrogen – 24.0, sulfate sulfur – 4.10, available phosphorus – 34.5, available potassium – 110.0 mg kg<sup>-1</sup> soil. The experiment was carried out in a randomized block design and comprised eight fertilization treatments in four replications. Three sulfate sulfur (S-SO $_4^{2-}$ ) and elementary sulfur (S-S $^0$ ) fertilization levels were applied: 40, 80 and 120 kg ha $^{-1}$ . The content of nitrate nitrogen (V) and ammonia nitrogen (III) was determined in soil samples by the colorimetric method using phenoldisulfonic acid and Nessler's reagent, respectively. In most cases, increasing sulfur doses caused an increase in the  $N-NH_4^+$  content of soil samples collected at a depth of 0-40 cm. The N-NH $_4^+$  content of the  $^4$ 0-80 cm soil layer varied. NPK+S fertilization, in particular the application of a single S-SO $_4^{2-}$  dose, contributed to an increase in N-NO $_3^$ concentrations in both sampled soil horizons in the majority of treatments, compared with the NPK treatment.

Key words: fertilization, sulfate sulfur, elementary sulfur, ammonia nitrogen, nitrate nitrogen, soil.

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# WPŁYW RÓŻNYCH DAWEK I FORM SIARKI NA ZMIANY ZAWARTOŚCI AZOTU MINERALNEGO W GLEBIE

#### Abstrakt

Azot, szczególnie forma azotanowa, należy do grupy tzw. pierwiastków mobilnych w glebie, może szybko rozpraszać się w naturalnym środowisku powodując jego zanieczyszczenie. Siarka może korzystnie wpływać na przemiany azotu w glebie i roślinie. Celem doświadczenia była ocena wpływu nawożenia siarką na dynamikę mineralnych form azotu (N-NH<sub>4</sub><sup>+</sup>, N-NO<sub>3</sub><sup>-</sup>) w glebie, z poziomu 0-40 i 40-80 cm, pobieranej wiosną i jesienią w każdym roku. Badania przeprowadzono w 3-letnim doświadczeniu polowym w Byszwałdzie, w pobliżu Lubawy. Gleba wyjściowa miała odczyn kwaśny (pH<sub>1 mol KCl dm</sub>-3 wynosiło 5,30), a zawartość składników mineralnych wynosiła: azot mineralny 24,0, siarka siarczanowa 4,10, przyswajalny fosfor 34,5 i potas 110,0 mg kg<sup>-1</sup> gleby. Doświadczenie stałe założone metodą losowanych bloków obejmowało 8 obiektów nawozowych w 4 powtórzeniach. Zastosowano 3 poziomy nawożenia siarką: 40, 80 i 120 kg ha<sup>-1</sup> w formie siarczanowej  $(S-SO_4^{2-})$  i elementarnej  $(S-S^0)$ . W przygotowanych próbkach glebowych oznaczono azot azotanowy (V) metodą kolorymetryczną z użyciem kwasu fenolodisulfonowego; azot amonowy (III) metodą kolorymetryczną z zastosowaniem odczynnika Nesslera. W poziomie gleby 0-40 cm wzrastające dawki siarki spowodowały na ogół zwiększenie zawartości N-NH<sub>4</sub><sup>+</sup>. W warstwie 40-80 cm zmiany zawartości  $N-NH_4^+$  w glebie nie podlegały określonym prawidłowościom. W większości obiektów nawożenie NPK + S, szczególnie pojedynczą dawką S-SO<sub>4</sub><sup>2-</sup>, przyczyniło się do zwiększenia zawartości N-NO<sub>3</sub><sup>-</sup> w obydwu warstwach gleby w porównaniu z obiektem NPK.

Słowa kluczowe: nawożenie, siarka siarczanowa, siarka elementarna, azot amonowy, azot azotanowy, gleba.

#### INTRODUCTION

Optimal rates of sulfur-based fertilizers contribute to improving the utilization of nutrients, in particular nitrogen. The beneficial effect of sulfur on nitrogen transformations and the sulfur to nitrogen ratio has been demonstrated for example by Schung et al. (1993), Fismes et al. (2000), Kozłowska (2002), Kaczor. Brodowska (2002). Sulfur reduces the content of undesirable and harmful compounds, including nitrate nitrogen and ammonia nitrogen, in plants. Sulfur deficiency inhibits protein synthesis, disturbs the process of photosynthesis and leads to the accumulation of non-protein nitrogen (Zhao et al. 1997, McGrath et al. 1996, Scherer 2001). Nitrogen is known to affect soil fertility (Bednarek, Reszka 2008). Since nitrogen, in particular nitrate nitrogen, is a mobile nutrient, it can be readily dispersed in soil, thus causing environmental pollution and groundwater contamination (Armstrong, Burt 1993, Borowiec, Zabłocki 1996). Due to a steady increase in commercial crop production and related environmental issues, relationships between nitrogen and sulfur compounds in soil remain an important consideration.

The objective of this study was to determine the effect of increasing doses of sulfate sulfur and elementary sulfur on the content of mineral nitrogen compounds in soil samples collected at a depth of 0-40 and 40-80 cm.

#### MATERIAL AND METHODS

The effect of sulfur fertilization on the dynamics of changes in mineral nitrogen (N-NH+ $_4$  and N-NO- $_3$ ) content of soil samples collected at a depth of 0-40 and 40-80 cm was studied. A three-year field experiment was conducted in Byszwałd near Lubawa, in 2000-2002. The soil had acidic reaction (pH $_1$  mol KCl dm-3 of 5.30) and contained the following concentrations of mineral nutrients: mineral nitro, gen -24.0, sulfate sulfur -4.10, available phosphorus -34.5, available potassium -110.0 mg kg $^{-1}$  soil. The experiment was carried out in a randomized block design and comprised eight fertilization treatments in four replications: 1) 0; 2) NPK; 3) NPK +  $+\rm S_1$ -SO $_4^{2-}$ ; 4) NPK +  $\rm S_2$ -SO $_4^{2-}$ ; 5) NPK +  $\rm S_3$ -SO $_4^{2-}$ ; 6) NPK +  $\rm S_1$ -S $_0^{3}$ ; 7) NPK +  $\rm S_2$ -S $_0^{3}$ ; 8) NPK +  $\rm S_3$ -S $_0^{3}$ . Three sulfate sulfur (S-SO $_4^{2-}$ ) and elementary sulfur (S-S $_0^{3}$ ) fertilization levels were applied: 40, 80 and 120 kg ha $_0^{-1}$ .

The following fertilizers were used: nitrogen – ammonium saltpeter or ammonium sulfate, phosphorus – triple superphosphate, potassium – 60% potash salt or potassium sulfate, sulfur – potassium sulfate + ammonium sulfate and elementary sulfur in the treatments with this form of sulfur. The tested crops were common cabbage, onion and spring barley. The above plants were selected based on their sulfur requirements and sensitivity.

Soil samples were collected in each plot, at a depth of 0-40 cm and 40-80 cm, before setting up the experiment, after harvest of each crop and prior to the sowing of the next crop. In the spring of 2001, soil samples were collected only from the 0-40 cm soil layer due to persistent precipitation. Air-dried soil samples were passed through a 1 mm mesh sieve. The content of nitrate nitrogen (V) and ammonia nitrogen (III) was determined in soil samples by the colorimetric method using phenoldisulfonic acid and Nessler's reagent, respectively. The results were verified statistically with analysis of variance for two-factorial experiments in a randomized block design. Experimental factor a was sulfur form, and experimental factor b – sulfur dose. Regression analysis was performed using Statistica 6.0 PL software. The significance of differences between datasets was determined with Duncan's test.

#### RESULTS AND DISCUSSION

In the fall, after cabbage harvest, the ammonia nitrogen content of soil samples collected at a depth of 0-40 cm ranged from 0.30 to 3.30 mg kg $^{-1}$  soil (Table 1). The application of a triple dose of elementary sulfur caused a significant increase in N-NH $_4$ <sup>+</sup> concentrations, in comparison with the remaining sulfur doses. In the spring of 2001, a considerable increase in the ammonia nitrogen content of soil was noted, compared with the samples

 $\label{thm:table 1}$  Effect of different rates and forms of sulfur on the content of ammonium nitrogen in soil (0-40 cm) (mg kg $^{-1}$  soil)

in boil (o' 10 cm) (ing ing boil)									
Treatment	After cabbage harvest	Before onion sowing	After onion harvest	Before barley sowing	After barley harvest				
0	0.30	39.8	6.40	7.50	4.40				
NPK	2.40	12.8	4.90	5.40	4.50				
NPK+ $S_1$ - $SO_4^{2-}$	2.70	18.8	3.90	6.40	2.90				
NPK+ S <sub>2</sub> -SO <sub>4</sub> <sup>2-</sup>	0.40	25.8	5.00	14.0	4.10				
NPK+ S <sub>3</sub> -SO <sub>4</sub> <sup>2-</sup>	0.30	28.7	6.50	9.00	7.10				
NPK+S <sub>1</sub> -S- <sup>0</sup>	2.60	22.0	4.90	17.3	4.00				
NPK+S <sub>2</sub> -S- <sup>0</sup>	2.60	20.7	6.40	8.0	5.40				
NPK+S <sub>3</sub> -S- <sup>0</sup>	3.30	26.3	4.70	12.5	6.50				
LSD-p-0.05 a b a x b	0.080 0.110 0.160	1.190 1.680 2.380	0.260 0.360 0.510	0.420 0.600 0.850	0.320 0.450 0.630				

 $\mathrm{SO_4^{2-}-sulfate}$  sulfur; S $^0$  – elementary sulfur; S $_1$  – 40 kg ha $^{-1}$ , S $_2$  – 80 kg ha $^{-1}$ , S $_3$  – 120 kg ha $^{-1}$ , a – form of sulfur; b – dose of sulfur; a x b interaction,

n.s. - no significant difference

analyzed in the fall. After harvest in 2001, the application of increasing sulfur doses led to an increase in the ammonia nitrogen content of soil, except in the treatment fertilized with 120 kg elementary sulfur. Soil samples collected in the spring of 2002 were characterized by a higher ammonia nitrogen content than the samples analyzed in the fall. Both the dose and form of sulfur had a significant effect on changes in N-NH<sub>4</sub><sup>+</sup> levels. In the third year of the experiment, elementary sulfur exerted a stronger effect, which could have resulted from its oxidation to sulfate (Zhou et al. 2002, Skwieraw-SKA, ZAWARTKA 2009). In the fall, after barley harvest, a decrease in the ammonia nitrogen content of the 0-40 cm soil layer was observed, in comparison with the same treatments studied in the spring. N-NH<sub>4</sub>+ concentrations were in the range of 2.90-7.10 mg kg<sup>-1</sup> soil. Soil samples collected each year in the spring (at a depth of 0-40 cm) were found to accumulate larger quantities of N-NH<sub>4</sub><sup>+</sup> than the samples collected in the fall of the preceding year. In most cases, increasing sulfur doses contributed to an increase in the N-NH<sub>4</sub><sup>+</sup> content of soil after harvest, which was particularly noticeable in the second and third year of the study. In a laboratory experiment, the application of elementary sulfur significantly reduced the leaching of watersoluble ammonia nitrogen (ZAWARTKA, SKWIERAWSKA 2005). The N-NH<sub>4</sub><sup>+</sup> content of the 40-80 cm soil layer varied (Table 2).

 $\label{eq:Table 2}$  Effect of different rates and forms of sulfur on the content of ammonium nitrogen in soil (40-80 cm) (mg kg^{-1} soil)

Treatment	After cabbage harvest	Before onion sowing	After onion harvest	Before barley sowing	After barley harvest
0	4.10	-	20.9	4.18	2.70
NPK	3.50	-	29.8	8.40	4.50
NPK+ S <sub>1</sub> -SO <sub>4</sub> <sup>2-</sup>	2.20	-	24.3	4.57	3.50
NPK+ S <sub>2</sub> -SO <sub>4</sub> <sup>2-</sup>	2.40	-	29.3	4.13	3.90
NPK+ S <sub>3</sub> -SO <sub>4</sub> <sup>2-</sup>	3.50	-	29.0	4.56	4.80
NPK+S <sub>1</sub> -S- <sup>0</sup>	2.10	-	28.8	4.57	6.80
NPK+S <sub>2</sub> -S- <sup>0</sup>	3.60	-	25.0	5.87	4.30
NPK+S <sub>3</sub> -S- <sup>0</sup>	3.90	-	22.8	5.00	5.20
LSD-p-0.05 a b a x b	0.080 0.120 0.160	-	0.720 1.020 1.450	n.s. 0.468 0.662	0.150 0.220 0.300

Explanation see Table 1.

In the fall, after cabbage harvest, the  $N-NO_3^-$  content of soil samples collected at a depth of 0-40 cm ranged from 2.60 to 6.20 mg kg<sup>-1</sup> soil (Table 3). The highest N-NO<sub>3</sub><sup>-</sup> content was noted in the treatment fertilized with 80 kg elementary sulfur. In the spring, before onion sowing, a significant increase in the N-NO<sub>3</sub><sup>-</sup> content of soil was observed, in comparison with the same treatments studied in the spring, in particular after the application of 120 kg sulfate and elementary sulfur. Both the dose and form of sulfur had a significant effect on changes in N-NO<sub>3</sub><sup>-</sup> levels. After the harvest in 2001, the N-NO<sub>3</sub><sup>-</sup> content of soil decreased, which could have been due to its leaching out and uptake by plants. Similar results were reported by Marcinkowski (1996) and Sykut (2000). In the spring, prior to spring barley sowing, a substantial increase in the nitrate nitrogen of soil was noted, compared with the samples collected in 2000 and 2001, in particular in the treatment fertilized with a triple dose of elementary sulfate, which oxidized over time. In the fall of 2002, after harvest, nitrate nitrogen concentrations fluctuated.

In the fall of 2000, in the 40-80 cm soil layer, nitrate nitrogen content was in the range of 2.60-3.90 mg kg $^{-1}$  soil. Both the dose and form of sulfur had a significant effect on changes in  $\rm N\text{-}NO_3^-$  levels. In the fall of 2001, the  $\rm N\text{-}NO_3^-$  content of soil was higher than in the previous year, in particular in treatments fertilized with 40 and 120 kg  $\rm S\text{-}SO_4^{2-}$  ha $^{-1}$ . Soil samples analyzed in the spring and fall of 2002 were characterized by a considerably lower nitrate nitrogen content than the samples collected in the fall of 2001.

Table 3 Effect of different rates and forms of sulfur on the content of nitrate nitrogen in soil (0-40 cm)  $\mbox{ (mg kg}^{-1}$  soil)

Treatment	After cabbage harvest	Before onion sowing	After onion harvest	Before barley sowing	After barley harvest
0	2.60	9.20	2.00	11.0	10.6
NPK	5.30	6.30	2.50	14.7	14.2
NPK+ S <sub>1</sub> -SO <sub>4</sub> <sup>2-</sup>	3.10	10.80	5.20	19.5	17.2
NPK+ S <sub>2</sub> -SO <sub>4</sub> <sup>2-</sup>	5.00	7.50	5.80	19.8	16.8
NPK+ S <sub>3</sub> -SO <sub>4</sub> <sup>2-</sup>	4.30	11.8	3.70	18.4	14.5
NPK+S <sub>1</sub> -S- <sup>0</sup>	3.70	5.00	3.40	14.5	12.7
NPK+S <sub>2</sub> -S- <sup>0</sup>	6.20	8.40	4.20	13.0	11.6
NPK+S <sub>3</sub> -S- <sup>0</sup>	3.00	18.5	2.90	25.2	12.3
LSD-p-0.05 a b a x b	0.110 0.160 0.230	0.72 1.02 1.44	n.s. 0.500 0.700	1.360 1.920 2.710	0.900 1.270 1.800

Explanation see Table 1.

Soil samples collected at a depth of 0-40 cm in NPK+S treatments were found to accumulate less of nitrates in the fall than in the spring (Table 3). In soil samples collected from the 40-80 cm layer, nitrate concentrations were higher in the fall (Table 4). As demonstrated by IGRAS and JADCZYSZYN (2008), the risk of nitrate leaching is the greatest in the fall, due to precipitation. The decrease in N-NO<sub>3</sub><sup>-</sup> concentrations in samples collected from the deeper soil layer in the spring could be related to translocation of nitrate nitrogen to groundwater. High mobility of nitrate ions was also observed by ZAWARTKA, SKWIERAWSKA (2004) in a lysimetric experiment.

No regular, unambiguous correlations between the form and dose of sulfur and nitrate concentrations in the soil were observed throughout the experiment. Nevertheless, NPK+S fertilization, in particular the application of a single S-SO<sub>4</sub><sup>2-</sup> dose, contributed to an increase in N-NO<sub>3</sub><sup>-</sup> concentrations in both sampled soil horizons in the majority of treatments, compared with the NPK treatment. The noted differences were statistically significant, as shown by the results of Duncan's test (Table 5).

In the spring and fall of 2000 and 2002, the predominant form of mineral nitrogen that accumulated in the 0-40 cm soil layer was N-NO<sub>3</sub><sup>-</sup>. According to some authors (Fotyma, Kryszkowska 1990, Mosiej, Tusiński 1993), higher nitrate content of deeper soil horizons results from high mobility of nitrate nitrogen in soil, particularly between the fall and spring, and from the antagonist effect of sulfate anions. In 2001, the predominant form of mineral

Table 4 Effect of different rates and forms of sulfur on the content of nitrate nitrogen in soil  $\,$  (40-80 cm) (mg kg^{-1} soil)

Treatment	After cabbage harvest	Before onion sowing	After onion harvest	Before barley sowing	After barley harvest
0	2.80	-	5.80	3.40	2.00
NPK	3.90	-	3.30	5.35	3.70
NPK+ S <sub>1</sub> -SO <sub>4</sub> <sup>2-</sup>	3.00	-	28.5	3.85	4.00
NPK+ S <sub>2</sub> -SO <sub>4</sub> <sup>2-</sup>	2.60	-	18.5	4.85	3.30
NPK+ S <sub>3</sub> -SO <sub>4</sub> <sup>2-</sup>	2.90	-	20.5	6.22	5.90
NPK+S <sub>1</sub> -S- <sup>0</sup>	2.90	-	16.0	4.75	2.50
NPK+S <sub>2</sub> -S- <sup>0</sup>	3.40	-	12.7	5.20	2.30
NPK+S <sub>3</sub> -S- <sup>0</sup>	3.80	-	7.80	3.42	3.10
LSD-p-0.05 a b a x b	0.060 0.090 0.130	-	n.s. 1.740 2.46	n.s. n.s. 1.454	n.s. 0.310 0.430

Explanation see Table 1.

Table 5 Significance of differences in the content of nitrate nitrogen in soil between particular objects according to Duncan's test. Differences statistically significant at  $(p \le 0.05)$ 

						,		
Treatment	0	NPK	$\text{I-S-SO}_4^{\ 2-}$	$\text{II-S-SO}_4^{2-}$	III-S- $\mathrm{SO_4}^{2-}$	$\text{I-S-S}^0$	II-S-S <sup>0</sup>	III-S-S <sup>0</sup>
0								
NPK	0.537							
S <sub>1</sub> -SO <sub>4</sub> <sup>2-</sup>	0.002*	0.014*						
$S_2$ - $SO_4^{2-}$	0.023*	0.086	0.437					
$S_3$ - $SO_4^{2-}$	0.013*	0.055	0.564	0.799				
$S_1$ - $S^0$	0.297	0.620	0.046*	0.196	0.136			
$S_2$ - $S^0$	0.256	0.549	0.058	0.228	0.163	0.883		
$S_3 - S^0$	0.044*	0.140	0.308	0.763	0.604	0.290	0.326	
$\overline{x}$	5.922	6.688	10.107	9.075	9.390	7.302	7.484	8.702

Explanation see Table 1;

<sup>\*</sup>  $\overline{x}$  average content of nitrate nitrogen in soil in particular objects for the years 2000-2003 (mg kg<sup>-1</sup> soil).

nitrogen in both sampled soil layers was N-NH $_4$ <sup>+</sup>. The above is consistent with the findings of Skowrońska (2004), who reported that in her study ammonia nitrogen had the highest share of total mineral nitrogen content.

#### CONCLUSIONS

- 1. In most cases, increasing sulfur doses caused an increase in the  $N-NH_4^+$  content of soil samples collected at a depth of 0-40 cm. The  $N-NH_4^+$  content of the 40-80 cm soil layer varied.
- 2. NPK+S fertilization, in particular the application of a single S-SO $_4$ <sup>2-</sup> dose, contributed to an increase in N-NO $_3$  concentrations in both sampled soil horizons in the majority of treatments, compared with the NPK treatments.
- 3. The predominant form of mineral nitrogen that accumulated in soil (0-40 cm) was nitrate nitrogen in 2000 and 2002, and ammonia nitrate in 2001.

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# PRELIMINARY EVALUATION OF THE INFLUENCE OF SOIL FERTILIZATION AND FOLIAR NUTRITION WITH IODINE ON THE EFFECTIVENESS OF IODINE BIOFORTIFICATION AND MINERAL COMPOSITION OF CARROT\*

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#### Abstract

Vegetables enriched with iodine can become an alternative to iodized salt as a way of introducing this element to human diet. Iodine is not a nutritional element for plants. Its influence on biochemical and physiological processes occurring in plants, including mineral nutrition, has not yet been diagnosed. In the available literature, no information can be found on the comparison of iodine biofortification efficiency of carrot storage roots through soil fertilization and foliar nutrition. The aim of this study was to assess the influence of pre-sowing soil fertilization with iodine (in the form of KI) and foliar application of this element (as  $\text{KIO}_3$ ) on the biofortification effectiveness and mineral composition of carrot storage roots. Carrot cv. Kazan  $F_1$  was cultivated in a field experiment in 2008 and 2009. The experiment comprised different variants of soil and foliar application of iodine: control (without soil or foliar application of iodine), combinations with pre-sowing soil fertilization with iodine in the dose of 0.5, 1.0 and 2.0 kg I ha<sup>-1</sup> as well as foliar nutrition with iodine in the concentration of: 0.0005%, 0.005% and 0.05% repeated four times. In total, using 1,000 dm³ of work solution per 1 ha, the following amounts of iodine were applied to plants in the latter variant: 0.02, 0.2 and 2.0 kg I ha<sup>-1</sup>, respectively. In carrot storage roots, iodi-

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ne as well as P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb were analyzed with the ICP-OES technique, whereas nitrogen was determined with Kiejdahl's method. In all the tested combinations, significant increase in iodine concentration in carrot was observed versus the control (2.1 mg I kg<sup>-1</sup> d.w). Storage roots of carrot treated with the highest doses of iodine (through soil and foliar application) contained comparable amounts of this element: 10.2 and 8.6 mg I kg<sup>-1</sup> d.w., respectively, which were also the highest quantities relative to the control and the other treatments. Soil fertilization in the dose of 1.0 and 2.0 kg I ha<sup>-1</sup> as well as foliar nutrition with 0.0005%, and 0.05% solution of iodine contributed to an increased content of nitrogen in carrot roots. Soil and foliar application of iodine, in relation to the control, resulted in a higher content of Mg, Fe, Al and K as well as a lower S concentration in carrot, except K and S in the combination with soil fertilization of 0.5 kg I ha<sup>-1</sup>. Diversified influence of the iodine dose, form and application method was observed in reference to concentrations of: P, Ca, Na, B, Cu, Mn, Zn, Cd and Pb in carrot storage roots. Iodine treatments included in the research had no significant influence on the Mo content in carrot.

Key words: biofortification, iodine, foliar nutrition, mineral composition, carrot.

#### WSTĘPNA OCENA WPŁYWU NAWOŻENIA I DOKARMIANIA DOLISTNEGO JODEM NA EFEKTYWNOŚĆ BIOFORTYFIKACJI MARCHWI W JOD ORAZ JEJ SKŁAD MINERALNY

#### Abstrakt

Warzywa wzbogacane w jod mogą stać się alternatywną, do jodowania soli kuchennej, droga wprowadzania jodu do diety człowieka. Jod nie jest składnikiem pokarmowym roślin. Jego oddziaływanie na procesy biochemiczne i fizjologiczne roślin, w tym na funkcjonowanie gospodarki mineralnej, nie zostało zdiagnozowane. W dostępnej literaturze brak jest informacji na temat porównania efektywności biofortyfikacji korzeni spichrzowych marchwi w jod poprzez nawożenie doglebowe i dolistną aplikację tego pierwiastka. Celem badań było określenie wpływu doglebowego przedsiewnego nawożenia jodem (w formie KI) i dolistnej aplikacji tego pierwiastka (w formie  ${
m KIO_3}$ ) na efektywność biofortyfikacji w jod oraz skład mineralny korzeni spichrzowych marchwi. Marchew odmiany Kazan  ${
m F_1}$  uprawiano w latach 2008-2009 w doświadczeniu polowym. W badaniach uwzględniono kombinacje ze zróżnicowanym nawożeniem doglebowym i dokarmianiem dolistnym jodem. Wyróżniono kontrolę (nienawożoną i niedokarmianą dolistnie jodem), kombinacje z przedsiewnym nawożeniem doglebowym jodem w dawkach: 0,5, 1,0 i 2,0 kg I ha<sup>-1</sup> oraz 4-krotne dolistne dokarmianie roślin jodem w stężeniach 0,0005%, 0,005% i 0,05% – sumarycznie po zastosowaniu 1000 dm<sup>3</sup> cieczy roboczej ha<sup>-1</sup> zaaplikowano roślinom odpowiednio: 0,02, 0,2 i 2,0 kg I ha<sup>-1</sup>. W korzeniach spichrzowych marchwi oznaczono: zawartość jodu oraz P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd i Pb techniką ICP-OES, a także zawartość azotu metodą Kiejdahla. We wszystkich badanych kombinacjach stwierdzono istotne zwiększenie zawartości jodu w marchwi w porównaniu z kontrolą (2,1 mg I kg-1 s.m.). Korzenie marchwi traktowanej najwyższymi dawkami jodu (doglebowo i dolistnie) zawierały porównywalną, najwyższą zawartość jodu – odpowiednio 10.2 i  $8.6~{\rm mg~I~kg^{-1}~s.m.}$  W odniesieniu do kontroli nawożenie doglebowe w dawkach 1 i 2 kg I ha-1 oraz dokarmianie dolistne 0,0005%, i 0,05% wpłyneto na zwiększenie zawartości azotu w marchwi. Dolistna i doglebowa aplikacja jodu, w porównaniu z kontrolą, wpłynęła na zwiększenie zawartości Mg, Fe i Al, a także K oraz obniżenie zawartości S w marchwi – oprócz K i S w kombinacji z nawożeniem 0,5 kg I ha<sup>-1</sup>. Stwierdzono zróżnicowane oddziaływanie dawki, formy i sposobu aplikacji jodu na zawartość P, Ca, Na, B, Cu, Mn, Zn, Cd i Pb w marchwi. Zastosowane zabiegi aplikacji jodu nie miały wpływu na zawartość Mo w marchwi.

Słowa kluczowe: biofortyfikacja, jod, dokarmianie dolistne, skład mineralny.

#### INTRODUCTION

Plant biofortification with iodine (or other biogenic elements) is defined as such an increase in the concentration of the element in edible parts of plant that efficiently improves the consumer's health (White, Broadley 2005, 2009, Yang et al. 2007, Zhao, McGrath 2009).

In Poland and in many other countries, the build-up of iodine content in human diet is achieved through salt iodization. It is an effective way of introducing iodine to people's diet in order to reduce health problems resulting from its deficiency. On the other hand, salt consumption in many countries is far too excessive and has led to greatly increased incidences of cardiovascular diseases. For that reason, WHO has developed the Global Strategy on Diet, Physical Activity and Health for the years 2008-2013. One of the main goals of this programme is to reduce salt intake while seeking alternative methods of introducing iodine to human diet. The need for a global development of an effective way of increasing iodine intake results from numerous functions that iodine plays in the human organism. Moreover, it should be mentioned that 35.2% of the global population has inadequate iodine nutrition (Winger et al. 2008).

Plant roots preferably take up the iodide (I<sup>-</sup>) rather than iodate (IO $_3$ <sup>-</sup>) form of iodine (Smith et al. 1999). In higher doses, however, iodates are less toxic to plants when compared to the reduced form of this element – in particular via foliar application of iodine. With respect to the effectiveness of plant biofortification, it is higher for foliar nutrition with this element rather than with soil fertilization (Altmok et al. 2003, Strzetelski et al. 2010), especially when long-term drought occurs during plant cultivation that favors strong iodine binding by the soil sorption complex (Dai et al. 2004).

In the last few years, many studies have been carried out on iodine biofortification of numerous plant species such as cabbage (Weng et al. 2008), lettuce (Bai et al. 2007, Blasco et al. 2008), tomato and spinach (Gonda et al. 2007), alfalfa (Altmok et al. 2003), pakchoi, celery, pepper and radish (Hong et al. 2008) as well as radish (Strzetelski et al. 2010). The scope of the mentioned research has included the assessment of the effectiveness of plant fortification with iodine depending on the dose, form and source type of the element – technical salts and organic matter rich in iodine (application of marine algae). The cited papers, however, contain no information on a documented effect of iodine on mineral nutrition of plants. Likewise, no data can be also found in the available literature on the comparison between the effectiveness of iodine biofortification of carrot achieved through soil fertilization and foliar nutrition.

The aim of the study was to determine the influence of pre-sowing soil fertilization with iodine (in the form of KI) and foliar application of this element (as  ${\rm KIO_3}$ ) on the effectiveness of iodine biofortification and mineral composition of carrot storage roots.

#### MATERIAL AND METHODS

In 2008-2009, a field experiment was conducted in Kraków, Poland, involving cv. Kazan F<sub>1</sub> carrot cultivation in crop rotation system on uniform soil complex. Carrot was cultivated on silt loam soil (35% sand, 28% silt and 37% clay) with the content of organic matter in the 0-30 cm soil layer: 2.84%-3.41% (in 2008 and 2009, respectively) and the following concentrations of the available nutrient forms soluble in 0.03 M acetic acid (in 2008 and 2009, respectively): N  $(NO_3-N+NH_4-N) - 8.1-3.8$  mg, P - 51.4-45.0 mg, K - 111.8-185.4 mg, Mg - 115.6-107.4 mg and Ca - 1255.8-837.9 mg dm<sup>-3</sup> soil. In 2008 and 2009, soil pH<sub>(H2O)</sub> was 6.98-7.10, while the total concentration of salt in soil (EC) was 0.12-0.11 mS cm<sup>-1</sup>, respectively. Carrot was grown on ridges, 40 cm wide and 30 cm high, where seeds were sown at a rate of 37 seeds m<sup>-1</sup> in a row (approximately 550,000 seeds per 1 hectare). Seed sowing was performed on 24 April in both years of the study. Nitrogen as ammonium nitrate was applied in a dose of 100 kg N ha<sup>-1</sup> pre-sowing and as top dressing. Pre-sowing nitrogen fertilization (and iodide application) was conducted immediately before ridge formation, whereas top dressing was performed at canopy closure.

Different iodine soil fertilization (as KI) and foliar nutrition (in the form of KIO $_3$ ) treatments were applied in the experiment, including: 1 – control (without soil fertilization and foliar nutrition with iodine); combinations with soil pre-sowing fertilization of iodine in the dose of 2-0.5 kg I ha $^{-1}$ , 3-1.0 kg I ha $^{-1}$  and 4-2.0 kg I ha $^{-1}$  as well as combinations with foliar application of iodine, repeated four times, in the following concentrations: 5 – 0.0005% (total – 0.02 kg I ha $^{-1}$ ), 6 – 0.005% (0.2 kg I ha $^{-1}$ ) and 7 – 0.05% (2.0 kg I ha $^{-1}$ ). Foliar nutrition was performed using 1,000 dm $^3$  of work solution per hectare on the following dates: 1st – 8 and 10 July, 2nd – 22 and 28 July, 3rd – 4 and 19 August, 4th – 18 August and 7 September (in 2008 and 2009, respectively).

The experiment was arranged in a split-plot design with four replications. Each experimental treatment was randomized in four repetitions on  $2.7 \text{ m} \times 5 \text{ m} (13.5 \text{ m}^2)$  plots. The total area under experiment was  $378 \text{ m}^2$ .

Carrot was harvested on 30 September 2008 and 23 September 2009. During harvest, about samples consisting of 5 kg of carrot storage roots were taken in four replications (from each plot) for further analyses. Additionally, soil samples were collected from the 0-30 cm layer with a soil drill.

In carrot storage roots, the content of iodine was assessed after incubation with 25% TMAH according to the standard protocol prEN 15111- R2-P5-F01 and the amount of N-total was determined with Kjeldahl's method (Persson, Wennerholm 1999). Concentration of P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb were determined after sample mineralization in 65% super pure HNO<sub>3</sub> (Merck no 100443.2500) in a CEM MARS-5 Xpress microwave oven (Paslawski, Migaszewski 2006).

In soil samples, pH was determined with a potentiometer and concentrations of I, N-NH<sub>4</sub>, N-NO<sub>3</sub>, P, K, Mg, Ca, S and Na were determined after soil extraction in 0.03 M acetic acid (Nowosielski 1988). The content B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb was assayed after extraction with 1 M HCl (Gorach et al. 1999).

Iodine as well as P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb in carrot and soil samples were determined with the ICP-OES technique using a Prodigy Teledyne Leeman Labs USA spectrometer. Concentrations of nitrogen forms in soil samples (N-NH $_4$ , N-NO $_3$ ) were determined by the FIA technique [PN-EN ISO 13395: 2001; PN-EN ISO 11732:2005 (U)].

The results were statistically verified using the ANOVA module of Statistica 9.0 PL programme for significance level P<0.05. Significance of changes was assessed with the use of variance analysis. When significant changes appeared, homogenous groups were determined with Duncan's test.

#### RESULTS AND DISCUSSION

Carrot storage roots from all the tested combinations with iodine soil and foliar treatments contained significantly higher amounts of this element than control plants (Table 1). When lower iodine doses through soil fertilization (0.5 and 1.0 kg I ha<sup>-1</sup>), carrot roots accumulated slightly more iodine than after foliar application of iodine in the total dose of 0.02 and 0.2 kg I ha<sup>-1</sup>. On the other hand, carrot treated with the highest doses of iodine (both soil and foliar application of 2.0 kg I ha<sup>-1</sup>) contained comparable concentrations of this element, i.e. 10.2 and 8.6 mg I kg<sup>-1</sup> d.w., respectively, which were also the highest quantities determined It is worth mentioning that the soil carrot harvest, in all the treatments, contained a comparable content of iodine soluble in 0.03 M acetic acid (Table 2). This observation indirectly indicates that iodine introduced to soil was either taken by cultivated plants or strongly sorbed by organic matter (thiol groups and polyphenols) or hydrous oxides of Fe and Al (WHITEHEAD 1984, MURAMATSU et al. 1996, Yamaguchi et al. 2005). Iodine desorption in soil is most profound under anaerobic conditions with the negative redox potential (Eh) resulting mainly from excessive humidity of soil (Muramatsu et al. 1996). For that reason, in cultivated soils (mostly characterized by aerobic conditions and the positive redox potential), iodine desorption as well its uptake by plants can be inhibited. This assumption can be an additional explanation for the fact that despite applying smaller doses of iodine in foliar nutrition (combinations 5 and 6) iodine concentration in carrot was only slightly lower comparing to soil fertilization in combinations 2 and 3.

In comparison to the control, soil fertilization with 1.0 and 2.0 kg I ha<sup>-1</sup> as well as foliar application of 0.02 and 2 kg I ha<sup>-1</sup> contributed to increased

Table 1

Mineral composition and the effectiveness of iodine biofortification of carrot depending on soil fertilisation and foliar application of iodine - means from 2008-2009

	own or so second did some or ness								
***************************************	Iodine content	ontent	Col	ntent of ma	acroelemer	nts in % d.v	v. of carrot	Content of macroelements in % d.w. of carrot storage roots	ots
Combinations	(mg I kg <sup>-1</sup> d.w.)	-1 d.w.)	Z	Ъ	K	$_{ m gM}$	Ca	ß	Na
1. Control	2.1 a	a	1.75 ab	0.368 b	$2.32 \ b$	0.115 a	$0.349 \ bc$	0.132 a	0.72 c
2. Fertilization 0.5 kg I ha $^{-1}$	6.2 bc	bc	1.70 a	0.355 a	2.29 a	0.123 c	0.342 ab	$0.130 \ a$	$0.70 \ b$
3. Fertilization 1.0 kg I ha $^{-1}$	9.9 d	q	1.83 c	0.408 d	2.69 e	0.126 d	0.338 a	$0.142\ cd$	0.64 a
4. Fertilization 2.0 kg I ha $^{-1}$	10.2 d	p 7	1.85 cd	0.381 c	2.38c	0.127 d	0.363 d	0.145 d	0.80 e
5. Foliar application 0.02 kg I $ha^{-1}$	4.8 b	9	1.85 cd	0.427 f	2.67 e	$0.122\ bc$	0.377 e	0.151e	0.78 d
6. Foliar application 0.2 kg I $ha^{-1}$	6.4 bc	bc	$1.77 \ b$	0.366ab	2.45 d	0.119  b	0.337 a	$0.138 \ b$	0.64 a
7. Foliar application 2.0 kg I ha $^{-1}$	8.6 cd	cd	1.89 d	0.406 d	2.36 c	0.127 d	0.355 c	$0.141\ bc$	0.82 f
Test F	*		*	*	*	*	*	*	*
Content of microelements, Al, Cd and Pb in mg kg <sup>-1</sup> d.w. of carrot storage roots	lements, Al	l, Cd and F	Pb in mg k	g <sup>-1</sup> d.w. of	carrot stor	rage roots			
	В	Cu	Fe	m Mn	Zn	$***^{\mathrm{o}}\mathrm{M}$	Al	рЭ	Pb
1. Control	24.9 a	9.9	34.1 a	7.8 c	35.8~ab	0.147	22.8 a	$1.41\ bc$	0.10 a
2. Fertilization 0.5 kg I ha $^{-1}$	25.5 b	5.6 a	42.7 b	7.4 a	34.3 a	0.158	32.2  b	1.33 a	0.24 ab
3. Fertilization 1.0 kg I ha $^{-1}$	24.8 a	6.1 ab	49.6 d	7.3 a	37.8 cd	0.178	43.3 e	1.45 c	$0.27 \ b$
4. Fertilization 2.0 kg I ha $^{-1}$	25.6  b	5.6 a	56.8 e	7.6  b	36.9 bc	0.176	51.3 f	1.39 b	$0.27 \ b$
5. Foliar application 0.02 kg I ha $^{-1}$	27.2 d	7.5 c	49.5 d	8.7f	38.3 cd	0.193	42.4 de	1.58 d	0.24 ab
6. Foliar application 0.2 kg I ha $^{-1}$	24.9 a	5.8 a	44.0  b	8.2 e	35.2~ab	0.151	36.3 c	1.34 a	0.11 a
7. Foliar application 2.0 kg I ha $^{-1}$	26.1 c	6.1 ab	47.3 c	8.1 d	39.3 d	0.126	40.0  d	1.45~c	0.21~ab
$\operatorname{Test} F$	*	*	*	*	*	n.s.	*	*	*

Means followed by the same letters are not significantly different at P<0.05, \*means are significantly different, \*\*in combinations nos: 5-7, the total dose of iodine applied in four foliar nutrition treatments was given, \*\*\*results for Mo only from the year 2009.

Table 2 Results of chemical analysis of soil after carrot cultivation (means from 2008-2009 for the 0-30 cm soil layer)

2000	II.				u)	(mg dm <sup>-3</sup> soil)	il)			
Combinations	$ ho_{ m HH_{20}}$	I	N-NH <sub>4</sub>	N-NO <sub>3</sub>	Ъ	K	m Mg	Ca	S	Na
1. Control	6.91 c	2.6	0.25	p9.2	31.4 bc	48.3 b	108.7	1052.1	$_{2.3c}$	4.4
2. Fertilization 0.5 kg I $ha^{-1}$	6.76 a	2.2	0.25	3.6 bc	25.0 a	38.8  a	101.8	918.3	4.3 ab	3.1
3. Fertilization 1.0 kg I $ha^{-1}$	7.03 e	3.1	0.25	3.0 ab	34.3 c	66.7~e	109.2	1039.2	3.8 a	3.5
4. Fertilization $2.0 \text{ kg I ha}^{-1}$	p 96.9	3.6	0.34	1.9 a	$32.8 \ bc$	64.1 de	101.9	974.6	3.6 a	3.5
5. Foliar application 0.02 kg I $ha^{-1}$	6.91 c	3.2	0.29	5.5 d	30.9 bc	57.4 c	104.2	982.7	5.0~bc	4.7
6. Foliar application 0.2 kg I $ha^{-1}$	6.95 d	2.6	0.33	4.5 cd	30.2  b	58.6 cd	106.9	992.9	4.6 bc	3.7
7. Foliar application $2.0 \text{ kg I ha}^{-1}$	6.87 b	3.8	0.45	5.5 d	26.3a	44.1 ab	104.9	963.1	7.2 d	3.9
Test F	*	n.s.		*	*	*	-	-	*	
					(mg kg	(mg kg <sup>-1</sup> soil)				
	В		Cu	Fe	Mn	Zn	Mo	Al	Cd	Pb
1. Control	1.94	9 1	5.5  b	2.6002	283.8~abc	52.5 a	$1.02\ cd$	$1280.0 \ b$	1.023~ab	30.4 a
2. Fertilization 0.5 kg I $ha^{-1}$	1.80 a	) a	5.3 a	1989.9	$286.7 \ bc$	53.3 ab	0.97~abc	1247.7~ab	$1.040\ bc$	31.3~abc
3. Fertilization 1.0 kg I $ha^{-1}$	1.87 ab	ab	5.4 ab	1997.2	288.0 c	53.7b	0.92 a	1227.1~a	$1.047\ bc$	31.7~bc
4. Fertilization 2.0 kg I ha <sup>-1</sup>	1.84 ab	ab	5.4 ab	1997.8	275.6  a	55.3 d	0.96~ab	$1251.8\;ab$	1.048c	31.8 c
5. Foliar application 0.02 kg I $ha^{-1}$	1.85 ab	ab	5.5 b	2037.2	281.8~abc	$54.0\ bc$	$0.99\ bcd$	$1273.7\ b$	$1.044\ bc$	31.2~abc
6. Foliar application 0.2 kg I $ha^{-1}$	1.79 a	) a	5.5 b	2028.3	275.9~abc	54.9 cd	0.97~abc	1265.4~ab	$1.045\ bc$	31.7~bc
7. Foliar application 2.0 kg I $ha^{-1}$	1.79 a	) a	5.4 ab	2028.1	278.8 ab	52.5 a	1.03 d	$1288.2 \ b$	1.014 a	30.8 ab
Test F	*		*		*	*	*	*	*	*

Means followed by the same letters are not significantly different at P<0.05, \* means are significantly different, n.s. – not significant.

content of N-total in carrot (Table 1). Positive effect of soil iodine fertilization on N-total concentration in carrot storage roots (enhancement of nitrogen utilization from mineral fertilizers) is confirmed by our previous results obtained from pot (Smoleń et al. 2009) and field cultivation of carrot (Smoleń et al. 2010). It should be mentioned that in the present study soil analyses after carrot harvest demonstrated a relatively low content of mineral nitrogen: N-NH $_4$  and N-NO $_3$  (Table 2). In the case of N-NO $_3$ , significant changes in the amount of this nitrogen form observed in soil did not correlate with its content in carrot storage roots.

The tested factors (soil fertilization and foliar application of iodine) had significant influence on the content of P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Al, Cd and Pb (Table 1) in carrot roots. Accumulation of Mo in carrot roots from all the tested combinations remained on a comparable level. Significant differentiation was revealed between the tested combination in respect of pH as well concentrations of P, K, S, B, Cu, Mn, Zn, Mo, Al, Cd and Pb in soil after carrot cultivation (Table 2).

In comparison to the control, foliar and soil application of iodine resulted in increased concentrations of Mg, Fe and Al as well as K and S, except K and S in carrot fertilized with 0.5 kg I ha<sup>-1</sup> (Table 1). The changes in K concentration in carrot were reflected by the changeable level of this element in soil (Table 2). When compared to the control, higher accumulation of Al in carrot roots, caused by soil application of iodine, was related to enhanced uptake of this element from soil. This observation is supported by the reduced content of Al in soil samples from these combinations. Changes in the Mg and Fe concentration in carrot cannot be explained on the basis of the results of soil analyses, as the levels of these elements in soil samples from all the tested combinations were comparable.

In the case of P, Ca, Na, B, Cu, Mn, Zn, Cd and Pb, the iodine dose, form and method of application produced varied effects on the accumulation of these elements in carrot storage roots (Table 1).

Taking into consideration soil fertilization alone, application of higher doses of iodine (1.0 and 2.0 kg I ha<sup>-1</sup>) led to increased concentrations of P, Zn Cd and Pb in carrot in comparison to the control (Table 1). A significant build-up in Ca, Na and Mn concentration in carrot roots fertilized with 2.0 kg I ha<sup>-1</sup> (combination no 4) as well as a reduction in the accumulation of these elements in carrot fertilized with 0.5 and 1.0 kg I ha<sup>-1</sup> were observed. In all the combination with soil iodine application, a lower concentration of Cu was found when compared to the control. As regards boron, a significant increase of its accumulation in carrot was observed when fertilized with iodine in the doses of 0.5 and 2.0 kg I ha<sup>-1</sup>. Among all the elements (P, Ca, Na, B, Cu, Mn, Zn, Cd, Pb), correlation consisting of an increased concentration in carrot (Table 1) and soil (Table 2) as a result of iodine fertilization was observed only for Zn, Cd and Pb. Lower accumulation of copper in carrot roots (Table 1) could be related to its reduced content in soil (Table 2).

The influence of foliar iodine nutrition with (combinations nos 5-7) on the content of P, Ca, Na, B, Cu, Mn, Zn, Cd and Pb in carrot storage roots was also interesting to trace (Table 1). It should be highlighted that, depending on the dose of iodine sprayed over leaves, the treatment produced different effects on the above elements was found, i.e. significant increase, reduction or no effect of iodine on the accumulation of P, Ca, Na, B, Cu, Mn, Zn, Cd and Pb in carrot. After foliar application of the lowest concentration of iodine (0.02 kg I ha<sup>-1</sup>), storage roots of carrot plants contained the highest levels of P, Ca, B, Cu, Mn and Cd, even when compared to the control. Noteworthy is the fact that alongside increased concentrations of the applied iodine, reduced Mn accumulation was observed. Notwithstanding, in all the tested combinations with iodine foliar nutrition, Mn content exceeded values obtained for the control. It should be mentioned that the differentiation of the carrot concentration of P, Ca, Na, B, Cu, Mn, Zn, Cd and Pb was not reflected by the content of these elements in soil (Table 2).

In the context of the above results, it was interesting to notice that the foliar application of iodine in the lowest concentration (combination no 5) contributed to significantly enhanced uptake of N, P, Ca, Na, B, Cu, Zn and Cd by carrot storage roots. This observation can indirectly bring explanation for yet unsupported positive influence of low concentration of iodine on improved plant growth and yielding (Kabata and Mukherjee 2007). Nevertheless, in the present study no significant impact of iodine applied to leaves or soil was found in reference to the yield of carrot storage roots or leaves – detailed data not shown.

To sum up, it can be stated that the influence of iodine on mineral nutrition of carrot plants is ambiguous. To much extent it can depend on agronomic conditions of cultivation, including the applied nitrogen fertilization (Smoleń 2009, Smoleń et al. 2009, 2010). In studies with pot carrot cultivation, nitrogen fertilization both in the form of Ca(NO<sub>3</sub>)<sub>2</sub> and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, in comparison to the control, contributed to a different iodine influence (in the form of KI and KIO<sub>3</sub>) on the uptake and accumulation of Ca, K, Mg, Na, P and S (Smoleń et al. 2009) as well as Al, B, Cd, Cr, Cu, Fe, Li, Ti and  $m V~(Smole\acute{n}~2009)$  in carrot storage roots. In the field experiments conducted by Smoleń et al. (2010), iodine nutrition (both as KI and KIO<sub>3</sub>) of plants not fertilized with nitrogen resulted in a significant increase of P, K, Ca content and a reduction in Fe concentration but had no effect on Mg, S, Cu, Mn, Zn, Mo, Al and Pb accumulation in carrot roots. However, KIO<sub>3</sub> application (in comparison to KI) to plants fertilized with ammonium sulphate led to higher concentrations of N, P, K, Mg, S, Na, B, Cu, Fe, Mn, Zn, Al and Cd in carrot. As far as the fertilization with calcium nitrate is concerned, soil application of KIO<sub>3</sub> contributed to increased accumulation of N, K, Fe and Zn in carrot storage roots when compared to a KI treatment.

### CONCLUSIONS

- 1. Foliar nutrition of carrot with iodine in the dose of 2.0 kg I ha $^{-1}$  (as  $\mathrm{KIO_3}$ ) allowed us to obtain a comparable effect of iodine biofortification of carrot storage roots in comparison to soil fertilization with this element in the same dose but applied in the KI form.
- 2. An increase in the N-total content in carrot was observed as a result of soil fertilization in the dose of 1.0 and 2.0 kg I ha<sup>-1</sup> as well as foliar nutrition of iodine in the concentration of 0.02 and 2.0 kg I ha<sup>-1</sup>).
- 3. The research revealed a synergistic effect of iodine applied both to soil and to leaves on the uptake of N, K, Mg, S, Fe and Al by carrot storage roots.
- 4. In the case of P, Ca, Na, B, Cu, Mn, Zn, Cd and Pb, different effects of the iodine dose, form and application method were observed in reference to the concentrations of these elements in carrot roots.
- 5. Soil fertilization with iodine resulted in an increased uptake of P, Zn Cd and Pb (synergistic effect) as well as a reduced uptake of Cu (antagonism) by carrot storage roots.
- 6. Foliar application of the lowest concentration of iodine (0.02 kg I ha<sup>-1</sup>) contributed to significantly higher accumulation of P, Ca, Na, B, Cu, Zn and Cd in carrot storage roots.
- 7. A significant effect of the tested factors was found in reference to the changes of soil pH as well as the content of N-NO $_3$ , P, K, S, B, Cu, Mn, Z, Mo, Al, Cd and Pb in soil after carrot cultivation.

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# VALUES OF QUALITY TRAITS OF OILSEED RAPE SEEDS DEPENDING ON THE FERTILISATION AND PLANT DENSITY

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### Abstract

The aim of this paper has been to evaluate yields and seed quality of winter oilseed rape depending on the plant density, foliar fertilisation with magnesium, sulphur, boron and Asahi growth biostimulant combined with constant NPK fertilisation. The research was performed in 2006-2008 as a two-factor field experiment. The first factor involved plant density (A - 20, B - 30, C - 40 plants per 1 m²) and the second one comprised fertilisation treatments (n=7). Foliar fertilisation was applied once or twice. In rape seeds, the following were determined: the content of total nitrogen, content of fat and its fractions.

The results show that the concentrations of total nitrogen and fat in rape seeds were significantly dependent on both the plant density and fertilisation. Significantly the highest content of those nutrients occurred after a double application of magnesium sulphate (VI), Solubor and Asahi biostimulant at the plant density of 40 and 30 plants  $\rm m^2$ , respectively. The composition of fatty acids extracted from seeds of the hybrid oilseed rape cultivar Nelson significantly depended only on fertilisation. The highest content of oleic acid and its increase, as compared with the control, was identified after a single application of magnesium sulphate (VI), Solubor and Asahi biostimulant. The fertilisation significantly decreased the value of the sum of polienic acids  $\rm C_{18:2}$  and  $\rm C_{18:3}$  in rape seeds against the control. It was only after a double application of magnesium sulphate (VI), microelement fertiliser (Solubor) and Asahi biostimulant that the accumulation of these fatty acids remained at the level of concentration reported in the control seeds.

Keywords: winter rape, quality traits, plant density, fertilisation variants.

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## OCENA CECH JAKOŚCIOWYCH NASION RZEPAKU W ZALEŻNOŚCI OD NAWOŻENIA I OBSADY ROŚLIN

### Abstrakt

Celem badań była ocena plonowania i jakości nasion rzepaku ozimego w zależności od obsady roślin, nawożenia dolistnego magnezem, siarką, boronem i biostymulantem wzrostu Ashai w połączeniu ze stałym nawożeniem NPK. Badania przeprowadzono w latach 2006-2008 jako dwuczynnikowe doświadczenie polowe. Pierwszy czynnik to gęstość obsady roślin (A - 20, B - 30, C - 40 roślin na 1  $\mathrm{m}^2$ ), drugi - to warianty nawożenia (n = 7). Nawożenie dolistne zastosowano jedno- lub dwukrotnie. W nasionach rzepaku określono zawartość całkowitą azotu, zawartość tłuszczu oraz jego frakcji.

Wyniki badań wskazują, że zawartość całkowita azotu oraz zawartość tłuszczu zależały istotnie od gęstości obsady roślin oraz nawożenia. Istotnie najwyższą zawartość wymienionych składników stwierdzono po podwójnym zastosowaniu siarczanu magnezu (VI) lub biostymulantu Ashai Solubor przy obsadzie roślin odpowiednio 40 oraz 30 roślin na m². Skład kwasów tłuszczowych ekstrahowanych z nasion mieszańcowej odmiany rzepaku ozimego Nelson był istotnie zależny od nawożenia. Istotnie najwyższą zawartość kwasu olejowego oraz wzrost jego zawartości, w porównaniu z kontrolą, określono po pojedynczym zastosowaniu siarczanu magnezu (VI) oraz Soluboru i Ashai. Nawożenie istotnie obniżyło wartość sumy wielonienasyconych kwasów tłuszczowych  $C_{18:2}$  i  $C_{18:3}$  w nasionach rzepaku w porównaniu z kontrolą. Jedynie po zastosowaniu dwukrotnie siarczanu magnezu (VI), nawozu mikropierwiastkowego Solubor i biostymulantu Ashai akumulacja tych kwasów tłuszczowych utrzymała się na poziomie odpowiadająym zawartościom określonym w nasionach kontrolnych.

Słowa kluczowe: rzepak ozimy, cechy jakościowe, obsada roślin, warianty nawożenia.

### INTRODUCTION

Owing to progress in quality breeding as well as production of high yielding population and hybrid cultivars, oilseed rape has become one of the most important plants in the moderate climatic zone. The acreage of oilseed rape and agrimony (winter and spring in total) in Poland was 796.8 thousand ha in 2006, being about 172.9 thousand ha higher (by 27.7%) than in 2005, and 317.4 thousand ha higher than the 2001-2005 mean (by 66.2%) (Rosiak 2006).

Rape seeds are most often used in oil and animal feed industry, although oil produced from improved cultivars can be also processed for non-food purposes (Bartkowiak-Broda 2005, Wałkowski 2002).

With the prospect of a continued growth in rape acreage and oilseed rape applications, a closer look should be paid at elements of the agronomic practise dedicated to this crop in order to ensure possibly highest yields of adequate quality while optimising costs. Among some important components of the cultivation technology are: preparation of the stand, the type of crop rotation, tillage method, cultivar selection, the application of certified seeds, sowing date and rate, chemical control of the plantation, as well as the

harvest date combined with a selected harvest method as well as fertilisation (Malhi, Leach 2000, Podleśna 2003, Szewczuk 2003, Bartkowiak-Broda et al. 2005).

With that in mind, research has been undertaken, which aimed at determining whether or not and - if so - to what extent winter rape yielding as well as seed quality depend on the plant density and foliar application of magnesium, sulphur and boron against constant NPK fertilisation.

### MATERIAL AND METHODS

The experiment was carried out in 2006-2008, in the village of Piecki near i Kruszwica (Figure 1) on soil classified as quality class soil V. The experiment was designed as a two-factor trial in three replicates.



Fig. 1. Location of the experiment

Fertilisation with nitrogen, phosphorus and potassium was constant. The first research factor involved the plant density (A -20, B -30, C -40 plants per 1 m<sup>2</sup>), and the second one - fertilisation treatments: 7 treatments with varied foliar fertilisation according to the following pattern:

- 0) control,
- 1) magnesium sulphate (VI) + microelement fertilisation (Solubor),
- 2) Asahi biostimulant,
- 3) magnesium sulphate (VI) + microelement fertiliser (Solubor) + Asahi biostimulant,

- 4) magnesium sulphate (VI) + microelement fertiliser (Solubor),
- 5) Asahi biostimulant.
- 6) magnesium sulphate (VI) + microelement fertiliser (Solubor) + Asahi biostimulant.

The plants in treatments 1, 2, 3 were fertilised and treated with the biostimulant once (April), while in treatments 4, 5, 6 – twice with a two-week break.

The following were applied pre-sowing: 120 kg  $\rm K_2O$ , 50 kg  $\rm P_2O_5$  and 25 kg N ha<sup>-1</sup>. In spring, at the beginning of the growing season, the following were applied as top dressing treatments: 70 kg S ha<sup>-1</sup> (Wigor), 80 kg  $\rm K_2O$  ha<sup>-1</sup> (potash salt) and 20 kg  $\rm P_2O_5$  ha<sup>-1</sup> (triple superphosphate) as well as 100 kg N ha<sup>-1</sup> (ammonium nitrate). The treatments were carried out in the first decade of March. Two weeks later, 90 kg N ha<sup>-1</sup> was applied.

Winter barley constituted the forecrop for oilseed rape. In each year, the plants were sown in the last decade of September, while the harvest took place in the last decade of July of the successive year. Each year, representative rape seed samples were taken for deetermination of total nitrogen with Kiejdahl method. The content of fat was determined with an analyzer NMR – MQA 7005 Oxford, while the fat fractions were assayed using gas chromatography.

The results underwent analysis of variance in a two-factor design with Tukey's semi-intervals of confidence.

### RESULTS AND DISCUSSION

The rape seed yield, as a result of the fertilisation applied, varied a lot and ranged from 4.66 t ha<sup>-1</sup> to 4.76 t ha<sup>-1</sup>, with the mean value of 4.69 t ha<sup>-1</sup> (Figure 2). The seed yield was significantly determined by the foliar application of the tested fertilisers.

The significantly highest rape seed yield was recorded after a double foliar application of magnesium sulphate (VI), Solubor, Asahi (treatment 6) at the density of 30 plants m<sup>-2</sup>. Szewczuk (2003) demonstrated that it was also justifiable to apply multi-component fertiliser on two dates with since foliar fertilisers usually enhanced the yield structure components, especially the 1000 seed weight, and thus raised the yield. Interestingly, in the present research the highest yields were reported from the plots with an increased dose of microelement fertiliser containing boron (treatment 6). A similar reaction of rape to boron is reported by Bowszys, Krauze (2000). Sienkiewicz-Cholewa (2005) also points to a yield-forming role of boron and claims that rape seed yield after microelement fertilisation in most of the experiments tended to increase noticeably.

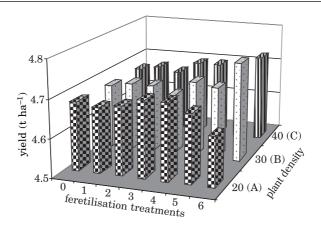


Fig. 2. Winter rape seed yielding (t ha<sup>-1</sup>)

The content of total nitrogen in oilseed rape seeds ranged from 35.6 g kg<sup>-1</sup> to 40.9 g kg<sup>-1</sup>, with the mean total of 38.12 g kg<sup>-1</sup>, and depended significantly on the plant density as well as on the fertilisation applied (Table 1).

Plant			Min	eral fertilisa	tion		
density	0	1	2	3	4	5	6
		Content of	total nitroge	en in rape se	eds (g kg <sup>-1</sup> )		
20 (A)	37.1Bd	37.8Bc	37.2ABc	37.9Bab	38.6Aa	36.9Ad	38.6Aa
30 (B)	35.6Cd	39.2Aa	37.8Abc	39.0Aa	39.1 <i>Aa</i>	37.3Ac	38.0Bab
40 (C)	38.2Ac	38.1Bc	38.1 <i>Ac</i>	39.8Ab	38.3 <i>ABc</i>	37.1Ad	40.9Ba
		Conten	t of fat in w	inter rape se	eds (%)		
20 (A)	44.7Cc	44.2Cd	45.0Bb	45.3Aab	43.9Be	45.4Aa	45.0Bb
30 (B)	45.1Bc	45.1Ac	45.6Ab	45.3Ac	44.5Ad	45.6Ab	46.4Aa
40 (C)	45.5Aa	44.6Bb	44.8Bb	43.7Bd	43.6Bd	44.2Bc	43.2Ce

a, b, c, d – means marked with different letters in the row differ significantly depending on the (p < 0.05); A, B, C – means marked with different letters in the column differ significantly depending on the plant density (p < 0.05)

In general, a significantly higher content of total nitrogen occurred in the seeds of plants growing at the density of 40 plants m<sup>-2</sup>, while the lowest N content was identified in the seeds growing at the density of 20 plants m<sup>-2</sup>.

On average, the significantly highest total N content was found following a double application of magnesium sulphate (VI), microelement fertiliser (Solubor) and Asahi biostimulant (treatment 6). It was also observed that

after a double application of magnesium sulphate (VI) and Solubor as well as after a single foliar application of magnesium sulphate (VI), microelement fertiliser (Solubor) and Asahi biostimulant (treatment 4), the content of nitrogen in rape seeds was significantly bigger than the control, on average by 5.8%.

Numerous authors (Fotyma et al. 2000, Wielebski, Wójtowicz 1998, 2001, Sienkiewicz-Cholewa 2001, Kotecki et al. 2001, Pałka et al. 2003, Podleśna 2004, Wielebski 2005) have demonstrated that the application of nitrogen and the treatment of rape with multi-component fertilisers as well as with sulphur enhance the content of total nitrogen, and thus the content of protein in seeds, an observation which is supported by the present research.

Technological usability of oilseed rape seeds for fat production depends on the content of fat and the percentage composition of fatty acids. The content of fat in the rape seeds ranged from 43.2% to 46.4% and it was determined by both experimental factors (plant density and fertilisation) as well as their interaction (Table 1).

Sienkiewicz-Cholewa (2001) reports a slightly lower (from 39% to 43%) content of fat in rape seeds as a result of microelement application. According to Muśnicki et al. (1999), it is the genetic factor which is decisive in creating the content of fat in rape seeds; the differences in extreme cases ranged from just 6.3% to 6.5%. Jedrzejak et al. (2005), on the other hand, claim that the productivity of crude fat depends mostly on the moisture and thermal conditions as well as on nitrogen fertilisation and, to a little extent, on the genetic factor.

The significantly highest content of fat was found in rape seeds collected from the plots of the density of 30 plants m<sup>-2</sup>, following the successive application of twice magnesium sulphate (VI), Solubor and Asahi (treatment 6), twice Asahi (treatment 5) as well as once Asahi (treatment 2). They were, respectively, 2.8%, 1.1% and 1.1%, higher than the control (treatment 0). Barlóg, Potarzycki (1997) found that foliar fertilisation of rape with magnesium, at a split dose, demonstrated a significant increase in the content of fat in rape seeds from 0.5 to 1.0%. Interestingly, the number of applications of Asahi biostimulant did not affect the content of fat in rape seeds. Rape obtained from the plots on which the density was 20 plants m<sup>-2</sup> accumulated significantly less fat in seeds as compared with the content produced from the plots where the density was 30 plants  $m^{-2}$  (by an average of 1.3%). The literature reports show that microelements have a favourable affect on the content of fat in rape seeds (Lääniste et al. 2004); positive effects were recorded for single microelements as well as for their mixtures.

The composition of fatty acids is highly cultivar-specific, so agrotechnical and weather factors do not actually change it (Muśnicki et al. 1999). Interestingly, the composition of fatty acids in oilseed rape seeds was typical and significantly determined only by fertilisation (the 2<sup>nd</sup> factor). In the

present research, it was found that winter rape seeds demonstrated the greatest share of oleic acid in crude fat, which – depending on the plant density – ranged from 62.66% to 62.87%. In dependence on the fertilisation applied, its share ranged from 61.93% to 63.53% (Figures 3, 4). The greatest increase in the content of oleic acid, as compared with the control, was found after a single application of magnesium sulphate (VI), Solubor and Asahi biostimulant (treatment 3).

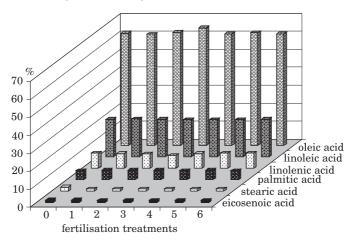


Fig. 3. Content of respective fatty acids depending on fertilisation

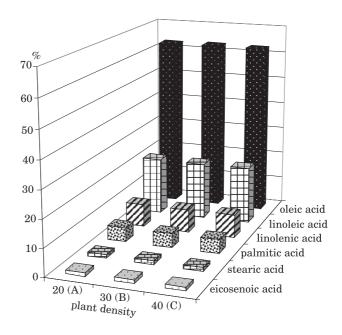


Fig. 4. Content of respective fatty acids depending on the plant density

The composition of fatty acids in rape seeds can be cultivar-specific dependent on the harvest date (Jackowska, Tys 2006, Murawa et al. 2000) or slightly depenent on nitrogen fertilisation (Kotecki et al. 2001). Moreover, Krauze, Bowszys (2001) demonstrated that fertilisation with sulphur increased the share of essential fatty acids (linolenic and linoleic acids) in fat, thus enhancing the nutritive value of fat. The fertilisation applied in the present research decreased the sum of polienic acids  $C_{18:2}$  and  $C_{18:3}$  (linoleic acid-linolenic acid) in rape seeds, as compared with the control. Interestingly, the values of the sum of the content of those acids were identical and the highest (29.73%) in rape seeds obtained from the control and after a double application of magnesium sulphate (VI), microelement fertiliser (Solubor) and Asahi biostimulant (Figure 4).

After a single application of magnesium sulphate (VI) and Solubor (treatment 1) an increase in the content of linoleic acid ( $C_{18:2}$ ) in rape seeds appeared, accompanied by a decrease in the content of oleic acid. Numerous results reported by other authors have shown changes in the composition of fatty acids as a result of fertilisation with microelements and multi-component fertilisers (Kotecki et al. 2001, Sienkiewicz-Cholewa 2002, 2005, Palka et al. 2003); they most often affected polyunsaturated acids, especially linoleic acid, which could indicate their lack of stability (Robak, Gogolewski 2002).

A weaker effect on the values of the sum of the content of acids  $\rm C_{18:2}$  and  $\rm C_{18:3}$  in rape seeds was identified for the plant density. The highest content of acids  $\rm C_{18:2}$  +  $\rm C_{18:3}$  (29.5%) was found in seeds from the treatments where the density was 40 plants m $^{-2}$ , remaining unchanged for the other two densities. In none of the rape seed samples significant changes in the value of the sum of acids  $\rm C_{18:2}$  and  $\rm C_{18:3}$  depending on the plant density were found.

### CONCLUSIONS

- 1. The significantly highest rape seed yield was ensured by a combined double application of magnesium sulphate (VI), microelement fertiliser (Solubor) and Asahi biostimulant. The plant density did not affect winter rape yielding.
- 2. The total nitrogen and fat in rape seeds were significantly determined by both the plant density and fertilisation. Significantly most of these nutrients were identified after a double application of magnesium sulphate (VI), Solubor and Asahi biostimulant in seeds collected from plots at the density of 40 and 30 plants m<sup>-2</sup>, respectively.
- 3. The composition of fatty acids extracted from winter rape seeds significantly depended on fertilisation. The significantly highest content of oleic acid and an increase in its concentration, as compared with the control, was

found after a single application of magnesium sulphate (VI), Solubor and Asahi biostimulant.

4. Similarly, fertilisation significantly depressed the values of the sum of polienic acids  $C_{18:2}$  and  $C_{18:3}$  in rape seeds, as compared with the control. A double application of magnesium sulphate (VI), microelement fertiliser (Solubor) and Asahi biostimulant were the only treatments which resulted in the accumulation of these fatty acids remaining at the level of the concentration recorded in the control seeds.

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# MAGNESIUM IN PATIENTS OPERATED DUE TO COLORECTAL OR SMALL INTESTINE CANCER AND RECEIVING TOTAL PARENTERAL NUTRITION (TPN) IN POSTOPERATIVE PERIOD

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### Abstract

Magnesium is fundamental to the existence of life. The consequence of altered magnesium homeostasis may be magnesium deficiency. It is well known that magnesium plays a role in tumour biology such as carcinogenesis, angiogenesis and tumour progression. In the field of gastrointestinal cancer surgery of the clinical importance, magnesium has not been specifically studied. Therefore, the aim of our study was to evaluate changes of magnesium concentrations in patients operated due to a small intestine or colorectal cancer parenterally nurtured in comparison with a group of patients submitted to surgical interventions due to gastrointestinal cancer but receiving standard nutrition after the operation. The study group involved 78 patients operated on for gastrointestinal cancer, who were divided into 3 groups: C - patients operated due to different types of alimentary tract cancers who were provided with normal feeding after the operation, I - patients operated due to colorectal cancer who were given TPN after the operation, II - patients operated due to small intestine cancer who were given TPN after the operation. Three measurements were performed in control group (C): the 1st measurement - a day before operation, the 2<sup>nd</sup> measurement - on the third day after the operation and the 3<sup>rd</sup> measurement - on the fifth day after the operation. In the group of patients receiving TPN, three measurements were performed as well: the 1st measurement - a day before operation, the 2<sup>nd</sup> measurement - on the third day after applying TPN and the 3<sup>rd</sup> measurement on the fifth day after applying TPN. Our studies revealed that application of TPN, conta-

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ining magnesium, in patients operated both due to colorectal cancer and small intestine cancer prevented decrease in the blood serum concentration of that element below the reference norm, which occurred in patients receiving standard diet.

Key words: magnesium, colorectal cancer, small intestine cancer, TPN.

### MAGNEZ U PACJENTÓW OPEROWANYCH Z POWODU NOWOTWORU ODBYTU LUB JELITA CIENKIEGO I OTRZYMUJĄCYCH CAŁKOWITE ŻYWIENIE POZAJELITOWE (CŻP) W OKREŚIE POOPERACYJNYM

### Abstrakt

Magnez jest pierwiastkiem niezbędnym do życia. Konsekwencją zaburzeń homeostazy magnezu może być jego deficyt. Magnez odgrywa rolę w biologii nowotworów, tj. w karcinogenezie, angiogenezie lub rozwoju guza nowotworowego. Dotychczas nie ma szerokich badań dotyczących znaczenia magnezu w operacjach nowotworów przewodu pokarmowego. Dlatego celem badań było zbadanie zmian stężenia magnezu u pacjentów operowanych z powodu nowotworów odbytnicy lub jelita cienkiego i otrzymujących całkowite żywienie pozajelitowe w okresie pooperacyjnym, w porównaniu z grupą pacjentów poddanych interwencji chirurgicznej z powodu nowotworów przewodu pokarmowego, ale otrzymujących po operacji standardową dietę.

Badaniem objęto 78 pacjentów operowanych z powodu nowotworów przewodu pokarmowego, których podzielono na 3 grupy: kontrolną (K) – pacjenci operowani z powodu różnych nowotworów przewodu pokarmowego, po operacji otrzymujący normalne żywienie, I – pacjenci operowani z powodu zaawansowanego nowotworu odbytnicy, po operacji otrzymujący CŻP, II – pacjenci operowani z powodu nowotworu jelita cienkiego, po operacji otrzymujący CŻP. W grupie K dokonywano trzech pomiarów w kolejnych okresach: 1. pomiar – doba przed operacją, 2. pomiar – trzecia doba po operacji, 3. pomiar – piąta doba po operacji. W grupach I, II – u pacjentów otrzymujących CŻP– dokonywano również trzech pomiarów: 1. pomiar – doba przed operacją, 2. pomiar – trzecia doba po zastosowaniu CŻP, 3. pomiar – piąta doba po zastosowaniu CŻP.

W badaniach wykazano, że podawanie CŻP zawierającego magnez, zarówno chorym operowanym z powodu nowotworów odbytnicy, jak i nowotworów jelita cienkiego, zapobiega obniżeniu stężenia tego pierwiastka w osoczu krwi poniżej norm referencyjnych, tak jak to ma miejsce w przypadku pacjentów otrzymujących standardową dietę

Słowa kluczowe: magnez, nowotwór odbytnicy, nowotwór jelita cienkiego, CZP.

### INTRODUCTION

In the 1970s, nutritional treatment became commonly applied in clinical practice. It is introduced when, due to clinical or/and biochemical symptoms of malnutrition or deficiency, it is necessary to administer appropriate nutrients for supplementation. Approximately 35–50% of hospitalized patients are malnourished and in many cases the condition develops or progresses during hospitalization while surgical interventions increase the incidence of complications and prolong hospital stay (Sungurtekin et al. 2004, Schnelldorfer, Adams 2005, Hall 2006, Stratton et al. 2006). Most patients with malignant

cancers suffer from malnutrition, decrease in body weight and fat-free body weight. Moreover, a decrease in body weight is the most frequent symptom of the presence of a tumour and, in over 60% of patients, development of the disease increases malnutrition. Total Parenteral Nutrition (TPN) is necessary in patients with alimentary tract cancers with metastases and stated malnutrition, who are unable to swallow or absorb food provided in a normal way for longer than 7-10 days (Echenique, Correia 2003). Despite numerous studies, we still lack a unanimous answer whether TPN application in patients with various tumours is beneficial or laden too many contraindications. Therefore, monitoring the malnutrition state and choosing an appropriate therapy may help to improve treatment conditions, accelerate recovery and successfully decrease costs of all medical procedures (Stratton 2005).

Magnesium plays a crucial role in many physiological and metabolic processes. Its homeostasis is therefore fundamental to the existence of life. The consequence of altered magnesium homeostasis is magnesium deficiency. Hypomagnesaemia may cause weakness, tremors, seizures and cardiac arrhythmias (Tang, Rude 2005). Altered plasma magnesium levels can in turn affect calcium and potassium levels (Huang, Kuo 2007, Alexander et al. 2008). Moreover, magnesium plays a role in tumour biology such as carcinogenesis, angiogenesis and tumour progression (Anastassopoulou, Theophanides 2002, Rubin 2005).

Therefore, the aim of this study has been to determine changes in blood serum magnesium concentrations in patients operated due to small intestine or colorectal cancer and parenterally nurtured in comparison with a group of patients submitted to surgical interventions due to gastrointestinal cancers but receiving standard nutrition after the operation.

### MATERIAL AND METHODS

The study group involved 78 patients operated on for gastrointestinal cancer who were divided into three groups:

- C patients operated on for different types of gastrointestinal cancer who were provided with normal feeding after the operation (on the day of an operation and during the 5 days afterwards, patients received intravenously typical amounts of liquids and essential electrolytes: ca 200 mmol of Na, 80 mmol of K and 300 mmol of Cl;
- I patients operated on for colorectal cancer who were given TPN after the operation, Lublin;
- II patients operated on for small intestine cancer who were given TPN after the operation.

The patients were chosen for the parenteral nutrition on the basis of a conducted screening examination (Table 1).

Table 1

Information	about the	patients

Parameter	Control $(n = 20)$	I(n = 34)	II $(n = 24)$
Sex (male/female)	14/6	22/12	18/8
Age (years ± SD)	65 ± 13.2	69 ± 10.4	58 ± 11
Body weight (kg ± SD)	$58.6 \pm 12.4$	50.8 ± 10.6	54.3 ± 11.3
Primary tumor (n):			
Colon/rectum	7	34	
Small intestine	2		24
Esophageal	3		
Pancreas	4		_
Stomach	4		

In the control group, particular measurements were conducted on the following days:  $1^{\rm st}$  measurement – one day before the operation,  $2^{\rm nd}$  measurement – three days after the operation,  $3^{\rm rd}$  measurement – five days after the operation.

In the experimental group, three measurements were taken as well:

 $1^{\rm st}$  measurement – one day before the operation,  $2^{\rm nd}$  measurement – three days after starting TPN,  $3^{\rm rd}$  measurement- five days after starting TPN.

The patients received TPN for a period of 5 or 7 days and afterwards normal nutrition was provided.

The study was approved by the Bioethical Commission of the Medical University in Lublin (No KE-0254/31/2006).

All patients were hospitalized in the I Chair and Department of General and Transplant Surgery and Nutritional Treatment of the Medical University in Lublin, and they fully agreed to participate in the research constituting this present work.

The patients were chosen for the parenteral nutrition on the basis of a conducted screening examination. Compositions of the mixtures given in TPN are presented in Table 2.

The plasma from each blood sample was collected immediately after centrifugation at  $2000 \times g$  for 15 minutes and then stored at  $-20^{\circ}$ C until analysis.

The concentration of magnesium was determined spectrophotometrically by the reaction with xylidyne blue at the wavelength 520 nm, using a Hitachi spectrophotometer. The results were expressed in mmol dm<sup>-3</sup>.

Statistical analysis of the results was performed with the use of SPSS 12.0 PL software.

 ${\it Table \ 2}$  Content of nutrients given in total parenteral nutrition

Group	Parameter	Mean	SD	Median	Min.	Max.	Interqu- artile range
I	nitrogen (g dm <sup>-3</sup> )	5.61	0.12	5.70	5.40	5.70	0.30
	amino acids (g dm <sup>-3</sup> )	38.25	3.11	40.00	33.00	40.00	7.00
	carbohydrates (g dm <sup>-3</sup> )	98.30	24.04	80.00	80.00	132.00	52.00
	fats (g dm <sup>-3</sup> )	47.60	3.76	50.00	42.00	50.00	8.00
	Na (mmol dm <sup>-3</sup> )	46.05	5.92	50.00	27.00	50.00	23.00
	K (mmol dm <sup>-3</sup> )	31.35	4.82	30.00	15.00	36.00	21.00
	Mg (mmol dm <sup>-3</sup> )	3.05	0.22	3.00	3.00	4.00	1.00
	Ca (mmol dm <sup>-3</sup> )	2.87	0.17	3.00	2.50	3.00	0.50
	Cl (mmol dm <sup>-3</sup> )	45.08	4.29	48.00	31.60	48.00	16.40
	Zn (mmol dm <sup>-3</sup> )	0.02	0.02	0.03	0.00	0.03	0.03
	acetates (mmol dm <sup>-3</sup> )	45.98	11.49	40.00	19.50	60.00	40.50
	phosphates (mmol dm <sup>-3</sup> )	11.09	5.22	7.50	5.70	18.00	12.30
II	nitrogen (g dm <sup>-3</sup> )	6.02	0.96	5.70	5.40	8.60	3.20
	amino acids (g dm <sup>-3</sup> )	39.80	7.63	40.00	33.00	60.00	27.00
	carbohydrates (g dm <sup>-3</sup> )	105.45	26.21	107.00	80.00	150.00	70.00
	fats (g dm <sup>-3</sup> )	51.05	8.85	50.00	42.00	75.00	33.00
	Na (mmol dm <sup>-3</sup> )	42.40	17.13	35.00	27.00	75.00	48.00
	K (mmol dm <sup>-3</sup> )	29.25	12.38	30.00	15.00	45.00	30.00
	Mg (mmol dm <sup>-3</sup> )	3.57	0.74	3.90	2.50	4.50	2.00
	Ca (mmol dm <sup>-3</sup> )	2.94	0.77	2.50	2.30	4.50	2.20
	Cl (mmol dm <sup>-3</sup> )	45.66	14.96	40.00	31.60	72.00	40.40
	Zn (mmol dm <sup>-3</sup> )	0.01	0.02	0.00	0.00	0.04	0.04
	acetates (mmol dm <sup>-3</sup> )	43.58	20.60	50.00	19.50	75.00	55.50
	hosphates (mmol dm <sup>-3</sup> )	11.63	6.18	11.25	5.70	23.00	17.30

The results were tested with a mixed-design variance analysis (ANOVA model). This approach, relying on the values of F test, allowed us to simultaneously analyze the influence of the group and the time of measurements on the examined parameters.

The level of statistical significance, which indicated statistically significant differences or interrelations, was p<0.05.

### RESULTS

The magnesium concentration and values of the arithmetic mean, standard deviation (SD), median, lower and upper quartile, maximum and minimum as well as interquartile range are presented in Table 3.

 ${\bf Table~3}$  Magnesium concentration in blood serum of the three groups of patient

Group	Mg (mmol dm <sup>-3</sup> )	Mean	SD	Median	Lower quartile	Upper quartile	Min.	Max.	Interquartile range
С	Mg (1)	0.76	0.26	0.72	0.55	0.89	0.47	1.44	0.97
	Mg (2)	0.63	0.19	0.62	0.46	0.77	0.32	1.28	0.96
	Mg (3)	0.61	0.17	0.62	0.47	0.74	0.32	0.91	0.59
I	Mg (1)	0.65	0.28	0.65	0.52	0.94	0.31	0.83	0.52
	Mg (2)	0.74	0.25	0.75	0.48	0.89	0.53	1.27	0.74
	Mg (3)	0.81	0.25	0.94	0.80	1.12	0.52	1.11	0.59
	Mg (1)	0.58	0.12	0.55	0.49	0.64	0.39	0.89	0.50
II	Mg (2)	0.66	0.17	0.71	0.65	0.90	0.49	1.09	0.60
	Mg (3)	0.78	0.24	0.79	0.61	0.79	0.54	1.11	0.57

A mixed-design variance analysis according to the ANOVA model was used to test the significance of differences between subsequent measurements of magnesium concentrations. The influence of the time of measurement on magnesium concentration was checked. The analysis of variance demonstrated statistically significant differences between average values of magnesium concentrations from all the measurements (F=12.246; p<0.05). The pairs of measurements that differed significantly wer 1-2 and 1-3 (post-hoc). A statistically significant decrease in the magnesium concentration between measurements 1-2 and 1-3 in the control group and a statistically significant increase in the magnesium concentration between measurements 1-3 in groups I and II were observed while comparing particular measurements (Table 4).

The influence of the affiliation to a group of patients on the magnesium concentration was also tested.

The variance analysis showed statistically significant differences between group C and groups I and II (F=4.241~p<0.05). Analyses of particular groups of patients showed that on the first date of determinations, the magnesium concentration was significantly higher in group C than in group II. On the second measurement date, the Mg concentration in group I was significantly higher than in group C, and in the third date – it was significantly higher in groups receiving TPN (I and II) than in group C (Table 4).

Table 4
Statistics of significance tests of differences for particular measurements and in groups of patients

Group	Test statistics 12.246	Significance level 0.000(*)	Post-hoc 1-2, 1-3	C 1-2, 1-3	I 1-3	II 1-3
Measurement	test statistics	significance level	post-hoc	1	2	3
	4.241	0.007(*)	C-I, C-II	C-II	C-I	C-I, C-II

### RESULTS AND DISCUSSION

Carcinogenesis is associated with disturbances of all metabolic processes in an organism, which results in a negative nitrogen balance, increase in glycogenesis, decrease in muscle protein synthesis and disturbances in water and electrolyte balance. There are many studies stating that hypomagnesaemia occurring in oncological patients is not only a consequence of asitia, anorexia and malnutrition but also a side-effect of drug administration and chemotherapy (Alberda et al. 2006). Cancer patients treated with a high dose or prolonged doses of cisplatin or cetuximab, both as monotherapy and in combination with chemotherapeutics, develop hypomagnesaemia (Tejpar et al. 2007, Vincenzi et al. 2008).

Magnesium tolerance test, which is based on the measurement of a parenterally administered Mg load retention, is a more accurate method for the detection of Mg deficiency (RICHETTE et al. 2007), but total plasma magnesium levels measurement, as used in this study, is a standard determination method for routine diagnostic purposes. In our study, the control group consisted of patients qualified to surgical intervention due to gastrointestinal cancers, whose magnesium concentration was below the reference norm but not equal the values corresponding to hypomagnesaemia (hypomagnesaemia is defined as any decrease in the serum magnesium concentration below 0.8 mmol dm<sup>-3</sup>). On the third day after an operation, the magnesium level was lower than before it and did not change on the fifth day. No symptoms of hypomagnesaemia, as cardiac arrhythmia, were observed in these patients. Numerous studies have confirmed that magnesium level decreases in patients undergoing gastrointestinal surgery (SAFAVI, HONARMAND 2007). ARMSTRONG et al. (2007) demonstrated that patients who underwent pancreaticoduodenectomy for peri-ampullary neoplasia, although generally well nourished, have lower serum micronutrient levels and a relative reduction of antioxidants versus paired controls. Schwarz, Nevarez (2005) have found in patients undergoing laparotomy, predominantly for upper gastrointestinal malignancies, that postoperative hypomagnesaemia occurred

in 42% of patients without bowel preparation and in 70% of patients having bowel preparation with sodium phosphate purgative. Evans et al. (2009), who studied statistically significant changes in the plasma magnesium in response to picolax bowel preparation and colorectal resection, observed that 34% patients became hypermagnesaemic following bowel preparation and 20% became hypomagnesaemic following resection.

In some studies, no measurements of magnesium concentration were performed after an operation, but some of the patients were observed to develop cardiac arrhythmia, which was probably caused by a decrease in the magnesium level (Atsmon, Dolev 2005, Walsh et al. 2006). Apart of potential cases of cardiac arrhythmia, hypomagnesaemia has been implicated in the pathogenesis of prolonged postoperative ileus and pseudoobstruction (Vincenzi et al. 2008).

In our research, hypomagnesaemia before surgical intervention was observed in patients with colorectal cancer and with small intestine cancer. On the third day of TPN application, an increase in the magnesium concentration in both groups was observed. On the fifth day of the therapy, the magnesium level was within the physiological reference range. There are only a few studies monitoring magnesium levels in the postoperative period or during the postoperative nutritional therapy. In our previous study, magnesium concentration in blood serum of patients with pancreatic cancer was within the lower limits of the reference range before an operation, and the application of TPN after the operation first caused some minimal fluctuations observed on the 3<sup>rd</sup> day but on the 5<sup>th</sup> day, the TPN therapy brought the Mg concentration back to the same level as before the surgical intervention (SZPETNAR et al. 2009). Machowska, Duda (2002) studied magnesium balance in patients operated due to gastric and colorectal cancer. These patients were divided into three groups: I - patients in whom postoperative magnesium concentration remained within the reference limits, II – patients in whom postoperative Mg concentration was found to be below the aforementioned reference limits for at least one day, III – patients receiving additional 2 mmol Mg<sup>2+</sup> per each 500 on intravenous fluid. Magnesium concentration decreases after surgery in groups I and II were minimal, although the Mg concentration in group I remained within the reference limits, while in group II it was below them for four consecutive days. Changes in the group receiving magnesium were minimal with positive Mg balance during the first two days after surgery. Papageorgiu et al. (2002) showed a significant decrease in the blood serum magnesium concentration in patients after surgery, when they were administered total parenteral nutrition. The mean value obtained during the first measurement decreased during the second and third one but rose again during the fourth measurement.

According to these studies, application of TPN containing appropriately balanced amounts of macro- and microelements may limit magnesium loss and help to improve the patients' condition after surgical intervention. This

effect is ever more important as the aim of curative surgery for small intestinal carcinoma is a complete resection of the neoplasm. In carcinomas with adhesion to neighbouring organs, surgical procedure may turn into multivisceral resection. Restitution of depressed serum magnesium postoperatively may have specific benefits. Postoperative magnesium supplementation has led to a significant reduction in cardiac dysrhythmias, improved postoperative analgesia and inhibition of platelet-dependent thrombosis (Shiga et al. 2004, Schwarz, Nevarez 2005).

Preparing appropriate TPN administrated just after the operation as well as controlling the balance of liquids and electrolytes are important steps also because of the risk of refeeding syndrome, which manifests itself by a rapid decrease in concentrations of phosphates, magnesium and potassium as well as fluid retention in the organism (Hearing 2004).

### CONCLUSION

TPN is recommendable for patients with neoplastic disease and diagnosed malnutrition subjected to surgical intervention, who are unable to swallow and absorb given food for a longer period. Thus, precise and accurate calculations of the amount of administered parenteral nutrition are mandatory. Our study has revealed that application of TPN, containing magnesium, in patients operated both due to colorectal cancer and small intestine cancer prevented decrease in the blood serum concentration of this element below the physiological reference norm, which occurred in patients receiving standard diet. In patients operated due to gastrointestinal cancer, administration of parenteral nutrition is necessary even for a long time after the surgical intervention, and monitoring of the magnesium level in these patients seems to be a requisite. Determination of the efficacy of Mg supplementation if hypomagnesaemia occurs is likewise recommended.

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# EFFECT OF BIOELEMENTS (N, K, Mg) AND LONG-TERM STORAGE OF POTATO TUBERS ON QUANTITATIVE AND QUALITATIVE LOSSES PART I. NATURAL LOSSES

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### Abstract

Storage is one of the most important and, at the same time, most difficult stages in potato production and potato economy. During long-term storage, potato tubers are affected by processes leading to quantitative changes. The present three-year field experiment (2003-2005) investigated the effect of varied mineral fertilization (N, K and Mg against the fixed P dose) and storage (3 and 6 months) on the occurrence of quantitative potato tuber losses. The research involved mid-early cultivars: Bila and Triada. The samples were stored in a storage chamber for 3 and 6 months (temperature +4°C, relative humidity 95%). The present research shows that natural losses were affected by all the experimental factors. The K and Mg fertilizer doses significantly decreased the amount of fresh weight losses as compared with the control; as for potassium, the most favourable in that respect was the dose of 160 kg K<sub>2</sub>O ha<sup>-1</sup>, and for magnesium - 60 kg MgO ha<sup>-1</sup>. Nitrogen fertilization, on the other hand, demonstrated a negative effect on the amount of losses throughout the storage period. The highest fresh weight losses occurred after 6 months of storage and for the nitrogen, potassium and magnesium fertilization experiments they reached 8.9, 6.4 and 7.3%, respectively. The best storage life was reported for cv. Triada potato tubers - potassium fertilization and cv. Bila - magnesium fertilization.

Key words: potato tuber, N, K, Mg fertilization, storage, natural losses.

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### WPŁYW BIOPIERWIASTKÓW (N, K, Mg) ORAZ DŁUGOTRWAŁEGO PRZECHOWYWANIA BULW ZIEMNIAKA NA STRATY ILOŚCIOWE I JAKOŚCIOWE Cz. I. UBYTKI NATURALNE

### Abstrakt

Przechowywanie należy do najważniejszych, a jednocześnie najtrudniejszych etapów w produkcji i gospodarce ziemniakiem. W okresie długotrwałego składowania w bulwach ziemniaka zachodzą procesy prowadzące do zmian ilościowych. W trzyletnim doświadczeniu polowym (2003-2005) badano wpływ zróżnicowanego nawożenia mineralnego (N, K i Mg na tle stałej dawki P) oraz przechowywania (3 i 6 miesięcy) na kształtowanie się strat ilościowych bulw ziemniaka. Do badań wybrano średnio-wczesne odmiany: Bila i Triada. Próby przechowywano w komorze przechowalniczej przez okres 3 i 6 miesięcy (temp. +4°C, wilgotność wzgledna powietrza 95%). Z badań wynika, że na ubytki naturalne miały wpływ wszystkie czynniki doświadczeń. Zastosowane dawki nawozów K i Mg zmniejszały istotnie ilość ubytków świeżej masy w stosunku do obiektu kontrolnego, przy czym dla potasu najkorzystniejsza w tym zakresie okazała się dawka 160 kg K<sub>2</sub>O ha<sup>-1</sup>, a dla magnezu 60 kg MgO ha<sup>-1</sup>. Natomiast nawożenie azotowe miało negatywny wpływ na wielkość ubytków w całym okresie przechowywania. Największe ubytki świeżej masy wystąpiły po 6 miesiącach przechowywania i wynosiły w doświadczeniach z nawożeniem azotowym, potasowym i magnezowym odpowiednio: 8,9, 6,4 i 7,3%. Stwierdzono, że najlepszą trwałość przechowalniczą miały bulwy ziemniaka odmiany Triada - nawożone potasem oraz odmiany Bila - nawożone magnezem.

Słowa kluczowe: bulwa ziemniaka, nawożenie N, K, Mg, przechowywanie, ubytki naturalne.

### INTRODUCTION

One of the largest segments of the farm produce market in Poland is the production of edible potato. Its importance is confirmed by the continuing high demand for both fresh potatoes and their products, with the potato processing growing all the time. Of the total potato produce, about 95% of potatoes annually are stored from 1 to 9 months (October through June) for various types of use (Gasiorowska 2000, Leszczyński 2000, 2006 Lisińska, Sowa--Niedziałkowska 2004a). Storage is one of the most important and yet most difficult stages in the production of potato and potato economy. During longterm storage, potato tubers are affected by processes leading to quantitative changes (Gasiorowska 2000, Leszczyński 2000, Sobol 2005). The magnitude of these changes depends on a number of factors, cultivar-specific ones, agritechnical practice during the vegetation period (mainly mineral fertilization) and the conditions in a storage room (Leszczyński 2000, Sowa--Niedziałkowska 2002, Sowa-Niedziałkowska, Zgórska 2005). The amount of losses is also affected by the handling of potatoes throughout storage preparation, such as the elimination of diseased, damaged and soil-covered tubers (CZERKO 2001) since only mature, healthy and mechanically undamaged tubers demonstrate good suitability for long-term storage (Sowa-Niedziałkowska 2002).

The aim of this study was to determine the effect of varied N, K, Mg mineral fertilization applied during the growing season and the length of a storage period, 3 and 6 months, on the occurrence of natural losses in the selected cultivars of edible potatoes.

### MATERIAL AND METHODS

The research material originated from three independent field experiments conducted at the Experimental Station in Mochelek (2003-2005), owned by the Faculty of Agriculture, the University of Technology and Life Sciences in Bydgoszcz (the Kujawy and Pomorze Province).

The experimental design involved:

- I. Nitrogen doses  $(0, 50 \text{ and } 100 \text{ kg N ha}^{-1})$  in the form of 34% ammonium nitrate against  $120 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$  and  $160 \text{ kg K}_2\text{O ha}^{-1}$ ; edible cultivar cv. Bila (medium early);
- II. Doses (0, 80, 160 and 240 kg  $\rm K_2O~ha^{-1}$ ) in the form of 50% potassium sulphate, against 100 kg N ha<sup>-1</sup> and 120 kg  $\rm P_2O_5~ha^{-1}$ ; edible cultivar cv. Triada (medium early);
- III. Magnesium doses  $(0, 20, 40, 60, 80 \text{ and } 100 \text{ kg MgO ha}^{-1})$  in the form of 26% magnesium sulphate against  $100 \text{ kg N ha}^{-1}$ ,  $120 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  and  $160 \text{ kg K}_2\text{O ha}^{-1}$ ; edible cultivar cv. Bila (medium early).

In autumn, each year before planting, manure was used in experiments I and II at the amount of 25 t ha<sup>-1</sup>. No manure was applied in the magnesium experiment (III) so as to attain a clearer view of the plants' response to the magnesium fertilization. In all the years, cereals were the forecrop for potato. Cultivation, weed-control and plant protection treatments were carried out in accordance with the requirements of the best agritechnical practice, adjusted to the actual weather conditions and potato development stages.

The samples were kept in a storage chamber for 3 and 6 months (temperature +4°C, relative humidity 95%). After storage, the loss of fresh weight, i.e. natural losses, was determined. The results of the 3-year research were statistically verified using the Sigma Stat software (SPSS Inc., Chicago, the U.S.) and Tukey's test to verify the significance of differences.

### RESULTS AND DISCUSSION

Reduction in the initial weight of potato tubers, the so-called natural losses, occurs in all storage conditions, and consequently brings about a reduction of marketable yield and economic losses. The loss of weight depends on many factors interacting with one another, both during the growth

of plants (fertilization) and storage (storage time, temperature, relative humidity and air gas composition) (Leszczyński 2000, Sowa-Niedziałkowska, Zgórska 2005). The present results support these relationships since both the mineral fertilization (N, K, Mg) applied during the growing season and the time of storage had a significant impact on fresh weight losses of the tubers stored (Figures 1,2,3). The longer the storage, the greater the natural losses, and the process is fully justified as resulting from physiological changes characteristic for plant products. The present research demonstrated that the highest fresh weight losses occurred after 6 months of storage, and for the nitrogen, potassium and magnesium fertilization experiments, they accounted for, respectively: 8.9, 6.4, 7.3% (means for the years and fertilization levels). Similar results were reported by Gasiorowska (2000) who noted that the longer the tuber storage, the higher the losses; and after 9 months the losses were nearly four times higher compared to a period of 3 months.

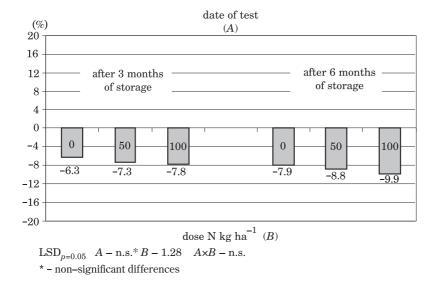


Fig. 1. Loss of fresh weight (%) of cv. Bila potatoes, depending on nitrogen fertilization and date of test (mean from 3 years)

In the potassium and magnesium fertilization experiments, the highest fresh tuber weight losses were observed for control treatments both after 3 and after 6 months of storage (Figures 2,3). The fertilizer doses applied significantly decreased the amount of fresh weight losses compared with the control; as for potassium, the most favourable in that respect was the dose of 160 kg  $\rm K_2O$  ha<sup>-1</sup>, and for magnesium it was 60 kg MgO ha<sup>-1</sup>. Reduction in the losses both after 3 and 6 months of storage, versus the control, by applying the above most favourable doses, accounted for 2.6 and 3.3% for potassium and 3.4 and 2.8% for magnesium, respectively. A positive effect

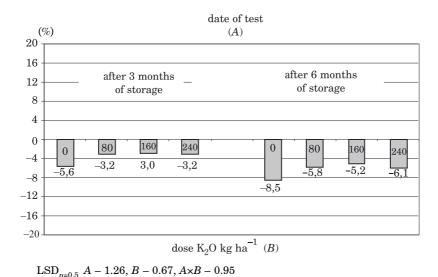


Fig. 2. Loss of fresh weight (%) of cv. Triada potatoes, depending on potassium fertilization

and date of analysis (mean from 3 years)

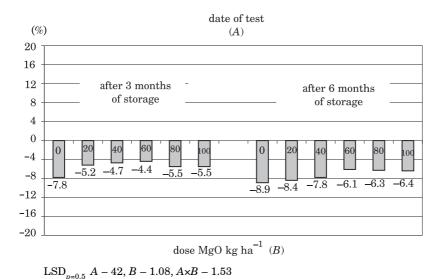


Fig. 3. Loss of fresh weight (%) of cv. Bila potatoes, depending on magnesium fertilization and date of test (mean from 3 years)

of magnesium on limiting natural losses was also reported by Ciećko et al. (2001) or Rogozińska and Jaworski (2001), who noted that it was the dose of 40 kg MgO ha<sup>-1</sup> which appeared significantly favourable.

A positive effect of potassium on limiting the quantitative losses, which coincides with the results of the present research, is reported by e.g. Jabloński (2000, 2001), Rogozińska, Jaworski (2001). Ciećko et al. (2001), on the other hand, when testing the same potassium doses as in the present research but different potato cultivar and storage conditions, did not identify any significant effect of that factor on the amount of storage losses.

In the experiment where varied nitrogen fertilization was applied, an opposite effect was recorded (Figure 1), since each of the doses increased the losses during storage as compared with the control. Irrespective of the storage time, maximum losses were reported at the dose of 100 kg N ha<sup>-1</sup>. A negative effect of nitrogen fertilisation on the storage life of tubers was also demonstrated in other research (Gasiorowska 2000, Jabloński 2006a,b, Rogozińska et al. 2002); it has been unanimously stated that nitrogen can lead to specific difficulties when storing potatoes, mainly delayed tuber ripening over the vegetation period and their increased susceptibility to storage diseases. Different results were reported by Jabloński (2006a,b), who demonstrated that levels of nitrogen fertilization (60 and 90 kg N ha<sup>-1</sup>) did not have any significant effect on natural losses after 6 months of tuber storage in a storage room (under optimal conditions); the average amount of losses reported did not exceed 7% irrespective of the cultivar and was almost the same as in the present research.

According to Sowa-Niedziałkowska (2004b) and Jabłoński (2004, 2006a,b), the cultivars in which the losses after a long-term storage period, irrespective of the affecting factors, do not exceed 12% are considered to be the material of good storage life, while the fresh weight losses exceeding 10% deteriorate the quality and result in an excessive loss of tuber turgor. The selection of cultivars is very important since, under the same conditions, much more commercial 'produce' of higher quality with lower financial outlays can be obtained from cultivars well surviving storage. The cultivars selected for the present research, Triada and Bila, demonstrated good storage life since fresh weight losses for those cultivars, irrespective of the factors analyzed, did not exceed 12% (Figures 1,2,3). Sowa-Niedziałkowska (2004b) stresses that each increase in natural losses, even by a single per cent, results not only in financial losses for the producer but also in worse quality of the tubers stored, and good quality is what the customer (the consumer, food industry) is interested in.

### CONCLUSIONS

- 1. Magnesium (60 kg MgO ha $^{-1}$ ) and potassium (160 kg  $\rm K_2O$  ha $^{-1}$ ) fertilization applied to potato growing limits the losses of fresh weight of potato tubers during storage, contrary to nitrogen fertilisation.
- 2. The best storage life was demonstrated for cv. Triada potato tubers, fertilized with potassium, and cv. Bila fertilized with magnesium.

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### REVIEW PAPER

## EFFECT OF MATERNAL SELENIUM SUPPLEMENTATION ON PREGNANCY IN HUMANS AND LIVESTOCK

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### Abstract

Following publications underlining the potential use of selenium (Se) in reducing the risk of prostate, skin, colorectal, liver, mammary and lung cancer, awareness of the importance of Se to human health has markedly increased. Moreover, Se status has been inversely associated with other health problems, such as impaired immune function, arthropathy and cardiomyopathy. The most important and well-known Se functions are represented by cell protection against oxidative stress and regulation of thyroid hormone metabolism. Recently, ongoing studies have focused on the relationship between Se intake and fertility and reproductive pathology, as demonstrated by the finding of low Se levels in blood and placenta of women suffering pre-eclampsia and intrauterine growth retardation. Similar problems have also been investigated in livestock and, since the concentration of Se in different soils and different geographical regions varies, addition of this element to animal feed is often required to prevent Se-deficiency diseases in production animal systems. Furthermore, Se supplementation in cattle and ewes is associated with increased embryo production, higher fetal mass and reduced incidence of retained placenta. However, the beneficial effects of supranutrional diet - above European Commission and Food and Drug Administration recommendations (0.5 and 0.3 mg kg<sup>-1</sup> dm, respectively), but below the maximum tolerable level established by the National Research Council (5 mg kg<sup>-1</sup> dm) - are affected by other environmental, nutritional and management factors (source of Se, time and length of the treatment, presence of interfering elements, diet feeding

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pattern). The relationship between Se supplementation and the risk of reproductive diseases are complex but recent developments represent promising results in order to refine dietary recommendations for both humans and livestock and develop effective health strategies.

Key words: selenium, pregnancy, placenta, fetus, humans, livestock.

# WPŁYW PODAWANIA SUPLEMENTU SELENU MATCE NA CIĄŻĘ U LUDZI I ZWIERZĄT HODOWLANYCH

### Abstrakt

Literatura opisująca zastosowanie selenu (Se) w celu obniżenia ryzyka wystąpienia nowotworów wyraźnie spowodowała wzrost świadomości znaczenia tego pierwiastka dla zdrowia człowieka. Ponadto interesujące jest, że poziom Se koreluje na zasadzie odwrotnej proporcjonalności z innymi problemami zdrowotnymi, takimi jak upośledzenie funkcjonowania układu odpornościowego, artropatia, kardiomiopatia, a nawet śmiertelność. Najważniejsze i najlepiej poznane funkcje selenu to ochrona komórek przed stresem oksydacyjnym i regulacja metabolizmu hormonów tarczycy. Ostatnie badania koncentrują się na związku między spożyciem selenu a płodnością i chorobami związanymi z procesem rozmnażania, który to związek wykazano poprzez oznaczenie niskich zawartości selenu we krwi i łożysku kobiet, u których występuje stan przedrzucawkowy i wewnątrzmaciczne zahamowanie wzrastania. Podobne problemy badano u zwierząt hodowlanych; ponieważ zawartość selenu w skorupie ziemskiej jest zróżnicowana w różnych miejscach, dodatek tego pierwiastka do karmy zwierząt jest wskazany celem uniknięcia chorób związanych z jego niedoborem. Ponadto suplementacja Se u bydła i owiec wiąże się ze zwiększoną produkcją embrionów, wysoka masą płodu i zmniejszoną czestością wystepowania zatrzymania łożyska. Jednak na pozytywne efekty diety wpływają inne czynniki środowiskowe, żywieniowe i związane z zarządzaniem, takie jak źródło selenu (organiczne lub nieorganiczne), czas i długość terapii, interakcja pierwiastków, skład diety. Potrzebne są dalsze badania mające na celu lepsze zrozumienie metabolizmu Se, szczególnie u przeżuwaczy, oraz wymagań dla optymalnego zdrowia. Związek między suplementacją Se i ryzykiem chorób reprodukcyjnych jest złożony, jednak wyniki ostatnich badań są obiecującym etapem na drodze do ustalenia dokładniejszych zaleceń dietetycznych zarówno dla ludzi, jak i dla zwierząt hodowlanych oraz opracowania efektywnych strategii prozdrowotnych.

Słowa kluczowe: selen, ciaża, łożysko, płód, ludzie, zwierzeta hodowlane.

### INTRODUCTION

Selenium (Se) is an essential trace mineral for normal growth and development in livestock and humans, primarily known for its antioxidant, anti-inflammatory and antiviral properties (Rayman 2000, McDowell 2003). Potential health benefits of supranutritional dietary Se in animals and humans include improved immune response and thyroid function, as well as cancer chemoprevention (use of natural or synthetic compounds to reverse, suppress or prevent cancer development) (Clark et al. 1996, El-Bayoumy 2004, Micke et al. 2005). Selenium's unique role in human physiology is also rep-

resented by the prevention of atherosclerosis, arthritis, diseases of accelerating aging, central nervous system pathologies and male infertility (RAYMAN 2000). Se deficiency in livestock leads to a nutritional myopathy (white muscle disease), ill thrift, infertility and impaired immunity (Van Metre, Callan 2001), while clinical cases are rare among humans (Burk, Levander 2006). In certain areas of China, Se deficiency predisposes patients to Keshan disease, an endemic viral cardiomyopathy primarily affecting children and young women (Koller, Exon 1986). In Siberian Russia and China, growing children with Se deficiency may develop chronic osteoarthropathy (Kaschin-beck disease) (Mo 1987). Since Se is required for the proper function of the immune system, it seems to be a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS (RAYMAN 2000). There is increasing evidence that Se supplementation of the maternal diet may be critical for progeny development and in humans a good Se nutrition has been suggested as an important factor for decreasing mortality and morbidity of preterm infants (Makhoul et al. 2004).

The present paper describes the results of numerous investigations into the effects of maternal Se supplementation on pregnancy and its role in normal placental and fetal development in both humans and livestock.

## SELENIUM FUNCTION AND SUPPLEMENTATION

Se is an essential nutrient that exerts its biological functions as a component of at least three groups of proteins: glutathione peroxidase that is responsible for the reduction of hydroperoxides in cells, plasma and gastrointestinal tract; the iodothyronine deiodinases, responsible for the peripheral deiodination of thyroxin (T3) to 3,5,3 triiodothyronine (T2) and other metabolites; and thioredoxin reductases, which are involved in many cell functions, including control of apoptosis and maintenance of the cellular redox state (Rooke et al. 2004). Glutathione peroxidase, the most well-known selenoprotein (Sel), is an antioxidant enzyme protecting tissue against oxidative damage (Khan et al. 1995, Rayman 2000) and, therefore, Se plays an important role in scavenging free radicals, in regulating prokaryotic and eukaryotic cell survival and maintaining the integrity of intracellular organelles (Hostetler et al. 2003). This antioxidant function is shared with Vitamin E and, although both can be synergistically beneficial, animals at grass or with other green foods should be of adequate vitamin E status, while dietary Se deficiencies are both more frequent and severe so that Se supplementation is strictly recommended (HEMINGWAY 2003). Se affects also cytochrome P450-dependent drug and xenobiotics metabolism (Shull et al. 1979) and counteracts the toxicity of several metals such as arsenic, cadmium, mercury and copper (Magos, Webb 1980). Moreover, Se plays a role in

increasing intracellular killing by neutrophils (Gyang et al. 1984), antibody production and lymphocyte function in ruminants (LARSEN et al. 1988). Unlike metals that interact with proteins in the form of cofactors, Se as a metalloid becomes cotranslationally incorporated into the polypeptide chain as part of the amino acid selenocysteine (Sec) and selenoproteins contain Sec as an integral part of their polypeptide chain (PAPPAS et al. 2008). Plants absorb Se from the soil in the form of selenite or selenate and synthesize selenomethionine (SeMet) (RAYMAN 2004). This means that Se in natural feed ingredients is mainly in the form of SeMet, which can also be synthesized by yeasts and some bacteria (Combs 2001). SeMet and Sec, the 21st amino acid (Böck et al. 1991), are identical to methionine and cysteine except for the sulphur (S) atom replaced by Se (Combs, Combs 1984). SeMet can be either reduced to form selenide or directly incorporated into proteins in place of Met (Butler et al. 1989). Vertebrates receive dietary Se in the forms of SeMet and other Se-amino acids (e.g. Se-methylselenocysteine, selenocystathionine) including Sec and its methylated forms, depending on their contents in food components. Although Se is widely distributed in the environment, Se content of food is greatly affected by the soil on which crops are grown or animals are grazed. Its concentrations and availability in soil in different countries are extremely variable and, specifically in Europe, the Se content of soil is low (McNeal, Balistrieri 1989). Therefore, currently, feeds for farm animals are supplemented with inorganic Se sources like sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>) and sodium selenate (Na<sub>2</sub>SeO<sub>4</sub>) as well as with an organic form of Se, e.g. selenized yeast (PAPPAS et al. 2008) at concentrations that do not exceed 0.5 mg kg<sup>-1</sup> (Commission Regulation EC 2006) and 0.3 mg kg<sup>-1</sup> dm (FDA 2004) in Europe and USA, respectively. The dietary requirement of Se for both ruminants and non-ruminants is 0.1 mg kg<sup>-1</sup> d.m. in the diet (Radostits et al. 2000), while the maximum tolerable level of Se has been increased from 2 (NRC 1980) to 5 mg kg<sup>-1</sup> d.m. (NRC 2005). However, it has been stated that lower value are necessary to avoid excessive accumulation in edible tissues (NRC 2005). The main problems with excess Se are due to mixing errors or overdosing with injectable preparations. In these cases, the toxicity is acute and is manifested by poor growth, high Se content in tissues, abnormal gait, vomiting, dyspnea, titanic spasm, laboured respiration, and death (NRC 2005). "Blind staggers" has historically associated with subacute to chronic Se toxicity, even if it cannot be reproduced with pure Se compounds alone and likely involves other factors, such as alkaloid poisoning starvation, or polioencephalomalacia (Gupta 2007). Chronic selenosis causes depression, weakness, anemia, hair/wool loss, diarrhea, weight loss, lameness, hoof wall abnormalities and death (GUPTA 2007). From first approval in 1974, either sodium selenate or sodium selenite could be used and, apparently, the two forms are of equal biopotency (Underwood, Suttle 2001). Indeed, it was found that, in the rat, 86% of a <sup>75</sup>Se-labeled oral dose of either sodium selenate or selenite is absorbed (Mason, Weaver 1986). However, when sodium selenite is orally given to ruminants, most of the Se is excreted in the feces (Cousins, Cairney 1961). Presumably, microorganisms and the reducing environment within the rumen promotes conversion of the selenite to less-soluble forms, such as elemental Se or selenides (Cous-INS, CAIRNEY 1961). Furthermore, numerous studies have shown the retention of the selenomethione form, as found in selenium yeast, to be greater than the inorganic sources in tissue and blood (Pehrson et al. 1999). Thus, while the use of inorganic Se may prevent severe selenium deficiencies, supplementation with selenium yeast could provide enhanced Se status and antioxidant function at times of the greatest stresses and disease challenge, such as in the periparturient period (WILDE 2006). Se may be supplemented in livestock as a free-choice Se mineral supplement, fertilization, injection, oral drenching, distribution in water or ruminal pellets (SZAREK et al. 1997). Alternatively, subcutaneous injection, usually as sodium selenite, or oral dosing with 10-30 mg of this compound for cattle and 1-5 mg for sheep are common means of preventing Se-responsive diseases in livestock. Heavy ruminal pellets, even in slow release bolus, prevent the occurrence of white muscle disease in sheep and cattle grazing Se-deficient pastures (UNDERWOOD, Suttle 2001). However, Se bioavailability and retention in ruminants is also influenced by diet composition, so that absorption of Se is greater in sheep receiving a concentrate-based diet than in sheep receiving a forage-based one (Koenig et al. 1997).

# EFFECTS OF SELENIUM SUPPLEMENTATION ON PREGNANCY IN HUMANS AND LIVESTOCK

Selenium seems to be an integral element for normal reproductive function (Allison, Laven 2000). It is known that the incidence of retained placenta, abortion, early embryonic death and ovarian cysts increase in Se-deficient cows (Segerson et al. 1977, Maas 1983, Harrison et al. 1984, Vipond 1984, Cook, Green 2007) and that Se supplementation overcomes some forms of infertility in ewes (Hartley 1963). These beneficial effects can also be observed in avians, since the inclusion of Se in the maternal diet improves the embryo viability, hatchability and growth of the progeny (PAPPAS et al. 2006). In animal models, it has also been demonstrated that Se may modulate ovarian granulosa cell proliferation and estradiol- $17\beta$  synthesis in vitro, affecting ovulation and the number of live embryos (Basini, Tamanini 2000). In humans, some studies have suggested that Se deficiency is related to adverse outcomes of pregnancy, miscarriages and preterm deliveries (Dobrzynski et al. 1998, Al-Kunani et al. 2001). In fact, both prenatal and postnatal Se supplementations are essential for proper functioning of antioxidant systems in offspring, already in place at the time of birth following prenatal supply and maintained after the first few days of progeny life by means of postnatal supplementation in maternal milk (PAPPAS et al. 2008). In man, several neonatal diseases (especially in preterm infants) are believed to be caused by oxygen free radicals, including bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis, patent ductus arteriosus and hypoxic-ischemic encephalopathy (Gathwala, Yadav 2002). Se is transferred via the placenta and the mammary gland, with the action of SelP (selenoprotein P), which contains Se in the form of Sec (selenocysteine) and it is the major Se transporting system (Burk, Hill 2005). Both inorganic and organic Se regulate the expression of SelP, but it seems that the transport process - bi-directional in placental tissue - may be a more complex action than a simple passive transport mechanism (Korpela et al. 1984). Se in the colostrum is one of the selenoamino acids that are well tolerated by the progeny (Przybylska et al. 2007). The importance of Se in pregnancy has been demonstrated in humans, where women suffering from preeclampsia have reduced levels of Se in umbilical venous blood compared to normal pregnancy (RAYMAN et al. 2003, MISTRY et al. 2008) and other studies have shown a decrease in maternal serum or toe-nail Se concentrations in preeclamptic patients (RAYMAN et al. 2003, ATAMER et al. 2005). Preeclampsia is a systemic and serious complication of pregnancy that affects from 2% to 7% of all pregnancies, leading to maternal and perinatal mortality and morbidity both in the western world and developing countries and it is often associated with intrauterine growth retardation (IUGR) (SIBAI et al. 2005). This disorder is characterized by hypertension, proteinuria, oedema and affects multiple organs, including the liver, kidney, brain and blood clotting system (Redman et al. 1999). Several lines of evidence support the oxidative hypothesis in the pathogenesis of preeclampsia, with placental underperfusion leading to an excessive production of reactive oxygen species and lipid peroxides causing oxidative stress and endothelial cell dysfunction (Hubel 1999). As expected, the activity of glutathione peroxidase is also decreased in preeclampsia as a function of Se depletion (Bulgan Kilicdag et al. 2005, Vanderle-LIE et al. 2005). HAN and ZHOU (1994) reports the beneficial effect of supplementation with 100 µg of Se per day in the prevention of pregnancy-induced hypertension and gestational oedema in a group of Chinese pregnant women. Moreover, Se supplementation in pregnant women effectively reduces the incidence of premature rupture of membranes (PROM) (TARA et al. 2010).

Similar findings have been reported in animals, although the effects of supplementation with Se on reproductive efficiency may be very variable (Hidiroglou 1979, Van Metre, Callan 2001, Hemingway 2003, Rooke et al. 2004), probably as a result of variations in factors such as the source of Se, the period of supplementation before mating, and the Se and vitamin E status of the animals. Moreover, parenteral Se supplementation of pregnant ewes between 15-35 days after mating results in reduced embryonic survival rate, so that supplementation of ewes during the first month of pregnancy with parenteral Se preparations is not recommended (Van Niekerk et al. 1996).

Regarding the effect of Se supplementation on placental development, cotiledonary tissue appears to be more susceptible to a greater amount of Se than caruncular tissue (Lekatz et al. 2010a, 2010b). In fact, it has been

demonstrated that there is an increased cellular proliferation and DNA concentration in cotiledonary tissue, but no effect on placentome number, mass and caruncular weight in supplemented ewes (Lekats et al. 2010a). Interestingly, a high amount of Se does not decrease proliferation in placental tissue - as in certain types of cancer in humans (Clark et al. 1996) - and the same finding occurs in the jejunal mucosa of pregnant ewes (NEVILLE et al. 2008) and beef steers (Soto-Navarro et al. 2004). Both placenta and small intestine consume a large percentage of maintenance energy in ruminants (Ferrell, Jenkins 1985, Reynolds et al. 1990), but the mechanism by which Se increases cellular proliferation in these nutrient-transferring and metabolically important tissues is unknown. How Se alters proliferation in metabolically active tissue such as placenta is, however, largely dependent on timing and length of Se treatment since Se supplementation from 21 days before gestation until near term in ewes (days 135 of gestation), as aforementioned stated, increases cotiledonary cellular proliferation (Lekatz et al. 2010b), while a supranutritional diet during mid- and late gestation decreases cell proliferation in the cotyledons (Lekatz et al. 2010a). Furthermore, high dietary Se leads to greater glutathione peroxidase activity in cotiledonary and caruncular tissue than in normal levels fed to sheep (Lekatz et al. 2010a). Se supplementation in ewes from 21 days before gestation until near term is also associated with increased Flt (VEGFR1) mRNA expression in cotiledonary tissue (Lekatz et al. 2010b). Flt increases vascular permeability (Peters et al. 1999), with following high fetal mass (Lekatz et al. 2010b). In dairy cattle, supplementation with Se reduces the incidence of retained fetal membranes (RFM) (failure of expulsion of the placenta and associated membranes within 24h of calving) (Allison, Laven 2000, Cook, Green 2007) and cows with RFM have lower glutathione peroxidase activity in maternal and placental tissues than cows without retained placenta (Kankofer et al. 1996). A study by Harrison and Conrad (1984) reported that animals supplemented with Se had no cases of RFM whereas the control animals have a 17% incidence. D'Aleo et al. (1983) reported similar results with control cows showing 20% incidence of RFM with a low Se diet against 0% among supplemented animals. Moreover, Se supplementation influences fetal development, as demonstrated by maternal supranutritional Se in ewes during mid- and late gestation that leads to increased fetal mass (Reed et al. 2007, Lekatz et al. 2010b, MEYER et al. 2010) and greater heart, lung, spleen, total viscera and large intestine weights compared to adequate supplemented foetuses (Reed et al. 2007). Fetal muscle RNA concentration and heart RNA content are also higher after supplementation (REED et al. 2007). Furthermore, the effect of Se on fetal body mass is much more evident in ewes subjected to nutrient restriction (REED et al. 2007), suggesting that Se may provide a sparing effect on fetal body mass, despite the low amount of nutrients (Godfrey, Barker 2000). In pigs, supplementation of pregnant sows with Se-Met also significantly increases the weaning litter weight and average piglets body mass (Hu et al. 2010). Fetal tissue cellularity, especially in intes-

tine, kidneys and liver, is responsive to maternal Se supplementation with selenate leading to a greater RNA: DNA ratio (index of cellular activity) in small intestine cells compared with High-Se wheat diet (Neville et al. 2008). These data also provide evidence of developmental programming of fetal internal organ cellularity in response to maternal Se supply (Neville et al. 2008). Regarding neonatal health and growth, Se supplementation of ewes' diet during early and mid-pregnancy, reduces the time taken for their lambs to stand and improves the immune status of the lambs, resulting in lower perinatal mortality and higher growth rates to weaning (Muñoz et al. 2009). Dietary Se intake seems to have an additional important effect on neonatal immune cell differentiation and function, since in neonates nursed by mothers fed a low-Se diet, the percentage of CD8 cytotoxic T cells, CD2 T cells, panB cells and natural killer cells are all decreased (Dylewski et al. 2002). Maternal and dietary Se may also influence antioxidant status in the testis of the offspring and the oxidative stress related to Se from the dam could modulate mRNA expression of apoptosis genes (Bcl-2, Bax and caspase-8) and programmed cell death of germ cells during spermatogenesis in the weaned kids (SHI et al. 2010).

In conclusion, the important effect of Se on animal and human reproduction underlines the role of dietary supplementation for increasing herd conception rates, lowering abortion cases in livestock and avoiding postnatal and prenatal feto-maternal disorders in humans. Nevertheless, the mechanisms of action of Se in many of these conditions are not fully known. In the context of ruminant production, data concerning the source and levels of selenium-rich feed are particularly important because of the rapid loading potential (Taylor 2005) and extended half-life of SeMet (Hawkes et al. 1994). SeMet-rich feed can be used to rapidly load animals and their foetuses or nursing offspring with Se before they are moved to remote Se-deficient ranges for an extended period of time. However, it should be taken into account that the variability in the herd response to supplementation may be due to a variety of dietary and management factors, including the concentration of interfering elements, the diet feeding pattern or the amount of concentrate which can affect the shrinking environment of the rumen, variations in soil characteristics and Se content between farms. In humans, providing antioxidants during pregnancy could decrease oxidative stress and may therefore prevent IUGR and preeclampsia. IUGR is a also major concern for the livestock industry because fetal growth restriction leads to negative impacts later in life on animal performance such as postnatal growth, body composition and reproductive performance.

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# Reviewers of the Journal of Elementology Vol. 16(1), Y. 2011

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- W przypadku stosowania skrótu po raz pierwszy, należy podać go w nawiasie po pełnej nazwie.
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